The gut immune system, inflammatory bowel diseases, and the body immune homeostasis: modern treatment strategies

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ABSTRACT

The digestive tract is nowadays conceived as a barrier organ constituted by a mucosal membrane separating the gut lumen from the inner milieu. The gut lumen is laden by a myriad of antigens brought about by the diet, but also pertaining to the overwhelming bacterial species of the gut microbiome. The mucosal cell population comprehends epithelial cells, and a variety of immune reactive cells. Of them, the mononuclear types effecting innate responses are endowed by membrane signaling receptors and, as a rule, are sensing the polysaccharides of bacterial cell walls; non-tolerated signals may then push the chain reaction on, to end in full activation of inflammation mediators. Acquired immunity is in turn mainly effected by T-cell types, some of them, behaving as autoreactive cells, may induce metastatic inflammation beyond bowel boundaries, partly explaining the so-called extra-intestinal manifestations of inflammatory bowel disease (IBD). The scenario is further complicated by the possible influence of epigenetic factors: diet, stress, smoking, drugs. Being IBD a low-penetrance disorder, for the full phenotype to develop, a critical mass of the above listed factors (typically, a disturbed membrane permeability, an immune stimulus, and an epigenetic factor) must occur. In the century since the full description of IBD, a variegated plethora of measures have been attempted. Some updated designs are now under scrutiny. Microbiota engineering, apoptosis modulation, and diet modification are just a few of the measures that we are arbitrarily describing here.

Key words: Inflammatory bowel disease, gut immunopathology, gut apoptosis, IBD modern treatment

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INTRODUCTION

The alimentary tract, devoted to life-keeping through the act of feeding, allows the highly antigenic outer world to largely enter the inner sterile milieu. To face such premises, a potent immune system has duly evolved to colonize the gut. This system is expected to exert two main functions: (a) the identification, elaboration, and eventual destruction of any antigenic invader; (b) the continuous control of such an immune patrol, with unchecked inflammation following loosening of the leash. These events may often assume the world-known phenotype of an "inflammatory bowel disease", which has long been familiar to clinicians under the species of Crohn's disease(CD), a ubiquitous granulomatous lesion plaguing patients with fistulae and abscesses, and Ulcerative Colitis (UC), a sort of Arthus-like phenomenon [1] restricted to the colon and mainly causing frequent bloody stooling. All gastroenterologists use to indicate the two entities with the acronym IBD (Inflammatory Bowel Disease) [2]. According to an underestimating guess, some 1 million people suffer from IBD in the US.

Functional anatomy

The gut-associated immune system is distributed between its primary mucosal/epithelial sites, and its secondary sites, including the Peyer patches and mesenteric lymph nodes, with dietary antigens [3] and the microbiome antigens [4] being the primary interlocutors. Generally speaking, disturbance of the intestinal immune reactions causing disease, may be expected when the gut luminal contents come erroneously in touch with the over-reactive immune system.

1. The errors at the base of a hypothetical IBD may thus be listed as :
    - Alteration of epithelial barrier;
    - Alterations of bacterial flora (microbiome);
    - Changes of innate immunity;
    - Changes of adaptive immunity.

Alteration of epithelial barrier

As a basic rule, control of the gut immune reactions is maintained if contact between the luminal antigenic load and the mucosa immune system is only allowed at specific spots (for example at the M cells area as seen below). The experimental basis of this tenet relies with work conducted in 1995 [5] which utilized chimeric mice for an altered N-cadherin, causing defective sealing of gut tight junctions. The animals showed obvious inflammation only in the lamina propria areas underlying the mutated cells, but not in the areas carrying no mutation. Anomalous inflammation may also be induced by purely functional modifications.

We first wish to refer to a family of cationic anti-bacteria peptides known as "defensins": the alpha-defensins released by Paneth cells, and the beta-difensins, as coming from colonocytes. In 2005, [6] Crohn's disease patients were shown to carry a 50% reduction of the expressed alpha difensin-5, whereas other defense factors were unaffected .

