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Co- and polymicrobial infections in the gut mucosa: The host–microbiota–pathogen perspective

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Abstract

Infections in humans occur in the context of complex niches where the pathogen interacts with both the host microenvironment and immune response, and the symbiotic microbial community. The polymicrobial nature of many human infections adds a further layer of complexity. The effect of co- or polymicrobial infections can result in enhanced severity due to pathogens cooperative interaction or reduced morbidity because one of the pathogens affects the fitness of the other(s). In this review, the concept of co-infections and polymicrobial interactions in the context of the intestinal mucosa is discussed, focusing on the interplay between the host, the microbiota and the pathogenic organisms. Specifically, we will examine examples of pathogen-cooperative versus -antagonistic behaviour during co- and polymicrobial infections. We discuss: the infection-induced modulation of the host microenvironment and immune responses; the direct modulation of the microorganism's fitness; the potentiation of inflammatory/carcinogenic conditions by polymicrobial biofilms; and the promotion of co-infections by microbial-induced DNA damage. Open questions in this very exciting field are also highlighted.

KEYWORDS
bacteria, co-infections, disease progression, fungi, microbiota, mucosal microenvironment, parasites, polymicrobial infections, viruses

1 | INTRODUCTION

The interface between the human body and the external environment represents a complex niche that accommodates a rich polymicrobial community and is the main port of adhesion and entry of pathogenic micro- and macroorganisms (Bomsel & Alfsen, 2003; Hirt, 2019; Ribet & Cossart, 2015). An additional layer of complexity is the site-specific mucosal immunological landscape necessary to fight infection, limiting the tissue damage and ensuring the re-establishment of microenvironment's homeostasis (McGhee & Fujihashi, 2012; Nagler & Feehley, 2013). Thus, it is clear that infections occur in very complex niches where more than one pathogenic agent can be present, and the sum of polymicrobial pathogens–host interactions define the course of the disease.

Several interesting aspects emerge from experimental data, including: (a) cooperative versus antagonistic behaviour of microorganisms (viruses, bacteria, fungi, unicellular parasites) and macroorganisms (helminths) either indirectly, via modulation of the host microenvironment or directly via alteration of the pathogen's fitness; (b) novel aspects where polymicrobial biofilms may potentiate inflammatory/carcinogenic conditions or microbial-induced DNA damage may promote co-infections.

The best characterized models of polymicrobial interaction are associated with the oral cavity and respiratory mucosa (Domingue, Drewes, Merlo, Housseau, & Sears, 2020; Lamont, Koo, & Hajishengallis, 2018; Peters, Jabra-Rizk, O'May, Costerton, & Shirtliff, 2012; Welp & Bomberger, 2020). Here, however, we will discuss the concept of co- and polymicrobial infections in the context of the intestinal mucosa, with specific focus on the interplay between the host, the gastrointestinal microbiota and the pathogenic organisms.

Collectively, four aspects will be highlighted: (a) cooperative behaviour; (b) antagonistic behaviour; (c) polymicrobial biofilms; (d) microbial-induced DNA damage and co-infections, as summarised in Figure 1. For a detailed analysis of mechanisms of inter-microbial interactions several excellent reviews are available (Deveau et al., 2018; Ghoul & Mitri, 2016; Stubbendieck & Straight, 2016).

2 | COOPERATIVE BEHAVIOUR

The cooperative behaviour among pathogens allows one organism to enhance the susceptibility and/or the severity of other infections. This effect can be exerted via two main mechanisms, which are not mutually exclusive: (a) modulation of the host microenvironment; (b) direct effect on the organism's fitness. Most of the experimental data in the literature have focused on the immunomodulatory properties, as summarised in Figure 2.

2.1 | Helminths and co-infections

Helminths are master modulators of both the host immune responses (Jackson, Friberg, Little, & Bradley, 2009) as well as the composition of the gut microbiota (Walk, Blum, Ewing, Weinstock, & Young, 2010), favouring colonisation and infection by other viral and bacterial enteric pathogens (Yap & Gause, 2018). The geographic distribution of these parasitic infections overlaps with high incidence of bacteria-virus-mediated gastrointestinal diseases. Therefore, the pathogenicity

of helminths can be the sum of a direct effect of the infection per se (due to tissue damage) and an indirect effect due to the increased severity caused by other types of infections.

