Chapter

Traditional Drugs: Mechanisms of Immunosuppressor and Corticosteroid Therapies for Inflammatory Bowel Diseases

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Abstract

The inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis are immunological dysfunctions of the gastrointestinal tract that develop because of multifactorial processes, including genetic predisposition, gut dysbiosis, and excessive inflammation in susceptible subjects. These pathologies affect millions of people worldwide, with substantial impact on healthcare systems and patients' quality of life. Considering the chronic inflammation that underlies the IBD presentation, the main treatment options are related to the control of patients' inflammatory response, through immunosuppressor and modulatory therapies. Therefore, in this chapter we reviewed the main mechanisms associated with the treatments that are aimed at suppressing mucosal immunity and the effects of corticosteroid therapies in Crohn's disease and ulcerative colitis.

Keywords: inflammatory bowel disease, Crohn's disease, ulcerative colitis, immunosuppressor, corticosteroid, therapy

1. Introduction

The treatment of Crohn's disease and ulcerative colitis has central purposes such as to induce and maintain the patients' remission, while restraining the disease's secondary effects and improving the quality of life of the affected subjects. Pharmacological therapy against these pathologies converges on controlling the exacerbation of immune response, either with systemic agents, such as corticosteroids, azathioprine (AZA), aminosalicylates, and methotrexate, or topical anti-inflammatory drugs. Traditionally, the treatment for CD and UC follows a "step-up" approach. However, in the last years, a "top-down" strategy was implemented in IBD therapy, beginning to treat patients with biological agents, especially for more aggressive diseases [1]. After the main control of the inflammation, biologicals can be withdrawn, and weaker immunosuppressor medicines can be used, such as AZA, aminosalicylates, or other drug alternatives for maintenance of disease remission [2], with different mechanisms of action, as discussed in the following section.

2. Mechanisms of action of IBD's therapies: from corticosteroids to immunosuppressor drugs

2.1 Glucocorticoids

Corticosteroids, a type of steroid hormones, are lipophilic molecules derived from cholesterol. Glucocorticoids, whose major representative is cortisol, play a role in the metabolism of lipids and carbohydrates and in the immune response, through immunosuppressive mechanisms. These hormones are synthesized by the adrenal glands in response to psychological or physiological stressful stimuli, such as excessive inflammation. The synthesis of glucocorticoids occurs after hypothalamic production of corticotropin-releasing hormone (CRH), which activates the pituitary secretion of corticotropin (ACTH) that, in turn, leads to adrenal release of cortisol, in a fine-tuned circadian rhythm [3].

Many of the immunosuppressive and anti-inflammatory functions of glucocorticoids occur after the binding of this hormone to the glucocorticoid receptor (GR). This molecule was described in the 1970s [4] and presents two isoforms of GR, GRα and GRβ, which differ in the C-terminal domain, being that the α forms the most prevalent in many human cells [5].

Glucocorticoids may exert their effects by non-genomic and mainly by the genomic signaling pathways [6]. One of the first evidences on the formation of a glucocorticoid-GR complex dated from 1972 in a study, which showed that free glucocorticoids penetrate hepatoma cells and bind to a cytoplasmic receptor, forming a complex which migrates to the nucleus shortly thereafter [7]. In the nucleus, the glucocorticoid/receptor complex binds to specific DNA sequences, named *glucocorticoid responsive elements* (GRE) [8]. Such binding to GREs may lead to repression and downregulation of target genes, especially those related to inflammatory response such as IFN-γ [9], TNF [10], and adhesion molecules [11], but may also lead to transcriptional activation of genes such as IL-10 [12], which plays an important anti-inflammatory activity. Another mechanism for gene transcription regulation by the glucocorticoid/receptor complex is the interference with other transcription factors, such as NF-κB, NFAT, and AP-1 [13], which also results in the inhibition of inflammatory responses.

Cortisol was first synthesized around 1937/1938 by Tadeusz Reichstein, who won the Nobel Prize about 10 years later for his work [14]. The first use of corticosteroids as an immunosuppressive and anti-inflammatory treatment occurred in the 1940s for rheumatoid arthritis in a study by Hench et al., who showed a decrease in symptoms when patients were treated with these hormones, besides disease relapse when treatment was stopped [15]. Since then, corticosteroids have been effective in treating other diseases, including intestinal inflammation [16].