A second series of functional changes apply to the genic complex named IBD5 (organic cation transport or OCTN): its mutants were shown to cause release of an anomalous carrier unable to sustain the trans-membrane transport of a variety of antigenic items [7]. Interestingly, this note may be seen as a harbinger of the current tenet that Crohn's disease is in fact an immune deficiency state [8].

Furthermore, CD patients were shown to produce an exaggerated amount of a changed mucus, as opposed to UC patients, who in fact are poor mucus producers [9]. Independently from these points, anyway, an increased number of epithelia - adherent bacteria is common to both CD and UC [10]. The onset of an IBD in the given patient may reasonably be seen as the chance co-existence of genetic and mucus production anomalies, creating the critical mass eventually leading to the IBD trigger contact between the flora and the mucosa immune system.

Alterations of bacterial flora (microbiome);

The microbiome of IBD patients is definitely different from that of normal. Impressively, anaerobes like *Bacteroides*, *Eubacterium*, and *Lactobacillus species* are significantly reduced, thus jeopardizing biodiversity, an essential prerequisite to maintain bacterial strain redundancy, and to keep correct epithelial metabolism and nutrition [11,12]; furthermore, pathogens including *H. Hepaticus* and *paratuberculosis* agents appear to be increased in IBD colons [13]; this topic will be touched on below, when dealing with interleukin-17 insofar as being stimulated by several bacterial species.

While the question as to whether such microbiome anomalies are the cause or an epiphenomenon of IBD, it seems to be relevant to stress that animal strains genetically predisposed to develop IBD (IL-2 or IL-10 knock-out mice) fail to experience IBD if kept in germ-free conditions [14]; antibiotics are effective in CD [15]; lowering of pathogenic flora by using probiotics has proved effective in spontaneous IBD and in post-surgical pouchitis [16]. Different bacterial strains can stimulate different inflammation pathways, as discussed in the following paragraph.

Alterations of Innate Immunity

Innate immunity senses the lipopolysaccharides of the bacterial cell wall, whether speaking of commensal or of pathologic strains. Two main orders of sensors exert this function: Toll-like transmembrane receptors (TLR)

[17], and NOD cytosolic systems (nucleotide oligomerization domains) [18,19]. Upon activation by bacterial products, both sensor types initiate the inflammatory cascade by unblocking the nuclear factor kappa-b (NF-kB) [20]. Monomeric, dimeric, or heterodimeric TLRs all signal through a toll-interleukin-1 receptor domain, ending up with the activation of the MyD88 adapter protein [21]; MyD88 then recruits the kinases associated to IL-1 receptor, eventually joining the NF-kB final common pathway. NODs in turn belong to a family of structurally related intracellular proteins, encoded for by genes on chromosome 16, including: two caspase recruiting domains; one oligomerization domain; a leucine-rich terminal region containing ten LRR leucine-rich repeats [22]. Binding endotoxin and bacterial peptidoglycans, this region impacts innate immunity potential by allowing NODs to respond to sub-cellular antigenic stimuli [23]. Worth noting, LRR deletion from NOD structures enhances homodimer formation and signal intensity, suggesting that LRR regions mainly exert a down-regulating function [24]. The aminoterminal end of NODs contains two caspase recruitment domains (CARD) [25]; caspases are intracellular proteinases that regulate programmed cell death (apoptosis, see below, Fig. 1.), a crucial event in both inflammation and tumorigenesis [26]. Furthermore, the NOD CARDS are required to activate the NF-kB pathway. The NOD sequence itself is endowed with three more functions: 1) ATP binding; 2) boosting homodimer formation; 3)easing the interaction with RIP-2 [27], a signaling molecule in the TLR/NOD inflammatory chain.

Beginning 2001, independent researchers have published evidence linking specific NOD mutations with a few chronic granulomatous disorders exhibiting arthritis, uveitis, and dermatitis known as Blau syndrome [28,29]; LRR mutations by contrast have been associated to CD [30].

