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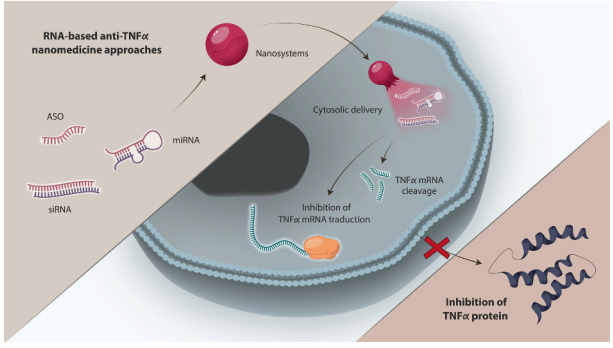
Tackling TNF-α in autoinflammatory disorders and autoimmune diseases: From conventional to cutting edge in biologics and RNA-based nanomedicines

Valentina Andretto [a,1], Silvia Dusi [b,1], Serena Zilio [a,c], Mathieu Repellin [a,d], David Kryza [a,e], Stefano Ugel [f,1,2], Giovanna Lollo [a,*,2]

[a] Univ Lyon, Université Claude Bernard Lyon 1, CNRS, LAGEPP UMR 5007, 43 Boulevard du 11 Novembre 1918, F-69622 Villeurbanne, France
[b] Istituto Oncologico Veneto IRCCS, Padova 35128, Italy
[c] SATT Ouest Valorisation, 14C Rue du Patis Tatelin 35708, Rennes, France
[d] PULSALYS SATT Lyon-Saint Etienne, 47 Boulevard du 11 Novembre 1918, 69625 Villeurbanne, France
[e] Hospices Civils de Lyon, 69437 Lyon, France
[f] Immunology Section, Department of Medicine, University of Verona, 37134 Verona, Italy

GRAPHICAL ABSTRACT



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ABSTRACT

Autoinflammatory disorders and autoimmune diseases result from abnormal deviations of innate and adaptive immunity that heterogeneously affect organs and clinical phenotypes. Despite having etiologic and phenotypic differences, these two conditions share the onset of an aberrant inflammatory process. Targeting the main drivers controlling inflammation is useful to treat both autoimmune and autoinflammatory syndromes. TNF-α is a major player in the inflammatory immune response, and anti-TNF-α antibodies have been a revolutionary treatment in many autoimmune disorders. However, production difficulties and high development costs hinder their implementation, and accessibility to their use is still limited. Innovative strategies aimed at overcoming the limitations associated with anti-TNF-α antibodies are being explored, including RNA-based therapies.

* Corresponding author.
E-mail address: giovanna.lollo@univ-lyon1.fr (G. Lollo).
[1] These authors share equal contribution.
[2] These authors share equal contribution and co-last author.

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Here we summarize the central role of TNF-α in immune disorders and how anti-TNF-based immunotherapies changed the therapeutic landscape, albeit with important limitations related to side effects, tolerance, and resistance to therapies. We then outline how nanotechnology has provided the final momentum for the use of nucleic acids in the treatment of autoimmune and autoinflammatory diseases, with a focus on inflammatory bowel diseases (IBDs). The example of IBDs allows the evaluation and discussion of the nucleic acids-based treatments that have been developed, to identify the role that innovative approaches possess in view of the treatment of autoinflammatory disorders and autoimmune diseases.

1. Introduction

Autoinflammatory disorders and autoimmune diseases originate from an abnormal immune response that fuels a pathological inflammatory state and organ-specific injuries [1,2]. Since the definition of autoimmunity generally depicts an impairment of adaptive immunity, whereas the umbrella definition of autoinflammatory diseases encompasses monogenic and multifactorial pathologies characterized exclusively by aberrant activation of innate immune cells, these two disorders have historically been studied as separate categories [3]. Despite the different pathogenic biology and the diverse immune actors involved, aberrant inflammation bridges the gap between autoimmune disease and autoinflammatory disorders. The intensity of pathological inflammation ranges from very mild to life-threatening pathologies. Indeed, excessive immune system activation can produce a lethal cytokine storm that can lead to multiorgan failure [4]. Therefore, targeting the key factors and molecular signaling pathways that control the pathogenesis of excessive inflammation may be useful for the treatment of both autoimmune and autoinflammatory syndromes. This review aims to investigate nanoparticles-based approaches to manipulate tumor necrosis factor (TNF)-α, a major player in the inflammatory immune response, using RNA-based strategies. Indeed, RNA-based nanomedicine is emerging as one of the most effective and flexible therapeutic strategies to control autoimmune and autoinflammatory pathologies to modulate those cytokines whose profile changes over the course of the disease or conventional therapy.