A common theme in the helminth viral/bacterial co-infections is the parasite-induced IL4-STAT6 axis, which skews the host immune responses towards a T Helper (TH) 2 profile (Allen & Maizels, 2011), with broad consequences on the control of infections that require a cellular TH1/IFN-γ-based immune response (Thakur, Mikkelsen, & Jungersen, 2019).

Reese and colleagues have investigated the impact of helminth infection on reactivation of the murine γ-herpes virus 68 (MHV68). Herpes viruses establish a latent infection in the host, characterized by a limited expression of viral genes, followed by reactivation of a productive cycle in order to spread to a new host (Sehrawat, Kumar, & Rouse, 2018). Several of the human γ-herpes viruses are well characterized oncogenic pathogens, including the Epstein–Barr virus (EBV) and the Kaposi Sarcoma virus (KSV). The helminths *Heligmosomoides polygyrus* (Hp) or *Schistosomiasis mansoni* (Sm) were used as models in this study. Acute infection with Hp or administration of Sm eggs reactivates the MHV68 lytic cycle (Reese et al., 2014). This effect is dependent on the activation of the IL-4-STAT6 pathway, which results in a concomitant STAT6-dependent transactivation of the viral transcriptional transactivator Rta/Orf50 and the IL-4-mediated suppression of IFN-γ. Exogenous administration of IL-4 was also sufficient to reactivate the KSV lytic replication in the human BCLB1 lymphoma cell line *in vitro* (Reese et al., 2014). Therefore, in the context of co-infections, changes in the cytokine levels in the tissue microenvironment can act as a rheostat for balancing the equilibrium between latent and lytic viral infections.

The helminth-induced IL-4-STAT6 pathway can also impair the antiviral immunity in response to acute infection with the murine norovirus (MNV) CW3. This was assessed by reduced cell activation and proliferation of CD8+ virus-specific cytotoxic T lymphocytes and increased viral loads in mice co-infected with *Trichinella spiralis* (Ts) and CW3 compared to mice only exposed to the viral pathogen. This effect is dependent on the IL-4-STAT6-mediated differentiation of alternatively activated macrophages (AAM), prevented in IL-4 or
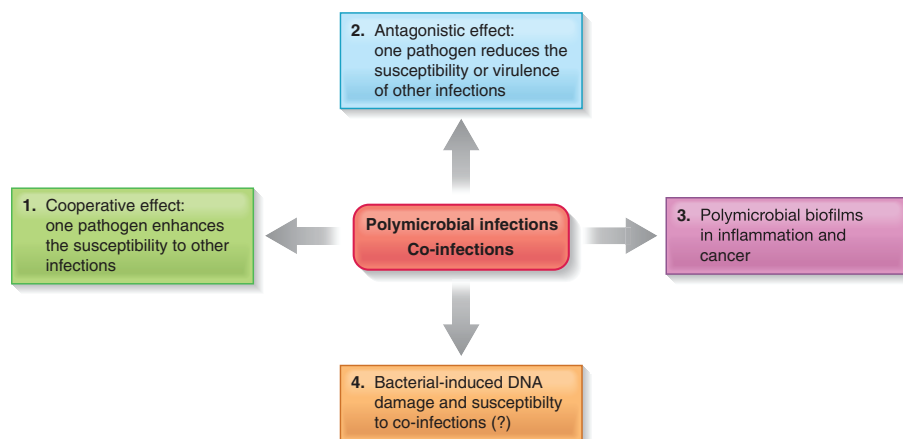

