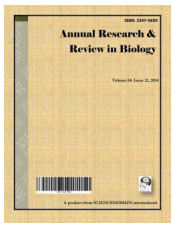

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Gastrointestinal Microflora in Radiation Injury and Countermeasure

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Authors' contributions

This work was carried out in collaboration between all authors. Author PKA designed the study, formulated the idea and critically revised the manuscript. Author AK interpreted the data and wrote the manuscript. Author NG gathered the initial data and performed preliminary data analysis. All authors read and approved the final manuscript

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ABSTRACT

Acute radiation syndrome (ARS) is a collection of pathological conditions as a result of exposure to high amounts of ionizing radiation (IR). Gastrointestinal (GI) system is highly sensitive to IR exposure and the symptoms include anorexia, nausea, vomiting and severe diarrhoea and can result in multiple organ failure. If remain untreated, it may result into death within 2 weeks with predominant cause being infection, dehydration and electrolyte imbalance. GI tract is inhabited by several commensal bacteria and damage to the GI system facilitates bacterial translocation to other organs due to loss in its integrity. Bacterial translocation results in conversion of commensals into opportunistic pathogens which secrete variety of lethal toxins culminating in multiple organ failure. Present review focuses on elucidating consequences of radiation exposure to GI system, the microbiota inhibiting GI and critical analysis of data from different studies done so far to counter those consequences. Using traditional therapeutics, there are no promising measures developed so far, to counter such radiation emergency to an acceptable extent. Review of existing literature urges development of innovative countermeasures and fecal transplant.

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1. INTRODUCTION

The microbiota in human body comprises of numerous microorganisms ranging from sizable diversity of bacteria, archaea and eukaryotes. Human body contains about 10^{13} cells and about 10^{14} bacterial, fungal and protozoan representing thousands of microbial species [1]. The most reliable and accepted 16S rRNA-based estimation of colonic mucosal and fecal microbial communities suggests the presence of 395 taxonomic units with 8 divisions, with the Bacteroidetes and Firmicutes divisions being highest [2,3]. The peculiar environment of GI tract (from oesophagus to rectum) favours the acquisition and colonization of diverse microorganisms. The human GI microbiota is just not simply involved in the digestion of food but has a vital role in the metabolism, strengthening and facilitating the development of immune system through strengthening of mucous membrane barrier and outcompeting potentially pathogenic microorganisms by providing strong competition for food and space. A variety of cellular pathways are influenced by regulation of gene expression patterns by intestinal commensal flora [4] affecting cell cycle progression, DNA repair, apoptosis, superoxide dismutase (SOD) control and immune response by activation of TLRs [5-10].

The severity of intestinal tissue toxicity [11] increases with dose and time of IR exposure. According to the law of Bergonie and Tribondeau, less differentiated cells with high mitotic activity are most susceptible for radio-injuries [12,13]. An inflammatory and degenerative consequence at higher doses of IR on intestinal tissues known as acute radiation syndrome (ARS) have been reported [14]. The intestinal microbial flora becomes important at this point. The possibility of intestinal flora to either turn hostile as an opportunist pathogen or act as shield (protector or mitigator) has recently been the research focus. The present review aims at summarizing the earlier and current studies to extract the meaningful information and to explore future perspectives of radiation-GI microflora interaction. The review emphasizes on (i) Effect of radiation on intestinal microflora i.e dynamics of microflora post radiation and its consequences, (ii) GI microflora as radiosensitizer and radiomitigator (iii) Current therapeutic approaches to counter radiation induced gastrointestinal syndrome (RIGS).

2. GASTROINTESTINAL MICROFLORA AS RADIOSENSITIZER

Chances of DNA aberration and mutations are maximal in rigorously dividing cells having high cell turnover due to their more proneness to radio-injuries. Intestinal mucosa is profoundly susceptible to radiation damages [15]. Occupational radiation exposure and patients undergoing radiotherapy are at greater risk of having radio-injuries in the form of ARS and radio enteritis as a result of disturbed intestinal microflora and damaged mucosal barrier. Intestinal microflora is suggested to have a role as radiosensitizer by enhancing the mitotic rate of epithelial cells [16,17]. Gram negative bacteraemia often have been reported [18,19] at the time of death of animal. This phenomenon is attributed to direct bacterial invasion of the intestinal wall after 3 days of x-ray (1.2Gy) irradiation [20-24]. It suggests that the intestinal tissue injury and subsequent villi degeneration or shortening paves the way for bacteria to enter deeper into tissues and ultimately blood. Other studies on the induction of bacteraemia with artificially infected irradiated animals [25-29] indicate that route to bacteraemia opens at intestine.

Further studies suggested that small numbers of bacteria cross the epithelial barrier at the time of greatest villi damage (three days after irradiation). By second week of exposure when immunological defences fail, bacteria may be found in regional lymph nodes, liver and spleen [30]. After exposure, bacteria proliferate in the colonic lumen and subsequently invade the intestinal wall producing bacteremia that may contribute to death of the organism. While death of mice by presence of endotoxins in blood has been studied [31], it is notable that susceptibility of mice to endotoxin increases after radiation exposure. Exposure to mid-lethal and lethal doses of radiation appeared to increase in the numbers of coliform organisms, streptococci and staphylococci in the feces [32]. Such changes were transient and less marked in animals that survived radiation. These findings, along with other studies suggest that these effects are directly related to radiation and do not represent an agonal event. Coliforms and other gram-negative members of the intestinal flora have been found to be the most common organisms causing bacteremia after radiation [33-38]. Qualitative and quantitative alterations in the

microbial population of the gut may be as significant as radiation injury to the intestinal mucosa in causing post irradiation syndrome and ultimately death. Damage of the mucosal barrier as an outcome of irradiation and compromised gut associated lymphoid tissue (GALT) leads access of bacterial toxins and bacteria to penetrate deeper into tissues. Commensal flora gaining access to other than their normal habitat becomes opportunist pathogen. Sepsis develops due to the action of certain "turned" pathogenic bacteria like *clostridium difficile* which is a gram positive strict anaerobe. This condition coincides with pancytopenia followed by weakened immunity and eventually results into sepsis formation [39]. An extensive study further advocated the role of gut microflora in increasing the radiosensitivity of intestinal mucosa and epithelium [40]. They reported that (*i*) mice harbouring a normal microbiota exhibit enhanced intestinal radiosensitivity as compared with germ free (GF) animals and that the enteritis is responsible for their increased mortality after total body irradiation-bone marrow transplantation (TBI-BMT) (*ii*) indigenous microbes alter the radioresponsive phenotype of the intestine.

