Drug Targeting of IBD

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Inflammatory bowel diseases (IBD) are disabling, noncommunicable, progressive and incurable immune-mediated inflammatory diseases (IMIDs). Crohn's disease (CD) and ulcerative colitis (UC) constitute the most prevalent forms of IBD. These diseases are highly prevalent worldwide, particularly in Europe and North America, and are spreading globally at an accelerated rate.

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1. Introduction to Inflammatory Bowel Diseases (IBD)

A westernized lifestyle, urbanization and industrialization are known as the driving forces of IBD initiation and endurance [1]. Regardless, IBD arises from intricate exchanges between host genetics, intestinal barrier function, the immune system, environmental factors, and the gut microbiome [2][3]. The cause remains unknown, but it appears to occur in individuals carrying specific genetic alterations, which develop an atypical immune response to certain bowel pathobionts following interaction with exacerbating environmental factors [3][4][5].

The intestinal mucosal barrier (with innate immune cells, epithelial cells (IECs), intraepithelial lymphocytes and the mucosal lining) constitutes the front wall, which is encountered by food antigens and intestinal commensal and pathogenic microorganisms, working with the intestinal luminal contents to maintain homeostasis [2]. Upon facing IBD, this barrier is compromised. Increased barrier permeability for dietary components and gut microbiota, inhibition of epithelium matrix remodeling and regeneration and antimicrobial activity, and a decrease in intracellular pathogen clearance and amplification of intestinal inflammation are known consequences [4][6][7][8].

IBD patients sustain multiple deviations from a normal inflammatory response to an antigen or cytokine [4][5][9][10]. Neutrophils, dendritic cells, macrophages, and innate lymphoid cells reinforce the intestinal mucosal barrier at the first level of defence of the mucosal innate immune system [11][12]. In healthy individuals, intestinal macrophages show attenuated proliferative rates and chemotactic abilities, while retaining the phagocytic and bactericidal function, effectively regulating adaptative T cell responses. Pathogenic Th1 and Th17 responses are restrained, and tolerogenic Treg cells are stimulated [12]. In IBD, a defective secretion of pro-inflammatory cytokines impairs neutrophil recruitment and pathogen clearance. Chronic inflammation occurs, and excessive pro-inflammatory cytokine production (e.g., TNF-α, IL-12, IL-17 and IL-23) includes an exaggerated and intolerant T cell-induced

response, unrestrained inflammation, and aggravated intestinal bowel damage [9][11][12][13][14]. Treg presence is reduced [9].

The socio-environmental factors influence IBD-associated cases at an (i) individual level: living habits (smoking—CD-exclusive, hygiene status), diet (poor in plant-based fibres), null or scarce physical exercise, psychological stress, medical history (childhood infections and vaccination, early-life antibiotic use, frequent intake of oral contraception and nonsteroidal anti-inflammatory agents, vitamin D deficiency, appendectomy), breastfeeding, etc.; (ii) population-level health interventions, such as differentiated access to healthcare and fluctuating IBD presence in migrants, among others. Physical components, with the air or water as vehicles of contaminants, such as: (i) air pollution or specific contaminants that can relate to the living areas or vehicle use; (ii) water contamination derived from deficient access to tap or hot water, leaching from pipes, food adjuvants, or by-products of industrial activities [15][7][16][17].

2. Gut Microbiota and IBD Progression

The resident gut microbiota are involved in several vital host physiological processes, including the development of the gut immune system, digestion of dietary factors, and colonization resistance against incoming pathogens, but it can also be associated with UC and CD pathogenesis [18][19]. Microbial antigens and their metabolic products are key promoters of barrier dysfunction in IBD, with a higher concentration of anaerobic bacteria in the distal ileum and colon, encouraging the appearance of IBD [20][19]. However, the presence of specific pathobionts within the bowel, and their correlation with the onset of IBD, remains unclear [20]. An imbalance in gut microbiota results in a change in the gut microflora-associated functions, such as changes in fermentation products, mainly carbohydrate, vitamins, and short-chain fatty acids (SCFA), and changes in biochemical processes, such as immune equilibrium imbalance [21]. Dysbiosis has been described as the root of IBD etiopathology, with differences between healthy and diseased gut microbiota regarding diversity and number [21][22][23]. For instance, Britton et al. showed that these microorganisms can modulate the immune system, namely, microbiota-specific anti- and pro-inflammatory activity. Anti-inflammatory RORγt + Treg cells are microbiota-dependent and are enhanced in the gut tissue, with a powerful, suppressive, unchanging phenotype. In a mice model, the deficiency of these cells demonstrated that they are essential to preserving tolerance to microbiota. Microbiota-induced Treg cells prevent colitis [24].