1.1. Pathogenesis of autoimmune disorders

Autoimmune diseases originate from a breakdown of tolerance in which lymphocytes whose B or T cell receptors (BCR and TCR respectively) able to recognize autoantigens are not completely eliminated due to an imperfect process of negative selection. Therefore, in humans, a considerable proportion of B and T cells escaping from either bone marrow or thymus are self-reactive [5,6]. Several checkpoints, such as induction of functional anergy or early apoptosis, limit the fitness and the activation state of these autoreactive cells. Indeed, a consistent group of inhibitory molecules (e.g., cytotoxic T-lymphocyte antigen 4 (CTLA-4), lymphocyte-activation gene 3 (LAG-3), T cell immunoglobulin and mucin domain-3 (TIM3), are normally absent in naïve lymphocytes but over-expressed on the surface of activated effector lymphocytes to abrogate their functionality and activation state when their function is no longer needed [7-10]. Deficiencies in the expression of these molecules result in an uncontrolled immune response with a high amount of autoantibodies in the blood and the release of several soluble pro-inflammatory mediators, such as cytokines (e.g. TNF-α and interferon (IFN-γ)) and interleukins (e.g. IL-23) that sustain both local and systemic immune activation [1,4,11]. The non-physiological blockade of activated lymphocytes may also be associated with a deficiency of immunosuppression elements such as T regulatory lymphocytes (Treg) and tolerogenic myeloid cells. Treg cells are commonly identified as $CD4^{+}CD25^{+}FOXP3^{+}$ cells that originate in the thymus (natural or thymic Treg cells) or in the periphery (induced Treg cells) [12-14] and affect different leukocytes by exploiting mechanisms of cell–cell contact (e.g., CTLA-4-dependent pathway) as well as the release of inhibitory molecules such as transforming growth factor TGF-β, IL-33, and IL-10 [15]. Therefore, one of the most convincing hypotheses on the pathogenesis of autoimmunity, supported by *in vivo* disease models, is that it originates from a defect in either the number or function of circulating Treg cells [16]. Recent advances in the field of Treg have enabled their more detailed molecular definition and a better understanding of their differentiation at molecular level. They have also clearly demonstrated that most autoimmune diseases, such as multiple sclerosis (MS) [17], systemic lupus erythematosus (SLE) [18], type 1 diabetes (T1D) [19], rheumatoid arthritis (RA) [20] and others [16], display defects on these immune regulators. In addition, the potential conversion of Treg cells into disease-inducing drivers under inflammatory conditions has been unveiled as an additional mechanism that sustains autoimmune disease progression [21,22], but this pathogenic process needs further investigation. Although tolerance failure is the *primum movens* in the pathogenesis of autoimmunity, innate cells such as dendritic cells (DCs), monocytes, and macrophages are responsible for priming autoreactive lymphocytes and, overall, exacerbating local inflammation that results in tissue damage [23]. These cells have a dual conflicting role, as they are responsible both for initiating the self-reaction process that leads to autoimmunity and for promoting and maintaining self-tolerance. Once recruited into the inflamed tissue, DCs orchestrate the inflammatory response by being a major source of pro-inflammatory cytokines (e.g. IL-1, TNF-α, IFN-α, and IL-6) and express costimulatory molecules that cause reactivation of self-reactive T cells. Additionally, DCs drive the recruitment and functional activation of circulating myeloid cells, including other DCs, neutrophils and monocytes with pro-inflammatory signature [24]. On the other hand, plasmacytoid DC subsets (pDCs) with tolerogenic functions inhibit T cell activation through the secretion of several anti-inflammatory factors (e.g. TGF-β) and the expression of immuno-regulatory enzymes such as indoleamine 2,3-dioxygenase (IDO-1) and heme oxygenase-1 (HO-1), which controls T cell proliferation, activation, and apoptosis [25,26]. Both conventional and pDCs are detected in the local inflammatory environment, such as the synovium of RA patients, where they effectively release a high amount of pro-inflammatory cytokines [27,28]. Interestingly, inflammation leads to the appearance of DC cell subsets different from those identified at steady state conditions characterized by specific deletions in key transcription factors and surface molecules that affect signal transduction events [29,30]. Deletion of signal transducer and activator of transcription 3 (STAT3) confer DCs with resistance to the anti-inflammatory effect of IL-10, promoting increased inflammation-associated factors and colitis in an experimental mouse model [31]. Similarly, a deficiency in the expression of αvβ8 integrin on DC membrane surface promotes the production of autoantibody and the establishment of colitis due to the inability of these cells to prime Tregs [32]. On the other hand, the same deficit in αvβ8 integrin expression on DCs' membrane protects against disease in an animal model of multiple sclerosis (MS), due to their impaired ability to activate a Th17 response [33]. These inflammation-reprogrammed DCs have been known for their robust ability to prime and activate T cells [34].

Macrophages (Mϕ) express different functional programs in response to microenvironmental signals. M1-polarized Mϕ are pro-inflammatory and contribute to local inflammation by secreting IL-12 and TNF-α, while M2-polarized Mϕ produce IL-4 and IL-10 fundamental for their immunomodulatory, wound repair and tissue remodeling functions. However, the M1/M2 dichotomy oversimplifies a more complex cell

biology [35]. Indeed, in some autoimmune disorders M1- and M2-polarized Mϕ are detected simultaneously where both M1- and M2-producing cytokines are present. The impact of Mϕ on autoimmune physiopathology is widely observed in many autoimmune diseases: Mϕ with defective phagocytosis have been linked with autoimmunity in SLE, as the ineffective clearance of apoptotic cells and debris can make their permanence in the inflamed tissue a source of autoantigens, just as Mϕ plasticity promotes their differentiation into bone-resorbing osteoclasts which are the major players in inflammatory joint destruction in arthritis [36].

Altogether, autoimmune diseases are characterized by a complex cell network in which elements of the adaptive immunity interact with cells of the innate counterpart resulting in a persistent and chronic pathological inflammation [37].