3. INTESTINAL MICROFLORA AS RADIOPROTECTORS

Intestinal microflora participates in countering DNA damage following irradiation possibly by activating toll like receptors (TLRs) present in intestinal tissues. Certain components like peptidoglycan and teichoic acid of gram positive bacteria and lipopolysaccrides, 'O' side chain, flagellins, lipopeptides of gram negative bacteria are potent activators of TLRs [41]. Activation of different TLRs is associated with different gene expression patterns which cumulatively results in strong immune system. TLR7/8 activation by an agonist, imiquimod, leads to the expression of nucleotide excision repair genes and enhances DNA repair in bone marrow derived cells. It further increases nuclear localization of DNA repair enzymes and resolution of pyrimidine dimers [42]. Decreased apoptosis, enhanced G2 phase cell accumulation and increased DNA repair in TLR9 stimulated $CD4^+$ T cells has been observed. Experimental evidence strengthening the concept of active involvement of the intestinal flora in reducing chromosomal aberration in bone marrow cells by activating TLR with agonists (CBLC502, LPS and lipopeptide) has been reported [43]. The agonists were administered in mice model prior to irradiation and hence the role of intestinal flora as radioprotectors becomes a hotspot for further extensive investigations. Expression of 25 different genes taking part in various pathways was markedly influenced by the agonists, 10 of which directly corresponded to DNA damage repair cascades (Table 3). In cases of bacterial translocation to the organs, specific cells in the concern organ provides first line of defence by activating innate immune response and thus local inflammation. Liver is the common site of infection after such translocations. A normal liver expresses low mRNA levels of TLRs such as TLR1, TLR2, TLR4, TLR6-10 implying a high tolerance of the liver to TLR ligands like LPS, flagellin, teichoic acid etc. from the GI microflora to which it is constantly exposed. Signalling through TLRs plays a major role in the physiology and pathophysiology of the liver.

The membrane component of gram-negative bacteria, LPS, is a potent activator of innate immune response through its binding to TLR4 complex. TLR4 is expressed by Kupffer cells, hepatic stellate cells, hepatocytes, biliary epithelial cells, sinusoidal endothelial cells and hepatic dendritic cells. There is a positive correlation between liver dysfunction and the occurrence of bacterial translocation and the clearance of LPS from the circulation is decreased in states of hepatic dysfunction, such as cirrhosis [44] suggesting the lowering of immunological response. A tightly regulated network of bacterial components and GI tissue associated TLRs in eliciting immunological response and complement proteins in clearance of LPS becomes crucial.

4. APPROACHES TO USE MICROFLORA FOR RADIATION COUNTERMEASURE

On exposure of IR higher than the 0.7 Gy dose in a short period of time, radiation sickness occurs which is characterize by an acute illness. The major cause of this syndrome is depletion of immature parenchymal stem cells in specific tissues. GI syndrome is one of the outcome of such exposure which occur with a dose approximately 6 -10 Gy although the the LD_{100} is about 10 Gy. Survival is extremely unlikely with this syndrome. Destructive and irreparable changes in the GI tract and bone marrow usually cause infection, dehydration and electrolyte imbalance and eventually death within 2 weeks.

Sepsis is characterized by neutropenia and thrombocytopenia [45]. In irradiated laboratory

animals (e.g, mice), circulating leukocytes drop precipitously within 2 days, begin to recover gradually after approximately 2 weeks and approach normal levels in 4 weeks or longer. The number of thrombocytes in mice decreases after 5 days and begins to recover within 10 to 12 days of IR exposure. As the intestinal microflora of mice and humans are more or less similar in terms of anaerobic and aerobic bacteria, the outcome of radio-injuries is also similar in terms of GI syndrome. Higher IR doses induce systemic infections caused by endogenous or exogenous microorganisms. Endogenous infections arise from facultative microorganisms that translocate from the upper and lower intestinal tract, which is normally colonized predominantly by anaerobic bacteria and lesser numbers of facultative bacteria. The anaerobic bacteria ordinarily provide colonization resistance against pathogenic exogenous microorganisms. On the other hand, nonlethal doses of IR enhance susceptibility to exogenous bacterial infections acquired from the environment and enhance mortality. Since, radiation of lower dose is known to affect the translocation of microflora, treatment with probiotics may replenish the commensal microfloral to govern defence against radio-injuries and acquisition by pathogenic microorganisms at the same time. Probiotics with a standard pre tested inoculum of mixed bacterial culture, particularly *Lactobacillus* species, on ingestion may help prevent GI infections [46]. Probiotics like VSL3 are generally prescribed for the reestablishment of gut microflora [47]. Despite much of work done on the development of probiotics, scientific efficacy evaluation and use in infants and immunocompromised individuals needs further investigation.

5. BACTERIAL TRANSLOCATION AND ROLE OF MICROFLORA FOLLOWING IRRADIATION

Bacterial translocation may be defined as the movement of viable bacteria from the GI tract through the epithelial mucosa into the lamina propria, mesenteric lymph nodes and then to other organs. This movement may sometimes lead to severe condition known as multiple organ failure (MOF). Long way back in 1890's, two independent investigators put forward a doctrine that viable bacteria could pass through the intact gut wall *in vivo* [48,49]. This phenomenon was defined as bacterial translocation [50]. Invasion of the host by endogenous bacteria and increased susceptibility to infection following irradiation was observed over 85 years ago [51,52]. Investigators [53,54] reported increased susceptibility of irradiated animals to pathogenic organisms and the complication of radiation mortality by infection [55,56]. Investigators systematically investigated the incidence of endogenous intestinal bacteremia in mice following whole body irradiation with 450 and 600 rad of X rays [57]. Their data showed that the highest incidence of positive cultures occurred during the period of greatest mortality [58] suggesting a direct relationship with bacteremia in animals irradiated with doses below 1000 r at 11 days after exposure. Such infection could be prevented by antibiotics or other means resulting into the increased chance for survival. This indicates that bacteremia is a contributory cause of death at this time. The percent of positive tissue and blood cultures was greatest during the median survival time. The median survival time and thus the onset of bacteremia was extremely dose-dependent. The lower the dose, the greater was the period of time between exposures and appearance of positive cultures. Further they explained that the mesenteric lymph nodes were the first tissues to show a positive culture followed by the liver and spleen and then heart blood. At higher doses, especially after X-ray, liver and spleen cultures showed a higher per cent of positive results than did the lymph nodes. Many investigators have shown that intestinal mucosa damaged by radiation was essentially repaired in 3 to 5 days [59-61]. Notably, their study showed that the incidence of infection following radiation doses in the lethal dose range occurred at about the median survival time which in most instances, was well over the time of maximum intestinal damage.