Today, corticosteroid therapy is one of the most widely used and most effective drugs in the treatment of IBD, especially in acute inflammation, to induce disease remission [17]. However, there are important limitations regarding their long-term use, because of the drug's side effects. In line with that, despite the anti-inflammatory role in experimental colitis, budesonide worsens the general status of the mice, leading to endotoxemia and impaired epithelial repair in the gut, which are findings that could partially explain the fails in long-term glucocorticoid therapy for intestinal inflammation [18]. In contrast, mice exposed to dextran sodium sulfate for colitis development and treated for short term with the glucocorticoid dexamethasone had decreased intestinal inflammation, with reduced expression of pro-inflammatory cytokines such as IFN-γ and IL-1, diminishment of IFN- γ-producing CD4$^+$ T cells and augmented frequency of anti-inflammatory cytokine-producing cells such as IL-10. Moreover, the increase in the frequency of regulatory markers such as GITR, CTLA-4, PD-1, CD73, and FoxP3 in treated mice pointed to a relevant role for this short-term therapy in the induction of immune regulation [19], despite the long-term adverse effects of these drugs. These findings corroborate the relevance of this hormone in the regulation of mucosal immunity. In fact, regulatory T cells deficient for glucocorticoid receptor fail to control intestinal inflammatory diseases, in vivo. In addition, these knockout regulatory T cells acquire Th1 phenotype and secrete IFN-γ, with a consequent failure to inhibit the proliferation of CD4$^+$ T cells. Then, not only the synthetic glucocorticoid is important to inflammation control, but the glucocorticoid receptor is critical for regulatory T cell functions neither [20].

Regarding the pivotal role of microbiota in the development of gut inflammation [21], it is known that the commensal intestinal bacteria may be involved in the mechanisms of action of glucocorticoid and mediate the anti-inflammatory effects of dexamethasone in the colon [22]. Indeed, the evaluation of mucosa transcriptomics of ulcerative colitis patients pointed to a corticosteroid-response gene signature that could predict response to this therapy, together with notable changes in gut microbiota [23]. In Crohn's disease or ulcerative colitis, the bacteria translocation in the gut is originally restrained by local phagocytic cells such as neutrophils, which in turn may contribute to tissue damage due to their excessive inflammation triggered in an attempt to control microbial invasion. Then, the mechanisms and efficacy of corticosteroids in IBD also involve the reduction in the chemokines responsible for the recruitment of neutrophils, besides natural killer cells and activated T lymphocytes to the gut, during ulcerative colitis [24]. There is also a decrease in adhesion and chemotaxis of these cells to the intestinal mucosa [25].

Although the efficacy of corticosteroid for the treatment of autoimmune and inflammatory diseases has been demonstrated, prolonged utilization of these drugs is associated with an increased risk of developing eye diseases such as glaucoma or cataract, hyperglycemia or insulin resistance, dermatological affections, and purpura [26]. Moreover, there is an increased risk of gastrointestinal problems such as peptic ulcer with perforations, bleeding, and acute pancreatitis [27]. The use of corticosteroids can also cause psychiatric and cognitive disorders [28], psychosis, and also sleep-related disorders [29]. Moreover, because of its immunosuppressive and anti-inflammatory effects, many patients who use corticosteroids may suffer from reduced effectiveness of the immune system and are at risk for opportunistic infections [30].

