Diogo André Andrade de Freitas

Infectious Etiology in Inflammatory Bowel Disease – Participation of *Mycobacterium avium* subsp. *paratuberculosis* and Adherent-Invasive Strains of *Escherichia coli*

1º Ciclo de Ciências da Nutrição
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(Diogo André Andrade de Freitas)

Trabalho Complementar apresentado à Universidade Fernando Pessoa como parte dos requisitos para obtenção do grau de licenciado em Ciências da Nutrição

Orientadora:

Prof. Doutora Amélia Assunção

Para a Luz da minha vida, para o Nélio e para o Afonso

Agradecimentos:

Os meus pais e ao meu irmão por me apoiarem ao longo desta longa caminhada! Obrigado! Adoro-vos com todo o meu coração!

Obrigado aos meus avós, pelo amor incondicional e preocupação!

Obrigado à Cátia por ser o melhor presente que a faculdade me deu!

Obrigado à Micaela que depois de tantos anos continua ao meu lado a apoiar-me!

Muito Obrigado à Doutora Amélia Assunção pela disponibilidade e dedicação ao longo do ano e na elaboração deste trabalho.

Obrigado à Doutora Rita Guerra, pela constante disponibilidade.

Obrigado a todos os professores por promoverem o meu crescimento pessoal e profissional ao longo destes quarto anos.

A Todos o meu Obrigado!

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Lista de Abreviaturas:

AIEC – Adherent-Invasive *Escherichia Coli*

CD – Crohn's Disease

CEACAM-6 - Carcinoembryonic Antigen Related Cell Adhesion Molecule 6

DC – Dendritic Cells

GPR43 - G-Protein-Coupled Receptor 43

IBD – Inflammatory Bowel Disease

IEC – Intestinal Epithelial Cells

IL – Inter-Leucine

INF-γ – Interferon-Gamma

MAP – *Mycobacterium Avium Paratuberculosis*

NF-kB - Factor Nuclear Kappa B

NOD2 – Nucleotide-binding oligomerization domain-containing protein 2

PCR – Polymerase Chain Reaction

SCFA - Short Chain Fatty Acids

Th - T Helper Cell

TNF-α – Tumor Necrosis Factor-Alpha

UC – Ulcerative Colitis

Infectious Etiology in Inflammatory Bowel Disease – Participation of Mycobacterium avium subsp. paratuberculosis and Adherent-Invasive Strains of Escherichia coli

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Short Title: Infectious Etiology in Inflammatory Bowel Disease

Word Count: 4307

Number of Tables: 0

Conflict of Interest: Nothing to Declare

Abstract:

Inflammatory Bowel Disease (IBD) includes Crohn's Disease (CD) and Ulcerative Colitis (UC), with its etiology still to be established. IBD has been suggested to be an interaction between genetic susceptible host, microbiome interactions and induction of an abnormal immune response. This study proposes to evaluate the multiple literature present and understand the possible activity of bacteria.

There have been identified multiple polymorphisms in CD and UC patients, from multiple agents related with immune response. These polymorphisms also seem to be useful for the colonization of specific bacteria, like *Mycobacterium avium. Paratuberculosis* (MAP) and Adherent-Invasive *Escherichia Coli* (AIEC) that seem to be involved in the IBD etiology as a causative agent or as an opportunist pathogen of the genetic susceptibility, however this involvement is still unclear and the studies until now are ambiguous when compared with each other.

Moreover, environmental factors like diet seem to be involved in the development of IBD, especially when considered the high prevalence and increase of IBD in western countries and newly westernized countries, respectively. Concluding that a western diet is a participant in the development of IBD. On the other side with the presence of plant-based diet it could be used as a therapeutic agent granting remission in IBD.

Keywords: IBD, Cronh's Disease, Ulcerative Colitis, *Mycobacteirum avium* subsp. *paratuberculosis,* Adherent-Invasive *Escherichia Coli* and Nutrition.

Resumo:

A Doença Inflamatória Intestinal (DII) inclui a Doença de Crohn(DC) e a Colite Ulcerosa(CU), estando a sua etiologia por estabelecer. DII encontra-se associada a uma interação entre hospedeiros geneticamente suscetíveis, interações do microbiota e a presença de uma resposta inflamatória exacerbada. Com este estudo pretendeu-se avaliar a literatura existente e estabelecer uma ligação entre a etiologia e a atividade de agentes microbianos.

Foram identificados com maior frequência em pacientes com DC e CU, a presença um leque variado de polimorfismos, relacionados com os agentes eretores da resposta imune. Estes apresentam-se com ferramentas uteis para a colonização de bactérias como *Mycobacterium avium. Paratuberculosis* (MAP) e Adherent-Invasive *Escherichia Coli* (AIEC), que tem sido ligada à etiologia da DII como agentes causadores ou potenciais patogénios oportunistas. Contudo, a informação existente é ambígua não permitindo concluir a causalidade destes microrganismos.

Adicionalmente, a o estudo de fatores ambientais com à dieta apresentam-se envolvidos no desenvolvimento da DII, especialmente quando considera a elevada prevalência e aumento da DII em "Western Countries" e em "Newly-Westernized Countires", respetivamente. Levando a concluir que uma dieta ocidental é um fator de risco para o desenvolvimento da DII, realçando-se assim a dietas de base-vegetal como potenciais agentes terapêuticos para a remissão da DII.