It is evident that bacterial translocation occurs in humans [62] and is associated with an increased incidence of septic complications [63]. Understanding the mechanism of bacterial translocation and factors influencing bacterial translocation therefore becomes important. Evidently, apart from certain bacterial species, the intestinal epithelium of adult mammals is considered to be permeable to small amounts of macromolecular substances [64-67]. The mucosal barrier integrity is a prime requirement for proper delivery of oxygen to the tissues and in conditions like disturbed microcirculation macrophages and leucocytes generate oxygen radicals that further leads to increased mucosal permeability. Exposure to IR generates such free radicals by photolysis of water and in a way enhances the mucosal damage and permeability, thereby increasing chances of bacterial

translocation. This permeability of intestinal epithelium further extends to pathogenic bacteria, such as certain *Salmonella* species, which readily penetrate the GI epithelium of mice and appear in the mesenteric lymph nodes [68-70]. Nonindigenous *Escherichia coli* [71-73], *Klebsiella pneumonia*, *Pseudomonas* and *Clostridium perfringens* [74] also disseminate from the GI tract to other organs in antibiotic treated mice. It has been reported that the intestinal lamina propria of specific pathogen free (SPF) mice was densely infiltrated with lymphocytes and plasma cells [75]. This infiltration has been termed "physiological inflammation". In fact, the morphology of the intestinal villi may be determined to a great extent by the presence of these lymphoid cells in the lamina propria. Amongst pathogenic bacteria, when *S. typhimurium* levels in the ilea of conventional mice reached high population, translocation to the mesenteric lymph nodes occurred as readily as in the gnotobiotic mice [76]. Population levels of indigenous *Escherichia coli* in the ceca of the gnotobiotic mice monoassociated with *E. coli* were 10,000 times greater than that of SPF mice inoculated with this *E. coli*. Therefore, translocation of indigenous *E. coli* to the mesenteric lymph nodes also might not occur unless the *E. coli* reaches to a critical high population level in mouse GI tract.

5.1 Pathways of Bacterial Translocation

Apart from the venous and lymphatic system, bacterial translocation occurs in gut after mucosal injury either by transcellualr, paracellular or in combination of both pathways. In more common transcellular pathway, translocation occurs through enterocytes and membrane pumps. Opening up the gaps between enetrocytes by loosening the intercellular tight junction may increase bacterial translocation [77]. Transcellular migration has been shown to occur in rats where in intact enterocytes certain pathogenic bacteria like *E.coli* and *P. Mirabilis* were seen. However, there is lack of evidences to confirm that changes in villi morphology are causally related to increased rates of translocation. Transportation of macromolecules occurs through apical and basolateral membranes. In paracellular pathway epithelial tight junctions open and close all the time in response to inflammatory mediators and microbial stimuli. It occurs through disruption of tight junction and damage to the cytoskeleton of the enetrocytes [78]. Table 1 depicts studies conducted to ascertain bacterial translocation as a result of GI damage followed by radiation.

The problem of bacterial translocation is just not limited to the possibility of infection in the organ where bacteria have reached but there are also other factors associated with the severity. Microbial regulation of radiosensitivity in intestine and resistance to radiation in certain pathogenic organism are few such factors. In such a study it was suggested that the enhanced lethality of TBI in conventional (CONV-R) mice is related to systemic infection and or greater susceptibility to intestinal damage. However, the precise nature of the cellular damage, its relationship to survival and the molecular pathways through which the microbiota operates to influence intestinal radiosensitivity remain poorly defined [79]. Further in this study, using adult germ free (GF), CONV-R and or CONV-D normal, knockout and chimeric mice treated with TBI and bone marrow transplantation (BMT) they could show that (i) gut microbes affect the radiosensitivity of endothelial cells and lymphocytes populating the mesenchyme of small intestinal villi and (ii) an epithelial-derived, secreted member of the angiopoietin family whose expression is normally suppressed by the microbiota modulates the radioresistant intestinal phenotype of GF animals. It was inferred from the study that there is microbial specificity to the lethal radiosensitive phenotype imparted by the microbiota. It was observed that mice harbouring a normal microbiota exhibited enhanced intestinal radiosensitivity compared with GF animals. They further showed that enteritis was responsible for their increased mortality after TBI-BMT and at the same time BMT cannot rescue lethality due to radiation enteritis. Also, indigenous microbes alter the radioresponsive phenotype of the intestine independent of systemic infection or functions that are provided by transplanted, bone marrow-derived, villus mesenchymal cells [80].

In those individuals with ill or compromised health status, mechanism of development of sepsis is shown in figure (Fig. 1) where if patient undergoing any radiotherapy, translocation of indigenous or and exogenous bacteria may occur to result into fatal problems multiple organ failure (MOF), septicaemia etc [81]. Some inflammatory compounds are responsible for the generation of systemic inflammatory response syndrome (SIRS) [82] which is a condition different from sepsis where the pathogenic microorganisms can be isolated. Hence in SIRS, the intestine starts generating cytokines due to ischaemia as

a result of sepsis, trauma and haemorrhagic shock. Intestinal submucosal oedema causes disruption of protective integrity of mucosal barrier due to portal hypertension and leads to abnormally increased gram negative bacterial population causing endotoxin mediated mucosal injuries. The radiation mediated GI syndrome may have similar consequences as it causes GI damage and submucosal injuries. Hence patients undergoing radiotherapy in the pelvic or abdominal region should be screened for any pathological changes and correlated with the radiation induced bacterial translocation and sepsis. After invading mucosa and associated membrane, chemokines, cytokines and other pro inflammatory intermediates are released as a result of bacterial or the endotoxins mediated immune response such that the gut becomes a proinflammatory organ [83]. It affects the systemic immune systems causing cytokine mediated SIRS or multi organ dysfunction syndrome (MODS) and death. This process is known as bacterial translocation and describes the so called 'gut origin of sepsis hypothesis' [84,85] as shown in Fig. 1.