2.2 Aminosalicylates

The aminosalicylates (5-aminosalicylic acid, 5-ASA, or mesalazine) are one of the most used therapeutic choices to control mild to moderate inflammatory bowel diseases (IBD). Sulfasalazine (SASP), balsalazide, and olsalazine are prodrugs in which an azo bond is added to the structure to connect the 5-ASA moiety to carrier molecules. Sulfasalazine was the first aminosalicylate used for IBD and provided the basis for this class of medications. It was developed in the late 1930s, by the Swedish physician Nanna Svartz for the treatment of patients with rheumatic polyarthritis. Interestingly, some of the patients who were treated with SASP had ulcerative colitis too, and, surprisingly, their condition became more stable [31]. Therefore, SASP was soon being chosen as a treatment option for patients with IBD. Later, metabolic studies revealed that when this drug reaches the colon, the azo bond is cleaved by bacterial azoreductase, liberating 5-ASA and sulfapyridine, which is responsible for most of the usual adverse effects related to sulfasalazine [32]. In fact, in earlier elegant studies from the 70–80 decades, 5-ASA was shown to be the therapeutically active compound in sulfasalazine, while sulfapyridine plays a role as a carrier molecule, not required for clinical efficacy of the drug. These works were very important to drive the development of pure 5-ASA preparations useful for the treatment of IBD. Therefore, since aminosalicylates are among the most common therapeutic agents for these diseases, many studies have been performed in an attempt to discover the mechanisms of action of these drugs in the gut inflammation.

When the initial triggers break the mucosal tolerance in IBD, there is a vast infiltration of leukocytes in the intestine, with consequent production of soluble mediators of inflammation such as cytokines, chemokines, and eicosanoids. Some of these mediators are significantly elevated in the inflamed mucosa of IBD individuals, corroborating the pathogenesis of the disease, due to their pro-inflammatory impacts upon the bowel. In fact, the increased levels of seven eicosanoids, including prostaglandin (PG)E2, PGD2, thromboxane (TBX)B2, 5-HETE, 11-HETE, 12-HETE, and 15-HETE are found on mucosal biopsies from patients with ulcerative colitis, being correlated with the severity of inflammation [33]. Similarly, prostacyclin I2, PGE2, and TBXA2 are increased in cultured gut biopsies of active colitis patients, and, notably, the levels of these inflammatory mediators are reduced in the presence of 5-ASA. In fact, the activated leucocytes in patients' mucosa release toxic reactive oxygen metabolites and harmful eicosanoids such as LTB4, which seems to be an essential chemotactic agent in these diseases [34]. Therefore, considering the therapy mechanisms, sulfasalazine can effectively repress LTB4 and 5-HETE production by human polymorphonuclear leukocytes [35], while sulfasalazine, 5-ASA, and olsalazine (a 5-ASA dimer) potently inhibit colonic macrophage chemotaxis toward LTB4 [36]. These data suggested that one of the mechanisms of action of these drugs could be the inhibition of eicosanoids and then it is plausible to infer that the therapeutic inhibition of LOX or COX pathways could be useful in both ulcerative colitis and Crohn's disease.

Platelet-activating factor (PAF) is another phospholipid mediator released early in inflammation by a diversity of cell types, playing important roles in inflammatory conditions, including IBD. In active Crohn's disease, PAF levels are significantly higher and more elevated in inflamed than in noninflamed areas [37]. In parallel, PAF is increased in the colon and ileum from Crohn's disease patients [38], while biopsies of inflamed areas taken from ulcerative colitis subjects produce PAF spontaneously [39]. In this context, sulfasalazine and 5-ASA greatly reduce the synthesis of this mediator when incubated with mucosal biopsy specimens, indicating that these drugs exert beneficial effects in the inhibition of inflammation induced by PAF [40].

Chronic gut inflammation is also related to enhanced production of reactive metabolites of oxygen and nitrogen, since both reactive oxygen species (ROS) and nitric oxide (NO) deeply modulate the inflammatory responses. The generation of these reactive species can be attenuated by sulfasalazine, as it inhibits the binding of N-formyl-methionyl-leucyl-phenyl-alanine (fMLP) to its receptor on neutrophils [41] and also the superoxide production [42]. Interestingly, olsalazine and sulfasalazine are both potent inhibitors of superoxide production and degranulation of human neutrophils stimulated with fMLP, in contrast to 5-ASA and sulfapyridine, which do not have this ability [43]. On the other hand, 5-ASA can be converted to the oxidation products salicylate and gentisate, when the drug is incubated with activated human mononuclear cells and neutrophils, indicating that 5-ASA may scavenge toxic oxygen and nitrogen metabolites [44]. Similarly, evidences from an in vivo study pointed once more to a scavenge role of sulfasalazine as a mechanism of action, thus reducing experimental intestinal inflammation induced by acetic acid [45]. In humans, 5-ASA oxidation products can be found in the stools of IBD patients using sulfasalazine, suggesting that this drug indeed plays a role as scavenger for ROS and NO in these diseases [46].