Mutations actually impacting the NF-kB pathway, hence leading to function down-regulation, can hardly be seen as causing enhanced inflammation such as in CD, and this apparent contradiction fueled intense scrutiny in those years. Two main working hypotheses were brought about. First, it was envisaged that the increase of host bacterial colonization following the reduced efficiency of the mutated NODs could enhance other defense stimuli fueling further inflammation. A second more elaborated and probably more likely hypothesis begins by underscoring that the Toll receptor 2 (TLR-2) responds to its ligand proteoglycan (PGN) and highly induces release of the pro-inflammatory interleukin IL-12, with an intact NOD down-regulating both this chain and NF-kB. A mutated NOD would no longer be able to down-regulating the TLR-2 –PGN reaction, thus explaining the paradoxically boosted inflammation in CD patients carrying the loss-of-function NOD mutation [31]. Interestingly enough, such omozygotic mutations have been found in some 10-15% of Caucasian patients [32], but in none of the Asian individuals [33].

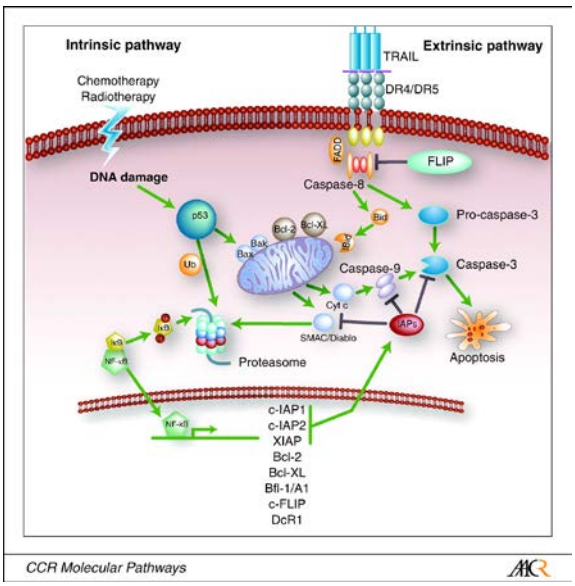

Figure 1. Please see text for details. The main event in the apoptosis sequence is structural and functional mitochondria disruption. In one pathway, it follows the caspase cascade, in the other, it is the amplifier event in the caspase cascade. Many antagonistic factors regulate apoptosis: The Bcl family proteins are inhibitors, the Bax/Bak are promoters. TRAIL: TNF-related apoptosis inducing ligand; FLIPS: FLICE inhibitory proteins; FADD: Fas-associated (MORT-1) protein with death domain Smac Diablo XIAP inhibitor: apoptosis inducer (an Open Access)

It must finally be emphasized that IBD patients do often exhibit a changed distribution of the TLRs, with subsequent anomalies of epithelial innate immunity. TLR-3 for example is under-expressed in CD patients; TLR-4 a pro-apoptotic factor is down-regulated on the epithelia of normal subjects, but is over-expressed on CD , inhibiting enterocyte turnover [34,35]. Furthermore, IBD epithelia over-express TLR-9 , a receptor that, if stimulated by bacterial DNA, can induce the granulocyte chemotactic IL-8. As a final note, one may emphasize that these changes work as non-specific enhancers of inflammatory reactions to ubiquitous stimuli: for example TLR-5 can increase its affinity for Flagellar antigens if placed on a previously damaged epithelium [36].

Alterations of adaptive immunity

The B cells. In the normal gut, B cells differentiate primarily into IgA secreting plasma cells. This IgA-supported immune reaction is fundamental in maintaining epithelial integrity despite ongoing immune reactions, insofar as IgA are known to fail fixing complement and are poor chemoattractants.

Interestingly enough, a vast array of antibodies circulating in the blood of IBD patients, and including Abs to the 40-kD protein, ANCA/ASCA reactions seem to be there to suggest that B cells are hyper-reactive in IBD.