FIGURE 1 Outcomes of co- and polymicrobial infections. Presence of one pathogen can enhance (cooperative behaviour) or reduce (antagonistic behaviour) the colonisation and virulence of other infections, respectively. The presence of polymicrobial biofilms can also contribute to the initiation and/or progression of inflammatory/carcinogenic conditions. The possibility that microbial-induced DNA damage may enhance the susceptibility to co-infections is a novel aspect, emerging in the recent literature
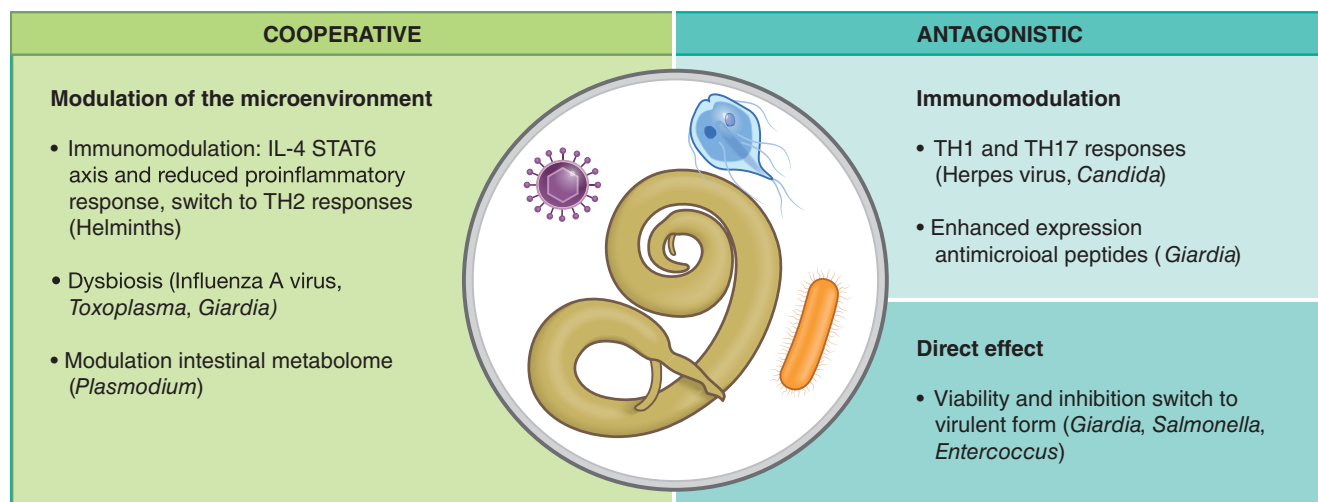
FIGURE 2 Mechanisms regulating cooperative and antagonistic behaviour. The cooperative/antagonistic behaviour in co- and polymicrobial infections can be regulated via two main mechanisms: (i) modulation of the host microenvironment (immune responses, gut microbiota and local metabolism); (ii) direct effect on the organism's fitness (regulation of viability or virulence features)

STAT6 deficient mice, rather than on the Ts-induced dysbiosis of the intestinal microbiota as demonstrated by co-infection experiments performed in germ-free mice (Osborne et al., 2014).

The synergistic effect of helminth colonisation of the intestinal mucosa has also been demonstrated in the context of enteropathogenic bacterial infections, specifically for *Citrobacter rodentium*, a murine model for enteropathogenic *Escherichia coli* (EPEC) and *Salmonella enterica* serovar Typhimurium. Co-infection of BALB/c mice with Hp and *C. rodentium* results in an enhanced bacterial-associated colitis, increased weight loss and mortality and enhanced systemic spread compared to mice exposed to the bacterium in a mono-infection model. Co-infection is associated with a significant decreased in IFN-γ mRNA with concomitant upregulation of TNF-α transcripts in the colonic tissue (Chen, Louie, McCormick, Walker, & Shi, 2005). These effects are dependent on STAT6, since they are prevented in a STAT6 knock out mouse model. Interestingly, the systemic response to the bacterial infection, measured by the levels of TH1- and TH2-specific antibacterial antibodies (IgG1 and IgG2a), is higher in the serum of co-infected mice. However, this is not sufficient to prevent intestinal tissue damage and bacterial clearance (Chen et al., 2005), highlighting the importance to study the effects of helminth-bacteria co-infection directly at the site of infection.

Similarly, enhanced colitis, higher bacterial burden and systemic spread are also observed in a model of co-infection of Hp and *S*. Typhimurium, and it is associated with a reduced pro-inflammatory and antimicrobial response in the colon mucosa (decreased levels of transcripts for IL-17, IL-22, IL-23 and the antimicrobial peptides Reg3β and Reg3γ) (Su et al., 2014). The overall result is a strong decrease of neutrophil infiltration, possibly due to the IL-10-mediated downregulation of the neutrophil-attracting chemokines KC and MIP2 (Su et al., 2014).