Several studies have recognized variances in gut microbiota biodiversity and species richness between healthy individuals and IBD patients, particularly in the phylum of Firmicutes and Bacteroides. Health gut microbiota are composed of Firmicutes < Bacteroidetes < Proteobacteria < Actinobacteria. IBD patients have fewer bacteria with protective proprieties, such as Bifidobacterium spp. , Bacteroides or Faecailbacterium prausnitzii and Roseburia spp. , and more with pro-inflammatory activities, mainly Veillomellaceae, Pasteurellacae, Escherichia coli (E. coli , adherent/invasive) and Fusobacteriaceae (**Figure 1**) [25][26][27]. Dysbiosis in UC showed a higher amount of Actinobacteria and Proteobacteria and a lower amount of Bacteroides (Firmicutes < Proteobacteria < Bacteroidetes < Actinobacteria) [28], whereas dysbiosis in CD has shown an even lower amount of Firmicutes phylum than in healthy individuals [29], such as F. prausnitzii , which is often proportionally decreased in the patients' stool [20][30].

A dysbiotic condition can be associated with prior use of antibiotics, and this can lead to the progression of IBD, at early stages. Changes in gut microbiota are the cause or consequence of the inflammation needed for an appropriate diagnosis, selection of therapy, and strategy to monitor response to treatment. Some studies show that dysbiosis may be a cause of IBD and T-cell-mediated chronic colitis [24][31]. The disequilibrium between anaerobe species (obligate and elective) and the oxidative stress induced by gut microbiota can be correlated [22]. The perpetuated inflammation of the intestinal tissue then begins, and enhances the release of haemoglobin, thereby transporting reactive oxygen species and oxygen into the inner intestinal wall, creating a microenvironment that is unfavourable to extremely oxygen-sensitive bacteria. This results in a reduction in obligate anaerobes, mainly F. prausnitzii , and causes a severe decrease in butyrate-producing obligate anaerobes and an increase in inflammation by thedepletion of anti-inflammatory proprieties of butyrate [22][32]. The IECs are fuelled by butyrate, which is needed to protect the gut epithelial barrier from becoming vulnerable to potential pathogens. Machiels et al. emphasized that a lower abundance of F. prausnitzii and Roseburia hominis exists in UC patients than in healthy individuals, which shows a reduction in the butyrate-producing bacteria of this Firmicutes phylum [27]. Depending on disease severity, gut microbial metabolites could encourage the pathogenic Th2 production by human dendritic cells, to the detriment of tolerogenic Th1 cells. Intestinal microbes of IBD patients also have decreased tryptophan-derived indole derivatives, which are known to induce production of the pro-inflammatory IL-22 owing to a gut imbalance [33]. Bergmann et al. showed that the uptake of tryptophan-metabolizing Lactobacillus species re-established IL-22 production within the gut and relieved its associated inflammatory status by producing IL-1β in the injured bowel and controlling the following IL-22 increase due to the activity of group 3 innate lymphoid cells. The potential of Lactobacillus strains to diminish colitis suggests that their gut metabolites are involved in IBD [33][34]. Sokol et al. also reported that F. prausnitzii can secrete metabolites that are able to block IL-8 production and NF-κB activation, as well as induce the production of IL-10 and limit the production of pro-inflammatory cytokines, mainly IFN-γ and IL-12 [35].

