

## Review

# Gut microbiome and anticancer immune response: really hot Sh\*t!

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The impact of gut microbiota in eliciting innate and adaptive immune responses beneficial for the host in the context of effective therapies against cancer has been highlighted recently. Chemotherapeutic agents, by compromising, to some extent, the intestinal integrity, increase the gut permeability and selective translocation of Gram-positive bacteria in secondary lymphoid organs. There, anticomensal pathogenic Th17 T-cell responses are primed, facilitating the accumulation of Th1 helper T cells in tumor beds after chemotherapy as well as tumor regression. Importantly, the redox equilibrium of myeloid cells contained in the tumor microenvironment is also influenced by the intestinal microbiota. Hence, the anticancer efficacy of alkylating agents (such as cyclophosphamide) and platinum salts (oxaliplatin, *cis*-platin) is compromised in germ-free mice or animals treated with antibiotics. These findings represent a paradigm shift in our understanding of the mode of action of many compounds having an impact on the host–microbe mutualism.

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## Facts

- The anticancer efficacy of cyclophosphamide (CTX) and platinum salts is reduced in germ-free (GF) mice and animals treated with antibiotics.
- Gut microbiota is involved in the reprogramming of intratumoral myeloid cells.
- Gram-positive commensal bacteria translocate during chemotherapy and prime pathogenic Th17 (pTh17) cells contributing to the tumoricidal activity of cytotoxic compounds.
- Intestinal microbiota is important for the bioactivity of immunomodulators (such as a combination of anti-IL-10R and Toll-like receptor 9 (TLR9) agonists).

## Open Questions

- To what extent gut microbiota interferes in the bioactivity of therapeutics?

- How to noninvasively explore microbial dysbiosis or mucosal barrier dysfunctions at the level of the small intestine (SI) and large intestine?
- How intestinal and systemic immunity are interconnected to modulate the effects of drugs?
- How anticomensal immune responses correlate with specific anticancer cellular immunity and patient prognosis?
- What are the most efficient probiotics capable of eliciting helper T-cell responses against cancer?

## Gut Microbiota, Health and Diseases

The distal intestine of humans contains tens of trillions of microbes (of thousands different species representing over 2 kg of material). These microbiota and associated genomes (called ‘microbiome’) have been characterized by metagenomic analyses combining next-generation sequencing of both targeted (16S rRNA hypervariable region) and random

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**Abbreviations:** AhR, aryl hydrocarbon receptor; AOM, azoxymethane; ASC, apoptosis-associated speck-like protein; ATB, antibiotics: streptomycin + ampicillin + colistin; ATBx, antibiotics: imipenem + vancomycin + neomycin; BCG, bacillus Calmette–Guerin; BM, bone marrow; CTX, cyclophosphamide; DC, dendritic cell; DSS, dextran sodium sulfate; GF, germ free; GI, gastrointestinal; HDAC, histone deacetylase; IEC, intestinal epithelial cell; FADD, Fas-associated protein with death domain; GC-C, guanylate cyclase C; hPepT1, human intestinal H-coupled oligonucleotide transporter; IDO, indoleamine 2,3-dioxygenase; Ig, immunoglobulin; LN, lymph node; LP, lamina propria; LPS, lipopolysaccharide; LTA, lipoteichoic acid; MHC, major histocompatibility complex; NEMO, NF- $\kappa$ B essential modulator; NLR, NOD-like receptor; NLRP, NLR family pyrin domain-containing protein; PAMP, pathogen-associated molecular pattern; PD-1, program death-1; PRR, pathogen recognition receptor; PSA, polysaccharide A; pTh17, pathogenic Th17 cells; ROS, reactive oxygen species; SFB, segmented filamentous bacteria; SI, small intestine; SPF, specific pathogen free; STAT, signal transducer and activator of transcription; TAK1, TGF- $\beta$ -activated kinase 1; TCR, T-cell receptor; Tg, transgenic; TLR, Toll-like receptor; TNBS, 2,4,6-trinitrobenzene sulfonic acid; VDR, vitamin D receptor; WASP, Wiskott–Aldrich syndrome protein; WT, wild type

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(whole-genome shotgun) bacterial DNA sequences.<sup>1,2</sup> The first extensive catalog of the intestinal metagenome outnumbers the size of the human genome by a factor of 150.<sup>3</sup> The human gut is colonized by microorganisms belonging to the domains Bacteria, Archaea, Eukarya and their viruses. The three main phyla characterizing the dominant human intestinal microbiota are *Firmicutes*, *Bacteroidetes* and *Actinobacteria*. Metagenomic studies revealed substantial interindividual variations at the genus and species levels, possibly because of genetic and environmental factors.<sup>3,4</sup> Recently, an interesting but still controversial notion has emerged as to the existence of 'enterotypes' characterized by dominant genera (*Bacteroides*, *Prevotella* and *Ruminococcus*) and their co-occurring phylogenetic traits that could be associated with long-term dietary habits.<sup>5,6</sup> The *Bacteroides* enterotype was associated with animal protein and saturated fats, whereas the *Prevotella* enterotype was predominantly observed with high fiber/plant-based nutrition and high carbohydrates (+ low meat and dairy consumption).<sup>5,7,8</sup> The third enterotype dominated by *Ruminococcus* often merged with the *Bacteroides* one.

The microbiome present in the distal gut performs myriad functions protecting the host against pathologies.<sup>3</sup> Indeed, the host-microbiota symbiosis has evolved in three directions. First, colonization by commensal microorganisms is key to immune development.<sup>9-12</sup> Second, the commensal community keeps in check invading pathogens and prevents them from expressing virulence.<sup>13,14</sup> Third, the intestinal microbiota appears to digest glycans and regulate fat storage in mice and potentially in humans.<sup>15,16</sup> Exemplifying the host-microbe mutualism, the microbial genome is highly enriched in hundred families of glycoside hydrolases and in more than 20 families of polysaccharide lyases, whereas the human genome is relatively devoid of these carbohydrate-metabolizing enzymes.<sup>17</sup> Finally, intestinal bacteria are essential for the postnatal development of the enteric nervous system in the mid-distal SI.<sup>18</sup>

The growing awareness of the importance of the gut microbiome in health and disease and recognition of the host-microbe mutualism at the immunological and metabolic levels become crucial for a better understanding of immunopathologies such as autoimmune and inflammatory disorders, allergy and obesity. Microbiome differences between controls and cases have been described for a variety of diseases such as inflammatory bowel disease (both Crohn's disease and ulcerative colitis), obesity, type 2 diabetes, autism and allergies, and involve abnormalities in the relative abundance and representativity of distinct commensal bacteria. A 'one microbe-one disease' algorithm has yet only been described for a limited number of pathologies, such as *Helicobacter pylori* and gastric ulcers.<sup>19</sup> However, it remains questionable whether a deviated repertoire of the intestinal microbiota, called 'dysbiosis', associated with an expanding list of chronic disorders<sup>20</sup> may be seen as a causative agent in disease or is just a by-product of the disease. Transplantation experiments in which microbiota of a disabled mouse is grafted into a GF healthy recipient have highlighted that several disease phenotypes (such as adiposity, metabolic syndrome, colitis eventually causing cancer) can be transmitted by gut microbiota.<sup>20-22</sup> Therefore, gut microbiota becomes progressively considered as a tractable environmental factor highly

quantifiable, relatively stable, resilient within an individual and potentially drug targetable (prebiotics, probiotics). Hence, it becomes increasingly important to decipher the genetic potential (metagenomics) as well as the functions (metatranscriptomics) of the gut microbiome and its causal relationship with diseases.

## Microbiome and Cancer

Cancer susceptibility and progression results from a complex interplay between gene regulation and the environment. Microbial communities inhabiting our intestine and other portals of entry represent so far unappreciated environmental factors that appear to have a role in carcinogenesis. Pioneering studies performed in GF mice or animals exposed to specific bacteria in specialized facilities (gnotobiotic mice) or in antibiotic-treated rodents revealed an unsuspected role of commensals or pathobionts in tumorigenesis driven or not by inflammation. In the genesis of colon cancer, at least in the 2% cases induced by a pre-existing inflammatory colitis, several studies demonstrated that microbiota can influence inflammation or innate immunity, genomic stability of intestinal epithelial cells (IECs) or the release of metabolites functioning as histone deacetylase (HDAC) inhibitors to regulate epigenetically host gene expression.<sup>22-34</sup>

Integrating all the current data, Tjalsma *et al.*<sup>35</sup> proposed a bacterial driver-passenger model for microbial involvement in the development of colorectal cancer, implying that bacteria must be incorporated into the genetic paradigm of cancer progression. According to this model, distinct indigenous intestinal bacteria, the 'driver bacteria' would create DNA damage and drive genome instability to initiate the first steps of tumorigenesis. Bacterial drivers may progressively disappear in favor of opportunistic bacteria, that is, 'passenger bacteria', which then overwhelm the intestinal niche alterations and corrupt the local innate immunity. For instance, the human colonic bacterium, enterotoxigenic *B. fragilis*, induces colitis and colonic tumors in multiple intestinal neoplasia (Min) mice following signal transducer and activator of transcription 3 (STAT3) induction and IL-23-dependent Th17/γδT17 immune responses.<sup>36</sup> Indeed, it appears that the NF-κB-IL-6-STAT3 cascade is a crucial regulator of the proliferation and survival of tumor-initiating IECs.<sup>37</sup> Defective expression of several barrier proteins (due to genetic defects initiating the first step of colon cancer) facilitates the adenoma invasion of microbial products, which in turn stimulate tumor-associated myeloid cells. Then, such inflammatory monocytes and precursors become potent producers of IL-23, a protumorigenic cytokine.<sup>38</sup>

## Microbiome and Immune Functions

Gut microbiota is critical for intestinal immune maturation, protecting the host against pathogens and damaging inflammatory reactions. GF mice have smaller Peyer's patches, fewer plasma cells and impaired immunoglobulin A (IgA) secretion, fewer intraepithelial lymphocytes, as well as compromised release of antimicrobial peptides among other immunologic deficiencies.<sup>39</sup> Many of these immune defects are corrected by recolonization with a healthy mouse

commensal microbiota. SI immune maturation depends on a coevolved host-specific microbiota. Although gut bacterial numbers and phyla abundance were similar after transplantation of human or rodent fecal material into GF animals, bacterial species among *Firmicutes* differed and were associated with lower numbers of dendritic cells (DCs) and T cells, mostly proliferating and memory T cells (among which Th17 cells) in the SI lamina propria (LP) and mesenteric lymph nodes (LNs) and weaker protection against pathogens.<sup>40</sup> These data underscore that exposure to just any gut commensal microbes or their pathogen-associated molecular patterns (PAMPs) (such as lipopolysaccharide (LPS) or lipoteichoic acid (LTA), etc.) is insufficient to induce intestinal immune maturation.