Infection with Hp promotes dysbiosis, specifically an expansion of *Lactobacillaceae* (Walk et al., 2010), known to have beneficial anti-inflammatory properties (Round, O'Connell, & Mazmanian, 2010): Therefore, it is plausible that the immunomodulatory feature of helminth infections is a combination of effects on the host immune responses and the gut microbiota. It would be interesting to assess whether the helminth-induced dysbiosis is also dependent of the IL-4-STAT6 axis.

It is also likely that Hp infection enhances *Salmonella* pathogenicity by additional mechanisms, such as alteration of the metabolic profile of the small intestine, and consequent modulation of the bacterial gene transcriptional programme. Indeed, Reynolds et al. have recently shown that Hp infection alters the profile of 362 intestinal metabolites. Exposure of *Salmonella* to the metabolites extracted from Hp-infected intestine regulates expression of virulence genes encoded by the *Salmonella* pathogenicity island (SPI) I, and affects the invasive capacity of the bacterium in an *in vitro* model (Reynolds et al., 2017). Interestingly, in this work the IL4-STAT6 axis seems not to play a key role in enhancing the *Salmonella* mediated pathogenicity, as demonstrated for *C. rodentium*, and the viruses MNV CW3 and MHV68. This different behaviour may be associated with the different life-styles of the pathogens: extracellular (*Citrobacter*), invasive (*Salmonella*) or exclusively dependent on the cellular microenvironment for replication (viruses).

2.2 | Malaria and *Salmonella* infection

Non-typhoidal *Salmonella* (NTS) infections, which normally cause a self-limiting gastroenteritis, is becoming one of the most common systemic bacteremia in young children in sub-Saharan Africa (Feasey, Dougan, Kingsley, Heyderman, & Gordon, 2012). Epidemiological studies have identified malaria (caused by several *Plasmodium* ssp) as a risk factor for the systemic dissemination of the disease (Bronzan et al., 2007). Co-infection experimental mouse models using

Plasmodium yoelii and *S.* Typhimurium as model for NTS demonstrates that underlying *Plasmodium* infection increases the risk of systemic *Salmonella* infection, as assessed by higher bacterial burden in the liver, spleen and blood of co-infected mice compared to mice exposed only to *Salmonella* (Cunnington, de Souza, Walther, & Riley, 2012; Lokken et al., 2014). Remarkably, the higher bacterial load in the liver of co-infected mice was associated with a reduced neutrophil infiltration, reduced activation of myeloid cells and mobilisation of immature granulocytes from the bone marrow (Cunnington et al., 2012; Lokken et al., 2014; Lokken, Stull-Lane, Poels, & Tsolis, 2018). These effects are due to the parasite-induced IL-10 production and upregulation of the haem-oxygenase-1 (HO-1) as consequence of the *Plasmodium*-induced haemolysis, since they were abrogated in conditional animals with myeloid cells deficient in IL-10 production or IL-10 receptor, or upon administration of an HO-1 inhibitor prior to *Salmonella* infection (Cunnington et al., 2012; Lokken et al., 2014). The combined action of IL-10 and HO-1 reduces the oxidative burst of *Salmonella*-infected myeloid cells limiting their bactericidal capacity, and simultaneously increasing the intracellular availability of iron, a key factor to sustain bacterial replication (Cunnington et al., 2012; Lokken et al., 2018).

2.3 | Dysbiosis and enhanced susceptibility to secondary infections

The gut microbiota is part of the innate immune defence, and any changes in its composition may influence the host susceptibility to gastrointestinal infections as shown for *Clostridium difficile* (Lagier, 2016). Therefore, it is not surprising that underlying infections may promote dysbiosis favouring secondary infections. An even more interesting facet is the possibility that infection on a distal site modulates the intestinal microbiota. Influenza A pulmonary infection promotes an increase in Proteobacteria, specifically *Enterobacteriaceae* (genus *Escherichia*) and a decrease in segmented filamentous bacteria (SFB). This effect is mediated by the activation of the type I interferon (IFN-I) pathway in response to the respiratory tract infection and is prevented in mice deficient for the IFNα receptor (Deriu et al., 2016). SFB represents an important group of commensals that contribute to the differentiation of mucosal TH17 CD4+ T cells, key adaptive effectors to confer protection against *C. rodentium* infection (Ivanov et al., 2009). Therefore, the virus-induced dysbiosis may promote susceptibility to enteropathogens. In support of this possibility, the viral-induced IFN-I-dependent dysbiosis enhances *S.* Typhimurium colonisation and invasive disease, and a significant reduction of the IFN-γ pro-inflammatory and antimicrobial peptide host response (Deriu et al., 2016). Whether the effect on *Salmonella* infection is a direct consequence of the dysbiosis or is dependent on the IFN-I-mediated remodelling of the intestinal microenvironment has not been assessed, and would require faecal transfer-based experiments.