Fungi, on another hand, represent <0.1% of the total amount of microbial species living in the intestine. In healthy people, Candida , Saccharomyces , and Cladosporium are the most predominant genera; however, in IBD, the gut microbiota reveal an elevated presence of fungi such as Basidiomycota , Ascomycota and Candida albicans [36]. Bacterial biodiversity decreases in CD and UC, while fungal biodiversity only decreases in UC [37]. CD patients exhibit a higher fungal burden over the inflammatory process, changing the ileal physiology in the terminal ileum, which impairs the inhibitory effect of antimicrobial peptides on bacteria and bile acid reabsorption. This explains why an enhanced load of Candida species is observed in CD patients, in parallel with disease severity [38]. Sokol et al. showed that Saccharomyces cerevisiae (S. cerevisiae) is a major component of the healthy fungal microbiota, with a reduction that is independently associated with IBD. S. cerevisiae is able to reduce the colitis induced by AIEC, opening a new approach to use fungi as a new therapeutic strategy due to its regulatory effects on the host, such as an anti-inflammatory IL-10 production [37]. Changes in IBD patients' microbiota include an enhanced fungi/bacteria diversity ratio and high abundance of C. albicans , showing an overgrowth of fungi over inflammation. Specific fungi/bacteria interactions may even be important in IBD. Hoarau et al. identified that the abundance of Candida tropicalis was high in CD samples and positively correlated with levels of anti- S. cerevisiae

antibodies (ASCA). Positive interkingdom correlations between C. tropicalis , E. coli ,and Serratia marcescens in CD patients were validated using in vitro biofilms, suggesting that these organisms interact in the gut [39].

3. IBD Symptoms and Treatment Options

CD provokes segmental, asymmetrical, and transmural lesions, affecting all the digestive tract, with 30% of the cases being installed within the distal parts of the small intestine, while UC only affects the superficial mucosa of the colon and occurs continuously, and circumferentially, from the anus [4][5][40][41]. Endoscopy in CD patients typically reveals a discontinuous distribution of longitudinal apthoid ulcers along the mesenteric aspect, wherein intestinal blood and lymphatic vessels assemble. In mild forms of the disease, superficial ulcers are formed, whereas deep serpiginous ulcers with modular oedematous mucosa are developed in moderate-to-severe cases, producing the so-called cobblestone appearance [4][42]. The non-necrotizing epithelioid and intralymphatic cell granulomas emerge in the focal points, juxta-positioned with endothelial lesions, with the damage suggesting an infectious setting, lymphatic endothelial cell death and granulomatous response, in and around the lymphatic, submucosal, muscular and subserosal layers [4][10][41][42]. This process is specific to CD and is not observed in other chronic forms of enteritis [41]. The extent of these lesions closely correlates with transmural inflammation, fibrosis, muscularization, and stricture formation, and is considered an active participant in intestinal inflammation, in a pathogenic process supporting the release of pro-adipokines and local amplification of inflammation in response to recurrent intestinal ulcerations, which are ineluctably accompanied by bacterial translocation [10][41]. Half of the patients may experience peri-anal complications such as strictures, as well as abscesses and fistulas, within the first decade after diagnosis [2][7].

On the other hand, UC lesions include clearly defined inflamed mucosa and sub-mucosa of the colon and rectum lining, instigating ulcer development [17]. The crypt architecture appears distorted, crypt length is shortened, more lymphocytes and plasma cells appear in the lamina propria, mucin is depleted, and Paneth cells transdifferentiate into other cell types. Severe UC may also comprise toxic megacolon, with colonic dilation visible through abdominal imaging. This is a surgical emergency, given the risk of potential perforation and sepsis [5][43]. Although normally shielded by a thick mucin coating that separates antigens and gut immune cells, mucosal injuries begin with the disruption of the epithelium, the peripheral mucosal layer, and exert antimicrobial activity. As mucin synthesis and secretion is diminished, mucosal internalization of luminal pathogens, antigen uptake and potential stimulation of the gut's handicapped and intolerant immune system increases [17]. UC can also evolve into dysmotility, anorectal incontinence, pseudopolyposis, bridging fibrosis, and strictures, either from disease progression or postoperative complications [44].

The current therapeutic approach includes 5-aminosalicylates (5-ASA), corticosteroids and immunosuppressants, indicated for mild-to-moderate IBD. More than 90% of UC patients are treated with oral or rectal administration of 5-ASA, shortly after disease diagnosis, particularly mesalamine [4][5][45][46]. If insufficient, oral or intravenous corticosteroids [5][47] may induce remission in mild-to-moderately active UC and CD and are used as a rescue therapy in disease flares [48][49]. Preference is given to the use of oral corticosteroids esuch asprednisone and budesonide [47][50]. Immunosuppressants such as thiopurines are used to maintain remission of UC and CD, after

surgery in CD, and as a maintenance strategy after rescue therapy [2][45][49]. Methotrexate presents advantages over thiopurines, such as only requiring a single dose per week, and possessing higher adherence rates and faster onset of action [49], and is, therefore, increasingly used to treat CD [4][5].