1.2. Pathogenesis of autoinflammatory disorders

Autoinflammatory disorders are caused by distinct dysregulations of the innate immune system [38,39]. One of the most relevant pathogenic mechanisms of these immunological disorders is linked to functional modifications of the inflammasome, a multiprotein cytoplasmatic complex assembled by the nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family including NLRP1, NLRP3, NLRP9. . Upon inflammasome activation, they convert pro-caspase-1 into the biologically activated form, which cleaves the proinflammatory cytokines pro-IL-1β, pro-IL-18, and gasdermin-D (GSDMD) [40]. The cell death induced by inflammatory caspases (caspase 1 and caspase 11 in mice, or caspase 4 and caspase 5 in humans) and GSDMD is named pyroptosis and is characterized by the pore formation in the cell membrane and, as a result, cell rupture and release of the cytoplasmic content

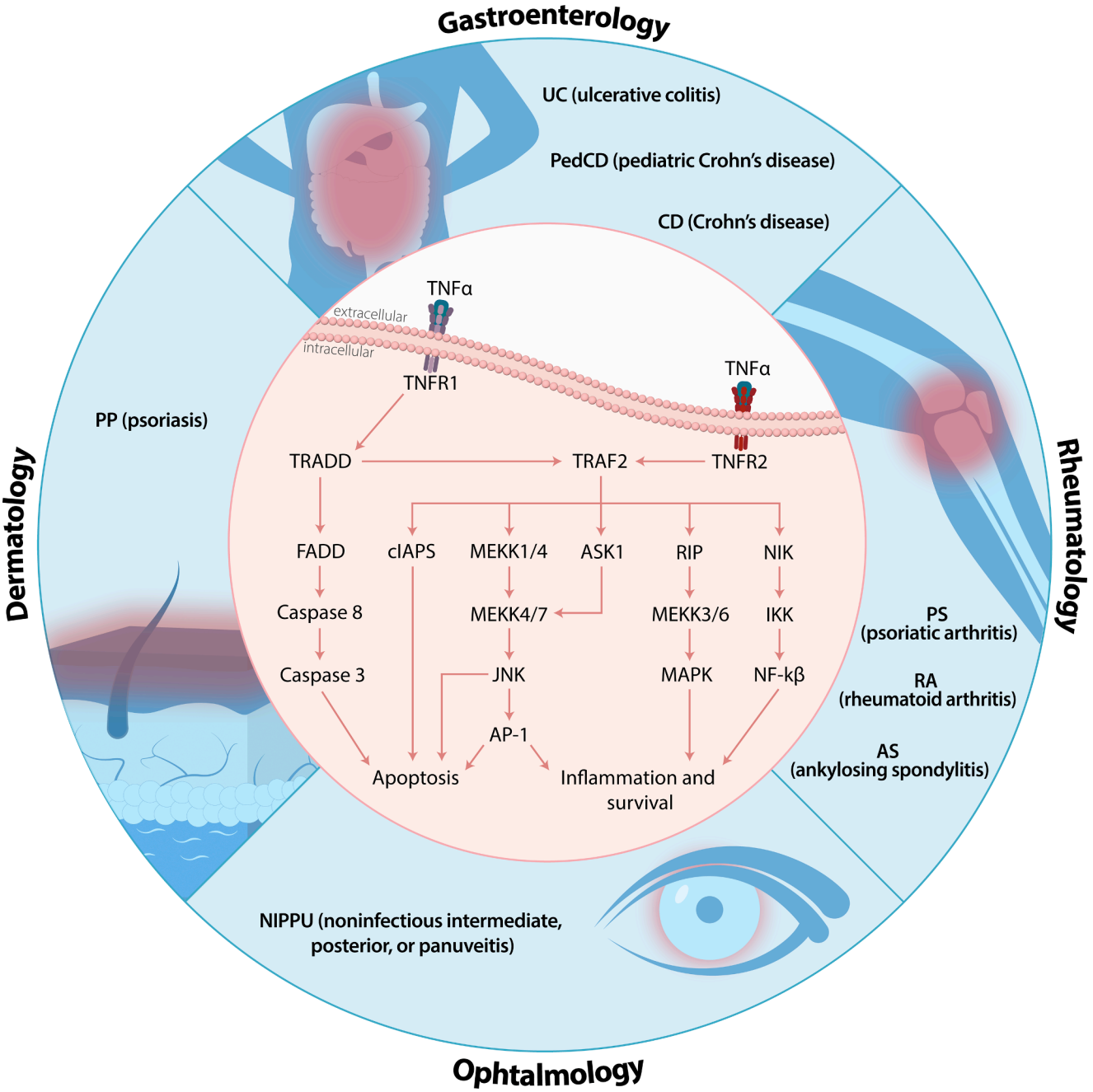

Fig. 1. TNF-α regulation and transcriptional pathways involved in different pathologies.

along with proinflammatory cytokines such as IL-1β and IL-18 [41]. Pyroptosis has the ability to disrupt immune system homeostasis by promoting autoinflammatory processes. Genetic mutations in the proteins constituting the inflammasome complex have been associated with a spectrum of diseases, including familial cold autoinflammatory syndrome and cryopyrin-associated periodic fever syndrome (CAPS) [42]. In Crohn's disease (CD), the inflammasome mutation concerns both intestinal epithelial cells and myeloid cells and promotes an aberrant innate immune response to bacterial peptidoglycan, fueling chronic inflammation and tissue damage [43-45]. The dysregulation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway is another central basis for autoinflammatory diseases [46]. Alterations of NF-κB-associated signaling pathway depend on either constitutive activation of NF-κB or loss-of-function mutations in the regulatory NF-κB system [38]. For instance, A20 protein haploinsufficiency (HA20), caused by heterozygous mutation or deletion of *TNFAIP3* gene, is characterized by persistent activation of the NF-κB pathway, as intracellular deubiquitinase A20 (also called TNF-α-induced protein 3) is unable to turn off TLR/TRAF6 molecular signaling [47]. Recently, we demonstrated that the anti-apoptotic molecule cellular FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein (c-FLIP), which plays an important role as modulator of caspase-8, is crucial in reprogramming both mature myeloid cells into immunosuppressive elements [48] and immature myeloid cells into pro-inflammatory cells that sustain fatal cytokine release disorders [49]. Therefore, aberrant NF-κB induction may not only depend on gene mutations but also on unpredictable protein–protein interactions or unexpected cellular localization [50].