Palavras Chave: IBD, Doença de Cronh, Colite Ulcerosa, *Mycobacteirum avium* subsp. *paratuberculosis,* Adherent-Invasive *Escherichia Coli* and Nutrição

I. Introduction

Inflammatory Bowel Disease (IBD) is indeed a group of diseases where the most common clinical presentations are Crohn's Disease (CD) and Ulcerative Colitis (UC)[1]. In UC, inflammation occurs most commonly in the mucosal and submucosal layers of the colon, whereas on CD, inflammation tends to be transmural and naturally discontinuous, with the capacity to affect any segment of the gastrointestinal tract, most frequently the terminal ileum, ascending colon and rectum[2,3]. There are also pathophysiological differences between CD and UC. Indeed. a number of studies refer that the immune response in CD is predominantly a Th1 response, mediated by the release of proinflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ), while in UC a predominance of Th2 response is observed.[4]

IBD was first described during the 20th century[5], exhibiting an exponential increase in prevalence in the westernized countries (Europe, North America, Australia and New Zeeland). Fortunately, in the 21st century its prevalence seems to be stabilized in these countries, although is still increasing in the newly westernized countries[6].

The etiology of both diseases is still debatable. Several studies have pointed out that the main cause of IBD is a combination of inherited susceptibility factors, environmental factors and altered mucosal immune responses[7]. These factors would allow the establishment of chronic inflammatory responses to microbes in genetical susceptible hosts[4]. Patients with CD tend to show diverse composition of their gut microbiota, with the increase in the number of mucosa-associated bacteria and the reduction in the overall biodiversity[8,9]. Specifically, many studies have reported an increase in *Proteobacteria* and *Bacteroidetes*, and a decrease in *Firmicutes,* which are considered beneficial bacteria.

Even though, until now, no single causative microorganism has been confirmed, there are two microbial agents that have been intensively investigated: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and adherent-invasive strains of Escherichia coli (AIEC)[9,10].

MAP belongs to the genus *Mycobacterium* and is a member of *Mycobacterium avium* complex. MAP is the causative agent of a chronic granulomatous inflammation of the ruminants named Johne's disease. Since Johne's disease shows similarities with CD, and MAP has been detected in higher frequency in CD patients than in controls[10,11], it has long been suspected to play a role in CD. On the other hand, AIEC are E. coli variants, with ability to adhere, invade intestinal cells (IEC) and replicate within macrophages[9].

Their high prevalence in the colonic mucosa of CD and UC patients suggests that AIEC may also participate in IBD establishment[9].

Nutrition has been extensively correlated with IBD, due to the frequent presence of multifactorial nutritional deficiencies in IBD patitents[4] and to its natural influence with microbiota composition. Diet is probably an important etiological factor, since western diet was shown to be correlated with the rising of IBD in the western and newly westernized countries. It was described that a high-sugar and high-fat diet stimulates the growth and metabolic activity of more aggressive commensal bacterial species resulting in dysbiosis [4,8,10] with changes in microbiota composition described above.

Due to the importance of microbial contribution to IBD onset (either as single microorganisms or the whole microbiota community) and the correlation between dysbiosis and diet, the objective of this study is to describe the current knowledge about MAP and AEIC involvement in IBD and also to investigate the role of nutrition, particularly western diet, in IBD establishment. I will also discuss possible nutritional interventions to prevent the development and progression of these diseases.

II. Methodology

This study is a bibliographical review of 51 articles present in the literature, recurring to multiple data base like PubMed, Scielo and ScienceDirect. The research for the articles was conducted from March to July, and the main key words used were: IBD, Crohn's Disease, Ulcerative Colitis, Mycobacterium Avium Paratuberculosis "MAP", Adherent/Invasive Escherichia Coli "AIEC", Ethology, Pathology, Nutrition. It was defined as exclusion factor any article published from more than 20 years, except articles with relevant findings (like Dalziel, 1913).

III. Inflammatory Bowel Disease

1. Definition and Epidemiology

IBD encompasses multiple diseases, with CD and UC as the most common clinical presentations. Patients who suffer from IBD frequently show fever, abdominal pain, diarrhea, rectal bleeding and weight loss[12]. Signs of malnutrition are also present. Perianal disease, abdominal mass and growth failure in children and adolescents are also

observed[12]. Most of the symptoms described are commonly present in CD but less are reported in UC[12]. Both CD and UC have been characterized as relapsing inflammatory diseases, since they alternate between relapse and remission phases, being heavily associated with the development of intestinal cancer[1,13].

CD has been characterized by the presence of transmural granulomatous inflammation commonly present in the terminal ileum, colon and perianal region being cable of affecting any segment of the gastrointestinal tract[14,15]. CD complications are usually presented as intestinal strictures, fistulas and abscesses[14]. UC affects the rectum and a variable length of the colon, it's confluent and it involves the mucosa and lamina propria, unlike CD that is not as superficial as UC[14,16]. Histologically, CD presents focal patchy chronic inflammation, focal crypt irregularity and non-caseating granulomata while UC presents a superficial confluent neutrophilic infiltration with loss of crypt architecture, basal plasmacytosis, goblet cell depletion and crypt abscess[15,16]. CD is normally associated with a Th1/Th17 immune response with the secretion of TNF-α, IFN-γ and IL-17 and UC a Th2 response with a predominant presence of IL-5 and IL-13 responses with a decrease in IL-33[1,16].

In the last decade the increase in IBD prevalence has been exponential, primarily in Western regions like North America and Europe, affecting about 2 million and 1.5 million of individuals, respectively. Since 1990, IBD prevalence in western countries has stabilized or decreased, while newly westernized countries in Africa, Asia and South America (for example Brazil) present an increasing prevalence representing a growing public health challenge worldwide[6].