Commensal microbiota actively shapes intestinal T-cell responses. Among the commensal bacteria, some organisms appear to have a greater impact than others on mucosal immunity. Some studies suggest that there might be a compartmentalization of the microbiota along the gastrointestinal (GI) tract dictating mucosal immune homeostasis with Th17-dominated immune responses in the ileum and Treg in the colon. Commensal bacteria (such as *B. fragilis*) have a positive effect on Treg numbers. *B. fragilis* induces IL-10 production by Treg in the colon in a polysaccharide A-dependent manner.<sup>41</sup> The *Clostridium*-forming spore (cluster IV and XIVa) induced robust differentiation of inducible Foxp3<sup>+</sup> and IL-10<sup>+</sup> Tregs in the mouse colonic LP.<sup>42</sup> Segmented filamentous bacteria (SFB), found in numerous vertebrate species but not in humans after 3 years of age, are potent inducers of SI Th17.<sup>43,44</sup> SFB colonization protects mucosae from aggression induced by the enteropathogenic bacterium *Citrobacter rodentium* through a mechanism involving IL-17 and IL-22.<sup>43</sup>

Moreover, the intestinal microbiota can also influence systemic immune responses. A recent work highlighted the role of certain metabolites (short-chain fatty acid butyrate and to a lesser extent propionate) produced by commensal bacteria in dictating the extrathymic differentiation of peripheral regulatory T cells.<sup>45</sup> Butyrate acts within T cells to enhance acetylation of the Foxp3 locus and protein, as well as DCs to decrease their proinflammatory NF- $\kappa$ B-dependent cytokine secretion profile through an HDAC inhibitory activity. Gut microbiota also controls the systemic Th17 pool. The incidence of an IL-17-dependent autoimmune arthritis developing in genetically susceptible hosts was reduced in GF conditions in which the autoantibodies titers as well as the splenic Th17 cells was also significantly decreased compared with specific pathogen-free (SPF) mice. Reintroduction of SFB into GF mice reconstituted the LP Th17 pool, raised the autoantibody titers and drove the development of arthritis.<sup>46</sup> In another example of autoimmune disease, type 1 diabetes (T1D) developing in SPF-free non-obese diabetic (NOD) mice, MyD88 protein was mandatory to drive T1D. The effect was dependent on commensal microbes because GF MyD88-negative NOD mice developed T-cell priming in pancreatic LNs and islet  $\beta$ -cell infiltration by T lymphocytes, whereas colonization of these GF MyD88-negative NOD mice with altered Schaedler flora attenuated T1D. Moreover, MyD88 deficiency changed the composition of the caecum microbiota (reduced *Firmicutes* to *Bacteroidetes* ratio, increased

*Lactobacilli*) and transmission of the microbiota of SPF MyD88-negative NOD donors attenuated T1D in GF NOD recipients.<sup>47</sup>

Altogether, the gut microbiota instructs the local and systemic immune system in a mutualistic/symbiotic manner.

### Microbiome and Therapeutics

Mucositis (mucosal barrier injury) is a major oncological problem caused by chemotherapeutic agents used against malignancies. Oral and small (and to a lesser extent large) intestinal mucositis translating into a variety of clinical symptoms (diarrhea, vomiting) can be worsened by neutropenia and antibiotics. As IECs do not regulate intestinal homeostasis in a solely intrinsic manner but require symbiotic coordination with commensal bacteria and local gut leukocytic cells, the role of intestinal microbiota in the development and severity of mucositis induced by chemotherapeutic products has been proposed.<sup>48,49</sup>

It is well established that chemotherapeutics elicit their proapoptotic activity against rapidly proliferating cell populations, meaning not only tumor cells but also intestinal stem/progenitor cells.<sup>50</sup> However, rapid regeneration occurs between 96 and 168 h after doxorubicin with increased numbers of CD45<sup>-</sup> stem/progenitor cells, goblet cells, Paneth cells and enteroendocrine cells, crypt fission and crypts.<sup>51</sup> The involvement of microbiota in the GI toxicity of irinotecan (CPT-11, 7-ethyl-10-(4-(1-piperidino)-1-piperidino) carbonyloxy-camptothecin) used to treat colorectal and other cancers has been reported.<sup>52</sup> *In vivo*, CPT-11 is converted to the pharmacologically active SN-38, which is responsible for both antitumor activity and dose-limiting toxicity. SN-38 undergoes hepatic glucuronidation and is secreted into the bile as an inactive glucuronide SN-38G.<sup>53</sup> Deconjugation of SN-38G in the colon by bacterial  $\beta$ -glucuronidases exposes intestinal epithelia to SN-38, mediating gut toxicity. Moreover, specific bacterial organisms translocate from the intestine of CPT-11-treated animals and cause systemic infection and sepsis. Prophylaxis with antibiotics reduced SN-38 concentration and/or diarrhea both in animal models and patients.<sup>54</sup> Glutamine, a key 'pharmaco-nutrient', protects the gut during a variety of stress conditions,<sup>55</sup> including cancer chemotherapy.<sup>56</sup> Oral glutamine reduced the incidence and severity of late-onset diarrhea following CPT-11 treatment in rats. Dysbiosis induced by CPT-11-based chemotherapy increased the abundance of intestinal *Enterobacteriaceae* and *Clostridium* cluster XI. Glutamine mediated several potentially protective responses (such as heat-shock protein induction, reduced  $\beta$ -glucuronidase activity in the caecum), increased in the ratio of reduced to oxidized glutathione and memory CD8<sup>+</sup> T cells in mesenteric LNs, eventually mitigating the dysbiosis induced by the tumor status and CPT11 administration.<sup>57</sup>

Besides the conventional cytotoxic compounds compromising cell cycle to facilitate apoptosis, a new class of therapeutic agents has emerged in the oncological armamentarium, whose mode of action is the effective blockade of T-cell inhibitory receptors (CTLA4, program death-1 (PD-1)). These antibodies also induce diarrhea and eventually colitis that could contribute to systemic inflammatory processes by

mitigating intestinal homeostasis. Fagarasan and co-workers<sup>58</sup> reported that PD1<sup>-/-</sup> mice presenting excess numbers of follicular helper T cells (overexpressing TGF $\beta$ 1 and impaired for IL-21 production) exhibit a reduced affinity maturation of IgA with reduced bacteria-binding capacity, causing a strong bias in the gut microbiota composition (loss of anaerobic bacteria, loss of *Bifidobacterium* and *Bacteroidaceae*, increase in *Enterobacteriaceae* and at the genera level, increase in members of the *Erysipelotrichaceae*, *Prevotellaceae*, *Alcaligenaceae* and TM7 genera incertae sedis). The skewed gut microbial communities and the leaky gut barrier leads to a generalized activation of self-reactive B and T cells and production of autoantibodies.<sup>59</sup>

Therefore, we surmise that any compound compromising the intestinal barrier integrity and/or the innate mucosal immunity and/or directly the gut microbiota will affect the functional equilibrium of this compartment and cause symptoms, as well as distant immunological perturbations.

### The Adjuvant Effects of Bacteria Against Cancer

Contrasting with the above-quoted exemplifications of bacteria driving carcinogenesis, other observations support a beneficial role of distinct bacteria against cancer. Hence, a prolonged combination of metronidazole and ciprofloxacin tripled breast cancer occurrence in proto-oncogene HER-2/*neu*-driven transgenic (Tg) mice.<sup>60</sup> In humans, some epidemiologic studies suggested a dose-dependent association between antibiotic use and risk of breast cancer.<sup>61,62</sup>

Since the first report in 1976, accumulated clinical evidence has supported intravesical bacillus Calmette–Guerin (BCG) therapy as one of the standard methods of management of intermediate- and high-risk non-muscle invasive bladder cancer.<sup>63</sup> Intravesical immunotherapy with BCG to prevent recurrence of these tumours has been shown to involve the participation of three different TLRs (TLR2, TLR4 and TLR9).<sup>64,65</sup> Despite the fact that BCG is viewed by the immune system as ‘pathogenic’, BCG inoculations performed as classical vaccines failed to prevent relapse of stage II metastatic melanoma in pioneering studies.<sup>66</sup>

Instead, distinct commensals could have a beneficial role in melanoma. Intratumoral inoculation of 3 mg of heat-killed *Propionibacterium acnes* in subcutaneous melanoma could promote local and systemic Th1 and Tc1 responses associated with *in situ* granuloma formation and tumor regression.<sup>67</sup> *P. acnes* is recognized by TLR2 on monocytes, macrophages and DCs, leading to the activation of IL-12 promoter.<sup>68</sup> Therefore, it is conceivable that certain commensals be involved in the natural immunosurveillance of malignancies exposed to the portals of entry (such as ulcerated melanoma). Hence, lung tumors presenting tertiary lymphoid organogenesis exhibit a more favorable prognosis, perhaps towing to chronic stimulation with environmental microorganisms.<sup>69</sup>