2. TNF-α as the orchestrator of the inflammatory pathway: Common ground to immune-mediate inflammatory diseases

Among the primary mediators of the systemic immune response, TNF-α has been identified as a "master regulator" of the pro-inflammatory cytokine cascade, responsible for pleiotropic events within cells during sepsis and infection (Fig. 1).

The importance of TNF-α as a central player in the establishment of many chronic inflammatory diseases is confirmed by the fact that neutralizing the presence or dysregulated action of TNF-α has revolutionized the history of cytokine research, with millions of patients treated since infliximab, the first approved monoclonal antibody approved against TNF-α, was shown to be effective in RA and is now used to treat CD and ankylosing spondylitis [51,52].

Aberrant TNF-α production and TNF receptor signaling are implicated in the pathogenesis of several inflammatory diseases. TNF-α is one of the first members of the TNF superfamily to be identified, and since its cloning in 1985, more than 40 members of the large TNF superfamily have been discovered. The ligands and receptors of this TNF superfamily have unique structural characteristics linking them to cell growth, survival, or death. Although many different cell types are responsible for TNF-α production, monocytes, and macrophages, as well as astroglia, microglia, Langerhans cells, and Kupffer cells stand out as the main producers of TNF-α [53].

TNF-α signals act through two transmembrane (tm) receptors: TNF receptor 1 (TNFR1), also known as p55 or p60, constitutively expressed in most mammalian tissues, and TNF receptor 2 (TNFR2), also known as p75 or p80, which expression is tightly regulated in the cells of the immune system. TNF-α can bind both TNFR1 and TNFR2 receptors with high affinity, leading to the regulation of cell proliferation, differentiation, survival, and apoptosis [53]. Notably, the cleaved extracellular domains of both receptors (sTNFR-I and sTNFR-II) retain the ability to bind TNF-α, function that leads to an endogenous inhibition of TNF-α signaling, as demonstrated *in vitro* and *in vivo* by Van Zee and coworkers in nonhuman primates with lethal sepsis, in which the administration of the recombinant protein sTNFR-I ameliorates hemodynamic complications and cytokine induction due to the hyperbolic TNF-α production [54].

Furthermore, as a pro-inflammatory cytokine, TNF-α is involved in most aspects of biology, from increasing lipid signal transduction mediators, such as prostaglandins and platelet-activating factors, to promoting cell activation and recruitment. Moreover, TNF-α is involved both in cell death and NF-κB-dependent cell survival pathways. On the one hand, TNFR1-associated signaling complexes can mediate a switch from inflammatory gene signaling to cell death via apoptosis or necroptosis. On the other hand, survival versus cell death is the result of NF-κB-dependent transcriptional activation of antiapoptotic genes functioning in the death-inducing signaling complex (DISC). Specifically, the above-described anti-apoptotic molecule c-FLIP blocks TNF-α-mediated apoptosis by preventing autocatalytic activation of procaspase-8 in the DISC.

3. Anti-TNF-α antibodies as therapy for immune-mediated inflammatory diseases: Pitfalls and side effects

TNF-α inhibitors are the first class of therapeutics with a selective mechanism of action, which play a crucial role in the management of many immune-mediated inflammatory diseases (IMIDs), such as RA, psoriatic arthritis (PA), and inflammatory bowel diseases (IBDs), inclusive of the two extreme phenotypes ulcerative colitis (UC) and Chron's disease (CD). These agents can act at different levels, inhibiting TNF-α transcription and TNF-α -mediated downstream signaling and binding to TNF-α receptors. Anti-TNF-α biological agents have led to important advances in the therapy of these diseases and have confirmed the concept of a common pathophysiology of IMIDs, with TNF-α playing a predominant role [55]. To date, five different anti-TNF-α biologics are available to patients (Table 1), four of which are monoclonal antibodies, and one is a circulating receptor fusion protein. All five agents block the biological effects of TNF-α but differ in structure, pharmacokinetics, and mechanisms of action (Fig. 2).

The monoclonal antibodies infliximab, adalimumab, certolizumab pegol, and golimumab exhibit high affinity for both the soluble and transmembrane form of human TNF-α, but not for TNF-β. The antigen-binding fragment (Fab) region of these antibodies competes for the TNF-α binding site and inhibits the binding of TNF-α to its cognate receptor, neutralizing its activity by hindering the initiation of the signaling cascade [56]. A second mechanism of action has been hypothesized for those monoclonal antibodies (infliximab and golimumab) also containing the crystallizable fragment region (Fc) as well as for the receptor fusion protein etanercept. This mechanism involves the removal of TNF-α expressing cells by the induction of Fc- or tmTNF-mediated effector mechanisms, such as antibody-dependent cellular cytotoxicity (ADCC). During ADCC, signaling initiated by FcR engagement of the Fc portion of an antibody bound to the target cell leads to the release of granzymes into target cells, causing activation of caspases cascade and cell death by apoptosis. ADCC as a secondary mechanism of action during monoclonal antibody therapy has been mainly reported for UC and CD.