5.2 Consequences of Bacterial Translocation in GI

Apart from the radiation induced bacterial translocation at gut other factors that are responsible for bacterial translocation are intestinal obstruction [86-90], jaundice [91], inflammatory bowel disease [92,93], malignancy [94,95], pre-operative total perenteral nutrition (TPN), emergency surgery and gastric colonization with microorganisms. These conditions act to disturb the natural and fragile homeostatic equilibrium between intestinal microflora and the gut barrier promoting access of bacteria to the intestinal barrier [96,97]. Investigators have reported the transcellular migration in rats with *Escherichia coli* and *Proteus mirabilis*, within intact enterocytes [98]. Opening up the gaps between enterocytes by loosening the intercellular tight junctions increases bacterial translocation. Identification and evidence for such bacterial translocations are never easy to produce, however in mice models culturing of bacteria from different organs like spleen, ceca, liver and mesenteric lymph nodes have been done.

Numerous risk factors have been identified for such bacterial translocations to other organs and blood. Although not fully studied, discussed following are the factors responsible.

5.2.1 Immune status

Immune status of an individual is one of the most influential factors in bacterial translocation. The only way a gut commensal microbe can invade the mucosa and mucosal membrane is either a severe tissue damage or/and suppressed immune status. This immune suppressive status or illness facilitates the translocation by providing conditions that can be availed by bacteria. Disturbed local homeostasis helps bacteria to counter anatomical barrier like secretory IgA, mucous, glycocalyx brush border of the intestine etc. As previously mentioned, leukaemia is found to be associated with increased bacterial translocation to blood. There are reports which suggest an association between different pathogenic and opportunistic pathogens being translocated to different organs [99] and an incidence of postoperative sepsis compatible with mentioned mechanism of bacterial translocation.

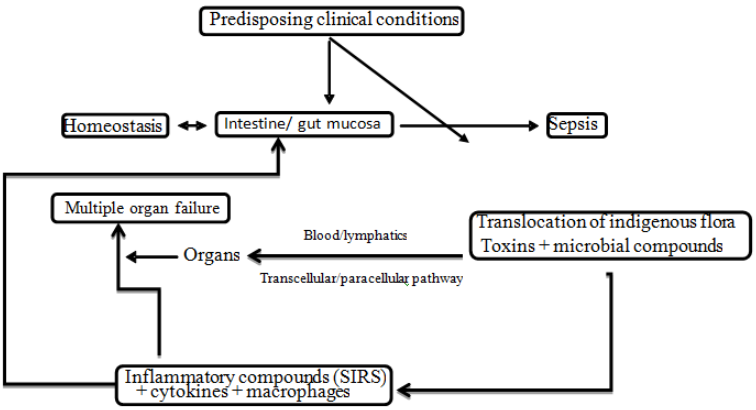


Fig. 1. Progression of gut sepsis and bacterial translocation

Table 1. Radiation induced gastrointestinal injuries and bacterial translocation

Radiation induced injury to GI	Radiation dose	Bacterial translocation in organs	Time after irradiation (days)	Predisposing factors involved	Reference
Decrease in absorption of water and sodium /chloride ions in the colon.	>8 Gy	-	4	Healthy	[128]
Two fold increase in potassium secretion.	>8 Gy		4		
Modifications of basal transepithelial electrical parameters	>10 Gy		4		
Bacterial translocation and bacteremia	5 Gy	Blood Liver Spleen Lymph nodes	7	Healthy but infected with *Citrobacter rodentium*	[129]
Liver infection Bacterial translocation	8 Gy	Liver	6	Healthy and G-CSF administered	[130]

Table 2. Different therapies for treatment of GI tract injuries

Therapy/ Drug	Complication	Outcome	References
Thermal coagulation	Late radiation proctitis, chronic rectal bleeding from rectal telangiectasia	In bipolar probe and the heater probe the mean fall in severe bleeds per case was statistically significant	[120]
Thermal coagulation	Late radiation proctitis	Statistically significant difference was observed in comparison to other untreated groups	[131]
4.0% – direct Formalin therapy	Radiation induced severe recurrent haemorrhage	Checked haemorrhage with some side effects in the form of ulcers.	[132]
Short chain fatty acids enemas (supplementation)	radiation proctitis	significant improvement for rectal bleeding	[133]
anti-inflammatory agents	Radiation-induced proctosigmoiditis	Clinical & endoscopical improvement	[115]
Probiotics	Radiation induced diarrhea	Significantly reduced the incidence of radiation-induced Diarrhea	[134]
Probiotics (by curbing bacterial pathogenesis in damaged GI tissues)	ulcerative colitis	remission maintenance of ulcerative colitis	[135] [136]

Table 3. Summary of studies using drug or agonist as radiation countermeasures

Radiation type and dose	Agonist/ Drug used	Dose concentration	Remarks	Mechanism involved	Model organism	Reference
X-ray (6.8 Gy)	Lactoferrin (*i.p*)	once at 4 mg/animal	Higher survival rate, improved Hb and hematocrit levels and hydroxyl radical scavenger activity.	By improving hematocrit levels	C3H/He mice	[137]
137Cs 8 Gy γ radiation	Flagellin purified from *Salmonella typhimurium* i.p.	50 µg 2 hr pre radiation + Bone marrow cells	Protected mice against radiation damage.	flagellin elicited radioprotection by TLR5 and MyD88 action	C57BL/6	[123]
137Cs 8 Gy	*Escherichia coli* O111:B4	5 mg/kg LPS	Inducible Radioprotection Depends on IKKβ	IKK_ protects crypt epithelial cells from both LPS- and IR-induced apoptosis, and mediates the radioprotective effects of LPS	transgenic *Vil-Cre mice and other knockout mice*	[138]
14 Gy	elemental liquid diet	Nutren 1.0 diluted in water 1:2 for 7 days after radiation	Improved survival, body weight recovery and normalization of intestinal epithelium	protective effects of liquid diet on ISC/intestinal epithelial cells at times later than 4 days post-radiation	C57Bl/6J	[139]
Cobalt 60 γ radiation	LPS injected subcutaneously 1 hr before radiation 3 µg/mouse 4 µg/mouse 3 µg/mouse	3 µg/mouse 4 µg/mouse 3 µg/mouse	Decrease in DNA damage	TLR5 activation	C57BL/6J CBLB502 CBLB502	[140]

5.2.2 Disturbing homeostasis of intestine

Maintaining the normal homeostasis is of utmost importance in the GI tract. It comprises of a numerous species of bacteria which in normal physiological condition are commensal but may turn into opportunist pathogen if the normalcy is disturbed. The gut flora is manipulated by various factors like diet, gastric acid, gastric and luminal secretions, bile salts, lysozyme, secretory IgA antibodies, antibacterial drugs, bacterial interactions and gut peristalsis [100]. A consistent increase in population of certain bacterial species is not necessary in bacterial translocation. It is evident by the fact that obligate anaerobes are significantly high in number but seldom show translocation. Different pathogenic bacteria with specific virulence factors contribute to natural disturbances and displacement of the normal flora leading to infection. Indigenous bacterial flora outnumbering the pathogenic bacteria shows rigid resistance to usual infection. Hence, some pathological conditions and oral antibiotics therapy are also responsible factors for the translocation as they can disturb the normal flora. In case of disturbances of indigenous flora by prolonged critical care therapy leads to imbalance between the host and the gut flora due to antibiotics [101].