The 40-kD protein belongs to colon, skin, and biliary epithelia: antibodies to this protein have mainly been suspected to be able to induce some of the extra-intestinal manifestations of IBD [37].

The pANCA (perinuclear anti-neutrophil cytoplasmic Ab) are circulated by some 70% of colitic patients [38] ; the ASCA reactivity (anti-saccharomyces antibodies) characterize CD [39]. Such reactivates are unlikely to fully be pathogenic; rather, they may correlate with a given disease subtype: for example the combination ANCA+/ASCA- seem to indicate IBD against indeterminate colitis with a 82% predictive value [40].

The T-cells. In homeostasis, the gut-associated lymphoid tissue includes the intestinal epithelium and the *lamina propria*, both of which contain non-naïve lymphocytes. Specifically, the lamina propria harbors IgA-secreting plasma cells, and CD4/CD8 alpha-beta T-cells in balanced proportions; CD8 positive T-cells, partly expressing the gamma-delta receptor are instead present in the epithelium [41]. In mice, the low frequency ratio between the precursors and their specific antigen (e.g. $1:2x10^5$), has made it necessary to evolve the so-called secondary lymphoid organs where naïve T-cells become educated [42]. The Peyer's patches and mesenteric lymph nodes are examples of such secondary immune stations [43]. In those areas various lymphocyte classes are allowed to come in contact with dendritic cells presenting modified dietary and bacterial antigens . The balance between naïve T-cells (Th0), Th1, Th2,Th17 and regulatory Foxp3 is maintained by pro- and anti-inflammatory drifts.

The IBDs may express a variety of anomalies of such immune steps, ranging from the mode of Ag presentation, to cytokine patterns, eventually causing prevalence of given pathogenic T-cell subsets.

Commensal antigens may be erroneously recognized and presented by **dendritic cells**, eventually leading to instruction of a TH1/Th17 response, as though they were dealing with pathogens [44]. Such a wrong pathway may derive from: (i) wrong recognition of bacterial patterns, possibly dependent on a wrong TLR display; (ii) inadequate ratio between mature (tolerization resistant) dendritic cells and immature subsets (tolerance responsive), with the former prevailing over the latter; (iii) aberrant reactivity to bacterial surrogates leading to loss of peripheral tolerance.

Non-professional cells (epithelial clones for example) may wrongly take over **as antigen presenting cells** [45]. Noteworthy, in the presence of pro-inflammatory cytokine signals (e.g. IFN-gamma) such surrogate cell types may acquire strong histocompatibility Ags and present Ag to activate T-lymphocytes in a non-tolerizable way.

This combination of factors may lead to an unbalanced maturation of pathogenic lymphocytes, prevailing over regulatory cells in IBD. Indeed, the CD immune scenario is Th-1 overexpressed with a gamma-IFN and alpha-TNF drift, whereas UC presents rather as a TH-2 phenomenon and IL4/IL5 hyperfunction [46]. NKT cells secreting IL-13 have recently been identified in UC as well [47].

Cytokine loops have further been targeted by recent studies. IL6, an effector of the hepatic acute phase response, may respond through STAT3 and favor inflammatory/tumorigenic pathways by blocking apoptosis [48]. IL8 behaves as a granulocyte recruiting factor [49]; IFN-gamma disturbs Ag presentation [50]; IL13 may cause severe colitis [51]. Quite recently, focus has been concentrated over the IL23/IL17 axis as a source of Th17 cells. The following paragraph deals with the TH17 cells potential to induce both gut and extra-intestinal pathology [52].