TNF-α levels are tightly regulated by several positive and negative feedback signaling loops. The receptor fusion protein etanercept acts as a decoy receptor, sequestering both soluble and transmembrane TNF-α molecules, thereby limiting their availability and binding to cell surface TNFRs, making TNF-α biologically inactive. Etanercept mimics the negative feedback loop naturally exerted by the presence of soluble TNF-α receptors cleaved from the cell surface.

Infliximab (Remicade®) is a recombinant DNA-derived chimeric antibody, consisting of a mouse variable region and human constant region [69], and is the first anti-TNF-α agent to achieve routine clinical use. It has been initially approved by the FDA in 1998 for the treatment of CD after a 12-year-old patient non-responsive to conventional therapies responded to a single injection of infliximab used as compassionate therapy [57]. Infliximab is administered intravenously, and its use has since been widened to different pathologies with the latest approval for

Table 1
List of anti-TNF biologics.

Antibody	Structure	Pathology	FDA Approval	Ref
Infliximab (Remicade®)	Chimeric mouse-human monoclonal anti TNF antibody Fc portion from Human IgG1k and murine Fv	CD RA + MTX AS PS UC Pediatric CR PP Pediatric UC	1998 1999 2004 2005 2005 2006 2006 2011	[57,58]
Adalimumab (Humira®)	Fully humanized IgG1k monoclonal anti-TNF antibody	RA PA AS CD PP Polyarticular JIA UC Pediatric CD HS NIPPU FP Pediatric UC	2002 2005 2006 2007 2008 2008 2012 2014 2015 2016 2017 2021	[59]
Certolizumab Pegol (Cimzia®)	Fragment antigen-binding (Fab) fragment of recombinant fully humanized monoclonal anti TNF antibody fused with 400 kDa Peg moiety	CD RA PA AS PP nr-axSpA	2008 2009 2013 2013 2018 2019	[60,61]
Golimumab (Simponi®)	Fully humanized IgG1k anti TNF antibody	RA SA AS Polyarticular JIA	2013 2017 2017 2020	[62,63]
Etanercept (Enbrel®)	Dimeric human recombinant fusion protein composed of 2 soluble TNF receptor extracellular domains with Fc portion of human IgG1	RA Polyarticular JIA PA AS PP Pediatric PP	1998 1999 2002 2003 2004 2016	[64–68]

+MTX: in combination with methotrexate; CD: Crohn's disease; RA: rheumatoid arthritis; AS: ankylosing spondylitis; PS: psoriatic arthritis; UC: ulcerative colitis; PP: plaque psoriasis; JIA: Juvenile Idiopathic Arthritis; HS: Hidradenitis Suppurativa; NIPPU: Noninfectious intermediate, posterior, or panuveitis; FP: Fingernail Psoriasis; nr-axSpA: Non-Radiographic Axial Spondyloarthritis.

pediatric plaque psoriasis in 2016. However, due to the presence of the mouse variable region, the induction of anti-drug antibodies (ADAs) is possible and is associated with the degradation of infliximab. The occurrence of ADAs was reported in 28.3% of patients treated with infliximab and presented low serum levels of the drug. Co-administration of methotrexate (MTX) has been shown to reduce immunogenicity and minimize the development of ADAs against infliximab [58].

Adalimumab (Humira®) is a subcutaneously self-injectable monoclonal human antibody approved in 2002 by the FDA for the treatment of RA. Despite being a fully humanized IgG antibody without mouse sequences, ADAs production have been detected in more than 40% of adalimumab-treated patients, with the serum level of the drug inversely correlated to ADA titer [70]. As with infliximab treatment, administration of adalimumab in association with MTX resulted in improved clinical response and reduced production of ADAs [59]. Adalimumab is currently approved for several IMIDs with its most recent approval dated 2021 for the treatment of pediatric patients with moderate to severe UC.

Although the introduction of infliximab and adalimumab brought significant improvement in the treatment of several IMIDs, the response of individual patients to TNF-α inhibitors is not homogeneous, and there have still been patients who have not achieved a satisfactory clinical response. Clinical experience has shown that patients who face a lack of efficacy or reduced response to a first anti-TNF agent are likely to respond to a second one [71,72]. For this reason, two additional TNF-α inhibitors have been developed to provide additional therapeutic options: certolizumab pegol and golimumab.

Certolizumab Pegol (Cimzia®) is a PEGylated Fab of a recombinant humanized anti-TNF-α monoclonal antibody. Certolizumab is engineered to be produced in *Escherichia coli* and it is compatible with subcutaneous administration [60,61]. Its half-life of up to 14 days is greatly increased by the addition of two 20 kDa PEG chains. Lacking the constant region Certolizumab is unable to fix complement or cause antibody-dependent cell-mediated cytotoxicity *in vitro*. Moreover, it has no detrimental activity on immune cells since it does not induce apoptosis in human monocytes or lymphocytes, or neutrophil degranulation (European Medicines Agency, 2009). Certolizumab has been first approved by the FDA in 2008 for the treatment of moderate to severe CD, and its use has subsequently been expanded to the treatment of RA, psoriatic arthritis, ankylosing spondylitis, and plaque psoriasis, with its latest approval dated 2019 for treatment of non-radiographic axial spondylarthritis.