### Beneficial Effects of Commensals During Cancer Therapy

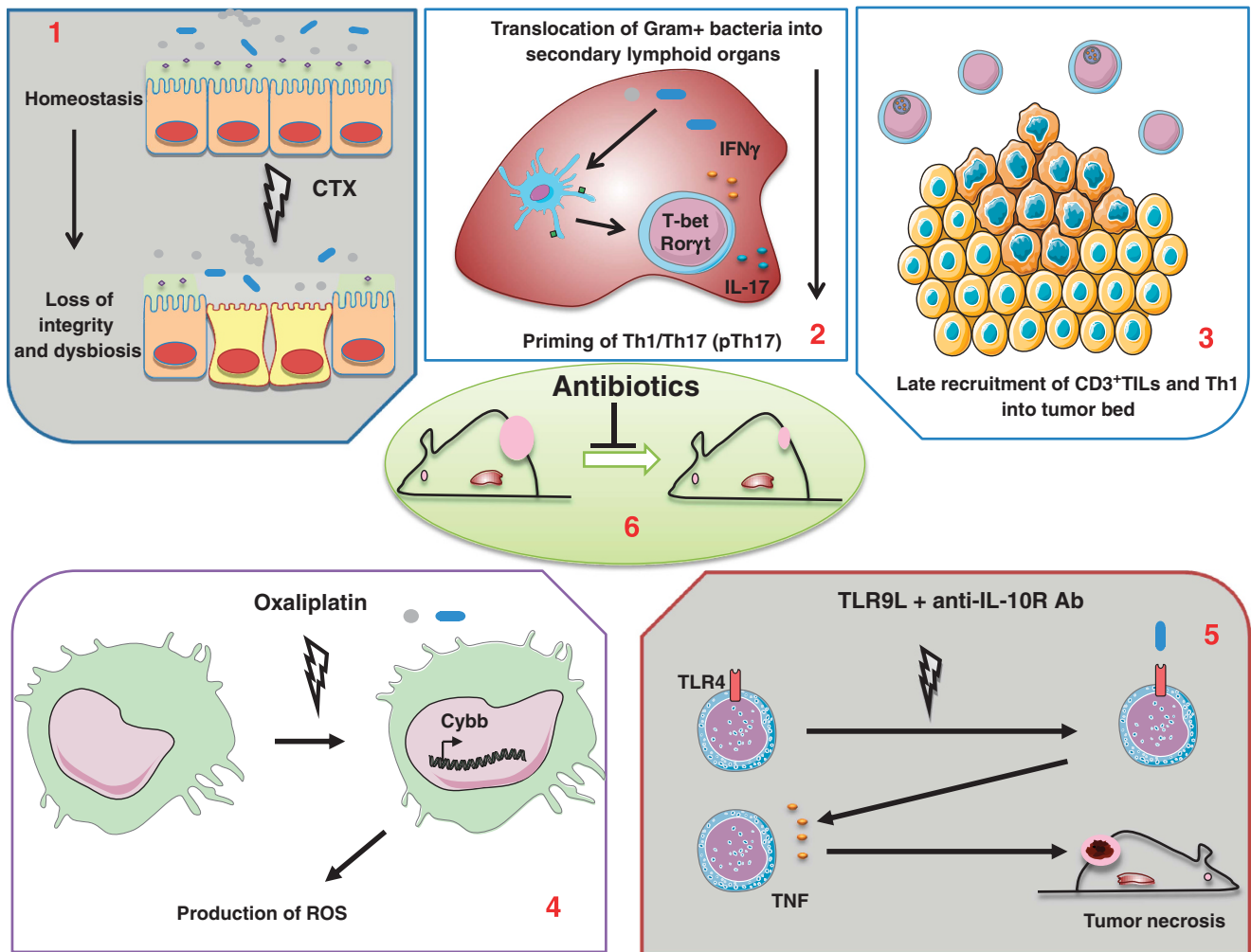
A role for gut commensals in dictating the response of subcutaneous tumors to cytotoxic or immunomodulatory

compounds was unsuspected so far. In recent reports, we and others demonstrated that gut microbiota is indispensable for the immunomodulatory and antitumor effects of certain anticancer therapeutics including CTX and platinum salts.<sup>70,71</sup> We surmise that the scenarii capturing the biology of these effects could be recapitulated as follows.

**Alkylating agent CTX mobilizes Gram-positive bacteria from the gut to secondary lymphoid organs.** CTX is a DNA-alkylating agent belonging to the family of nitrogen mustards, which entered the clinical practice not only as an anticancer agent but also for the therapy of autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis. Its pharmacodynamic profile indicates that CTX mediates immunosuppressive properties at high doses, whereas metronomic CTX regimens exert immunostimulatory effects.<sup>72</sup> Several lines of evidence underscore that CTX can also induce a systemic differentiation of Th1 and Th17 cells, as well as  $\gamma\delta$ T17 cells in a dose-dependent manner, in mice and humans, which may interfere in several immunopathologies or malignancies.<sup>73</sup> Recently, we and others reported that gut microbiota is indispensable for the immunomodulatory and antitumor effects of certain anticancer therapeutics including CTX and platinum salts (Figure 1).<sup>70,71</sup>

First, we observed that certain bacterial species of the SI can selectively and efficiently translocate within 24–48 h after exposure to an alkylating agent, CTX, yet administered at a metronomic regimen only reducing B-cell numbers.<sup>70,73</sup> Indeed, cultivation on blood agar plates (in both aerobic and anaerobic conditions) of mesenteric LNs and spleens of CTX-treated mice revealed the specific outgrowth of high numbers of colonies, identified as *Lactobacillus johnsonii* or *Enterococcus hirae* using mass spectrometry. Second, at these early time points, the permeability of the intestinal barrier was readily increased, whereas the number of Th17 cells and CD103<sup>+</sup> DCs accumulating in the LP significantly decreased, setting the stage for bacterial translocation. Third, within 7 days after CTX administration, the polarity of splenic T cells was geared toward a mixed Th1 and Th17 pattern, whereas a small proportion of CCR6<sup>+</sup>IL-17<sup>+</sup>ROR $\gamma$ t<sup>+</sup> CD4<sup>+</sup> Th17 cells became CXCR3<sup>+</sup>, T-bet<sup>+</sup> and IFN $\gamma$ <sup>+</sup>, suggesting the acquisition of a ‘pTh17’ phenotype. Interestingly, the elicitation of pTh17 cells was significantly reduced in GF mice or animals treated with broad-spectrum antibiotics (ATB; antibiotics: streptomycin + ampicillin + colistin) or vancomycin (killing Gram-positive bacteria), supporting the notion that gut microbiota was involved in the CTX-mediated splenocyte polarization. Reinforcing this notion, pTh17 cells could not be increased in *Myd88*<sup>-/-</sup> mice treated with CTX, whereas somewhat enhanced in *Nod2*<sup>-/-</sup> counterparts. Of note, CTX-induced IFN $\gamma$ -producing  $\alpha\beta$ <sup>+</sup>TCR (T-cell receptor) CD8<sup>+</sup> T cells and  $\gamma\delta$ TCR<sup>+</sup> T cells were not dependent on gut microbiota in the same experimental conditions.

**Reprogramming of intratumoral myeloid cells in the absence of gut microbiota.** Iida *et al.*<sup>71</sup> compared the gene expression profiling of three transplantable tumor models growing in mice treated with ATBx (*versus* no ATBx) (Figure 1). The ATBx therapy profoundly downregulated



**Figure 1** Mechanisms involved in the action of gut microbiota during cancer therapies. Certain chemotherapeutics (presumably those affecting the integrity of the gut barrier (1), e.g. CTX and oxaliplatin) facilitate the translocation of distinct bacteria or bacterial products (2) (Gram-positive for CTX), causing a cascade of systemic immune effects translating in the control of tumor outgrowth (3). Viaud *et al.*<sup>70</sup> (2, 3) showed the role of specific Gram-positive commensal bacteria in the elicitation of pTh17 responses (2) associated with Th1 accumulation in tumor beds (3). Iida *et al.*<sup>71</sup> demonstrated the role of ROS and TNF-producing myeloid cells conditioned by the presence of gut commensal bacteria in the efficacy of oxaliplatin and immunomodulators (4, 5), respectively, in triggering early tumor cytotoxicity. These effects account for the reduced efficacy of cytotoxic compounds in animals cotreated with broad-spectrum (or vancomycin or colistin) antibiotics (6)

genes related to inflammation, phagocytosis, antigen presentation and adaptive immunity while upregulating those encoding tissue development, cancer and metabolism. Notably, ATBx decreased the recruitment of monocyte-derived major histocompatibility complex (MHC) class II<sup>+</sup> Ly6C<sup>high</sup> and Ly6G<sup>+</sup> cells in tumors and spleens. Based on previous work showing the TNF $\alpha$ - (and CD8<sup>+</sup> T-cell-) dependent antitumor efficacy of a combined therapy associating CpG oligodeoxynucleotides and anti-IL-10R antibodies,<sup>74</sup> the authors went on to demonstrate that commensal bacteria were required to prime tumor-associated innate myeloid cells for the CpG-induced inflammatory cytokine (IL-1 $\alpha$ , IL-1 $\beta$ , IL-12 $\beta$ , CXCL10) production and TNF-mediated necrosis, both indispensable for the tumor regression. In accordance with these data, Tlr4<sup>-/-</sup> mice failed to fully respond to CpG + anti-IL-10R combinatorial regimen, whereas LPS could partially restore TNF production by intratumoral antigen-presenting cells in ATBx-treated

wild-type (WT) mice. Principal component analysis of the microbiota composition *versus* TNF production in tumors showed a codependence. Among the positive correlates stood out the *Alistipes* and *Ruminococcus* genera, whereas *Lactobacilli* negatively associated with TNF release. Importantly, reconstitution of mice preexposed to ABTx with *Alistipes shahii* restored the capacity of myeloid cells to secrete TNF, whereas *L. fermentum* failed to do so.