A series of studies have demonstrated that sulfasalazine and its metabolites, at clinically relevant concentrations, also inhibit the release of cytokines produced by multiple cell types, including T cell mediators such as interleukin (IL)-2 [47] and those produced by monocytes or macrophages, like IL-12 [48], IL-1β, and tumor necrosis factor (TNF) [49]. Precisely, how sulfasalazine represses the release of cytokines has not been fully elucidated yet, but some studies have shown, for example, that sulfasalazine inhibits TNF expression in macrophages by inducing apoptosis [49] or inhibiting nuclear factor kappa B (NF-KB), a transcription factor crucial to the production of inflammatory mediators [50]. In the last years, the effects of sulfasalazine have been extensively studied in experimental models of intestinal inflammation. The chemically treated animals develop inflammation signs similar to those of human IBD, such as severe bloody diarrhea, body weight loss, colon length shortening, and gut pathological changes. In general, sulfasalazine treatment is able to reduce these signs and the colitis severity. Moreover, the drug significantly decreases the levels of inflammatory markers such as ROS [51], NF-KB, COX-2 [52], IL-6, TNF, IL-1 [53], NO [53], inducible nitric oxide synthase (iNOS) [52], myeloperoxidase (MPO) [54], monocyte chemoattractant protein-1 (MCP-1) [51], intercellular adhesion molecule-1 (ICAM-1) [51], and LTB4 [55], which are frequently overexpressed in IBD and widely known to be involved in chronic inflammatory disorders. Taken together, these experimental findings pointed to different mechanisms of action of sulfasalazine in the control of innate inflammatory reactions in gut mucosa, with outstanding relevance to the disease outcome.

Regarding adaptive and regulatory responses, it is known that a close relationship exists between colonic inflammation and T helper 1 (Th1) or Th17 immune reactions, which are related to the severity of inflammation in both human and experimental IBD [56]. In accordance, in a colitis model, mesalazine is able to inhibit Th1 and Th17 responses in contrast to an induction of regulatory immune profile, as observed by the disease amelioration, reduced expression neutrophil activity, IL-1β, TNF, IL-12, IFNγ, IL-17, IL-6, and RORγt, along with an augment in the suppressive cytokines IL-10 and TGF-β and in the transcription factor Foxp3 [57]. These data indicate that another mechanism of action of aminosalicylate drugs could be by decreasing pathogenic while increasing regulatory responses in intestinal inflammation.

The peroxisome proliferator-activated receptor ligand-γ (PPARγ) plays a significant role in the immune control through its capacity to repress the expression of inflammatory cytokines and induce the differentiation of leukocytes toward anti-inflammatory phenotypes. Importantly, by using experimental approaches with epithelial colon cell lines and human biopsies, Rousseaux et al. showed that 5-ASA activates PPARγ, pointing to the receptor as an important drug's target for the control of intestinal inflammation [58]. In line with that, regulatory T cells (Tregs) play an indispensable role in suppressing exacerbated inflammatory immune responses that can be harmful to the host, such as in IBD [59]. Recently, Oh-Oka et al. proposed a new anti-inflammatory mechanism for mesalamine (5-ASA) in colitis, involving colonic Tregs. The oral treatment with this drug leads to the accumulation of Tregs in the colon lamina propria associated with increased levels of the active form of the anti-inflammatory cytokine TGF-β. These alterations attributed to mesalamine are dependent on the activation of aryl hydrocarbon receptor (AhR), a transcription factor that regulates several immune processes, including Treg activation and differentiation [60].

Altogether, these studies show that aminosalicylates play an important role in the regulation of IBD responses.