2. Etiology of IBD

i. Genetic factors

Genetic susceptibility is believed to be one of the main etiological factors of IBD[1]. Genetic variants of multiple genes involved in immune responses have been identified, which in association with microbial virulence factors would potentiate bacterial infection leading an abnormal immune response[1,12]. These polymorphisms have been identified in higher frequency in CD patients rather than healthy controls, indicating a possible relation with CD etiology.

The presence of a defective mucosal barrier and increased intestinal permeability has long been observed in CD and UC patients[1]. The first physical barrier in the gut is formed by the mucus layer, produced by polymerization of gel-forming mucins, secreted by goblet cells. The mucus layer enables the prevention of bacterial break-through and the subsequent intestinal inflammation. CD patients with inflamed ileum have presented a reduced expression of MUC1 mRNA as well as MUC3, and MUC5B, reflecting a disruption in the mucous layer[1,10]. The second physical barrier is composed by epithelial cells firmly connected by tight junctions maintaining its integrity. Epithelial cells, besides its physical barrier capacity, are also responsible for secreting bactericidal agents like defensins[1]. A defective expression of defensins by Paneth cells has been described in CD patients.

Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) is an intracellular receptor present in epithelial cells as well as in phagocytes and is responsible for activating the Factor Nuclear Kappa B (NF-kB) pathway, inducing the autophagy pathway, secretion of cytokines and secretion of antimicrobial peptides. The first gene variants identified as risk factor for CD were observed in NOD2 gene [1,10].The risk alleles present loss-function mutations leading to reduced NF-kB activation, weakened inflammatory cytokine response towards muramyl dipeptide (a pathogen-associated molecular pattern (PAMP) from gram-positive bacteria), ineffective autophagy and IL-10 transcription (an anti-inflammatory cytokine)[1,10,12]. Defective production of bactericidal agents like defensins has been related to risk polymorphisms of NOD2[1]. Recent Studies showed that NOD2 and Autophagy Related 16 Like 1 (ATG16L1) [the latter highly involved in autophagy] gene variants induce altered autophagic process, antigen presentation and intracellular bacterial handling in innate response cells like macrophages[1] and dendritic cells resulting in defective clearance of bacteria.

IL-23 is cytokine responsible for the crosswalk between the innate immune response and the adaptive immune response [1]. The presence of polymorphisms in IL23R has been identified in cohorts of CD and UC patients, suggesting that IL-23 might play a role in the inflammatory pathway in chronic intestinal inflammation. It has been demonstrated that IL-23 is responsible for inducing cytokine secretion in Th17 cells from the adaptive immune response[1], and so promote inflammation.

CD adaptive immune response has been characterized by an imbalance of effector T cells (Th1 and Th17, responsible for releasing high amounts of IFN-γ, TNF-α, IL-17 and IL-22) and regulatory T cells (Treg, responsible for secretion of anti-inflammatory cytokines like IL-10,transforming growth factor[TGB] and IL-5)[3]. Crucial Loci involved in Treg, Th1 and Th17 differentiation may contribute to the immune imbalance present in Crohn's disease[3]. Additionally, mutations in IL-10R gene derange the highly regulated T cell balance and are present in early onset Crohn's disease[3].

ii. Bacterial Triggers

The role of microbiome in IBD has been intensively investigated and the concept of dysbiosis has been associated with these diseases[17]. Dysbiosis is an alteration in the composition and function of luminal and mucosal-associated bacteria[17,18]. It has been established that that CD and UC patients share common dysbiosis features, consisting in a reduced diversity, decrease of beneficial bacteria like *Faecalibacterium prausnitzii* (from *Firmicutes* family with anti-inflammatory properties) while there has been observed an increse in bacteria from *Bacteroides* and *Proteobacteria*[18,9]. From these two groups of bacteria there are a lot of possible pathogenic agents (MAP, *Clostridium difficile*, *helicobacter* species) but also from the altered commensal bacteria (AIEC and enterotoxigenic *Bacteroides fragilis*)[10]. Drs Sartor et established that the alterations of gut microbiota in their composition and metabolic function can contribute to aggressive immune responses[19]. Even though there haven't been no specific agents identified yet, there have two intensively investigated agents: MAP and AIEC[10].

a) *Mycobacterium avium paratuberculosis*

Mycobacteria have been correlated to IBD even before CD was first described. It was back in 1913 that Kennedy Dolziel[5] firstly identified a mycobacteria as a possible agent in IBD. MAP belongs to the *Mycobacteria* genus, along with *Mycobacterium tuberculosis* and *Mycobacterium leprae*, which are responsible for tuberculosis and leprosy, respectively[20]. MAP is included in the *Mycobacterium avium* complex (MAC) which is comprised by *Mycobacterium avium-intercellulare* and *Mycobacterium avium* including the subspecies *paratuberculosis*[20,21]. MAP is a rod-shaped facultative

intracellular, bacterial pathogen, that resists to decolorization with acidic alcohol, just like other mycobacteria, that are known to be acid-fast[20,22]. It is also defined as an obligate intracellular pathogen, due to its inability to synthesize mycobactin from environmental sources (essential for iron uptake and cell replication)[20,22]. In human tissues MAP often presents as a spheroplast, a cell-wall-deficient state that, differently from other mycobacteria, cannot be highlighted by Ziehl-Nielsen staining[20]. This, the paucibacillary infection (with a small number of bacterial units) and the difficulty in establishing MAP *in vitro* cultures from biological samples, are major obstacles in establishing a definitive link between MAP and IBD [20,22].