Golimumab (Simponi®) is a fully humanized IgG1 antibody produced by a mouse hybridoma cell line originating from splenocytes of transgenic mice engineered to express human IgG. Golimumab's amino acid sequence matches those of germline IgG1 and its high affinity for TNF-α, high conformational stability, and high solubility allow subcutaneous administration every 4 weeks. Golimumab has been first approved in 2013 for the treatment of RA, psoriatic arthritis, ankylosing spondylitis, and UC. Because intravenous administration of a single dose, Golimumab appears to produce a higher serum concentration than subcutaneous administration while maintaining a median terminal half-life [62], Golimumab has been approved also for infusion since 2013. As whit Infliximab and Adalimumab, concomitant administration of MTX increases the mean steady-state trough serum concentration and half-life, presumably limiting ADA production [63].

Etanercept (Enbrel®) is a genetically engineered TNF-α antagonist composed of a dimer of the extracellular ligand-binding portions of TNFR fused with the human IgG1 Fc portion [64,65]. After subcutaneous injection, Etanercept shows a shorter plasma half-life compared to IgG1 mAbs Infliximab and Adalimumab [66], despite having identical aminoacidic sequence in its Fc regions. Since antibody half-life appears to be largely determined by the binding ability of their Fc regions to the neonatal Fc receptor (FcRn) on endothelial cells [67], it is possible that etanercept shortened half-life is due to a different conformation or steric accessibility of its Fc regions. Etanercept was first approved by FDA in 1998 for the treatment of RA and subsequently, its use was expanded to the treatment of juvenile idiopathic arthritis, psoriatic arthritis, ankylosing spondylitis, and plaque psoriasis, with the latest approval in 2016 for the treatment of pediatric plaque psoriasis. Compared with other TNF inhibitors, particularly Infliximab and Adalimumab, Etanercept is less affected by the development of ADAs [68].

There is no doubt that the introduction of anti-TNF biologics has greatly improved the outcome and management of immune-mediated autoinflammatory diseases [73]. However up to 40% of patients do not respond to anti-TNF treatment [74], and several types of adverse effects may occur in association with anti-TNF therapy. All anti-TNF biologics are administered parenterally, intravenously, or subcutaneously, resulting in a systemic way of action. While this can be beneficial for patients with the most severe course of the disease, it can also lead to deleterious and possibly life-threatening side effects, including reactions at the site of infusion and injection, increased susceptibility to infections, and in particular reactivation of tuberculosis, autoantibody formation and drug-induced lupus erythematosus, liver function abnormalities, decompensation of cardiac failure, hematologic and solid

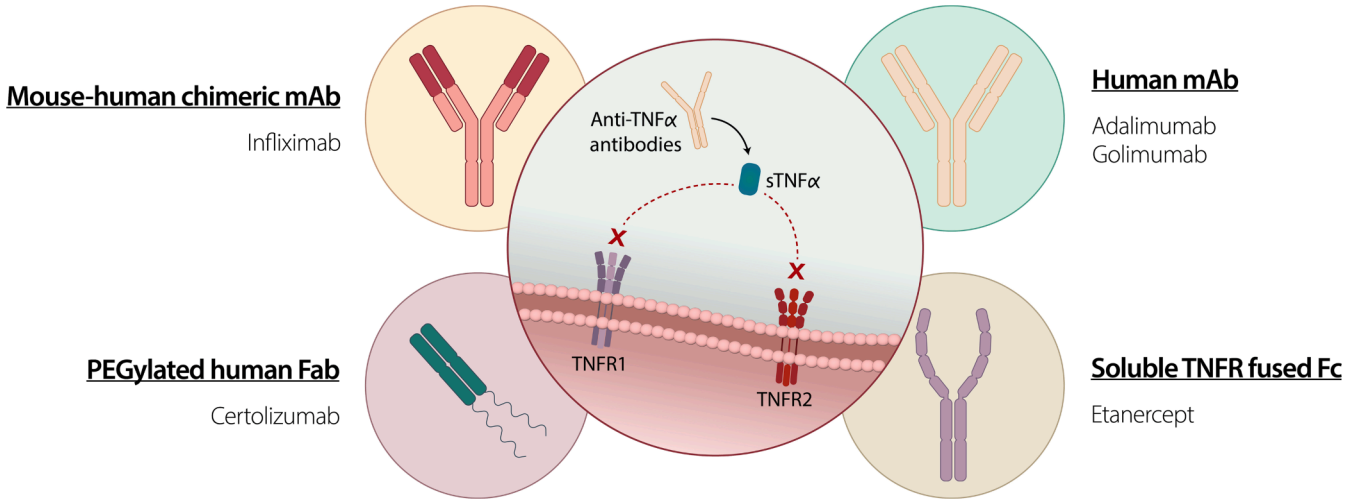

Fig. 2. The different categories of TNFα antibodies available for immune-mediated inflammatory diseases.

organ malignancies [75,76]. Both parenteral routes are also associated with pain, distress, and discomfort and are not considered completely patient-friendly [77]. Not secondarily, parenteral drug administration can require regular and direct contact with healthcare providers, and sometimes can even involve hospitalization. These circumstances have been particularly highlighted as limitations in the recent context of restrictions and social distance regulations related to the coronavirus disease-19 (COVID-19) pandemic [78-80]. The association of anti-TNF therapies with increased incidence of malignancies is a debated topic, as the chronic inflammation associated with the conditions treated with anti-TNF drugs is itself considered a hallmark of cancer [81] and the direct contribution of anti-TNF therapeutics is still unclear [82]. However, most treatment guidelines recommend limiting their use in patients diagnosed with cancer in the previous 5 to 10 years due to the possibility of cancer recurrence or second malignancy development [83]. The neurological system can also be affected by anti-TNF biologics, as demonstrated by disease exacerbation in MS patients [84]. Moreover, anti-TNF therapy has been associated with the development of psoriasis in patients with IBD, indicating that TNF-α blockade may paradoxically induce *de novo* some new autoimmune diseases [85]. Finally, it is important to consider that exposure to TNF-α inhibitors induces the appearance of TNF-independent inflammatory pathways that mediate resistance to anti-TNF-α therapy [86].