The Th17 lymphocytes

- Cell source: CD4+ T-lymphocytes and CD4+CD25-Foxp3- reg T-cells [53]
- Permissive environment: IL-17 positive environment after IL23 dependent STAT3 activation [54].
- Production of cytokines: IL17; TNF-alpha; IL6 [55]
- Physiological roles. Differentiation into Th17 progenies was first demonstrated in the case of bacterial/fungal infection, thus suggesting a primary protective role for Th17 cells. The infection list arousing Th17 cell clones includes *Klebsiella*, *Candida*, and *Mycoplasma* [56].
- Pathophysiological roles. Th17 cells can be found in the gut lamina propria, where they also home to, if injected [57]. When injected into Rag-1 deficient mice, Th17 cells promptly home to the gut, inducing local inflammation in some 7 days. In human spontaneous pathology, IL17 and Th17 cells can be retrieved from inflamed, but not healthy areas. Such homing is signaled by the CCR6 chemokine, with it being less pronounced in CCR6 deficient animals [57]. In view of the frequent rheumatic involvement in IBD, it is worth knowing that CCR6 and its receptor CCL20 are crucial in the development of Th17-dependent joint pathology [58].
- The Th17-dependent arthritis. Th17 t-lymphocytes have been shown to be able to induce bone resorption and autoimmune arthritis, behaving as osteoclastogenic helper T-cells. IL17 (--) animals are resistant to

experimental arthritis, and develop a milder uveo-retinitis. Again, articular homing proves to be enhanced by the presence of CCL20 and its ligand CCR6, with treatment with anti-CCR6 antibodies being inhibitory [59].

In conclusion, Th17 lymphocytes are biased to gut, where, in addition to regulating interaction between commensals and local immune tissue, can also manage various infections by releasing IL6/TNF-alpha upon stimulation in the loop IL23-IL17. In these premises, escaping autoreactive clones may well trigger autoimmune phenomena. Specifically, the interaction CCR6-CCL20 can induce arthritis, as frequently encountered in the clinics of IBD.

The gut immune system is continuously swinging between the need to restrain an invader within the defense trench of inflammation, and the opportunity to down-grade response to save tissue integrity. A high grade of resilience is needed to do the job. Balance is however fragile, and the system often sways towards local (IBD) and metastatic (extra-intestinal manifestations) inflammation. Since the first description of IBD, tons of measures and strategies have been tried to control the problem. We have arbitrarily chosen to speak of modulating programmed cell death (apoptosis) a highly conserved phenomenon; of ways to modify gut immune response by the use of genetically engineered bacteria; and finally of diet , as a primary antigen deliverance to gut.

FUTURE AVENUES IN IBD TREATMENT

Apoptosis as a target (reviewed in [60])

Apoptosis from the ancient Greek "to fall apart" is nowadays often indicated as "programmed cell death". It is very initial description may be traced back to a manuscript of C. Vogt, who, in the middle of the 19th century described the cellular aspects of amphibians metamorphosis [61]. The apoptotic program is latently present in all body cell types and can be found at work in the most variegated conditions, from sculpting of tissue during development to selection of immunocompetent cells in immune reactions (the latter being most relevant to this review). Researchers have often emphasized the differences between necrosis and apoptosis; we like to underscore only two among the many: (i) necrosis often affects contiguous groups of cells, whereas scattered single cells are preferentially hit by apoptosis; (ii) necrotic cell debris can attract phagocytes giving birth to full-blown inflammation, tissue reaction to apoptosis is often inconspicuous, easing tissue reorganization.

Brief morphological description of an apoptotic process. Once triggered by a variety of physiologic or paraphysiologic stimuli, the program goes through a defined series of steps. Morphological changes imply shrinkage of cell volume, paralleled by endoplasmic reticulum dilatation, and convolution of the plasma membrane. A series of membrane-bound spherical bodies containing compacted organelles do appear on the one hand, while on the nucleic side, chromatin condenses around the nuclear periphery. At a profound discordance with necrosis, many apoptotic cells fall apart from neighbors in the absence of any inflammatory reaction.

The protagonists of apoptosis.