A similar change of the gut microbiota composition, with a marked expansion of *Proteobacteria* is observed upon infection with *Toxoplasma gondii* (Wang et al., 2019) and *Giardia lamblia* (Barash,

Maloney, Singer, & Dawsona, 2017), suggesting that these pathogens may favour secondary bacterial infections in a cooperative modality.

Conversely, it is also possible that enteropathogenic bacteria favour parasitic infection. It has been recently shown that EPEC protects *Entamoeba histolytica* against oxidative stress induced *in vitro* by exposure to hydrogen peroxide (Shaulov et al., 2018; Varet et al., 2018). Based on this evidence, it would be relevant to assess whether co-infection with EPEC or dysbiosis associated with expansion of *Enterobacteriaceae* would enhance the host susceptibility to *Entamoeba* infection.

3 | ANTAGONISTIC BEHAVIOUR

Co-infection can also result in an antagonistic behaviour, where one pathogen confers protection against other microorganisms. Modulation of the host microenvironment, in particular the host immune response, plays a key role in this protective effect (Figure 2). To date, only a limited number of studies have addressed the possibility of a direct effect of one microbe over the other (Figure 2).

3.1 | Pathogen-induced modulation host microenvironment

Latent infection with the murine γ-herpes virus MHV68 reduces colonisation and mortality upon challenge with the enteropathogenic bacteria *Listeria monocytogenes* and *Yersinia pestis* (Barton et al., 2007). These effects are associated with a higher serum levels of IFN-γ and TNF-α, and independent on the adaptive host response, since they are still observed upon depletion of CD4+ and CD8+ T cells, suggesting a role for a viral-dependent systemic effect on the innate immunity. In support of this possibility, peritoneal macrophages isolated from MHV68 latently infected mice present characteristics of activation, such as upregulation of MHC class II, vacuolisation, membrane ruffling and enhanced bactericidal capacity *in vitro* against *L. monocytogens*. The MHV68-induced protection is long lasting and can be detected as late as 3 months after the establishment of the latent viral infection. These data indicate that co-evolution of the herpes viruses with the host has led to an immune modulation, beneficial for both the host (protection from infections) and the virus (establishment of a persistent infection and successful spread within the reservoir). Possibly, this explains the successful nature of this virus family, which infect asymptomatically and long-lived the majority of the human population (Sehrawat et al., 2018).

Infection with the unicellular parasite *Giardia muris* also attenuates the severity of colitis and the bacterial burden upon *C. rodentium* infection in an experimental model. This is associated with the transcriptional upregulation of the antimicrobial peptides (AMPs) β-defensin 3 and trefoil factor 3 (TFF3) in the colonic tissue, and significant reduction of *C. rodentium* attachment to the mucosa and translocation of the commensal microbiota in the lamina propria and to distal organs (Manko et al., 2017). In addition, the

same study has demonstrated that *G. muris* or *G. duodenalis* directly inhibit the growth of *C. rodentium* or EPEC, respectively in *in vitro* experiments. These data indicate that the protozoan effect on the enteropathogenic bacterial infection can be multifaceted and dependent both on immune-modulation of the microenvironment and on a direct effect on the bacteria viability, via their cathepsin-like cysteine proteases.

Pre-colonisation of mice with *Candida albicans* has been shown to reduce the mortality upon *C. difficile* infection in a mouse model. Colonisation with the fungus does not reduce the extent of intestinal colonisation by the enterobacterium, but it promotes increased levels of transcripts for IL-17A and IFN-γ and changes in the intestinal microbiota, with expansion of the beneficial genera *Akkermansia* and *Bifidobacterium*. Intraperitoneal administration of recombinant IL-17A is sufficient to recapitulate the protective effect of the *Candida* pre-colonisation (Markey et al., 2018). Another study, however, reports that *C. albicans* pre-colonisation worsens the *C. difficile* disease severity (Panpetch et al., 2019). This discrepancy highlights the complexity and the multifactorial aspects of polymicrobial infections, including microbiota diversity in different facilities, age of infection, strains used, status of the host.