MAP is the causative agent of paratuberculosis, also known as Johne's disease, which occurs in beef cattle as well as other mammals including non-human primates, causing chronic granulomatous enteritis, reginal lymphangitis and lymphadenitis[11,23]. Its clinical and histological findings show some similarities to CD, partly suggesting an association between MAP and IBD[22]. Infected animals shed the bacilli through milk and feces. Possible routes of human exposure are fecal-oral transmission from the use of manure as fertilizer, the consumption of milk and dairies[20,22], and the consumption of contaminated, treated and untreated water, as well as the use of contaminated water for recreational purposes[24]. Pierce E [24] also states that the consumption of MAP in milk represents a higher risk of infections than MAP in water.

Since its first association with IBD, and more specifically with CD, the link between MAP and CD has become tighter. Since MAP detection is difficult, molecular methods have been developed. A specific insertion element (IS900) can be used to detect and differentiate DNA MAP from other mycobacteria, via Polymerase chain reaction (PCR)[20]. Another method to identify the presence of MAP in tissues is *in situ* hybridization [20,22]. Many studies have demonstrated that the presence of MAP DNA tends to be more frequently detected in patients with CD in contrast to healthy controls[25,26]. Such tests have used intestinal biopsies and surgical specimen as well as blood and fecal samples[22]. These results were observed by Pistone *et al* [27] who found a frequency of 82.3% CD patients positive for MAP DNA, in biopsies collected from different intestinal sites. However, taking into consideration that in the area studied a wide circulation of water and other products takes place, it may influence MAP concentrations[27]. A study by Ryan *et al* [28] where MAP prevalence was evaluated by PCR from bovine intestinal granulomas (main area of macrophage-MAP-infected aggregation) obtained by laser

capture microdissection, found high prevalence of MAP, leading to the conclusion that this technique is efficient and clears some of the previous questions arose. Nazareth *et al* [29] also found high MAP DNA frequency in the peripheral blood of IBD patients. Although in healthy subjects MAP DNA in also detected [30], MAP may act as an IBD causative agent in genetically susceptible subjects. Pierce E[11] and Liverani E *et al* [22] suggests that since MAP is a mycobacteria, its pathogenicity could follow a similar pattern as observed in other mycobacterial diseases such tuberculosis, where one third of the world is infected with *M. tuberculosis* with but only 5%-10% develop clinical disease.

Several studies suggest that MAP infection results in host immunomodulation. In 2007, Clancy *R et al* [31] studied the secretion of TNF-α in cultured intestinal tissue biopsies of MAP positive (identified via PCR) CD patients using paired antibodies. These patients presented higher TNF-α levels in comparison to MAP negative CD patients. These findings suggested that in CD a specific defect in NOD2 may affect the handling of MAP by macrophages/dendritic cells[31]. Indeed, MAP was detected in granulomata in CD, particularly localized inside macrophages and myofibroblasts[31]. So, genetic defects that contribute to an impaired capacity of innate immune cells for bacterial clearance, results in infection establishment[31]. The high TNF-α secretion found may be explained by this impaired macrophage capacity to eliminate MAP [31]. However, Clancy R et al [31] discarded the possibility that such defect could be related with mutations in *NOD2* because haplotype mutations were only seen in 30% individuals and TNF-α levels were similar in patients with and without NOD2 mutations suggesting that another macrophage defect could be related the high TNF-α. These findings, however, are not corroborated by others (e.g. Clancy *et al* [31]) concluding that there is still ambiguity in the understanding of the immunomodulatory activity of MAP in CD.

Many studies have reported that CD patients positive for MAP DNA, show a higher prevalence of Th1 and Th17, as well as their pro-inflammatory cytokines IFN-α and IL-17[32]. MAP-infected macrophages secrete higher levels of pro-inflammatory cytokines like IL-23 and IL-12 that in consequence will induce inflammation by recruiting inflammatory cells, like Th1 and Th17 causing tissue injury and granuloma formation[32]. The N-glycolyl muramyl dipeptide (MDP) – present in the bacterial wall of mycobacteria - and on MAP cell wall, is a natural ligand of NOD2 cytoplasmic receptor[33]. Since MAP persists in macrophages, the continuous NOD2 activation possibly results in potent release of pro-inflammatory cytokines, such as TNF-α and IL-23[33]. It is also

possible that *NOD2* risk polymorphisms are more prone to MAP activation[33]. IL-23 induces Th17 to secrete IL-17, promoted by NOD2[33]. The presence of risk variants in IL-23R and increased IL-23 secretion may induce an expansion of IL-17 production by Th17 cells leading to an abnormal high presence of this cytokine[33].

Campos *et al* [32], also found that MAP infection of macrophages was associated with a decreased release of IL-10, an anti-inflammatory cytokine and a deficient secretion of TNF-α, going against some of the studies previously mentioned. However, these authors hypothesized that even though there was a low secretion of TNF-α from infected/colonized macrophages, other cell types secrete TNF-α as a result of the adaptive immune response, contributing to the inflammatory response[32]. TNF-α also acts as an anti-inflammatory cytokine, being already demonstrated that it can inhibit macrophage and dendritic cells from secreting IL-12 and IL-23, which allowed *Campos* to conclude that its low prevalence could implicate in a continuous promotion of inflammatory T cell mediated responses[32].