5.2.3 Mucosal permeability

The first line of defence in the GI tract comprised of mucous coating which contains mucin and antimicrobial peptides. Beneath the mucin lies the epithelium lining on which TLR are expressed to elicit the host defence response after recognizing the pathogens. Once the pathogens pass the mucous and epithelial barriers they are phagocytosed by submucosal macrophages [102]. Bacterial translocation occurs as a result of hypoperfusion which causes movement of blood toward more vital organs. Villi injury due to reperfusion is followed by release of pro-inflammatory factors, mucosal disruption and increased intestinal permeability. The integrity of gut mucosa does not remain intact when there is a disturbance in the microcirculation. Oxygen radicals released from radiation induced injury, reactive oxygen species and abrupt increase in oxygen radicals released from macrophages and leucocytes may lead to increased mucosal permeability. As mentioned earlier, pathological conditions like colonic ulcers and jaundice may further help in bacterial translocations. Such conditions impair luminal tissue permeability by immunological disturbance and by inhibitory effect of bile on bacterial invasion of enterocytes. The physical integrity is usually restored by a rapid migration of specialized cells to the site of injury.

Several other factors like stress, radiation, intestinal peristalsis and some drugs can influence intestinal permeability and thus bacterial translocation. Immunosuppressive agents, non-steroidal anti-inflammatory drugs and certain antibiotics increase the translocation.

5.2.4 Multi-organ failure (MOF)

Bacteria and their components, products or both cross the intestinal barrier and cause infection leading to excessive inflammation in the local region and eventually organ damage and death [103]. During stress conditions gut injury may occur due to decreased blood flow to the intestines. This allows bacteria and endotoxin to enter the circulation causing MODS. Infection in the blood occurs by bacterial translocation across the epithelial mucosa.

6. COUNTERMEASURES (SYNTHETIC DRUGS AND PROBIOTICS)

Parker used the term probiotics to describe organisms and substances that improve microbial balance in the intestine [104]. A probiotic is a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract. This definition, however, was initially intended for use with animal feed products. For human nutrition probiotics are defined as live microbial food supplements or components of bacteria which have been shown to have beneficial effects on human health. The intestinal normal microflora is a metabolically active but as yet unexplored area of host defence. The bacterial flora (microbiota) of the gut is significant in relation to inflammation and so favourable influence on its composition can be a strategy to mitigate inflammation. Ingesting probiotics can affect the composition of the resident gut microbiota that may have effect on the immune system and the permeability of the mucosa. The better the barrier effect of the mucosa the smaller the risk of translocation of pro-inflammatory components originating from the gut microbiota [105]. Probiotics introduce new microbes to the GI tract to enhance microbiota maintenance and modification, while most prebiotic components have been shown to enhance the growth of *Bifidobacterium* biota. Intestinal exposure to specific bacterial strains

may either suppress an undesired immune response, for instance, allergic and autoimmune reactions, immune stimulatory way, associated with adjuvanticity and increased intestinal non-specific IgA secretion [106].

Many bacterial species like *lactobacillus, L. paracasei*, *L. rhamnosus*, *L. acidophilus*, *L. johnsonii*, *L. fermentum*, *L. reuteri*, *L. plantarum, bifidobacteria, E.coli, species of bacillus, yeast, enterococcus and bravibacillus*. [107] are known to have potential to be used as probiotics. For radiation induced enteritis, there have been human trials using certain probiotics with different markers affected such as VSL#3, *L. rhamnosus, L. rhamnosus GG, L. acidophilus, L. casei* DN-114 001 [108-112]. VSL#3 is a mixture of *L. casei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *Bifidobacterium longum*, *B. breve*, *B. infantis* and *Streptococcus salivarius* subsp. *thermophiles*. Probiotic supplement not only helps establishment of intestinal bacterial flora but also restores the immunologic homeostatsis by means of these bacterial component's binding with TLR. Microbial stimulation by probiotic bacteria may modulate the immune response differently in healthy individuals stimulating a nonspecific immune response to pathogens, while in hypersensitive subjects it down-regulated the inflammatory response [113]. However some other factors are to be taken care of like age, immune status and health status of individual. Intestinal bacteria and its components, such as LPS, may traverse through the portal blood flow. From circulation it reaches the hepatocytes, non-parenchymal cells, Kupffer cells, hepatic stellate cells and disturbs the functioning of such cells [114].

Several drugs have been investigated for their possible role as therapy for radiation induced GI injuries. Oral sulfasalazine plus rectal steroids were studied for their potential as choice of therapy and found to have appreciable results [115].

The efficacy of Metronidazole (3 x 400 mg orally per day) in comparison to other two drugs, mesalazine (3x1 g orally per day) and betamethasone enema (daily) or mesalazine and betamethasone enema, was studied for 1 year in the treatment of chronic radiation injury. It was observed that incidences of rectal bleeding and mucosal ulcers were significantly lowered in the metronidazole group at 4 weeks, 3 months and 12 months. There was also a significant decrease in diarrhea and oedema in the metronidazole group after 4 weeks, 3 months and 12 months. A reduction in the grade of their rectal bleeding compared to 5 out of 12 in the group treated with mesalazine and betmethasone was observed after a year of treatment showing metronidazole as an effective drug for such radiation induced injuries in GI and lower abdomen [116]. Other researchers used short chain fatty acids (SCFA) for the treatment of radiation proctitis [117,118]. SCFA are oxidative fuel of colonic mucosa and their use may be impaired in chronic radiation proctitis. SCFA are produced in the colon by anaerobic bacterial fermentation of non-absorbed carbohydrates in dietary fibre.