The authors extended the role of microbiota in modulating the intratumoral myeloid innate cell phenotype in tumors treated with platinum salts. Both oxaliplatin- and cisplatin-mediated tumoricidal activity against MC38 and EL4 tumors were more efficient in SPF mice than in ATBx-treated or GF counterparts. Gene expression analysis revealed that the induction of inflammatory mediators was reduced in the absence of gut microbiota at 18 h after oxaliplatin. ATBx therapy attenuated the oxaliplatin-induced expression of Cybb encoding reactive oxygen species (ROS)-generating

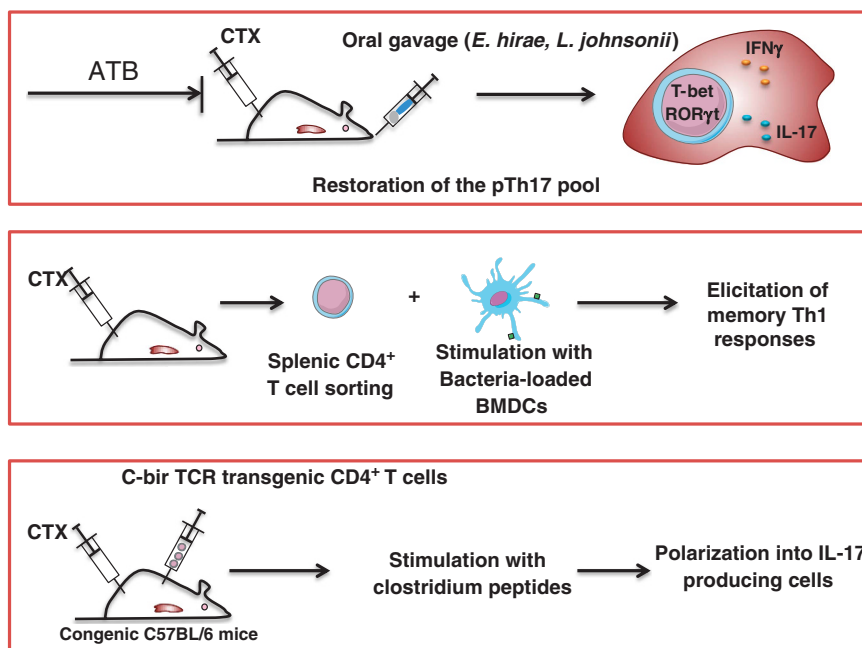
nicotinamide adenine dinucleotide phosphate oxidase (NOX2) that mainly resided in intratumoral neutrophils and macrophages. Inhibition of ROS by genetic (*Cybb*<sup>-/-</sup>) or pharmacological (*N*-acetyl cysteine) maneuvers, as well as depletion of Gr1<sup>+</sup> cells, interfered in the oxaliplatin-mediated tumoricidal activity. These findings underscore that the reduced efficacy of oxaliplatin in ATBx or GF mice could be attributed, at least partially, to an impaired ROS production by myeloid cells. Altogether, Lida *et al.*<sup>71</sup> unraveled the unsuspected role of commensal bacteria in affecting the type of inflammatory microenvironment required for a TNF- or ROS-dependent therapeutic effect mediated by various immunomodulators or cytotoxics.

**Host's immunization against commensal bacteria in the course of chemotherapy.** To address whether ignorance or tolerance toward gut commensals has been broken after CTX, as described following oral infection with *Toxoplasma gondii*,<sup>75</sup> we used two different experimental approaches (Figure 2). First, we performed an adoptive transfer of CBir-specific TCR Tg CD4<sup>+</sup> T cells prone to recognize a flagellin epitope of a *Clostridium*<sup>76</sup> into congenic C57BL/6 mice. Then, we monitored their proliferation and polarization as effector cells as well as their memory response directed against the *Clostridium* peptides. Indeed, CBir-specific TCR Tg lymphocytes accumulated and differentiated into IL-17-producing cells in CTX but not into sham-treated mice. Moreover, following restimulation of splenocytes with CBir peptides, IFN $\gamma$  secretion was markedly enhanced in CTX-treated recipients but not in control recipients. Second,

we addressed whether the Gram-positive translocating bacterial isolates (that could be cultivated and reused in transplantation studies) could mediate pTh17 responses in the spleen after gut recolonization of SPF animals sterilized by a 21-day broad-spectrum ATB regimen (ampicillin, streptomycin, colistin). Indeed, the cocktail of *L. johnsonii* + *E. hirae* restored the pTh17 splenic pool generated after CTX, whereas *L. plantarum* or *L. reuteri* failed to do so.

Third, we analyzed memory T-cell responses directed against a variety of Gram-positive bacteria including the translocating bacterial species as well as others (such as *E. coli*, *E. faecalis* and LPS). In 50% and 30% cases, CTX could elicit memory Th1 responses against *L. johnsonii*, and *E. hirae* or *E. faecalis*, respectively. Altogether, these data indicate that CTX facilitates the priming of effector pTh17 and memory Th1 cell responses directed against distinct commensals in distant secondary lymphoid organs.

**Tumor invasion by Th1 cells is affected by antibiotics, which concomitantly compromised the efficacy of cytotoxic compounds against cancers.** We next addressed whether broad-spectrum ATB or vancomycin- or colistin-based antibiotherapy would affect the anticancer efficacy of CTX in various tumor models. In two transplantable tumor models (P815 mastocytoma and MCA205 sarcoma) syngeneic of DBA2 and C57BL/6 mice, respectively, the CTX-mediated control of tumor outgrowth was significantly impaired by either one or all ATB regimen, supporting a beneficial role for intestinal commensals in the tumoricidal activity of CTX. Of note, at this metronomic



**Figure 2** Three ways to demonstrate that naive mice can get immunized against their commensal bacteria during CTX therapy. (Upper panel) Reconstitution (by oral gavage using *L. johnsonii* + *E. hirae*) of mice presterilized with broad-spectrum antibiotics (ATB) restores the pool of splenic pathogenic Th17 cells (coexpressing the transcription factors T-bet and ROR $\gamma$ t). (Middle panel) Restimulation of splenic T cells from mice treated with CTX using syngeneic bone marrow-derived DCs loaded with distinct commensal bacteria (such as *L. johnsonii* or *E. hirae*) reactivates memory T cells that produce high levels of interferon- $\gamma$  (IFN $\gamma$ ). (Lower panel) Adoptive transfer of TCR Tg CD4<sup>+</sup> T cells recognizing a flagellin peptide of *Clostridium* into naive C57BL/6 mice treated or not with CTX. After 1 week, Tg T cells harboring a congenic marker can be analyzed by FACS to determine IL-17 production (in intracellular staining) as well as IFN $\gamma$  release following restimulation of splenocytes with MHC class II-restricted flagellin peptides

dosing, CTX induces T-cell-dependent antitumor effects.<sup>72</sup> Vancomycin indeed altered the CTX-mediated recruitment of CD3<sup>+</sup> TILs into the MCA205 sarcoma and markedly compromised the accumulation of Th1 TILs. We corroborated these results in a spontaneous lung carcinoma model (as initially described by Jacks and co-workers<sup>77</sup> and Cortez-Retamozo *et al.*<sup>78,79</sup>). Eight-week-old KP (*Kras*<sup>LSL-G12D</sup>/WT; *p53*<sup>Flox/Flox</sup>) mice received an adenovirus-expressing Cre recombinase (Ad-cre) by intranasal instillation to initiate lung adenocarcinoma (d0). Mice were either left untreated or received CTX-based chemotherapy (d84, d91 and d98) in the absence or presence of 0.25 mg/ml vancomycin (mixed into drinking water) starting on d77 after Ad-cre and until the end of the experiment to test the inhibitory role of vancomycin-based antibiotherapy on the anticancer efficacy of a successful chemotherapy. In this preclinical model mimicking human tumorigenesis, we validated the concept that the eradication of Gram-positive bacteria by vancomycin compromised the efficacy of CTX-based chemotherapy, correlating with a reduced intratumoral CD8<sup>+</sup> T effector/Foxp3<sup>+</sup> regulatory T-cell ratio. Thus, Gram-positive bacteria appear to be necessary for the optimal efficacy of the CTX-induced anticancer immune response and tumor mass reduction. Finally, to demonstrate a cause-effect relationship between the lack of elicitation of pTh17 cells by commensals and the loss of chemotherapeutic efficacy observed because of vancomycin cotreatment, we used a transplantable tumor model in which we transferred *ex vivo*-expanded Th17 derived in various cytokine media to exhibit a regulatory *versus* pathogenic Th17 phenotype. Indeed, infusion of pTh17 could restore chemosensitivity in vancomycin and CTX cotreated animals, whereas that of regulatory Th17 failed to do so.

**Concluding remarks and discussion.** These data support the concept that distinct commensals (such as *L. johnsonii* + *E. hirae*) niching in the SI of tumor bearers could elicit pTh17 cells in the spleen after translocation into secondary lymphoid organs, such as pTh17 appearing capable of developing into memory Th1 cells eventually accumulating in inflammatory lesions such as growing tumors. These adaptive T-cell responses directed against commensals are occurring consecutively to earlier events modulating the functions of antigen-presenting cells in tumor beds. Several important points are intriguing and remain to be investigated.