The immunopathology resulting from MAP infection in IBD is still under controversy, with some conflicting results. However, genetic susceptibility is surely a key factor for MAP-induced exacerbated inflammatory response of the gut mucosa, contributing for IBD establishment[6].

b) Adherent-Invasive Strains of *Escherichia coli*

AIEC strains are considered pathobionts, because of their ability to promote inflammatory response due to the adaptive evolution of their genome in a specific and susceptible host[9]. These strains have the ability to adhere and invade intestinal epithelial cells and macrophages, survive and replicate within the latter, without inducing cell death. AIEC strains can translocate across the human intestinal barrier, move to deeper tissues, continue activating macrophages and eventually induce the formation of granulomata[9,34,35].

Many studies have reported the presence of a higher prevalence of AIEC in IBD patients, particularly in CD [29,35]. Darfeuille-Michaud A *et al* [35] observed that AIEC strains are deeply associated with the ileal mucosa of CD patients using AIEC detection by PCR and DNA *in situ* hybridization in ileal biopsies. Another study detected a high prevalence

of E. Coli DNA in blood samples of CD patients by nested PCR[29]. Among these, around 80% carried the *fimH30* allele, bearing a mutation strongly associated with AIEC[29].

It is still unclear whether AIEC is a CD causative agent or an aggravating one by colonizing the already inflamed mucosa. AIEC presents multiple virulence genes that encode for general pathogenic traits like motility, capsule and lipopolysaccharide (LPS) expression, serum resistance, iron uptake, adhesion and invasion capacity, biofilm formation and the production of long polar fimbriae[9]. Other virulence genes encode for specific traits essential for survival and replication within macrophages, such as stress protein HtrA, the thiol-dissulfide oxireductase DsbA, the RNA-binding protein Hfq and the FAD-dependent oxyredutcase IbeA, crucial agents in AIEC resistance to phagosome acidic pH, oxidative stress, proteolytic enzymes and antimicrobial compounds of phagocytes[9,36].

Before adhering to epithelial cells, AIEC need to cross the existing mucous layer that protects the epithelial barrier. If a deficiency mucin production (by presence of certain polymorphisms) is not present, AIEC can produce a protease, concomitantly by the presence of two specific genes in a plasmid-encoded genomic island, *arla* (related to defensin resistance) and *arlc*, from the OmpT family outer membrane protease [37]. These genes grant AIEC the ability to alter the secretion of anti-microbial peptides by host Paneth cells[1,37].

Adherence is mediated via the interaction between bacterial type 1 pili, long polar fimbriae (LPF) and the host glycoprotein carcinoembryonic antigen related cell adhesion molecule 6(CEACAM-6)[9,38,39]. Type 1 pili express fimH adhesin, that efficiently adheres to intestinal epithelial cells[39]. LPF induce the secretion of pro-inflammatory cytokines (IL-8) and chemokines (CCC20) in intestinal epithelial cells, which will lead to macrophage and dendritic cell recruitment and further pro-inflammatory cytokine secretion of IFN-γ and TNF-α, increased CEACAM-6 expression and amplification of AIEC colonization [9,39]. CEACAM-6 is abnormally overexpressed in CD patients and its expression is positively regulated by adhesion of AIEC and by pro-inflammatory cytokines [9,38,39,]. It was already described that once AIEC invades macrophages, promotes the secretion of pro-inflammatory cytokines, leading to an amplification loop of colonization and inflammation[9,38,40].

Vimentin, an intermediate filament expressed on cell surfaces, also acts as a receptor for AIEC and in addition interacts with NOD2, promoting its recruitment to the plasma membrane and facilitating NOD2-dependent antigen detection[8,39]. However, when CD patients bear the NOD2 risk variants interaction with vimentin is inhibited, resulting in defective inflammatory response, autophagy induction and a mishandling of AIEC in CD[9,39].

AIEC adherence promotes the expression of the pore-forming tight junction protein claudin-2 in intestinal epithelial cells (IEC) and induces a reorganization of the tight junction from the apical side by displacing zonulae occludens-1 and E-cadherin which causes a decrease and loss of barrier function[9,38].

AIEC invasion, also named internalization, has been described to occur during tight junction reorganization [9,38]. It occurs by micropinocytosis and vacuolization of AIEC into IEC and macrophages[38]. The outer membrane vesicles of AIEC containing the transmembrane protein OmpA are shed and have the ability to interact with the endoplasmic reticulum stress response chaperone Gp96 of IEC [41-43]. Gp96 is commonly overexpressed on apical surface of CD patients IEC [41,43]. The interaction of OmpA with Gp96 promotes fusion of the outer membrane vesicles with the cell membrane and subsequent release of other bacterial components that are involved in actin polymerization and microtubule reorganization, inducing AIEC internalization [41-43].In association, flagellin binds to the Toll-Like Receptor 5 which induces a IL-8 pro-inflammatory response, also promoting the internalization into the internal mucosa [44]. Besides, M cells have the ability to transport foreign antigens and microorganisms to organized lymphoid tissue within the mucosa (Peyer's Patch)[38,39]; AIEC fimH also binds to M cells, by the recognition of glycoprotein 2, allowing the translocation of AIEC from the lumen to the mucosa. Cieza *et al*[36] reported that the protein IbeA is associated with the invasion of M cells by AIEC, with this study demonstrating in its absence a reduction of AIEC invasion. After the internalization, AIEC endosomes will eventually follow a maturation process into phagolysomes[38]. Recent studies have observed that AIEC has the capacity to escape autophagy in T84 cells and mouse enterocytes, through the up-regulation of expression of microRNA (MIR) 30C and MIR130A levels by the activation of the NF-κB pathway, resulting in the decrease expression of Autophagy related 5 (ATG5) and ATG161 levels (two autophagy proteins), inhibiting autophagy and also enhancing inflammatory response[31,45,46]. These defects have been frequently identified in CD leading to the conclusion that AIEC pathogenesis is a combination of host defects

and the microbe survival mechanisms[39]. When AIEC vacuoles fuses with lysosomes AIEC resist and take advantage of these harsh conditions, like the acidic pH, through the secretion of diverse proteins, as described above[32]. One of those is IbeA, a protein that is a FAD-dependent-oxidoreductase agent, present in AIEC that confers resistance to oxidative stress[36,39].