Upon failure of these drugs due to steroid dependency or unresponsiveness, conventional step-up pharmacological intervention strategy considers targeted biologic therapy as the standard of care [44][51], either used alone or as a co-adjuvant therapy [52]. These targeted therapies (via monoclonal antibodies or small molecules) have been effective in achieving remission and complete mucosal healing in a significant portion of moderate-to-severe cases of CD and UC [53][54], despite their only being effective in a proportion of patients [51]. Some clinicians additionally claim that an early introduction of biologics can, in some cases, further benefit the patients, compared to the traditional treatment course [52]. Anti-TNF-α drugs, specifically adalimumab, infliximab, certolizumab pegol (CD-exclusive) and golimumab (UC-exclusive), are used to treat IBD [5][53][55]. These are widely known monoclonal antibodies which work against TNF-α [47][56][57] and are capable of inducing remission in nearly 50% of patients [56]. Following anti-TNF agents, given their non-negligible rate of loss of response, contraindications, adverse events, and intolerance [51][57], biological therapy can resort to anti-integrins, especially vedolizumab and natalizumab. Integrins are transmembrane receptors that act upon various leukocyte signalling pathways, including cell adhesion, proliferation, and migration [58]. These drugs comprise monoclonal antibodies targeting α4β7 integrins (proteins responsible for the regular migration of leukocytes, preventing leukocyte migration to the gut) and/or α4β1 integrins (with known roles in leukocyte adhesion, spreading, and motility, as well as T cell recruitment to intestinal and non-intestinal inflamed tissues) can be used [45][47][58][59]. Moreover, a recently approved anti-interleukin agent, namely, ustekinumab, may be directed towards the p40 subunit of pro-inflammatory interleukin-12 (IL-12) and interleukin-23 (IL-23) of CD and UC patients [56][60]. The induction dose is administered intravenously, and the following maintenance doses are subcutaneous, which is an advantage for the patient [56]. The inhibition of activated T cells using small molecules that inhibit the enzyme calcineurin–cyclosporine and tacrolimus has also been useful to UC patients who are unresponsive to thiopurines or anti-TNF as an induction therapy in the prevention of UC-induced colectomy, or combined with vedolizumab to stabilize the disease. It may also be used in cases of drug contraindications and rescue therapy in IBD [49][61]. In patients in which conventional and/or biological therapies have not worked, Janus kinase (JAK) inhibitors have been considered as an alternative for UC management. Tofacitinib, with a small-molecule JAK inhibitor, was recently licensed for oral treatment of moderate-to-severe active UC [47][62]. It inhibits all JAKs (preferably JAK1 and JAK3, members of the tyrosine kinase family, which are involved in cytokine signalling), affecting cytokine production and enabling immunomodulation in IBD [54][57]. The simultaneous inhibition of multiple cytokines leads to a lower risk of immunogenicity, which is an advantage compared to the aforementioned therapies, which are associated with monoclonal antibodies [57]. A large number of small-molecule JAK inhibitors are currently under investigation [63], constituting, in parallel with sphingosine-1-phosphate receptor 1 (S1PR1) agonists (e.g., ozanimod and etrasimod), new and attractive treatment tools for parenteral administration [2][64]. Modulation of S1PR activity is needed for lymphocyte blood circulation, additionally enabling lymphocyte entrapment in lymphatic structures [65]. Antisense oligonucleotides (AGO), short nucleotide sequences, inhibit RNA or DNA transcription or translation through complementary base pairing. Alicaforsen specifically binds to ICAM-1 mRNA, thereby reducing the mRNA levels and inhibiting ICAM-1

translation. ICAM-1 is a glycoprotein expressed on the surface of intestinal epithelial cells and vascular endothelial cells, with promising results in terms of UC management, including safety and potentially long-lasting effects. Cobitolimod, an AGO-simulating bacterial DNA used to activate Toll-like receptors 9, is another relevant example [66]. To date, the clinically approved targeted therapies (i.e., monoclonal antibodies and small molecules) constitute the standard of care for moderate-to-severe IBD; however, they are only effective in a portion of the patients [51].