3.2 | Direct effect of one pathogen over the other(s)

A heat stable factor secreted by *S.* Typhimurium can prevent *C. albicans* transition from yeast to the filamentous form (filamentation), a key virulence step, in the model organism *Caenorhabditis elegans*. The effect of the heat-inactivated filtered cultures supernatant is dose-dependent and maximal inhibition of filamentation is observed when the supernatant from the bacterial stationary culture is used (Tampakakis, Peleg, & Mylonakis, 2009). Co-culture experiments performed *in vitro* in planktonic conditions further demonstrated that *S.* Typhimurium reduces *Candida* viability, which is more pronounced at 37°C, a parameter that promotes the switch from the yeast to the filamentous form. The cytotoxic activity of *Salmonella* is also observed when the fungus is grown in biofilm-forming conditions.

A very interesting interaction between *C. albicans* and the Gram-positive *Enterococcus faecalis* has been described, where the co-infection by both these opportunistic pathogens of the oral and gastro-intestinal tract reduces the host mortality and enhances long term colonisation in a *C. elegans* model (Cruz, Graham, Gagliano, Lorenz, & Garsin, 2013) This interaction represents a clear example on how the polymicrobial infections may reduce the virulence of both microorganisms, possibly allowing them a long-term colonisation of a favourable niche. Similarly to the *Salmonella* co-infection model (Tampakakis et al., 2009), the reduced virulence of *Candida* is associated with an inhibition of filamentation, due to secretion of a heat stable product with a molecular mass between 3 and 10 kDa. Deletion of the Frs quorum sensing system and two of the downstream regulated proteases GelE and SprE partially inhibits the *E. faecalis* protective

effect (Cruz et al., 2013). A subsequent study has identified the bacteriocin EntV[68], whose expression is regulated by the Frs system and requires the GelE-mediated cleavage into the active form, as the molecule that inhibits *Candida* filamentation in an *in vitro* biofilm model, but not in planktonic conditions (Graham, Cruz, Garsin, & Lorenz, 2017). Administration of a synthetic EntV[68] reduces the cytotoxic effect of *C. albicans* on macrophages, by inhibiting the hyphal morphogenesis and fungal viability within the phagocytic cells, and preventing the development of oropharyngeal candidiasis in a mouse model (Graham et al., 2017). It would be very interesting to investigate the effect of *C. albicans* on *E. faecalis*, since in the *C. elegans* model the co-infection also reduced the severity of the bacterial disease.

3.3 | Effect of the gut virome on intestinal infections

An emerging player in the maintenance of the intestinal homeostasis and susceptibility/resistance to intestinal infection is the gut virome, largely composed by bacteriophages (*Caudovirales*, *Microviridae* and the recently identified cross-assembly phage, crAssphage, belonging to the *Podoviridae* family) and a small percentage of animal viruses (anellovirus, parvovirus, adenovirus and papillomavirus) (Mukhopadhy, Segal, Carding, Hart, & Hold, 2019; Neil & Cadwell, 2018).

Study the human virome poses many challenges compared to the analysis of the bacterial component of the tissue microbiota, due to the lack of conserved regions in viral genomes that can be amplified as the bacterial 16S rDNA, the technical challenges associated with loss of genomic material during the isolation of virus-like particles (VLP), the limited number of reference genomes and the lack of standardise methodologies (Mukhopadhy et al., 2019; Ogilvie & Jones, 2015). However, in spite of these limitations, some indirect evidences suggest that intestinal viruses may contribute to enhance colonisation resistance of vancomycin-resistance *Enterococcus faecium* (VRE). Administration of resiquimod (R848), an agent that mimic viral-derived single-stranded (ss) RNA, known to trigger activation of the Toll-like receptor (TLR) 7, strongly reduces VRE colonisation in ampicillin treated C57BL/6 mice. This effect is associated with the TLR7-mediated upregulation of IL22 mRNA by the innate lymphoid cells (ILCs), leading to enhanced gene expression of the antimicrobial peptide Reg3γ (Abt et al., 2016).