With the increased TNF-a production induced by AIEC in macrophages, inflammation and formation of granulomata takes place in the lymphoid follicles. AIEC is able to prevent induction of host cell death by apoptosis, through AIEC-induced alteration of caspase-3, leading to its degradation in the proteasome [35,39]. Besides macrophages and IEC, AIEC is also capable to replicate within neutrophils, however in these cells, AIEC induces autophagic death [9,39].

Finally, Th17 immune response has been linked with chronic inflammation, being characterized as a CD secondary event due to poor bacterial clareance[39]. However, a recent study from Zhang HJ *et al* [47] observed that IL-17 enhanced expression of antimicrobial peptides related to host defense. Using IL-17 knock-out mice, they demonstrated that IL-17 maintained colonic epithelial integrity under colonization of AIEC[47], constituting an important host defense agent, suppressing AIEC-dependent exacerbation of experimental colitis. However, other studies also refer that a dysregulated expansion of Th17 population, in association with the induction by IL-23, are deleterious for the host, due to inflammatory exacerbation[48].

iii. Nutrition and Diet

Diet plays a dominant role in influencing the composition of the gut microbiota and so may influence the pathogenic potential of microorganisms like MAP and AIEC[8]. In a recent study by Roberts *et al* [49] it was observed that the ingestion of soluble fibers reduced *E. coli* translocation to M cells, whereas the presence of emulsifiers regularly present in western diet, showed an increase in the translocation, potentiating E. coli colonization of the intestinal mucosa[49]. This suggested that a western diet may promote AIEC pathogenesis while diets rich in fiber may act as a therapeutic agent. In 2010, Mitsuto Chiba *et al* [50] observed a high rate of remission of CD in a group of subjects who had a semi-vegetarian diet, comparing to subjects with an omnivore diet [50]. Very recently a case report by Sandefur K *et al* [51] disclosed a full remission of CD in a patient on a plant-based diet[39]. Both studies allowed us to understand that there are components on these diets that act as anti-inflammatory. Fiber ingestion seem to correlate with an anti-

inflammatory role. Indeed, Agus A *et al* [8] described that fiber has the ability to induce the production of short chain fatty acids (SCFA), by commensal bacteria[8]. SCFA have been reported to be anti-inflammatory, reducing the production of TNF-α, IL-6 and IFN-γ, through the binding with G-protein-coupled receptor 43 (GPR43) present in intestinal immune cells[8]. Mice fed on western-diet regimen (low in fiber and high in sugar and fat), showed a lower expression of GPR43, correlating with a failure in induction of acute inflammatory responses responsible for pathogen clearance in an early state, indicating that the presence of a western diet could potentiate the overgrowth of AIEC in ileal mucosa of CD[8]. This information comes in agreement with the epidemiology studies referring that newly westernized countries as well, showed an increase in the development of IBD, leading researchers to conclude that environmental factors like diet have a strong impact in such diseases[8]. These studies present some of the potential effects of western diet and show alternative diets associated with IBD remission, reinforcing the importance of healthy dietary habits as potential therapeutic agents.

IV. Conclusion:

Throughout this study it's was possible to acknowledge the presence of potential factors in the development of IBD, that could independently act as causative agents or induce the disease via a complex interaction between them. MAP and AIEC, from the studies reviewed, there still ambiguity in defining their pathogenicity and possible etiology in the disease. Further investigation and development of new producers are needed to clearly access their role.

Furthermore, studying environmental factors and they're impact on IBD, is crucial for understanding their association with the etiological agent but also for the possible capability of preventing and treating this disease.

Acknowledgments:

This review was elaborated in the context of an investigation, where I took part, related to the study of AIEC and MAP potential pathology in the etiology of IBD.