2.3 Thiopurines

One of the most prescribed strategies for IBD therapy is the use of thiopurines, mainly azathioprine (AZA) and 6-mercaptopurine (6-MP). AZA is a prodrug that is metabolized by nonenzymatic mechanisms to be converted to 6-MP and other metabolites. Therefore, patients could be treated with AZA or directly with 6-MP, but the final metabolites produced from the thiopurines are the same. Also, both drugs generate endogenously active products able to interfere on DNA and RNA synthesis [61].

The discovery of AZA and 6-MP yielded a Nobel Prize in Medicine in 1988 for Gertrude B. Elion and George Hitchings. At first, the thiopurines were used in cancer therapy, in order to stop cell proliferation. Nonetheless, the immunosuppressive effect of thiopurines was evident as well as their efficiency in prolonging renal allograft transplant survival [62]. Thereafter, AZA and 6-MP began to be used in the clinics for inflammatory and rheumatic diseases. Since then, many mechanisms of action of thiopurines were proposed, mainly involving immunological axis in an attempt to unravel their immunosuppressive effects.

Some thiopurine metabolites, such as deoxyguanosine triphosphate (dGTP) and 6-thioguanine (6-TG), can be incorporated to DNA, replacing the natural purines adenine (A) and guanine (G). Then, during the DNA replication, a high level of substitution 6-TG could be particularly cytotoxic [63]. These DNA modifications are not restricted to cancer cells, and lymphocytes can be affected by the purine analogue 6-TG as well [64]. Besides that, some evidences point to the inhibition of de novo synthesis, which produce purines, by the thiopurine therapy. Then, the lack of abundant nitrogenous bases impairs the lymphocyte replication either, which contributes to the immunosuppression [65].

The thiopurines have the capacity to downregulate the expression of inflammatory genes in activated T lymphocytes [66]. One of these genes is the TNF-related apoptosis-inducing ligand (TRAIL), which is important to induce apoptosis and is upregulated in activated T lymphocytes. Despite being apparently contradictory, TRAIL could increase T cell proliferation and IFN-γ production [67], a phenomenon that is pathogenic for Crohn's disease patients. It is important to state that IFN-γ is a cytokine that accompanies the Th1 response, which increases gut inflammation. Also, CD27, which is a member of TNF superfamily, is downregulated by AZA [66]. This receptor is required to T cell maintenance and for B cell activation. Consequently, a low expression of CD27 could facilitate the lymphocyte death [68]. Besides, CD27 is involved in the NF-κB activation and IFN-γ production [69]. In fact, the 6-TG incorporation into T cell DNA is correlated to the decreased IFN-γ production in CD patients [70]. Lastly, the thiopurines could reduce the expression of the α4-integrin as well [66]. This integrin is mandatory to the lymphocyte accumulation in the gut and the chronic inflammation [71].

It is clear that the accumulation of T lymphocytes in the gut mucosa is one of the main hallmarks for the exacerbated inflammation and disease worsening. Accordingly, thiopurines also reduce T cell proliferation and the consequent excessive inflammatory mediators produced by this population. Indeed, 6-MP that impairs the A and T purine integration into the replicant DNA and replaces them for mimetic purines compromises the cell cycle and T cell proliferation. 6-MP interferes in the G1 to S phase transition and progression through S phase in cell cycle, with consequent increase in lymphocyte death [72]. Thereby, it is unquestionable that the thiopurine metabolites incorporate into the genetic material and negatively influence the DNA integrity or stability, which causes cellular death. In the last decade, the first conclusive and detailed studies about the thiopurines' molecular mechanism of action in T lymphocytes explained better the delayed effects of these drugs, besides the incorporation of mimetic purines, as described above.

The Ras-related C3 botulinum toxin substrate 1 (Rac1) is a GTPase protein that activates MEKK/IκB/NF-κB (mitogen-activated protein kinase kinase/IKK/nuclear factor kappa-light-chain-enhancer of activated B cells) and signal transducer and activator of transcripition-3 (STAT-3) pathways, both of which lead to the accumulation of B-cell lymphoma-extra large (Bcl-xL) in the mitochondria. The enhancement of this protein results in an anti-apoptotic effect to cell survival. However, AZA and the 6-MP metabolite 6-thioguanine triphosphate (6-Thio-GTP) bind to Rac1, which impairs MEKK and STAT-3 phosphorylation, and consequently the anti-apoptotic effect by Bcl-xL is lost. Instead of that, there is an enhancement of Caspase-9, an apoptotic pathway of human cells involving mitochondria [73]. Interestingly, these mechanisms require the co-stimulation by CD28 in T cells.