Considering all the limitations and problems associated with currently available formulations of anti-TNF-α biologics it is clear that new solutions need to be developed to improve therapeutic outcomes and safety of TNF-targeted treatment. As an example, several attempts have been made to design oral delivery systems for mAbs, with a more direct targeting and potent anti-inflammatory effect at the intestinal level, sparing some of the side effects associated with systemic exposure. Many of these strategies include the formulation of nanoparticles and have been recently reviewed by Eder et al [87]. Another line of research is investigating small molecules binding to TNFRs with potentially less severe side effects, which could be orally administered [88]. Another strategy includes the use of RNA therapeutics combined with site-targeted delivery, which could represent a winning choice for the future of autoimmune-mediated and autoinflammatory diseases treatments.

4. RNA-based therapeutics and their formulation strategies as an alternative to anti-TNF-α biologics

RNA-based therapeutics offer important advantages over small molecules and recombinant proteins and antibodies, such as the ability to interact and modulate targets that were considered inaccessible, the efficient development and large-scale production, the ability to modulate multiple targets, and the absence of the need for post-translational modification and folding ability [89]. Since the beginning of the 21st century, with the progress of the Human Genome Project and the genomics field overall, the medical potential of gene-based therapies has been gaining momentum, which has peaked with the recent development of the Sars-Cov-2 vaccines [90–92]. Gene-based therapies represent a novel approach to overcoming the limitations of biologics providing safe and long-lasting gene regulation [93]. DNAs, messenger RNAs (mRNAs), small interfering RNAs (siRNAs), micro RNAs (miRNAs), and anti-sense oligonucleotides (ASOs) are the genetic materials that enable specific elimination of autoreactive cells without extensive suppression of the entire immune system [94].

DNA drugs delivered as plasmids or embedded into viral vectors access the nucleus to integrate into the genome and be transcribed into RNA, carrying the risk of insertional mutagenesis. On the other hand, RNA targets are mainly cytoplasmatic, with rare exceptions [95]. RNA drugs are based on antisense RNAs (RNAi), where short oligonucleotides recognize and hybridize with complementary endogenous RNA sequences, and on mRNAs that elicit temporary peptides or protein expression [96]. However, regardless of their nature, RNAs can be rapidly degraded by ubiquitous RNase, present difficulties in crossing the cell membrane to reach the cytoplasm and can cause strong immunogenicity [97]. To make the advancement to the market more realistic, RNAs require chemical modifications involving alterations of the ribose group, phosphate backbone, RNA terminus, or modification of the nucleotides themselves [89]. While these modifications help with increase the stability and reduce the immunogenicity, cellular barriers remain tough hurdles to overcome. Progress can come from formulating approaches, such as conjugation to active targeting moieties that can help RNA accumulation and internalization into target tissues and cells, although they do not protect it against degradation. For instance, a siRNA conjugated to three N-acetylgalactosamine (GalNAc) molecules (givosiran, Givlaari™) able to bind the asialoglycoproteins receptor (ASGR) and promote the internalization into hepatocytes has recently been approved by the FDA for the treatment of acute hepatic porphyria, holding promise for the treatment of hepatic diseases [98].

Other formulation strategies include RNA complexation with synthetic or viral nanoparticles, both to enhance the protection of the genetic material and to facilitate cellular uptake [99]. Nanomedicine provides novel therapeutic approaches for many active compounds which cannot be administered or delivered as conventional formulations. Polymer- and lipid-based delivery systems, often with the addition of surface modifications, have been extensively studied in the recent decades to protect and deliver different RNAs, as outlined in Fig. 3 [95].