- Death receptors and ligands. The subfamily of death receptors is part of the TNF/NGF receptor superfamily, characterized by a sequence of two to five cysteine-rich extracellular repeats, as opponents to an intracellular death domain, essential for transduction of the apoptotic signal. Death receptors mostly recognize their ligands in the TNF family. CD 95 is the best characterized death receptor [62].
- Caspases. Caspases are a family of cysteine proteases. We functionally distinguish the two "gatekeeper proteases" caspase8 and caspase 9, activated by either CD95, or by the cytochrome *c* released from mitochondria in the intrinsic pathway (see below). Basically, these proximal caspases activate down-stream caspases in a waterfall fashion, leading to the changes of organelles and nuclear chromatin that hallmark the apoptotic process. This is known as the execution phase of the process.
- The Mitochondria. The mitochondria are a sort of final common target of all apoptotic pathways. The main event of mitochondrial permeabilization is reached in three phases of cell death: (a) initiation; (b) decision; (c) degradation. The initiation phase marks the cell accumulation of the effector molecules that will eventually lead to membrane permeabilization; during the decision phase, permeabilization actually takes place, determining cell' s fate; then protein leakage, activation of lytic enzymes, and irreversible loss of function characterize the final degradation phase. The crucial patho-physiological event in mitochondrial death is the loss of the inner membrane integrity, causing permeabilization, loss of the transmembrane potential ($\Delta\Psi m$), and block of the respiratory chain. In apoptotic pathway 1, mitochondrial breakage follows the caspase chain reaction; in pathway 2, mitochondrial dysfunction is the primary event, eventually amplifying the executionary apoptosis caspase cascade.
- Regulation of apoptosis: the BCL-2 family. The BCL-2 family members include anti-apoptotic proteins (Bcl-2 and Bcl-xl) and pro-apoptotic proteins (Bax and Bak). There are

still uncertainties as to the mechanism of action of these regulators. One hypothesis holds that an anti- or pro-apoptotic action may depend on the ratio of the two functional messages on the polydimers that these proteins can form; alternatively, the effectiveness of Bcl-2 and Bcl-xl would imply their ligation with the adapter protein Apaf1, cytochrome c, and caspase 9.

A model of apoptosis at work: The CD95 system. CD95 trimerization is the leading event. Then, the adaptor molecule Fas-associated death domain/Mort-1 [FADD], pro-caspase-8, and CAP 3 are recruited to oligomerized CD95 to form a death-inducing signaling complex (DISC). The proper apoptotic pathway is initiated by a caspase-8 tetramer . Then mitochondria become activated entering the breakage program. As said above, in program 1 mitochondrial death follows the caspase cascade, in program 2, it actually serves as an amplifier of the caspase events [Fig.1]

Clinics of apoptosis with relevance to IBD. We have already alluded to the importance of apoptosis in IBD on at least two occasions. Firstly, antigen handling by non-professional dendritic cells which resist apoptosis may be one way leading to IBD chronicization; secondly, failure of T-cells to die out in apoptosis may perpetuate epithelial damage. Three of the routinely used drug classes in IBD treatment are known to be apoptosis activators. Sulphasalazine (but interestingly not aminosalicylic acid) [63] can induce mitochondria activated apoptosis. Through their metabolite 6-thioguanine, the thiopurines azathioprine and 6-mercaptopurine can inhibit RAC-1 and the efficacy of anti-apoptotic factors in lymphocytes [64]. The anti-TNF monoclonal antibody Infliximab induces caspase 8-9-3 activation, and mitochondrial and BAX-BAK response [65]. Last but not least, one should mention the results of recent animal studies on the natural cyclic peptide beauvericin [66]. Among other actions, this peptide was shown to promote apoptosis of activated T-cells by suppressing Bcl-2.