Another strategy in the form of administration of fecal solution has been investigated to counter GI related damages in mice model. Several other studies are available with different therapy strategies like thermal coagulation therapy [119], hyperbaric oxygen therapy [120-122] which allows re-epithelialisation of damaged tissue (Table 2).

7. DYSBIOSIS FOLLOWING IRRADIATION

The state of shift in distribution pattern of local bacterial community or change in the microbial community or/and alteration in the metabolic activity is known as dysbiosis. All of the mentioned conditions are possible after irradiation and hence the different aspects of disturbance in the gut flora following radiation needs to be addressed. The mechanism of action of radiation to cause dysbiosis and its treatment strategies are yet to be studied in order to understand dysbiosis following irradiation as a whole.

LPS administration can be quite dangerous inducing rapid sepsis at high doses and causing severe lung inflammation in mice at doses as low as 1 mg/kg body weight. The TLR5 agonist flagellin is a potent activator of innate immune signalling pathways in epithelial cells but has generally observed to be a poor activator of hemopoietic cells, such as macrophages and dendritic cells (DC). The effect of bacterial flagellin in protection against chemicals, bacteria, viruses and radiation was well studied earlier [123]. They were able to prove that flagellin on systemic administration elicits a non-pathologic profile of cytokine with no acute injury unlike. They reported that compared with LPS, an equal or 5-fold greater amount of systemically

administered flagellin by mass (an equimolar dose) induced very little TNF, IL-1 and RANTES. It induced only a modest level of IL-6. Flagellin induced similar levels of G-CSF and induced markedly greater levels of the human IL-8 homologue KC compared with LPS. Thus flagellin is not necessarily a weaker agonist than LPS but rather induces a distinct response that might have less potential to cause injury. In case of radiation mediated injuries, the study highlighted that the flagellin protects mice against irradiation by a mechanism requiring TLR5 and MyD88. Administration of 1 μg of flagellin, 2 h before irradiation, protected 75% of mice against this challenge. On the other hand mice lacking MyD88 or TLR5 could not be protected against radiation by flagellin. It was verified that mice lacking TLR5 could still be protected by the TLR4 agonist LPS. Thus flagellin-elicited radioprotection is mediated by TLR5-mediated innate immune signalling.

Epithelial cells produce certain cytokines which play key role in flagellin elicited radioprotection. For example, G-CSF boost production of innate immune cells that protects mice from opportunistic infections arising from irradiation due to leukopenia and loss of intestinal mucosal barrier. Although flagellin induces both pro and anti apoptotic gene expression, its induction of anti-apoptotic gene expression generally prevails *in vitro* while *in vivo* flagellin acts as a potent cytoprotectant of intestinal epithelial cells. Also, flagellin induces expression of genes with direct antibacterial activity and activates heat-shock protein expression which better equip these cells to survive a bacterial challenge which is usually a consequence of radiation induced GI syndrome.

8. INTESTINAL MICROBIOTA AND DERIVED METABOLOMICS BLOOD PLASMA AS BIOMARKERS OF RADIATION EXPOSURE

To lower the burden of examining, admitting and treatment of each possibly radiation exposed individual and avoiding the crucial time in attending only suspicious patients would be the aim of first responders in any radiation accident scenario. First few hours or days after radiation exposure are very crucial in terms of development of anomalies and survival in subjects and hence immediate attention should be given to the actually exposed individuals. An effective assay is hence required to serve the purpose. A research conducted on rat model to investigate (i) the possibility of development of such biomarker assay and (ii) the potential for intestinal microbiota to provide a non-invasive measurement to rapidly identify prior exposure to ionizing radiation suggested that intestinal microbiota provided a sustained level of reporting signals persisting over 21 days following exposure. The ratio of two individual bacteria increased 64-fold at day 21 compared with day 0 and that may have utility as a biomarker of prior exposure. However, microbial fluctuation in intestine may also be indicative of early GI injury following fractionated therapeutic radiation. They further analysed taxa in 373 stool samples from the Human Microbiome Project and compared them with their findings. Fourteen of the 15 bacterial genera that were up, down and stable following irradiation in rats were found in the human samples. It was thus concluded from the study that using intestinal microbiota as biomarkers of prior radiation exposure represents a novel approach that can complement conventional chromosome aberrational analysis and may significantly enhance biological dose assessments [124].

Earlier investigation on fecal microbial diversity after radiation revealed that certain bacterial populations remain unchanged, some were sensitive and some showed variation. For instance, total-body irradiation of 13.6 Gy or local intestinal radiation alone (19.4 Gy) in the rat resulted in bacterial overgrowth of fecal-type organisms in the small intestine.

Despite limited studies characterizing serum/ plasma metabolomic response to ionizing radiation as a function of varied doses and times after irradiation, recently there are studies which give insight into the possibility of using microbiota derived metabolites as biomarker for prior radiation exposure.

9. FUTURE PERSPECTIVES AND CONCLUSION

Gut microflora apparently helps in developing innate immune system as reported in earlier studies [125] (Table 4). The intestinal epithelium constitutes the host's first line of defense against exogenous agents such as food antigens, live bacteria, bacterial products and others present in the intestinal lumen. Along the length of intestine, the dynamics and diversity of microbial flora changes and serves as the stimulators of TLRs to help in maintaining local homeostasis. Under physiological conditions the digestive tract