First, what molecular or metabolic cues support the cell stress and damage of the intestinal barrier triggered by pharmaceutical compounds (such as CTX, oxaliplatin, TLR9L, anti-IL-10R, etc.) that generate such a 'helper' immunity? Wide investigations at several levels (apoptosis, necroptosis, autophagy, activation of the NF- $\kappa$ B pathway, inflammasomes, TLR/NOD-like receptors (NLRs), cytokine receptors (TNF $\alpha$ , IL-17, IL-22, IL-18, IL-22BP, etc.), hematopoietic and/or epithelial-driven signaling pathways (Tables 1 and 2) will be mandatory to nail down the principal components leading to this favorable dysbiosis.

Second, how can microbial structures be recognized by pattern recognition receptors expressed by immune or parenchymal cells and generate innate immune responses,

which in turn shape adaptive immunity against microbial and tumoral antigens? These considerations have been largely discussed in the context of organ transplantation by Alegre *et al.*<sup>80</sup> and Chong and Alegre,<sup>81</sup> where infections and/or tissue damage directly or indirectly affect alloreactivity and the outcome of transplanted allografts. Hence, three scenarios can be envisioned to explain how T-cell responses elicited by commensals could influence antitumor immunity. First, antigen cross-reactivity or superantigen-driven responses could account for T-cell-dependent tumor regression. Second, cell-intrinsic effects such as direct activation of antigen-presenting cells located in tumor beds by bacterial cell walls or microbial components could reset myeloid cell functions or reprogram Treg bearing TLRs and/or parenchymal cells that respond to TLRs by chemokine or inflammatory cytokine release or apoptosis.<sup>82</sup> Third, the differentiation of anticommensal T cells could provide helper cytokines or costimulatory factors for anticancer T cells to be driven. The role of IL-2/IL-15, type 1 or type 2 IFNs or CD40/CD40L interactions could be explored among other candidates.

Third, the precise mode of selection of distinct commensal bacteria/pathobionts by CTX remains obscure. Mucosal integrity (bacterial dysbiosis, loss of Th17 and CD103<sup>+</sup> CD11b<sup>+</sup> DCs, upregulation of lysozyme M) was affected at the level of the SI (more than in the colon) with a positive gradient from the ileum to the duodenum. Many *Lactobacilli* and *E. hirae* strains have been described to be highly resistant to acidic pH and bile salts (compared with *Lactococci*), explaining their relative abundance in the SI downstream of the stomach.<sup>83</sup> Physical properties might account for the translocation of distinct bacterial species. Hence, mucosal bacteria that adhere to intestinal mucosal surfaces and epithelial cells<sup>84</sup> might be more prone to translocation in case of loss of mucosal permeability. Many *Lactobacilli* strains and *E. hirae* belong to this category of 'mucosal bacteria', which are more prone to translocation or invasion upon loss of the barrier function of tight junctions<sup>85</sup> than 'luminal' bacteria. The metabolic properties of distinct bacteria might also explain their selective accumulation or resistance after CTX. Thus, in our study, the identified translocating bacteria were all facultative anaerobes (or microaerophilic ones) and not strict anaerobes, perhaps reflecting the presence of a relative hypoxia (but not total anoxia) during the translocation.

Fourth, the links between bacterial translocation and SI dysbiosis remain unsolved. The kinetics relationship between bacterial translocation and gut dysbiosis indicate that dysbiosis detected in the SI mucosa established late when pTh17 are already primed and might influence other parameters than those described in the first study.

Finally, the functional links between the two pioneering studies<sup>70,71</sup> have to be deciphered. It is conceivable that bacterial products or bacteria could modulate the tone of the tumor microenvironment through metabolic changes, setting the stage for restoration of T-cell functions, anergized in the context of tumor-induced tolerance. pTh17 cells and memory Th1 cells elicited against commensal bacteria might preferentially accumulate in inflammatory tumor microenvironment, already primed by bacterial products or ligands for pathogen recognition receptors. Hence, various

**Table 1** Parenchymal or epithelial molecular defects associated with colitis

Default molecule or pathway	Colitis model	Microbiota modification	Macroscopic consequence	Histopathological consequence	Biological consequence	Pathways or molecules involved	Reference
NEMO (NF- $\kappa$ B essential modulator, also called I $\kappa$ B kinase- $\gamma$ (IKK $\gamma$ )) or both IKK1 (IKK $\alpha$ ) and IKK2 (IKK $\beta$ )	NEMO <sup>IEC-KO</sup> mice or IKK1/2 <sup>IEC-KO</sup> mice	ND	Severe colitis, diarrhea and rectal bleeding, thickening and shortening of the colon	Thickening of the mucosa, enlarged crypts with loss of goblet cells, early infiltration of innate immune cells and later presence of T-cell infiltrates	Epithelial cell apoptosis (colon), localized disruption of epithelial integrity, reduced expression of defensin-3, bacterial translocation into the mucosa, inflammatory response (DCs and neutrophils, then T cells)	MyD88, TNF (TNF receptor-1)	Nenci <i>et al.</i> <sup>97</sup>
RelA (subunit of NF- $\kappa$ B)	IEC-RelA <sup>-/-</sup> mice + 3% DSS	ND	Acute colitis, diarrhea, gross rectal bleeding, weight loss	Increased mucosal damage (monolayer ulceration and crypt loss of the colon) and immune cell infiltration	Enhanced levels of IL-6, MCP-1, CXCL1, TNF $\alpha$ (in ceca), COX-2, PGE2 (in distal colon), increased apoptosis in IECs, greater proliferation index of IECs		Steinbrecher <i>et al.</i> <sup>98</sup>
A20 (NF- $\kappa$ B target gene)	A20 <sup>IEC-KO</sup> mice + 1.5% DSS	ND	Severe colitis (gross rectal bleeding, diarrhea, colon shortening)	Increased mucosal damage, crypt loss, immune cell infiltration	Increase of serum IL-6 levels, no recovery after DSS cessation, high sensitivity of IECs to apoptosis	MyD88, TNF (TNF receptor-1), commensal bacteria	Vereecke <i>et al.</i> <sup>99</sup>
STAT3 (signal transducer and activator of transcription 3)	STAT3 <sup>IEC-KO</sup> mice + 2.5% DSS	ND	Weight loss, bleeding	Colonic tissue damage, severe epithelial erosions, disruption of the normal vessel structure, diminished numbers of colonic crypts and IECs	Less epithelial turnover (less proliferation), increased apoptotic of IECs	Mucosal wound healing, IL-22	Pickert <i>et al.</i> <sup>100</sup>
TAK1 (TGF- $\beta$ -activated kinase 1)	<i>villin-Cre TAK1<sup>FL/FL</sup></i> mice and <i>villin-Cre ERT2 TAK1<sup>FL/FL</sup></i> mice (tamoxifen-inducible KO)	ND	<i>villin-Cre TAK1<sup>FL/FL</sup></i> mice: severe intestinal bleeding within 1 day of birth and death by postnatal day 1; <i>villin-Cre ERT2 TAK1<sup>FL/FL</sup></i> mice: 3 days after tamoxifen: weight loss, mild diarrhea	<i>villin-Cre TAK1<sup>FL/FL</sup></i> mice: by E18.5, normal villi and crypts; at birth, disruption of epithelium architecture and enhanced immune cell infiltration; <i>villin-Cre ERT2-TAK1<sup>FL/FL</sup></i> mice: disruption of the SI structure at day 2 and total absence by day 3	Enhanced cell apoptosis and upregulation of proinflammatory factors in SI and colon	TNF/TNFR1	Kajino-sakamoto <i>et al.</i> <sup>101</sup>
TAK1 and TNFR1	<i>villin-Cre TAK1<sup>FL/FL</sup> TNFR1<sup>-/-</sup></i> mice	ND	Ileitis and severe colitis (40–50% of the mice), reduced weight gain, shorter colon length	Enlargement of crypt cellularity in the SI, decrease of mature goblet cells	Increased cellular proliferation in the SI and colon, abundant apoptotic cells, upregulation of proinflammatory factors	TNF-dependent and -independent mechanisms	Kajino-sakamoto <i>et al.</i> <sup>101</sup>
FADD (Fas-associated protein with death domain)	<i>villin-Cre TAK1<sup>FL/FL</sup> TNFR1<sup>-/-</sup></i> mice + 2.5% DSS	ND	Significant weight loss, rectal bleeding, colon shortening	Complete loss of crypt architecture, severe ulceration	Upregulation of MIP2 and S100A9 in the colon, abundant apoptosis in the intestinal epithelium, marked reduction of cell proliferation	IL-6, COX2	Kim <i>et al.</i> <sup>102</sup>
FADD (Fas-associated protein with death domain)	FADD <sup>IEC-KO</sup> mice	ND	Reduced body weight and diarrhea, thickening and shortening of the colon	Enteritis: mucosal thickening, severe transmural inflammation, epithelial erosion, death of crypt epithelial cells, immune cell infiltration, epithelial hyperproliferation and loss of Paneth cells	Severe colon inflammation-driven primarily by an innate immune response (F4/80 <sup>+</sup> and Gr-1 <sup>+</sup> myeloid cells), spontaneous epithelial cell necrosis, impaired expression of antimicrobial factors (lysozyme, $\alpha$ -defensin 20, $\alpha$ -defensin-related sequence 1 and angiogenin 4) in the ileum	Colon damages: RIP3, deubiquitinating enzyme CYLD catalytic activity, TNF partly involved, MyD88, commensal bacteria; Paneth cell loss and enteritis: RIP3	Welz <i>et al.</i> <sup>103</sup>
Caspase-8	<i>Casp8<sup>ΔIEC</sup></i> mice	ND	Spontaneous ileitis	Marked destruction of intestinal architecture, villous erosions in the terminal ileum, bowel wall thickening, crypt loss and increased cellularity (CD4 <sup>+</sup> T cells and granulocytes) in the LP, complete absence of Paneth cells, reduction of goblet cells	Increased expression of <i>S100a9</i> and <i>Tnf</i> genes, downregulation of genes belonging to the family of antimicrobial peptides, high number of necrotic Paneth cells, increased expression of <i>Rip3</i> mRNA in IECs	RIP3, necroptosis	Günther <i>et al.</i> <sup>104</sup>