Engineered Pro-biotics. We now firmly know that the human gut indwells some 10^{14} bacterial entities, currently renamed as "the microbiome". A recent paper of ours [4] reviews the matter and the reader is referred to it for an exhaustive data presentation and discussion. The microbiome may contain several potentially harmful species. *Prevotella copri* may arouse rheumatoid arthritis pictures, mutated strains of *Campylobacter concisus* may contribute in the increase of gut permeability, perhaps triggering IBD-like deviations, *Bilephila wadsworthi* may thrive in response to fatty diets, and induce epithelial ulceration by raising pro-inflammatory Th1 cell clones. Beside this, a number of "positive" effects are ascribed to the microbiome individuals: control of glucose metabolism, control of body weight, control of neurologic disease. Noteworthy, the microbiome thrives on a fiber-rich diet, releasing SCFA (short chain fatty acids)as by-products that in turn fuel gut epithelial integrity. On this background, no wonder that attempts have been made to take advantage of genetically modified strains to sharpen their therapeutic potential. In 2011, the development [67] of a mutant variety of Lactobacillus Acidophilus was described. Genetically engineered, this strain lacked functional lipoteichoic acid, a surface-expressed component whose absence deprives the strain of the ability to be immunogenic through dendritic cell presentation, down grading bacterial adherence properties. In an initial series of co-culture experiments, these engineered strains showed a scarce ability to induce TLR-2 expression on antigen-presenting dendritic cells, with concurrent reduction of TNF-alpha production, and increased release of the down-grading signal from IL10. In a second series of experiments, involving animals, three classic models were used: the model of dextran-sodium-sulphate (DSS) induced colitis, the colitis mediated by CD4+ $CD45RB^{high}$ T-cells, and the IL10 knock-out mouse models. Inoculation of the animals with the mutated strains prevented colitis in the DSS model, and was accompanied by IL10 release and reduced levels of pro-inflammatory signals including IL12 and TNF-alpha. The engineered strains showed also a therapeutic potential, insofar as established DSS colitis was found to be mitigated by injection of the relevant strains. We like now to conclude this paragraph by a succinct review of the use of genetically modified lactic acid bacteria (LAB) to treat IBD [68].

LABs that are genetically modified to produce anti-oxidant enzymes. It is known that in IBD a significant oxidative stress is going on as mediated by continuous release of ROS. Since a few micro-organisms do spontaneously exert poor anti-oxidant activities, it looked rational to experimentally tackle the problem. Briefly, it was found that strains of *L. plantarum* and *L. lactis*, modified to release super oxide dismutase (SOD) were able to mitigate TNBS induced colitis

Modified LABs that produce the anti-inflammatory cytokine IL-10. Back in the year 2000, it was shown that strains of *L. lactis* secreting IL-10 were effective in mitigating DSS related colitis. Unfortunately, preliminary experiments in humans affected by spontaneous colitis have not been that rewarding.

Strains of LABs were further modified to express various anti-inflammatory complexes, including monovalent and bivalent murine TNF neutralizing antibodies, the helper-T-cell regulating IL27, and TGF-beta. All of these measures were shown to virtually exert a down-grading potential of colitis in animal models.

As a general caveat, a deal of work has still to be done to fully assess the safety of such genetic

contractions in human use. In any way, it will take time before consumers accept to ingest genetically engineered bacterial products that continue to be perceived as "non-natural".

The role of the diet in gut pathophysiology and induction of IBD [69,70]

Several matters of fact, discussed in the preceding pages of this review as well as in the literature, point to genetic factors being crucial for development of an IBD: increased disease frequency in relatives of affected patients; IBD concords in a greater measure in monozygotic than dizygotic twins; co-segregation of IBD-like syndromes in families with rare genetic disorders; identification of 47 risk alleles for UC, and 71 for CD as of 2011. Genetic signatures, however, do not satisfactorily account for two orders of observations. Firstly, genetic polymorphisms often do not account for more than some 30% of CD cases, and may be absent from non-Caucasian subjects, or those with UC (see for example the case of NOD polymorphisms discussed in the preceding paragraphs). Genetic arguments, in addition, do not explain the dramatic increase of the incidence of IBD (together with that of diabetes, obesity, and colorectal cancer) over the past half-century. In the same time period, instead, it is easy to identify profound changes in our feeding habits, and food accessibility. The advent of modern agriculture and animal husbandry on the one hand, and the industrial revolution on the other hand, all led people to rapidly abandon consumption of nearly raw plant products, in favor of highly refined food, mostly of a sugar and fat nature. The advent of large-scale market distribution has eventually tipped the balance towards preference for packaged food and beverage, easy-to-store-, easy-to-eat, nicely colored to look at. Among other variables, this kind of nutrients have allowed for the addition of a large number of chemical additives. All-in-all, it is intriguing to note that independent sources have now pinpointed that easy access to hyper-caloric/ industrially manipulated food has posed the basis for the rise of childhood obesity. This early pathologic weight gain, in turn, has been recognized as the one factor in the increase of the inflammatory tune [71], a chronic bias that seems to pervade us since early in life. Indeed, this inflammatory background might be the reason to thrive for IBD and the tens of the inflammatory "Western" disorders named above