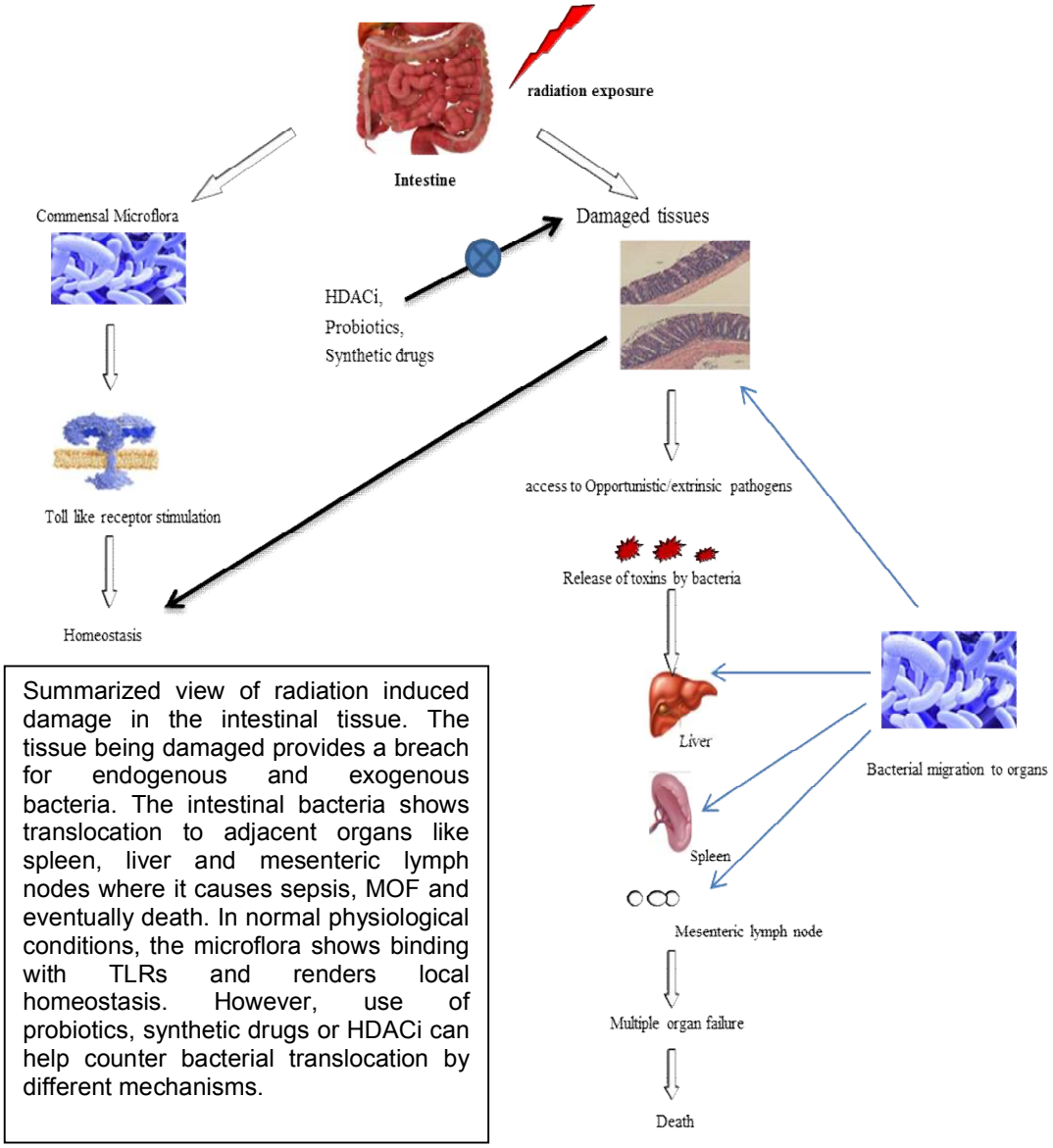
epithelium serves as a strong, selective barrier against potentially harmful bacteria. The histological alterations observed after 10 and 12 Gy are consistent with changes in basal electrical transepithelial parameters. The abolition of epithelial potential difference and the increased conductance observed 4 days after irradiation with 10 Gy are clearly associated with a disruption of epithelial integrity. Exposure of the digestive system to IR induces a series of cellular and functional alterations that lead to diarrhea after high doses (10 Gy). Increased loss of water and electrolytes has been attributed to denudation of intestinal epithelium subsequent to disruption of mitosis and cell death. Increased evidence has suggested that radiation exposure to GI tract results into bacterial translocation across the intestinal barrier. The translocation leads to dysbiosis and infection in organs to which it has translocated and eventually death due to MOF. It is observed that in times, bacterial translocation may occur through other mechanisms where the mucosal barrier might not have lost its integrity. TLR signaling is associated with increased DNA repair and activation of innate immune response. In some cases, activation of certain TLRs also mediate the opposite effect i.e. the inhibition of inflammation like as in a study it was observed that blockade of TLR 3 protected mice from lethal radiation induced GI syndrome. It is evident from the published studies that the survival or apoptosis of the concerned cell is linked with the TLR signaling via DNA repair pathway. Activation of TLRs is associated not only with the commensal flora of the gut but also with the potent pathogens. This leads to influence variety of cellular pathways which further results in regulation of cell cycle, DNA repair, apoptosis, superoxide dismutase control and immune response is consistent with the data discussed in this review.

Hence, change in the commensal bacteria under the response of radiation and expression of TLRs accordingly may be the hotspot for future research in designing countermeasures against radiation induced GI damage. There are several countermeasures studied so far to mitigate and subsidize the after effects of GI syndrome with measurable success. Varieties of probiotics have been tested for the recovery of radiation induced GI syndrome using almost same principle of replenishing the gut microflora and hence bringing the local homeostasis back. Data in the review evidently suggests that various components of bacteria have been used to illicit the TLR response under the radiation stress and subsequent gene expression were studied. Genes responsible for DNA repair, SODs were found to be influenced collaterally with TLR activation by different microbial components like lipopolysaccride and flagellin. In a study it was observed that transplantation of TLR9 agonist-stimulated macrophage induced radio-mitigation against 9.4 Gy WBI (whole body irradiation), suggesting that TLR9 agonist-activated macrophages could secrete growth factors that might mediate the radioprotective effects of TLR9 agonist and TLR activation. Histone deacetylase inhibitors (HDACi) like Sulforaphane, trichostatin A and Diallyl sulphides are the regimen to look for, as earlier published reports have explored and verified the promising nature of these substances as countermeasures for radiation induced injuries [126]. Limited reports are available for the application of HDAC inhibitors in mitigating GI syndrome and very rarely its direct effect on GI microbiota. From several evidences mentioned in this review, it is evident that no concrete treatment is yet available which ensures replenishment of natural microflora by repairing GI tissue damage. It is hence recommended that studies are urgently needed on application and potential of HDACi as potent countermeasure for radiation induced GI damage and subsequent disturbance in microflora. A summarized line diagram depicting the endogenous microbial translocation, consequences and therapies is shown (Fig. 2).