The bind of CD28 by costimulatory molecules leads to lymphocyte's lamellipodia formations, which are projections of the cytoskeletal protein actin, necessary for T cell movement and membrane readjustment to make contact with antigen-presenting cells (APC). GTPase Rac1 also mediates this process [74]. Later, it was observed that thiopurines also bind to and block Rac2 activation, while the treatment with these drugs impairs the lamellipodia formation. Additionally, upon binding to Rac proteins, AZA and its metabolites reduce ezrin-radixin-moesin protein (ERM) desphosphorylation and subsequently the formation of APC-T cell conjugates, necessary for an effective immune adaptive response. Likewise, that was dependent on CD28 activation too [74]. Taken together, these results suggested that AZA and its metabolites binding Rac1 promote T cell apoptosis, by decreasing Bcl-xL and increasing caspase-9, but also interfere in T cell function or activation. Recently, a Bcl-2 inhibitor was suggested as a novel therapy to patients refractory to AZA treatment, despite Bcl-2, as a biomarker, cannot predict AZA treatment response in IBD patients [75].

In 2009 a study confirmed that 6-MP and 6-TG decrease the lymphoproliferative capacity of T cells, but in a physiological concentration (5 μM) [76]. The thiopurine therapy causes, in vivo, specifically depletion of T CD4 memory cells, thus reducing the capacity of response to a recurrent antigen. Considering that in IBD there is continuous microbial translocation and antigen presentation [77], this should explain, at least in part, the delayed onset of the drug's effect on the disease.

Thiopurine metabolites are also capable to inhibit the inflammatory response of macrophages and epithelial cells. These drugs significantly reduce the activity of c-Jun N-terminal kinase (JNK) and STAT3, as well IL-6, IL-8, CCL2, and CCL5 and inducible nitric oxide synthase (iNOS) expression. However, only iNOS in macrophages and IL-8 in epithelial cells are decreased dependent on Rac1 [78]. In fact, AZA restores the paracellular permeability after TNF-induced apoptosis. The treatment improves the expression of tight junctions and adherens junctions, such as occludin and E-cadherin [79]. Thus, the reduction of Rac1 is proposed as a biomarker for effectiveness of thiopurine treatment in patients with IBD [80].

It seems that the use of thiopurines can modulate the frequency of diverse immune cell populations, even by an indirect pathway. For example, patients treated with AZA have increased CCR5 expression in circulating monocytes. These $CCR5^+$ cells are considered to have an anti-inflammatory profile, with increased CD163 and diminished TLR4-induced TNF and IL-6 secretion, probably in an attempt to achieve immunoregulation under AZA treatment [81]. Moreover, thiopurine therapy decreases CD160 expression [82], as well as natural killer (NK) cells and the population of B lymphocytes in the peripheral blood of IBD patients [83]. Indeed, the reduction in B cells is one of the reasons for using combo therapy with AZA plus infliximab (IFX), instead of IFX alone. AZA diminishes the antibody formation against IFX and then improves the patients' responsiveness to the biological treatment [84].

The presence of variant Tγδ cells, specifically the TCR Vδ2, in the gut mucosa of Crohn's disease patients is associated with worse clinical prognosis and inflammation [85]. However, AZA is able to ablate this population in the blood and mucosa of patients treated with this drug, suggesting other potential mechanisms of action of AZA in the control of intestinal inflammation [86].