V. References:

1. Geremia A, Biancheri P, Allan P, Corazza GR, Sabatino AD. Innate and adaptive immunity in inflammatory bowel disease. Autoimmun Rev. 2014 Jan; 13(1):3-10;
2. Danese D, Fiocchi C. Ulcerative colitis. N. Eng. J. Med. 2011. 365:1713-1725;
3. Baumgart DC, Dandborn WJ. Crohn's disease. The Lancet. 2012. 380:1590-1605;
4. Neuman MG, Nanau RM. Inflammatory Bowel disease: role of diet, microbiota, life style. Translational Research. 2012 Jul. 160(1):29-44;
5. Dalziel TK. Chronic interstitial enteritis. British J Med 1913; 2:2756;
6. Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, Panaccione R, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st centuray: systematic review of population-based studies. The Lancet. 2017 Dec 23; 390(10114):2769-2778;
7. Neurath MF. Cytokines in inflammatory bowel disease. Nat. Rev Immunol. 2014. 14: 329-342;
8. Agus A, Denizot J, Thévenot J, Martinez-Medina M, Massier S, Sauvanet P, et al. Western diet induces a shift in microbiota composition enhancing susceptibility to Adherent-Invasive E. Coli infection and intestinal inflammation. Scientific Reports. 2016. 6(19032);
9. Palmela C, Chevarin C, Xu Z, Torres J, Sevrin G, Hirten R. et al. Adherent-invasive Escherichia coli in inflammatory bowel disease. Gut. 2017;0:1-14;
10. Sartor RB. Bacteria in Crohn's disease: mechanisms of inflammation and therapeutic implications. J Clin Gastroenterol. 2007 Jul;41(6): S37-S43;
11. Pierce E. Where Are All the Mycobacterium avium Subspecies paratuberculosis in Patients with Crohn's Disease?. PLoS Pathog. 2009 Mar;5(3);
12. Podolsky DK. Inflammatory bowel disease. N Engl J Med. 2002 Aug 8;347(6):417-29;
13. Baumgart DC, Sandborn WJ. Crohn's disease. Lancet. 2012 Nov 3;380(9853):1590-605;
14. Ray C, Sagar P. Management of Crohn's disease and ulcerative colitis. Surgery (Oxford). 2017 Aug;35(8):439-442;
15. Hart AL, NG SC. Crohn's disease. Medicine. 2015. 43(5):282-290;
16. Ho GT, Boyapati R, Satsangi J. Ulcerative Colitis. Medicine. 2015. 43(5):276-281;

17. Colombel JF, Mahadevan U. Inflammatory Bowel Disease 2017: Innovations and Changing Paradigms. Gastroenterology. 2017 Feb;152(2):309-312;
18. Beaugerie L, Langholz E, Nyboe-Andersen N, Pigneur B, Sokol H. Differences in epidemiological features between ulcerative colitis and Crohn's disease: The early life-programmed versus late dysbiosis hypothesis. Medical Hypotheses. 2018; 115:19-21;
19. Sartor RB, Wu GD. Roles for Intestinal Bacteria, Viruses, and Fungi in Pathogenesis of Inflammatory Bowel Diseases and Therapeutic Approaches. Gastroenterology. 2017 Feb;152(2):327-339.e4;
20. McNees AL, Markesich D, Zayyani NR, Graham DY. Mycobacterium paratuberculosis as a cause of Crohn's disease. Expert Rev Gastroenterol Hepatol. 2015;9(12):1523-34;
21. Ormaechea JI, Gisbert JP, Marín-Jiménez I. Papel de Mycobacterium avium paratuberculosis en la etiopatogenia de la enfermedad de Crohn. Gastroenterol Hepatol. 2009 May;32(5):353-63;
22. Liverani E, Scaioli E, Cardamone C, Dal Monte P, Belluzzi A. Mycobacterium avium subspecies paratuberculosis in the etiology of Crohn's disease, cause or epiphenomenon?. World J Gastroenterol. 2014 Sep 28;20(36):13060-70;
23. Constable P, Hinchcliff K, Done S, Grünberg W, editors. Veterinary Medicine. 11th ed. New York: Elsevier Ltd;2017;
24. Pierce ES. Ulcerative colitis and Crohn's disease: is Mycobacterium avium subspecies paratuberculosis the common villain?. Gut Pathog. 2010 Dec 17;2(1):21;
25. Sechi LA, Scanu AM, Molicotti P, Cannas S, Mura M, Dettori G. Detection and Isolation of Mycobacterium avium subspecies paratuberculosis from intestinal mucosal biopsies of patients with and without Crohn's disease in Sardinia. Am J Gastroenterol. 2005 Jul;100(7):1529-36;
26. Scanu AM, Bull TJ, Cannas S, Sanderson JD, Sechi LA, Dettori G. Mycobacterium avium subspecies paratuberculosis infection in cases of irritable bowel syndrome and comparison with Crohn's disease and Johne's disease: common neural and immune pathogenicities. J Clin Microbiol. 2007 Dec;45(12):3883-90. Epub 2007 Oct 3;

27. Pistone D, Marone P, Pajoro M, Fabbi M, Vicari N, Daffara S. Mycobacterium avium paratuberculosis in Italy: commensal or emerging human pathogen?. Dig Liver Dis. 2012 Jun;44(6):461-5;
28. Ryan P, Bennett MW, Aarons S, Lee G, Collins JK, O'Sullivan GC. PCR detection of Mycobacterium paratuberculosis in Crohn's disease granulomas isolated by laser capture microdissection. Gut. 2002 Nov;51(5):665-70;
29. Nazareth N, Magro F, Machado E, Ribeiro TG, Martinho A, , Sarmento A. et al. Prevalence of Mycobacterium avium subsp. paratuberculosis and Escherichia coli in blood samples from patients with inflammatory bowel disease. Med Microbiol Immunol. 2015 Dec;204(6):681-92;
30. Singh AV, Singh SV, Makharia GK, Singh PK, Sohal JS. Presence and characterization of Mycobacterium avium subspecies paratuberculosis from clinical and suspected cases of Crohn's disease and in the healthy human population in India. Int J Infect Dis. 2008 Mar;12(2):190-7;
31. Clancy R, Ren Z, Turton J, Pang G, Wettstein A. Molecular evidence for Mycobacterium avium subspecies paratuberculosis (MAP) in Crohn's disease correlates with enhanced TNF-alpha secretion. Dig Liver Dis. 2007 May;39(5):445-51;
32. Campos N, Magro F, Castro AR, Cabral J, Rodrigues P, Sarmento A, et al. Macrophages from IBD patients exhibit defective tumour necrosis factor-α secretion but otherwise normal or augmented pro-inflammatory responses to infection. Immunobiology. 2011 Aug;216(8):961-70;
33. Olsen I, Tollefsen S, Aagaard C, Reitan LJ, Bannantine JP, Andersen P. Isolation of Mycobacterium avium subspecies paratuberculosis reactive CD4 T cells from intestinal biopsies of Crohn's disease patients. PLoS One. 2009 May 22;4(5):e5641;
34. Ellermann M, Huh EY, Liu B, Carroll IM, Tamayo R, Sartor RB. Adherent-Invasive Escherichia coli Production of Cellulose Influences Iron-Induced Bacterial Aggregation, Phagocytosis, and Induction of Colitis. Infect Immun. 2015 Oct;83(10):4068-80;
35. Darfeuille-Michaud A1, Boudeau J, Bulois P, Neut C, Glasser AL, Barnich N. High prevalence of adherent-invasive Escherichia coli associated with ileal mucosa in Crohn's disease. Gastroenterology. 2004 Aug;127(2):412-21;