A recent remarkable study in this very context summarizes the basic concept of this review that the intestinal commensal microbes not only can be looked for biomarkers but also in first place for protection against radiation injuries. They reported that repair of chromosomal DNA lesions induced by high LET radiation occurred more efficiently in conventional than in restricted intestinal microbiota mice model. Based on different phylotype densities after WBI, bacterial indicator phylotypes were found to be more abundant in restricted than in conventional microbiota. As per their conclusion (a) restricted microbiota phylotypes when correlated with persistent DNA double strand breaks (DSBs) were found to orchestrate oncoprotective controlled cell death after radiation, (b) restricted microbiota composition reduced proinflammatory extracellular stimulated immune responses, but specifically increased antineoplastic cytolytic memory $CD8^+$ T cells by low taxonomic diversity and (c) DNA damage repair efficiency induced by a model of conventional microbiota most likely

initiates an adaptive response to radiation [127]. These responses are made through microbiota induced intestinal sub symptomatic inflammation. This makes it clear that the intestinal microbiome (commensal) is associated with immune regulation in a certain way to reduce risk of high LET radiation induced injury.

Hence, with analysis of all the relevant data available and studies done so far we conclude that extensive research on development of chemical substances as radiomitigators and radioprotectors needs to be done. However, more attention should be focused on the naturally available regimen in the form of intestinal microbiota as far as the radiation induced GI syndrome is concerned. In depth study on immune mechanisms including the regulation of TLRs and their association with microbial ligands is warranted. Several beneficial therapeutic options might originate from a better understanding of the relationship among intestinal microbiota and immune regulation during radiation injury.



Summarized view of radiation induced damage in the intestinal tissue. The tissue being damaged provides a breach for endogenous and exogenous bacteria. The intestinal bacteria shows translocation to adjacent organs like spleen, liver and mesenteric lymph nodes where it causes sepsis, MOF and eventually death. In normal physiological conditions, the microflora shows binding with TLRs and renders local homeostasis. However, use of probiotics, synthetic drugs or HDACi can help counter bacterial translocation by different mechanisms.

Fig. 2. Overall picture of radiation induced disturbances in GI tract, consequences and therapies

Table 4. Role of gut microflora in development of immune system

Gut flora type	Model	Remarks/outcome	Methodology	Reference
Enteric virus and bacteria	murine	Enhanced self limiting humoral response. Activation of natural killer cells & constitutively cytotoxic T cells. Chronic germinal centers reaction.	Oral administration of enteric reovirus. Colonization of the gut with segmented filamentous bacteria.	[141]
Probiotics.(*lactobacillus* and *Bacillus* spp.)	Human trials	NK-cells, IgM, IgA and IL-10 affected	Daily oral admin of L.salivarius CECT5713	[142]
Lactobacillus casei shirota	Human volunteers	enhance NK cell activity *in vivo* and *in vitro* in humans, and IL-12 may be responsible for enhancement of NK cell activity triggered by LcS.	Placebo-controlled cross over trial. Administration of fermented milk with strain for 3 weeks.	[143]
Combination of Lactobacillus acidophilus (L. acidophilus) 74-2 and Bifidobacterium animalis subsp lactis DGCC 420 (B. lactis 420)	A placebo-controlled, double-blinded, randomized crossover trial was conducted. Human volunteers.	Percentages of granulocytes and monocytes showing phagocytic activity were significantly elevated from 92 to 95%	Consumption of 300 g/day of yoghurt supplement containing probiotic strains L. acidophilus 74-2 and B. lactis 420 by treatment group.	[144]
Lactobacillus rhamnosus GG (LGG), *Bifidobacterium animalis ssp. lactis Bb12* (Bb12), or *Propionibacterium reudenreichii ssp. shermanii JS* (PJS)	randomized, double-blind and placebo-controlled parallel group intervention study on healthy humans	serum hsCRP expressed as the median AUC0-21 (minus baseline) was 0.018 mg/L in the placebo group, -0.240 mg/L in the LGG group, 0.090 mg/L in the Bb12 group and -0.085 mg/L in the PJS group. In vitro production of TNF-α from in vitro cultured peripheral blood mononuclear cells (PBMC) was significantly lower in subjects receiving LGG vs placebo. IL-2 production from PBMC in the Bb12 group was significantly lower compared with the other groups.	volunteers randomized to receive a milk-based drink containing strains	[145]

Gut flora type	Model	Remarks/outcome	Methodology	Reference
Immunostimulating probiotic *Bifidobacterium lactis* HN019	healthy elderly human volunteers	Increases in the proportions of total, helper (CD4+),and activated (CD25+) T lymphocytes and natural killer cells. ex vivo phagocytic capacity of mononuclear and polymorphonuclear phagocytes and the tumoricidal activity of natural killer cells were also elevated	3-stage dietary supplementation trial lasting 9 wk. During stage 1 (run-in), subjects consumed low-fat milk (200 mL twice daily for 3 wk) as a base-diet control. During stage 2 (intervention), they consumed milk supplemented with B. lactis HN019 in a typical dose (5 × 1010 organisms/d) or a low dose (5 × 109 organisms/d) for 3 wk. During stage 3 (washout), they consumed low-fat milk for 3 wk	[146]
Lactobacillus gasseri PA 16/8, Bifidobacterium longum SP 07/3, B. bifidum MF	A randomized, double-blind, placebo-controlled intervention study on healthy adults.	A significantly higher enhancement of cytotoxic plus T suppressor cells (CD8+) and a higher enhancement of T helper cells (CD4+) reported in the probiotic-treated group	Volunteer's diet supplemented daily with vitamins and minerals with or without the probiotic bacteria	[147]
Lactobacillus casei Shirota	Patients with alcoholic cirrhosis	Baseline neutrophil phagocytic capacity in patients was significantly lower compared to healthy controls (73% versus 98%, $p < 0.05$), but normalised at the end of the study. TLR2, 4 and 9 were overexpressed in patients. TLR4 expression normalised by the end of the study.	patients with alcoholic cirrhosis (n = 12) received Lactobacillus casei Shirota (6.5 × 109) 3 times daily for 4 weeks	[148]
Lactobacillus plantarum 299v	critically ill patients	On day 15, serum IL-6 levels were significantly lower in the treatment group compared to controls and hence associated with late attenuation of the systemic inflammatory response	Oral preparation containing L. plantarum 299v (ProViva)	[149]

Gut flora type	Model	Remarks/outcome	Methodology	Reference
Lactobacillus rhamnosus GR-1 and *L. reuteri* RC-14	20 healthy controls and 20 subjects with inflammatory bowel disease	The proportion of CD4+ CD25high T cells increased significantly in IBD patients after treatment, but non-significantly in controls. The increase in CD4+ CD25high T cells correlated with the decrease in the percentage of TNF-α- or IL-12-producing monocytes and Dendritic cells.	Oral administration of strains supplemented yogurt for 30 days	[150]

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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