Table 1 (Continued)

Default molecule or pathway	Colitis model	Microbiota modification	Macroscopic consequence	Histopathological consequence	Biological consequence	Pathways or molecules involved	Reference
XBP1 (UPR signaling)	XBP1 flox/floxVCre (XBP1 <sup>-/-</sup> ) mice (deletion in small and large intestinal epithelium)	ND	Spontaneous colitis (SI)	Spontaneous enteritis, cryptitis with villous shortening, apoptosis, crypt regeneration and architectural distortion, neutrophilic crypt abscesses, duodenitis with surface ulceration and granulation tissue	Spontaneous SI mucosal inflammation, increased ER stress, loss of Paneth cells, reduction of mRNA expression of cryptdins-1, -4, -5 and lysozyme, reduced numbers and size of goblet cells reduced mRNA for Muc2 (SI), impaired antimicrobial function, IEC hyperproliferation	ER stress, JNK	Kaser <i>et al.</i> <sup>105</sup>
	XBP1 <sup>-/-</sup> mice + 4.5% DSS	ND	Wasting and rectal bleeding	Mucosal erosion, edema, cellular infiltration and crypt loss in colonic tissue	Inflammation of colonic tissue, ulcers, increase of TNF $\alpha$ mRNA expression in colon	ER stress, commensal flora, TNF $\alpha$	Kaser <i>et al.</i> <sup>105</sup>
	<i>Xbp1</i> <sup><math>\Delta</math>IEC</sup> mice	ND	Spontaneous enteritis	Numerous autophagosomes and degradative autophagic vacuoles in hypomorphic Paneth cells	Broad evidence of ER stress, relative increase in LC3-II, stable amounts of ATG7, elevated levels of ATG16L1 and beclin 1, increased p-eIF2 $\alpha$ , ATF4 in IECs, IEC-associated NF- $\kappa$ B activation, progressive increase of IRE1 $\alpha$ in IECs	ER stress and autophagy, commensal microbiota, IRE1 $\alpha$ -regulated NF- $\kappa$ B activation, TNF	Adolph <i>et al.</i> <sup>106</sup>
XBP1 and defensin 6	<i>Xbp1</i> <sup><math>\Delta</math>PC</sup> mice	ND	75% of <i>Xbp1</i> <sup><math>\Delta</math>PC</sup> mice: spontaneous enteritis	Increased cell death in crypts, intestinal epithelial cell turnover	ER stress and autophagy activation, structural defects in granule morphology in Paneth cells	ER stress and autophagy	Adolph <i>et al.</i> <sup>106</sup>
XBP1 and ATG16L1 or ATG7 (autophagy)	Atg16l1/Xbp1 <sup><math>\Delta</math>IEC</sup> mice or Atg7/Xbp1 <sup><math>\Delta</math>IEC</sup> mice	ND	Exacerbated spontaneous ileitis	Discontinuous sub-mucosal or transmural inflammation extending through muscularis propria into serosa	Correlation between apoptotic IECs and enteritis severity, IEC-associated NF- $\kappa$ B activation, progressive increase of IRE1 $\alpha$ in IECs	ER stress and autophagy, commensal microbiota, IRE1 $\alpha$ -regulated NF- $\kappa$ B activation, TNF	Adolph <i>et al.</i> <sup>106</sup>
Epithelial protein tyrosine phosphatase SHP-2 (PTPN11)	SHP-2 <sup>IEC-KO</sup> mice	ND	Severe pancolitis, growth retardation, reduction of body mass index, diarrhea and rectal bleeding with high mortality rate	Marked immune cell infiltration, inflammation from the rectum to the colon, reduction of goblet cells and mucin, crypt abscesses, enlarged crypts in the colon	Enhanced intestinal permeability (decrease protein levels of occludin, claudins 1, 4, 8 and 15 in colonic epithelium), altered production of chemokines and cytokines (Th1 bias), deregulation of epithelial ERK, STAT3 and NF- $\kappa$ B signaling pathways	Commensal bacteria, ERK, STAT3 and NF- $\kappa$ B	Coulombe <i>et al.</i> <sup>107</sup>
MUC2	Muc2 <sup>-/-</sup> mice	ND	After weaning: growth retardation, severe weight loss and gross bleeding, diarrhea, occasional rectal prolapses	After weaning: lack and change in morphology of goblet cells, epithelial flattening, superficial erosion in the distal colon, increase of distal colonic crypt lengthening, infiltration and persistence of inflammatory cells in the mucosa	Rapid and permanent increase of CD3e <sup>+</sup> T cells in the colonic mucosa after birth, increase in <i>Cd45</i> , <i>Cd3e</i> , <i>Foxp3</i> , <i>Il12 p35</i> , <i>Ebi3</i> , <i>Tgfb1</i> , <i>Tnfa</i> and <i>Il10</i> gene expression in the distal colon before weaning, proinflammatory responses and decreased expression of TLR2 after weaning	Protective capacities of mother's milk, composition of the intestinal microbiota?	Van der Sluis <i>et al.</i> <sup>108</sup> and Burger-van Paassen <i>et al.</i> <sup>109</sup>
Guanylate cyclase C (GC-C)	GC-C <sup>-/-</sup> IL-10 <sup>-/-</sup> mice	ND	Accelerated appearance of colitis in GC-C <sup>-/-</sup> IL-10 <sup>-/-</sup> mice: diarrhea and rectal prolapse	Severe epithelial hyperplasia and apoptosis, frequent crypt abscesses, disruption of crypt-surface architecture, significant mixed inflammatory infiltrate	Goblet cell depletion, clear indications of inflammation-associated epithelial transformation and progression toward adenocarcinoma	Guanylin, TNF $\alpha$	Harmel-Laws <i>et al.</i> <sup>110</sup>

Table 1 (Continued)

Default molecule or pathway	Colitis model	Microbiota modification	Macroscopic consequence	Histopathological consequence	Biological consequence	Pathways or molecules involved	Reference
Vitamin D receptor (VDR)	<i>hVDR</i> Tg mice (transgenic mice expressing human VDR specifically in IECs) + TNBS	ND	Transient and mild weight loss	None or few abnormalities	Protection of mucosal epithelial barrier integrity, relatively normal tight junction protein transcripts in the colonic mucosa		Liu <i>et al.</i> <sup>111</sup>
	<i>hVDR</i> Tg mice (transgenic mice expressing human VDR specifically in IECs) + 3% DSS	ND	Delay and reduction of colitis	Decrease colonic immune cell infiltration and ulceration	Low colonic transcript levels of proinflammatory cytokines, reduced mucosal barrier damage	T cells	Liu <i>et al.</i> <sup>111</sup>
	VDR KO mice + TNBS	ND	Marked weight loss, highly shortened and swollen colon, death after 5 days of treatment	Severe ulcerations, complete crypt depletion in the distal colon	Very high myeloperoxidase activity (neutrophils) and proinflammatory cytokine expression in the colonic mucosa, abundant apoptotic colonic epithelial cells	Apoptosis through PUMA-dependent and p53-independent mechanisms	Liu <i>et al.</i> <sup>111</sup>
	<i>Vdr</i> -null × <i>hVDR</i> Tg mice (transgenic mice expressing only human VDR specifically in IECs) + TNBS	ND	Little weight loss, subnormal colon	Relatively normal crypt architecture	None or low myeloperoxidase activity (neutrophils) and proinflammatory cytokine expression in the colonic mucosa	Protection by VDR in gut epithelial cells despite a VDR-deficient immune system (epithelial VDR signaling inhibits PUMA expression by blocking NF-κB activation)	Liu <i>et al.</i> <sup>111</sup>
NLRP6, ASC or caspase-1	<i>NLRP6</i> <sup>-/-</sup> (NLRP6 expressed in primary colonic epithelial cells of WT mice), <i>ASC</i> <sup>-/-</sup> or <i>caspase-1</i> <sup>-/-</sup> mice + 2% DSS or 3.5% DSS/AOM + % DSS	Increased representation of members of <i>Prevotellaceae</i> and TM7 and reductions in members of genus <i>Lactobacillus</i> , transmissible colitogenic microbiota	Weight loss, increased mortality, more and larger tumors within the distal rectum (AOM + DSS)	Increased histologic score (mucosal ulceration, submucosal edema and inflammatory cell infiltration)	Increased intestinal permeability, marked increase in proinflammatory cytokine levels, sustained proliferation in IECs and dysplastic changes within the regenerating epithelium	IL-18, CCL5	Chen <i>et al.</i> <sup>112</sup> Elinav <i>et al.</i> <sup>113</sup> and Normand <i>et al.</i> <sup>114</sup>
MyD88	<i>pVillin</i> -dnMyD88 (dominant-negative mutant of MyD88 under villin promoter) and <i>MyD88</i> <sup>ΔIEC</sup>	Decrease in abundance of <i>Bacteroides</i> families, increase in abundance of <i>Proteobacteria</i> , abundant OTUs in the candidate phylum TM7	Age-dependent spontaneous intestinal inflammation, severe ileitis (54 weeks)	Diffuse thickening and edema, mainly in the SI wall (ileum), epithelial cell hyperplasia and inflammatory cell infiltration in the LP and submucosa of the ileum, villus atrophy and crypt elongation (hyperplasia), enlargement of the lymphatic vessels and lymphedema in the LP, crypt microabscesses, depletion of goblet cells	Release of inflammatory cytokines and inflammatory cell infiltration (CD4 <sup>+</sup> T, CD8 <sup>+</sup> T, neutrophils and macrophages), mild apoptosis in the ileum IECs, decreased barrier functions, decreased release of antimicrobial peptides, increased commensal bacterial translocation and bacterial adherence to the epithelial surface, reduced fecal IgA	Antimicrobial peptides, commensal bacterial translocation	Gong <i>et al.</i> <sup>115</sup> and Frantz <i>et al.</i> <sup>116</sup>
	Villin-MyD88/IL-10 KO mice	ND	Spontaneous colitis comparable to <i>IL-10</i> <sup>-/-</sup> mice, unformed stools, MLN hypertrophy	Intestinal wall thickening, epithelial hyperplasia, massive leukocytic infiltration	No change in cytokine levels in colonic tissue: IL-12 p40, IL-1β, IL-6, TNFα and in cytokine secretion (IFNγ and IL-17A) by MLN/LP T cells	MyD88 signals in colonic CD11c <sup>+</sup> and LysM <sup>+</sup> cells	Hoshi <i>et al.</i> <sup>117</sup>
HDAC1 and HDAC2 (histone deacetylase 1 and 2)	HDAC1/2 IEC-deficient mice	ND	Spontaneous colitis, loss of weight, loose stools, increased SI length and weight, colon shortening	Intestinal wall thickening, colonic infiltration of immune cells, dysplasia and hyperplasia of jejunal and colonic mucosa (presence of expanded crypts, branched villi in the jejunum, villus-like structures in the colon)	Increase of proliferating cells and apoptosis, decrease of goblet and Paneth cells, change from a secretory to an absorptive IEC phenotype, increase expression of cleaved Notch1, decrease of claudin 3 expression, increase of intestinal permeability, increase of phosphorylated STAT3, chronic inflammatory response in IECs	Notch, mTOR?	Turgeon <i>et al.</i> <sup>118</sup>