These profound changes, as expected, have significantly impacted our overwhelming microbiome population, which, as emphasized above, crowds our gut with some 10^4 microbic individuals. In balanced conditions, the microbiome behaves as resilient and stable, under the influence of a stabilizing selection. In the presence of the social and feeding changes delineated above, directional selection takes over, not only moving our human physiology to a new set point, but also forcing the microbiome to adapt. At this point, it is still hard to clearly unfold the intricate interplay between genetics, germs, and environment **(diet)** in the induction of human IBD. Data from IL-10-/- mice (colitis permissive) can be oversimplified as follows: (1) If kept germ-free (commensal free) these IL-10 negative animals do not develop any experimental colitis; (2) the milieu of enteric microbiota decides the type and severity of spontaneous colitis, with for example , penetrance attaining nearly 100% when the population contains *Helicobacter hepaticus* inducing a Th1 response; (3) in such IL-10 minus mice disease phenotype is manifestly decided by the gut bacteria present: *B. wadsworthia* can induce low-grade distal colitis with a Th1 mediated response – *E.coli* by contrast, leads to mild-moderate cecum inflammation.

A specific look at the role of diet. We know that diet components can alter the composition and virulence of commensals: as emphasized above changing of source, nature, and elaboration of nutrients may partly explain the sharp rise of Westernized disease over the last 50 years. Increased consumption of fat has easily been blamed in recent studies, but soon things have turned out to be more intricate. Devkota *et al.* [69,70] have shown that microbe re-shaping, microbe-host interaction perturbation, and eventual colitis appearance can efficiently and reliably be driven only by milk fat, through favoring *B. wadsworthia* growth in the permissive IL10-/-mice model. Noteworthy, the authors themselves emphasized the "individualistic" limits of this finding, whereby other genetically marked individuals can well be expected not to develop colitis in the same condition. The same words of caution must apply to the well-known issue of short-chain-fatty acids. Several studies have emphasized use of pre-biotics to enhance growth of *Bifidobacterium* and *Lactobacillus* that in turn release the SCFA on which colonocytes may thrive and enhance their physiological barrier functions. Again, we lack evidence that invariably all IBD patients may utilize SCFA in such a way to mend their disease.

From the above data, a concept of IBD may stand as a sort of three-hit disorder, whereby the three hits include genetics, the microbiome, and a plethora of adding factors with diet in the chief rows. The individual and reciprocal weight of these elements still elude our search. As it stems from the paragraphs above, when dealing with the immunopathology of IBD and struggling to find a causative treatment, no single policy can be pretended to bear a universal value. The call, as finally discussed below, is for an individualistic approach

A futuristic vision in IBD management (in specific) and in internal medicine (in general) ***Signals*** *deriving from the IBD fields, but also from other medicine areas, are beginning to call*

clinicians towards the envisaging of treatment plans at the individual level, rather than at a one-size-fits-all level in order to specifically tailor their prescriptions. This is evident also when dealing with prescriptions of "conventional" drugs, wherein physicians are called to identify peculiar disease signatures to lead them to choose options in a "precise" medicine fashion [72].

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