Besides the cellular changes, thiopurines are also capable of modulating soluble mediators, by decreasing IL-1β, TNF, and IFN-γ or increasing IL-10 *production* in vivo [87]. Likewise, the higher expression of inflammatory cytokines in detrimental to anti-inflammatory mediators may dictate the augmented production of matrix metalloproteinases (MMPs) in contrast to inhibitors of metalloproteinases (TIMPs), which are correlated to the control of the disease and improvement of intestinal barrier [88]. In line with that, the treatment with thiopurines reduced the pro-inflammatory effects, with decreased neutrophil MMP-9 and MMP-26 production, besides increased TIMP-3 expression by enterocytes [89].

Finally, a last mechanism of immune regulation was recently described involving AZA's use. This drug can induce autophagy, which is a natural mechanism to recycle cellular components and to promote cell survival, depending on PERK sensor and mTORC1 in lymphocytes. Hence, modulation of autophagy could represent an additional mechanism of inflammation control through AZA treatment in IBD [90].

2.4 Methotrexate

Methotrexate (MTX), originally known as amethopterin, is a folate antagonist. Its history and clinical use refers to Faber and Diamond [91], who reported the utilization of aminopterin, the first folic acid antagonist, as a treatment for acute leukemia in children. MTX, which is a derivative of aminopterin and is distinguished by having an additional methyl in its structure, subsequently replaced aminopterin after a study reported its lower toxicity in an experimental model of acute leukemia in rats [92]. The idea behind the use of antifolates for the treatment of neoplasias was based on the knowledge that folates function as cofactors for DNA biosynthesis. Subsequently, the ability of MTX to interfere in DNA synthesis was proven experimentally [93], and years later lower doses of MTX also began to be studied for other conditions such as psoriasis [94] and rheumatoid arthritis [95].

For IBD, Kozarek et al. [96] were the first to report the ability of this drug to induce clinical and histological remission in patients with Crohn's disease, but it was only after two randomized controlled trials (RCTs) of the North American Crohn's Study Group (NACSG) that MTX was formally established as a possible therapy for this disease [97]. On the other hand, there is no strong scientific basis for recommending the use of MTX as a monotherapy for UC. Nevertheless, the utilization of high or low doses of MTX in combination with anti-TNF has been shown to be effective in disease control at the same extent in both Crohn's disease and ulcerative colitis patients [98]. In summary, because of these and other results, MTX is usually recommended in specific conditions, especially depending on disease outcome and response to other therapies [99].

MTX acts as an antineoplastic drug when used at high doses and as immunosuppressive at low doses [100]. This led to the investigation of other possible mechanisms capable of inducing immunosuppression, in addition to interfering in cell proliferation. In line with that, there is a lack of specific investigation unraveling the exact mechanisms of action of MTX in IBD, but this drug is capable of inducing apoptosis in activated T cells [101], inhibiting IL-8 production by peripheral blood mononuclear cells [102], and increasing extracellular adenosine levels. This metabolite has potent anti-inflammatory properties [103] in patients with rheumatoid arthritis [104] and potentially in IBD [105]. Clearly, more experimental studies are needed to better understand the action of MTX in IBD, but those mentioned above represent possible mechanisms that could explain the relative success of MTX as an immunomodulatory therapy, especially for Crohn's disease.

2.5 Cyclosporine

The cyclosporine A (CsA) is an immunosuppressor drug initially used for organ transplantation on the late 70 and 80 decades [106]. Some years later, it was utilized as an alternative treatment for ulcerative colitis (UC) patients refractory to glucocorticoids, because of its strong immune regulatory effects [107].

CsA is a lipophilic cyclic peptide that is metabolized by hepatic enzymes of cytochrome P450 pathway [108]. Its immunosuppressor activity depends on the intracellular binding to cyclophilins with further inhibition of the calcium-calcineurin pathway and the resulting blockage of the nuclear activated T cell factor (NFAT) translocation to the nucleus [109], thus avoiding cellular activation. Consequently, there is reduction in the transcription of genes related to cytokine production such as IL-2, IL-4, and IFN-γ [110], inhibition of CD4 expression, cell proliferation [111], and activation of CD8 lymphocytes [112]. Therefore, the blockage of NFAT is considered one of the main effects of this immunosuppressor drug [113].