36. Cieza RJ, Hu J, Ross BN, Sbrana E, Torres AG. The IbeA invasin of adherent-invasive Escherichia coli mediates interaction with intestinal epithelia and macrophages. Infect Immun. 2015 May;83(5):1904-18;
37. McPhee JB, Small CL, Reid-Yu SA, Brannon JR, Le Moual H, Coombes BK. Host defense peptide resistance contributes to colonization and maximal intestinal pathology by Crohn's disease-associated adherent-invasive Escherichia coli. Infect Immun. 2014 Aug;82(8):3383-93;
38. Shawki A, McCole DF. Mechanisms of Intestinal Epithelial Barrier Dysfunction by Adherent-Invasive Escherichia coli. Cell Mol Gastroenterol Hepatol. 2016 Oct 22;3(1):41-50;
39. Martinez-Medina M, Garcia-Gil LJ. Escherichia coli in chronic inflammatory bowel diseases: An update on adherent invasive Escherichia coli pathogenicity. World J Gastrointest Pathophysiol. 2014 Aug 15;5(3):213-27;
40. Chassaing B1, Rolhion N, de Vallée A, Salim SY, Prorok-Hamon M, Neut C. Crohn disease--associated adherent-invasive E. coli bacteria target mouse and human Peyer's patches via long polar fimbriae. J Clin Invest. 2011 Mar;121(3):966-75;
41. Rolhion N, Barnich N, Bringer MA, Glasser AL, Ranc J, Hébuterne X, *et al.* Abnormally expressed ER stress response chaperone Gp96 in CD favours adherent-invasive Escherichia coli invasion. Gut. 2010 Oct;59(10):1355-62.
42. Elliott TR, Hudspith BN, Wu G, Cooley M, Parkes G, Quiñones B, *et al.* Quantification and characterization of mucosa-associated and intracellular Escherichia coli in inflammatory bowel disease. Inflamm Bowel Dis. 2013 Oct;19(11):2326-38;
43. Rolhion N, Hofman P, Darfeuille-Michaud A. The endoplasmic reticulum stress response chaperone: Gp96, a host receptor for Crohn disease-associated adherent-invasive Escherichia coli. Gut Microbes. 2011 Mar-Apr;2(2):115-9;
44. Subramanian S, Rhodes JM, Hart CA, Tam B, Roberts CL, Smith SL, *et al*. Characterization of epithelial IL-8 response to inflammatory bowel disease mucosal E. coli and its inhibition by mesalamine. Inflamm Bowel Dis. 2008 Feb;14(2):162-75;
45. Lapaquette P, Glasser AL, Huett A, Xavier RJ, Darfeuille-Michaud A. Crohn's disease-associated adherent-invasive E. coli are selectively favoured by impaired autophagy to replicate intracellularly. Cell Microbiol. 2010 Jan;12(1):99-113;

46. Nguyen HT, Dalmasso G, Müller S, Carrière J, Seibold F, Darfeuille-Michaud A. Crohn's disease-associated adherent invasive Escherichia coli modulate levels of microRNAs in intestinal epithelial cells to reduce autophagy. Gastroenterology. 2014 Feb;146(2):508-19;
47. Zhang HJ, Xu B, Wang H, Xu B, Wang GD, Jiang MZ, et al. IL-17 is a protection effector against the adherent-invasive Escherichia coli in murine colitis. Mol Immunol. 2018 Jan; 93:166-172;
48. Agus A, Massier S, Darfeuille-Michaud A, Billard E, Barnich N. Understanding host-adherent-invasive Escherichia coli interaction in Crohn's disease: opening up new therapeutic strategies. Biomed Res Int. 2014; 2014:567929;
49. Roberts CL, Keita AV, Duncan SH, O'Kennedy N, Söderholm JD, Rhodes JM. Translocation of Crohn's disease Escherichia coli across M-cells: contrasting effects of soluble plant fibres and emulsifiers. Gut. 2010 Oct;59(10):1331-9;
50. Mitsuro Chiba, Toru Abe, Hidehiko Tsuda, Takeshi Sugawara, Satoko Tsuda, Haruhiko Tozawa, *et al*. Lifestyle-related disease in Crohn’s disease: Relapse prevention by a semi-vegetarian diet. World J Gastroenterol. 2010 May 28; 16(20): 2484–2495;
51. Sandefur K, Kahleova H, Desmond AN, Elfrink E, Barnard ND. Crohn's Disease Remission with a Plant-Based Diet: A Case Report. Nutrients. 2019 Jun 20;11(6).