4. Benefits of a Nanomedicine-Based Therapy for IBD

Nanomedicine approaches allow for the development of therapeutic formulations designed to enhance drug uptake (absorption) into diseased tissues in the colon or other regions of the GIT [67], thus contributing to localized therapy [68]. Nanoparticles (NPs) can access the intestinal mucosa for site-specific drug delivery. Different compositions, sizes, surface charges and coatings have been shown to successfully reach the inflamed intestinal tissues [69]. The adhesion of NPs to the mucus layer results in a prolonged intestinal transit time. Stimuli-responsive delivery systems also display improved drug delivery, directed at the diseased tissues [70].

Another problem associated with IBD is the high expression levels of myeloperoxidase (MPO), which, through a cascade of events, may cause damage at the site of inflammation [71]. In this sense, Iwao et al. [72], developed 5-ASA-loaded human serum albumin (HSA) NPs, to take advantage of the communication between MPO and HSA. The formulation presented 190 nm as averaged particle diameter, a polydispersity index of 0.35 and zeta potential of ≈−11 mV. The specific affinity between NPs and MPO was explored in the imaging of colonic tissue sections, after being collected from the used DSS-induced colitis mice model, demonstrating that HSA NPs and MPO were co-localized in the colonic tissue. Mild inflammatory damage could also be perceived, but this still suggests mucosal repair.

A polysaccharide bacteria-degradable hydrogel comprising alginate and chitosan was employed to facilitate the delivery of active agents to the inflamed colonic tissues [73][74][75]. Laroui et al. [74] used this hydrogel as a matrix to deliver polylactide NPs containing CD98 small interfering RNA (CD98siRNA) with colon-homing properties. CD98 expression in the intestine is crucial in the local management of immune responses and homeostasis. The designed CD98 siRNA/polyethyleneimine-polylactic-acid-loaded NPs (ca. 480 nm) were cytocompatible towards intestinal cells. Upon oral administration, the NPs, enclosed in a hydrogel, decreased CD98 expression in colonic cells and reduced colitis parameters in the DSS-induced colitis in a mouse model. Given the crucial role of cytokines and chemokines in IBD progression, Frede and co-workers [76] designed a delivery system for local interference in the signalling pathways. The study evaluated the therapeutic potential of siRNA-loaded calcium phosphate (CaP)/PLGA NPs to modulate gene silencing in epithelial cells. Multi-shell NPs of a CaP core were coated with siRNA directed at mediators of inflammation, such as TNF-α, then encapsulated in PLGA coated with an outer layer of polyethyleneimine. This prevented nanoparticle degradation and conferred them with a cationic surface to enhance cellular uptake. The non-toxic siRNA-loaded calcium phosphate /PLGA NPs were rapidly taken up by MODE-K intestinal epithelial cells; subsequent in vitro gene silencing was observed. Upon intrarectal application of the NPs in a DSS-induced colonic inflammation mouse model, a substantial decrease in the targeted

genes (e.g., TNF-α, IP-10) was found in the colonic biopsies and the mesenteric lymph nodes. Amelioration of the intestinal inflammation was achieved with specific management of the inflammatory response using polymeric NPs.

A different approach was followed by Xu and colleagues [77], in which TNF-α siRNA and DEX sodium phosphate were loaded into a TKPR peptide-functionalized, reversibly crosslinked polymersomes constituted by poly(ethylene glycol)-b-poly(trimethylene carbonate-codithiolane trimethylene carbonate)-b-polyethylenimine (PEG-P(TMC-DTC)-PEI) triblock copolymer. The cationic PEI segments enabled drug encapsulation via electrostatic interactions, while PEG promoted NP furtivity. The pendent dithiolane rings in the P(TMC-DTC) block can form redox-sensitive disulphide bonding, thus conferring enhanced colloidal stability and responsiveness to the NPs. TKPR, a macrophage-targeting peptide, was grafted to PEG terminal moieties for targeting action. These neutral and serum-stable NPs exhibited a spherical and hollow vesicle structure with a diameter of nearly 108–138 nm. About 98% of NPs were efficiently internalized by macrophages. A glutathione-induced drug released was observed, along with efficient gene silencing and anti-inflammatory effect. Intravenous injection of the NPs revealed potent anti-inflammatory action in inflamed colons of UC mice, substantially reducing colonic injury.

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