Upon in vitro treatment of peripheral blood mononuclear cells (PBMCs), from ulcerative colitis or Crohn's disease patients with CsA, there is reduction of TNF, IL-17, and IL-10 in samples from all donors, besides an exclusive significant IL-13 decrease in subjects with UC. Also, CsA stimulates the cellular apoptosis of PBMC from patients with UC, though not by the mitochondrial route [114]. In an experimental colitis model, the treatment with CsA reduces the clinical activity of the disease and mRNA expression of several inflammatory cytokines such as IL-1β, IL-6, and TNF [115].

Hence, though the therapy with CsA has shown to be beneficial, the systemic treatment can be limited due to its side effects such as nephrotoxicity, hypertension, seizures, production of ROS or hydrogen peroxide, and opportunistic infections [116].

2.6 Tacrolimus

Tacrolimus (Tac) was isolated in 1984 from the fungus strain *Streptomyces tsukubaensis*. It was initially used in the treatment of transplants and later in therapies for inflammatory or autoimmune diseases [117]. This drug is a substrate for cytochrome P450 isoenzymes (CYP3A), and the expression or activity of these enzymes in liver and intestinal cells may vary between individuals, thus contributing to different pharmacokinetic profile of Tac therapy [118].

The Tac, compared to CsA, has a more potent inhibitory action against T cell activation, leading to immunosuppression. It binds to FKBP-12, with further inhibition of the calmodulin-dependent phosphatase activity of calcineurin [119]. Thus, it inhibits the action of activated nuclear T cell factor (NFAT), reducing the production of IL-2. In line with that, Tac can also decrease the activity of NF-κB [120]. Therefore, besides IL-2, Tac is a calcineurin inhibitor that leads to reduced production of IL-3, TNF, IFN-γ, and IL-17, as well as the release of histamine from mast cells and proliferation of $CD4^+$ or $CD8^+$ T cells in a variety of inflammatory processes [121]. Tac treatment in bone marrow-derived macrophages also leads to reduced IL-12p40, IL-12p70, and IL-23 during LPS stimuli [122].

As described, in vitro treatment with Tac inhibits the activity of leukocytes such as T lymphocytes, NKT, and antigen-presenting cells, usually present on colon tissue. Moreover, the administration of Tac in trinitrobenzene sulfonic acid (TNBS) colitis results in the reduction of neutrophil infiltrate in the intestinal mucosa associated with inhibition of T cell activation, as well as decreased expression of CXCL1 and CXCL2 chemokines [123]. Most interestingly, Tac is able to inhibit the expression of IL-17 and TNF [124], suggesting that this drug could assume therapeutic effect on diseases mediated by Th17 responses, such as IBD. Furthermore, the rectal treatment in mice leads to better results than oral administration of the drug [125].

In experimental granulomatous colitis, treatment with Tac results in the reduction of intestinal permeability, neutrophil activity, as well as extra-intestinal manifestations of the disease, such as hepatic and splenic granulomas, caused by the colitis-inducing agent [126]. On the other scenario, myofibroblasts isolated from normal gut tissues and stimulated in vitro with TNF show increased phosphorylation of the p38 subunit of MAP kinase, leading to augmented CCL2 and CXCL10 expression. However, in vitro treatment with Tac suppresses the expression of CCL2 and CXCL10 mRNA by inhibiting phosphorylation of MAP kinase, indicating that these effects could be one of the mechanisms of therapeutic action of Tac on intestinal inflammation [127].

Hence, although this therapy may result in satisfactory IBD outcome, research has pointed that after mucosal healing, it is desirable to change this therapeutic intervention to other immunosuppressor drugs, in order to reduce the long-term adverse effects caused by Tac, such as nephrotoxicity [128].

3. Conclusions

The introduction of pharmacological therapies for IBD is of high importance to achieve remission and maintenance of quiescent disease in affected patients. Nonetheless, although these drugs act by diverse mechanisms, all of them are relevant in constraining the activation and perpetuation of the exacerbated immune-inflammatory responses that underline the gut inflammation in Crohn's disease and ulcerative colitis. Then, the balance between adequate control of inflammatory responses and drugs' adverse effects dictates the efficiency of corticosteroid and suppressor treatments in IBD.

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