It is very likely that with the exponential development of the sequencing techniques and bioinformatic tools, our knowledge regarding the role of the virome in the modulation of infections will significantly expand in the near future.

4 | POLYMICROBIAL BIOFILMS IN HEALTH AND DISEASE

The combination of *in situ* hybridisation analysis and 16S rDNA sequencing allows visualisation and characterization of mucosa-

associated bacteria in healthy subjects and in patients suffering from different diseases, such as inflammatory bowel disease (IBD) or colorectal carcinoma (CRC).

Swidsinski and colleagues have demonstrated that mucosa-associated bacteria forming biofilm-like structures were at least 100-fold more concentrated in biopsies from IBD patients compared to healthy controls. These structures were of polymicrobial origin, however *Bacteroides fragilis* represents more than 60% of the biofilm biomass in IBD patients, while a more diverse composition with presence of *Eubacterium rectale* and the *Bacteroides* groups is detected in biopsies from patients suffering from spontaneous colitis (Swidsinski, Weber, Loening-Baucke, Hale, & Lochs, 2005). A different composition of the mucosa-associated microbiota in IBD patients compared to healthy subjects was also confirmed by Nishino et al. (2018) by 16S rDNA sequencing. The authors have identified a different pattern of mucosal microbiota in the two different subgroups of IBD patients: Crohn's disease (CD) and ulcerative colitis (UC). Expansion of Proteobacteria (including *Escherichia* and *Fusobacterium*) is detected in CD patients, compared to UC patients and healthy subjects, while *Blautia*, *Veillonella* and *Bifidobacterium* are more abundant in UC patients compared to healthy individuals (Nishino et al., 2018).

These two studies (Nishino et al., 2018; Swidsinski et al., 2005) have applied two different methods for the analysis (in situ fluorescent in situ hybridisation of Carnoy's fixed biopsies to preserve the tissue spatial organisation versus bulk 16S rDNA sequencing). However, in spite of the non-completely overlapping results in terms of the identity of the genera, it is obvious that the mucosa-associated microbiome is complex, and clearly differs in inflammatory-prone compared to healthy conditions.

Similarly to IBD, polymicrobial biofilms have also been characterized in proximal CRC and in polyps that develop in patients with familial adenomatous polyposis (FAP), a hereditary condition caused by germline mutations of the tumour suppressor gene APC (Dejea et al., 2014;Dejea et al., 2018; Drewes et al., 2017). Interestingly, patients with biofilm-positive tumours, also present similar structures in distal tumour-free regions. This is associated with significant pro-tumorigenic changes on the underlying colonic epithelium, including reduced expression of E-cadherin, increased activation of the IL-6/STAT3 pathway and enhanced proliferative capacity of the crypt epithelial cells (Dejea et al., 2014). These data suggest that the alteration of the microbiota may occur prior tumour initiation.

Biofilms in FAP patients are enriched in Proteobacteria and Bacteroidetes, specifically *E. coli* and *B. fragilis*, while CRC-associated biofilms are enriched in *B. fragilis* and oral pathogens including *Fusobacterium nucleatum*, *Parvimonas micra* and *Peptostreptococcus stomatis* (Dejea et al., 2018; Drewes et al., 2017).

Bacteroides fragilis seems to be a common denominator in inflammatory or cancer-prone conditions. A proportion of *B. fragilis* strains expresses a zinc-dependent metalloprotease toxin (BFT) and is defined as enterotoxigenic *B. fragilis* (ETBF). Chronic colonisation with ETBF promotes a low grade TH17-mediated colonic inflammatory condition (Wick et al., 2014). Therefore, it is possible that this alteration of the tissue microenvironment promotes favourable conditions for the formation of specific polymicrobial biofilms, contributing to disease progression. The synergistic effect of co-colonisation by *B. fragilis* and *E. coli* has been confirmed in two CRC-experimental models, one mimicking sporadic CRC (Azoxymethane, AOM, treatment) and one representing familiar form of CRC (mouse model carrying a mutated version of the APC gene). In both models, co-colonisation is associated with an enhanced morbidity and mortality as well as increased colonic tumorigenesis (Dejea et al., 2018). The carcinogenic properties are associated with two key virulence factors: the BFT from *B. fragilis* (Sears, 2009) and the expression of a functional *psk* island in *E. coli*, producing a well characterized bacterial genotoxin that promotes DNA damage, known as colibactin, which can promote genomic instability (Martin & Frisan, 2020). In the presence of ETBF, the DNA damaging effect of the *pks + E. coli* in the colon epithelium is significantly higher. This is possibly due to the *B. fragilis* mucolytic effect, which may favour an easier access of *E. coli* to the mucosa, enhancing delivery of colibactin into the host cells (Dejea et al., 2018).