Table 1 (Continued)

Default molecule or pathway	Colitis model	Microbiota modification	Macroscopic consequence	Histopathological consequence	Biological consequence	Pathways or molecules involved	Reference
HDAC3 (histone deacetylase 3)	HDAC3 <sup>ΔIEC</sup> mice or HDAC3 <sup>ΔIEC-IND</sup> mice (inducible tamoxifen-dependent IEC-specific HDAC3 KO mice)	Increased levels of <i>Proteobacteria</i> , intestinal phenotype not transmissible by microbiota	Spontaneous intestinal inflammation, rectal prolapse with age	Normal intestinal architecture, crypt elongation in colon, decreased numbers of Paneth cells	Dysregulated gene expression in the large intestine in glutathione metabolism, mitochondria, lipid biosynthesis, PPAR signaling, antigen processing and defense response, reduced lysozyme expression, increased IEC proliferation, elevated cell death in crypts, impaired intestinal barrier (increased LPS in MLN, permeability and bacterial translocation), impaired crypt bactericidal activity		Alenghat <i>et al.</i> <sup>119</sup>
	HDAC3 <sup>ΔIEC</sup> mice + 2.5% DSS	ND	Profound weight loss, disease severity, shortened colon	Extensive intestinal ulceration, loss of crypt architecture, edema, inflammation (specific of IECs and not LysM)	Increased infiltration of the LP by macrophages and neutrophils, profound apoptosis of IECs at the bottom of the crypts	Intestinal dysbiosis necessary but not sufficient	Alenghat <i>et al.</i> <sup>119</sup>
hPepT1 (human intestinal H-coupled oligonucleotide transporter)	Villin-hPepT1 mice + 3% DSS	ND	Severe weight loss, high total clinical score, increase in inflammation, massive mucosal erythema and bleeding, shortened colon	Complete crypt disruption, inflammatory infiltration	Increase of mRNA levels of proinflammatory cytokines (IFN $\gamma$ , IL-1 $\beta$ , IL-6 and TNF $\alpha$ )	Bacterial peptides, NOD2	Dalmasso <i>et al.</i> <sup>120</sup>

commensal or pathobionts may act in concert to modulate systemic and local immunity during cancer regimens.

### Future Challenges for the Exploitation of the Host–Bacteria Symbiosis/Dysbiosis During Oncogenesis

Interfering against inflammation has been a source of inspiration for chemoprevention. Apart from aspirin that reduces the incidence of distinct colon cancers and other adenocarcinomas, other strategies based on our expanding knowledge of gut microbiota are being pursued.<sup>86</sup> Although a beneficial role for broad-spectrum antibiotics has been shown in many experimental settings to reduce inflammation-induced cancers, this approach would select antibiotic-resistant strains and eliminate species involved in gut homeostasis. Instead of killing indiscriminately all bacteria, restoring an ‘ideal’ microbial composition could theoretically be a more suitable option. Fecal microbiota transplantation was shown to be effective in diarrhea caused by a dysbiosis dominated by *Clostridium difficile* by suppressing or displacing *C. difficile*.<sup>87,88</sup>

Probiotics and prebiotics represent more common ways to establish/maintain healthy microbiomes. In individuals presenting with lactose intolerance (5–15% frequency in northern Europe), the beneficial effect of live *Lactobacilli* residing in non-pasteurized yogurt relies on the provision of  $\beta$ -galactosidase activity.<sup>89</sup> Genetically modified bacteria may even have stronger effects. A *L. acidophilus* strain harboring a deletion in the phosphoglycerol transferase gene and unable to synthesize LTA prevented the progression of colonic polyps in Apc<sup>Dflox</sup> mice.<sup>90</sup> Elafin-overexpressing *L. casei* and *L. lactis*

reduced colitis in mice and *ex vivo* in inflamed epithelial cells from human colitis.<sup>91</sup> *L. gasseri* genetically modified to overexpress superoxide dismutase decreased colitis in IL-10-deficient hosts.<sup>92</sup>

Prebiotics refer to indigestible food ingredients that selectively promote the colonization of healthy commensals such as the dietary fiber inulin that promotes *Bifidobacteria* growth. More specifically, cancer-preventive antioxidants include dietary polyphenols (flavonoids, phenolic acids, lignins present in tea, wine, nuts, fruits, and so on, and ellagic acid metabolized by colonic microbiota into urolithins exhibiting antiestrogenic and anti-COX2 activities).<sup>93</sup> Another polyphenol called ‘daidzein’, a soy isoflavone metabolized by gut microbiota into equol and only detected in a fraction of individuals (harboring sulfate-reducing bacteria), may protect against breast and prostate cancer, mostly in Asia.<sup>94</sup> The fiber has been involved in the prevention of colorectal cancers and butyrate, one of the most abundant short-chain fatty acids resulting from the bacterial fermentation of fibers and selectively transported into the colon epithelium is the most compelling tumor-suppressive molecule. Butyrate has both cell autonomous and cell extrinsic antitumor effects. It decreases proliferation and promotes apoptosis of tumor cells, ameliorates inflammation associated with colitis and favor expansion of peripheral Treg. Most of these effects result from epigenetic regulation, butyrate acting as an endogenous HDAC inhibitor.<sup>95,96</sup>

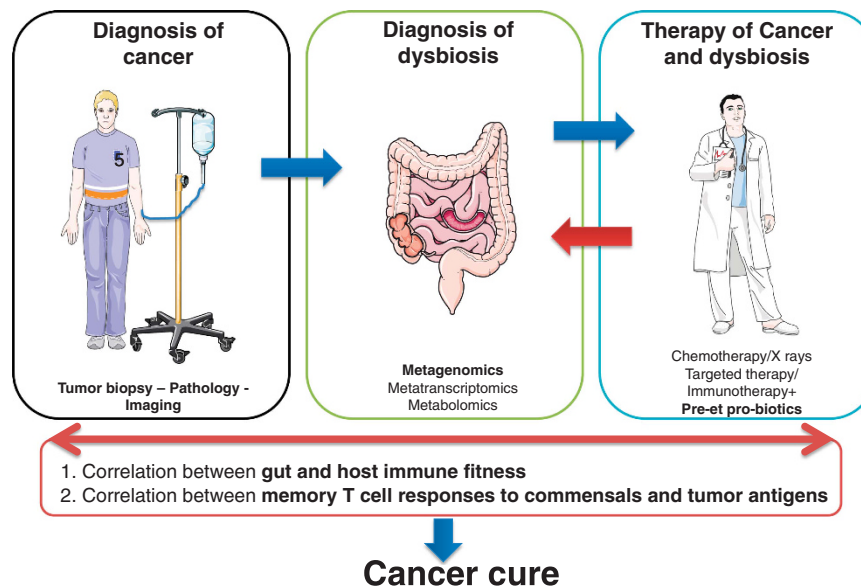
Assuming a causal and linear relationship between translocation of Gram-positive bacteria, pTh17 responses and antitumor immunity leading to tumor control, one might think about exploiting the adjuvanticity of gut commensals to