5 | MICROBIAL-INDUCED DNA DAMAGE AND CO-INFECTIONS

A novel aspect emerging from studies of the pathogen–host interaction field is the role of senescence (Humphreys, ElGhazaly, & Frisan, 2020). Senescence is a process by which cells, in response to diverse stress conditions, enter a permanent cell cycle arrest, but maintain a high metabolic state characterized by secretion of a plethora of soluble mediators, a feature known as senescence-associated secretory phenotype (SASP) (Gorgoulis et al., 2019). Chronic induction of DNA damage is one of the triggers of cellular senescence (Wang, Kohli, & Demaria, 2020). Microbial infections have been shown to promote DNA damage either indirectly (oxidative stress-induced by activation of the innate host response) (Weitzman & Weitzman, 2014), or directly via dedicated microbial virulence factors that can damage the host DNA (bacterial genotoxins) or inhibit DNA repair (Bezine, Vignard, & Mirey, 2014; Chumduri, Gurumurthy, Zietlow, & Meyer, 2016; Martin & Frisan, 2020).

Why is this relevant in the context of polymicrobial infections? Recent studies have demonstrated that induction of cellular senescence can enhance invasion and replication of *S.* Typhimurium and *F. nucleatum*, as well as increased susceptibility to *Streptococcus pneumoniae*, Influenza virus and Varicella Zoster virus infection (Ahn et al., 2017; Ibler et al., 2019; Kim, Seong, & Shin, 2016; Lim et al., 2010; Shivshankar, Boyd, Le Saux, Yeh, & Orihuela, 2011). Several enteric pathogens produce genotoxins (including typhoid and non-typhoidal *Salmonella*, the *E. coli* phylogroup B2, *Shigella* ssp, *Campylobacter* ssp), which can exert a strong immunomodulatory effect on the intestinal mucosa (Del Bel Belluz et al., 2016; Miller et al., 2018). The genotoxin-induced senescence may promote colonisation and intracellular invasion by other microorganisms. Thus, this additional mechanism may contribute to the establishment of specific polymicrobial biofilms as those detected in IBD, FAP and CRC patients,

explaining the association between *E. coli* and *F. nucleatum* observed in CD patients (Nishino et al., 2018).

6 | CONCLUDING REMARKS

The mucosal microenvironment is a microcosm where three key players interact with each other: the local host microenvironment/immune system, the microbiota and invading pathogens. The microorganisms present in this tissue coexist in polymicrobial communities with intra- and interspecies interactions, which have important implication on disease onset and severity.

In spite of the recent advances, there are still many questions that need to be addressed to understand the intricate molecular mechanisms governing these interactions. This knowledge can be exploited to develop better, more specific and targeted therapeutic approaches.

The exponential growing technological developments now allows *in situ* and *ex vivo* multiplex transcriptomic and phenotypic analyses (single cell sequencing, mass cytometry, multiplex transcriptomics) as well as the possibility to use complex 3D/organotypic culture. These tools can be used to answer still open questions such as: (a) what determines the switch from a commensal to a pathogenic life-style; (b) how one pathogen or changes in the mucosal microbiota modulate the fitness of another pathogen during co-infections in term of nutritional availability, rapid mucus turnover, resistance to oxidative stress, physical stress and host defence mechanisms; (c) what is the role of the tissue microenvironment on the polymicrobial infections; (d) how these complex interactions alter the course of infection (e.g., morbidity or definition of acute versus chronic infections). The characterization of the gut virome role in maintaining the intestinal homeostasis, shaping the bacterial microbiota in health and disease, and modulating the interaction with pathogenic organisms adds a novel twist in our understanding of co-infections. With all these possibilities to explore, infection biology and cellular microbiology will remain very exciting fields of research for many years to come.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
Data sharing is not applicable to this article since it is a review and no new data were created or analysed in this study.

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