**Table 2** Immune molecular defects associated with colitis

Default molecule or pathway	Colitis model	Microbiota modification	Macroscopic consequence	Histopathological consequence	Biological consequence	Pathways or molecules involved	Reference
T-bet and RAG2	T-bet <sup>-/-</sup> × RAG2 <sup>-/-</sup> mice (or TRUC mice for T-bet <sup>-/-</sup> × RAG2 <sup>-/-</sup> ulcerative colitis)	Colitogenic microbiota: vertically and horizontally transmissible, the presence of <i>Klebsiella pneumoniae</i> and <i>Proteus mirabilis</i> correlates with colitis	Spontaneous colitis, anorectal prolapse, rectal inflammation	Colonic thickening, surface ulceration, crypt distortion and hyperplasia, dense mixed inflammatory cell infiltrate in the LP	Continuous inflammation of the rectum and left colon, marked inflammation, increased colonic permeability	TNF $\alpha$ (colonic DCs)/ TNFR1/p55, commensal microbiota (anaerobic bacteria)	Garrett <i>et al.</i> <sup>121,122</sup>
IL-10	IL-10 <sup>-/-</sup> mice	ND	Spontaneous chronic enterocolitis of the entire intestinal tract, weight loss, splenomegaly, anemia, lethality for 30% of the animals	Mucosal inflammation, epithelial hyperplasia, abnormal crypt and villus structures, crypt abscesses, ulcers, mucin depletion, erosions of the mucosa, thickening of bowel wall	Aberrant immune cell activation and increase of immune cells, Th1 polarization, deposits of fibrinoid material and IgA, aberrant expression of MHC class II molecules in intestinal epithelia, reduction of Treg in the large intestinal LP, increase of B1 lymphocytes (CD19 <sup>+</sup> CD5 <sup>+</sup> ), enhanced levels of serum IgG and IgA	Enteric microbial flora, MyD88-dependent, IL-23	Kühn <i>et al.</i> , <sup>123</sup> Rakoff-Nahoum <i>et al.</i> <sup>124</sup> and Gomes-Santos <i>et al.</i> <sup>125</sup>
IL-2	IL-2 <sup>-/-</sup> mice or IL-2 <sup>-/-</sup> Myd88 <sup>-/-</sup>	ND	Spontaneous colitis, rectal prolapse, wasting, mortality, thickening of the bowel wall, shortening colonic length, unformed or absent stool	Multifocal, transmural leukocytic infiltrate, severe epithelial hyperplasia, destruction of crypt architecture	Aberrant CD4 <sup>+</sup> T-cell activation, increase of DCs in MLN, Th1 polarization,	Commensal microflora-dependent, MyD88-independent, IL-12- or IL-23-independent factors	Rakoff-Nahoum <i>et al.</i> <sup>124</sup>
IL-15	IL-15 <sup>-/-</sup> mice + 2–3% DSS	ND	Attenuated induced chronic colitis, increased survival rate, less weight loss, bleeding and diarrhea than WT mice, inhibition of colon shortening	Reduced numbers of infiltrating cells, degree of mucosal injury and edema	Low levels of IFN $\gamma$ , TNF $\alpha$ and IL-12p40 in the large intestine LPs	CD8 <sup>+</sup> T cells, NK cells, DCs?	Yoshihara <i>et al.</i> <sup>126</sup>
IL-22	IL-22 <sup>-/-</sup> mice + 2 or 3% DSS	High diversity microbiota, 7 reduced genera ( <i>Lactobacillus</i> , <i>Bacteroides</i> , <i>Ruminococcus</i> , <i>Turicibacter</i> , <i>Anaerobacter</i> , <i>Parabacteroides</i> and <i>Hespellia</i> ) and 7 increased genera ( <i>Coprococcus</i> , <i>Allobaculum</i> , <i>Barnesiella</i> , <i>Alistipes</i> , <i>Xylanibacter</i> , <i>Butyricimonas</i> and <i>Helicobacter</i> ), transmissible colitic microbiota	Induced colitis, more weight loss and higher rate of mortality than WT mice		Reduced expression of RegIII $\beta$ and RegIII $\gamma$	NK cells, dependent and independent role of intestinal microbiota	Zenewicz <i>et al.</i> <sup>127,128</sup>
IDO (indoleamine 2,3-dioxygenase)	Transplantation of <i>Ido1</i> <sup>-/-</sup> BM cells in <i>Ido1</i> <sup>+/+</sup> mice + TNBS (A) or <i>Ido1</i> <sup>-/-</sup> mice + TNBS or 1-mT (IDO inhibitor) in <i>Ido1</i> <sup>+/+</sup> mice + TNBS (B)	ND	(A) More severe colitis than with <i>Ido1</i> <sup>+/+</sup> BM cells transplanted; (B) more severe colitis in <i>Ido1</i> <sup>-/-</sup> mice + TNBS than WT + TNBS, phenotype of WT mice treated with 1-mT + TNBS similar to <i>Ido1</i> <sup>-/-</sup> mice + TNBS and decrease of survival and colonic dilation with stool retention	Severe colonic transmural inflammation, changes in mucosal architecture (extensive ulceration and coagulation necrosis) in <i>Ido1</i> <sup>-/-</sup> mice + TNBS and 1-mT + TNBS and TNBS-treated WT mice	(A) Higher expression of <i>Irfng</i> and <i>Tnf</i> and decreased Foxp3/CD4 ratio in the colons than with <i>Ido1</i> <sup>+/+</sup> BM cells transplanted; (B) Increased expression of IL-12, IFN $\gamma$ and IL-2 in TNBS + 1-mT-treated mice	Suppression of inflammation mainly by IDO-expressing colonic inflammatory cells and contribution of IDO-expressing colonic epithelium, IFN $\gamma$ (STAT1)	Takamatsu <i>et al.</i> <sup>129</sup> and Gurtner <i>et al.</i> <sup>130</sup>
WASP (Wiskott–Aldrich syndrome protein)	WASP KO mice (WKO mice)	ND	Frequent signs of colitis (wasting, rectal prolapse, diarrhea), thickening and shortening of the colon, extensive enlargement of mesenteric LNs and spleen	Crypt elongation, epithelial hyperplasia, extensive LP infiltration of inflammatory cells, occasional crypt abscesses, depletion of goblet cells	LP infiltration by CD4 <sup>+</sup> T cells, CD8 <sup>+</sup> T cells, F4/80 <sup>+</sup> macrophages, neutrophils and CD11c <sup>+</sup> dendritic cells, increase in activated CD4 <sup>+</sup> T cells in mesenteric and subcutaneous LNs and spleen, increase of IL-4, IL-13 and IFN $\gamma$ in colonic LP cells	CD4 <sup>+</sup> T cells, Treg, IL-10, tolerogenic DCs	Nguyen <i>et al.</i> <sup>131,132</sup>

Table 2 (Continued)

Default molecule or pathway	Colitis model	Microbiota modification	Macroscopic consequence	Histopathological consequence	Biological consequence	Pathways or molecules involved	Reference
Runx3	Runx3 KO mice	ND	Spontaneous chronic colitis, cecal wall thickened, rigid and opaque, colon with tubular thickening, enlargement of the mesenteric LNs	Multifocal and coalescing mixed mucosal and submucosal infiltration of plasma cells, lymphocytes, histiocytes and eosinophils, mucosal hyperplasia, crypt loss, increased mitotic figures, late-onset progressive proliferative gastritis	Increase of T lymphocytes, macrophages and DCs in the mucosa, of lymphocytic clusters with B cells in the large intestine, IgA production, IFN $\gamma$ , TNF $\alpha$ , IL-12, Tim-3, IL-4, T-bet and GATA-3 expression in the colon	Leukocytic cell-autonomous function of Runx3	Brenner <i>et al.</i> <sup>133</sup>
AhR (aryl hydrocarbon receptor)	AhR KO mice + 3.5% DSS	ND	Severe induced colitis, severe decrease in body weight, colon shortening	More severe histological scores and severe inflammation for the colon tissue	Increased mRNA expression level of TNF $\alpha$ , IL-6, IL-1 $\beta$ and IL-8		Furumatsu <i>et al.</i> <sup>134</sup>
PD-1 (program death-1)	Adoptive transfer of naive CD4 <sup>+</sup> CD25 <sup>-</sup> T cells from PD-1 <sup>-/-</sup> mice to Rag1 <sup>-/-</sup> mice	ND	T-cell transfer-induced colitis, body weight loss	Severe lymphocyte infiltration, crypt dropout, epithelial regeneration, overall crypt architectural alteration in the colons	Defect in <i>de novo</i> iTreg development, increase of Th17 cells in draining LNs	Akt-dependent mechanism of iTreg development	Qiao <i>et al.</i> <sup>135</sup>



**Figure 3** Future prospects and clinical implementations of this work. To date, patients who are being diagnosed with cancer undergo a pathological biopsy and imaging tomography/magnetic resonance to analyze spreading of the malignant process. In the near future, we will need to investigate their intestinal microbiota and their systemic anticancer and antimicrobial immunity to be able to adapt/personalize the oncological therapy according to their microbial dysbiosis or immune dysfunctions. Specific food intake as well as probiotics composed of immunogenic (and safe) commensal/pathobionts could precede chemotherapeutics to facilitate their tumoricidal activity through bacterial adjuvantization

ameliorate the effects of chemotherapeutics. Understanding the molecular cues underlying the immunogenicity of *L. johnsonii* + *E. hirae* may unravel novel PAMPs and/or novel patterns of T-cell differentiation that could shape the design of future cancer vaccines. Modifying these bacteria to uncouple their potential pathobiontic from their commensal properties may be mandatory for a future development of such probiotics as ‘adjuvantizers’ of chemotherapeutics.

Future prospects for a better management of cancer patients aim at (i) diagnosing patients dysbiosis (metagenomics, metatranscriptomics, epidemiology on diet, medications and exercise, and so on), (ii) compensating dysbiosis by appropriate ‘immunogenic probiotics’, WT or genetically

modified to overexpress specific functions, (iii) prebiotics synergizing with probiotics to set the stage for a healthy intestine that has been compromised by DNA-damaging agents, (iv) monitoring the immune responses raised against the relevant commensals to establish a correlation with long-term benefit and immune fitness (Figure 3).

**Conflict of Interest**

The authors declare no conflict of interest.

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