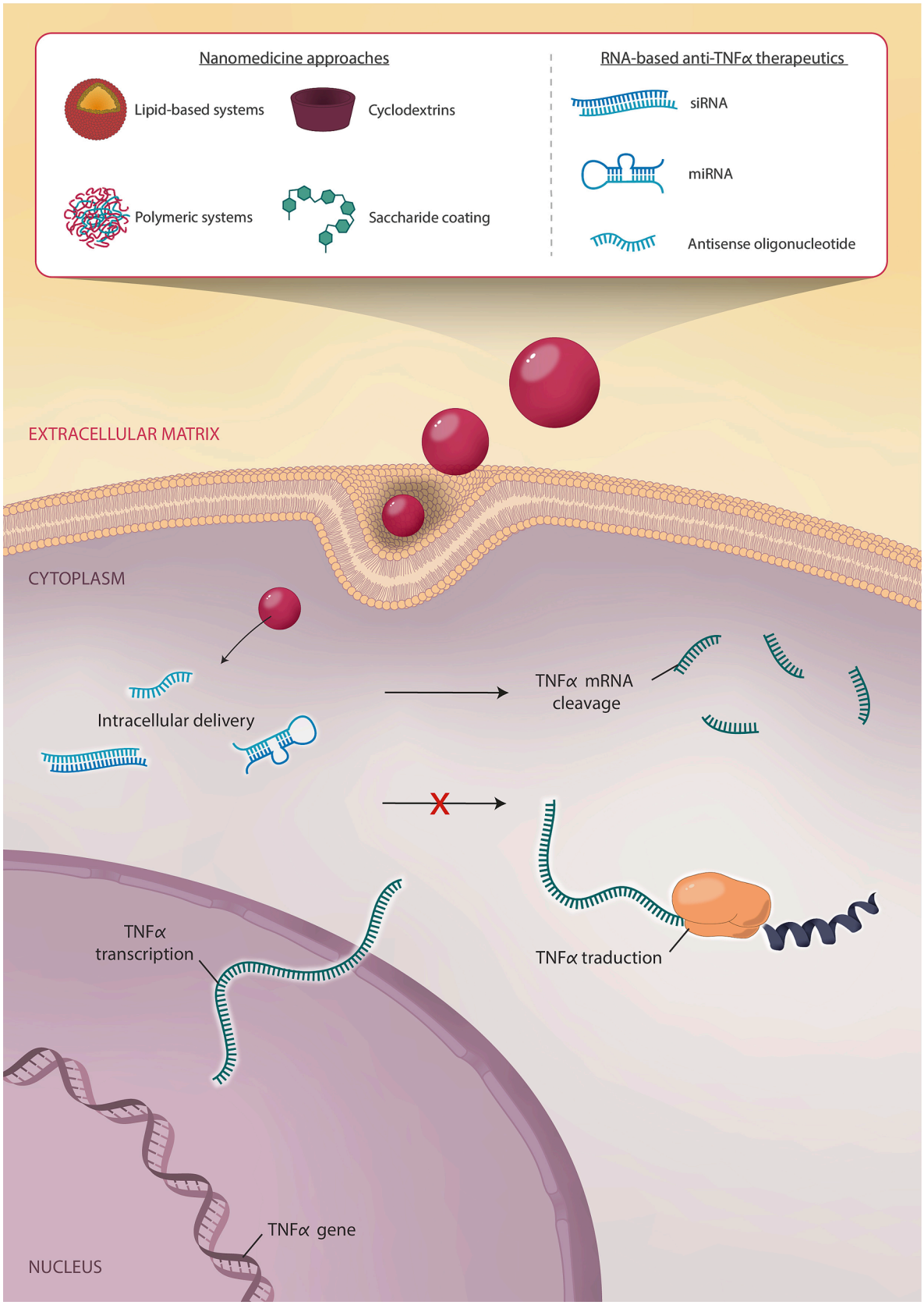

Fig. 3. RNA-based nanomedicine and their mechanisms of actions for inhibiting TNF-α in inflammatory bowel disease.

In both cases, the best strategy in terms of complexation and encapsulation efficiency, as well as RNA protection and enhanced *in vitro* internalization, has been identified in exploiting the presence of positively charged moieties in the nanoparticles (NPs), which can interact with the negatively charged phosphate groups of RNAs.

In the manufacture of optimized polymeric NPs, the incorporation of polyethyleneimine (PEI) has been one of the most studied approaches, as it can allow the formation of tailored systems with a well-defined number of positive charges [100]. The use of cationic dendrimers is another well-studied method for the complexation with RNAs since their size can be easily tuned by forming monodisperse structures [101]. Recently, cyclodextrins have also been modified by incorporating cationic charges through hydroxyl group substitution, while in other cases the host/guest interactions have been exploited to self-assemble cationic polymers [102]. Cationic biopolymers such as chitosan and its derivatives have also been used to complex genetic material to improve the stability and cell penetration abilities of RNAs [103]. Cationic liposomes are among the first approaches used for gene transfer, first described by Felgner et al. in 1987. The system design arises from different combinations of the nitrogen/phosphate ratio (N/P) to obtain liposomes with a tunable surface potential [104]. The main cationic polymers used for this purpose are 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) and its precursor N-(1-[2,3-dioleoyl]-propyl)-N,N,N-trimethylammonium chloride (DOTMA) [105,106]. Next, the researchers synthesized ionizable lipids exhibiting positive charges to interact with RNA molecules when protonated under acidic conditions, while they are neutral under physiologic conditions. To tailor the lipid system for optimized conjugation or encapsulation of different nucleic acids, several innovative lipids have been developed and are categorized into cationic lipid, helper lipid, ionizable lipid, and gene lipid-conjugates. Recent achievements with lipid nanoparticles enabling gene therapy, resulting in the approval of a treatment for a rare disease such as Onpattro® [107] and the development of mRNA-based vaccines during the Sars-Cov-2 pandemic [108,109] highlight the fast-growing interest in the RNA nanoparticles-based delivery approach as a possible improvement for the treatment of autoinflammatory and autoimmune diseases.

However, even if nanotechnology has been proven to be an effective delivery system for different diseases, the complex mechanisms behind inflammations in both autoinflammatory and autoimmune diseases are still under study and still undisclosed pathways can be implicated in the therapeutic response and deserve future investigation. To allow RNA-based nanomedicines to successfully enter the clinical practice, some points result of key importance: the necessity (i) to develop drug delivery systems with an improved shelf life by minimising the need for a cold chain, (ii) to improve the currently used nanosystems to obtain a targeted and precise delivery and effect by further developing the formulation design, (iii) to implement the current manufacturing technologies to generate modular, scalable GMP-level manufacturing units, and (iv) to reduce the side effects profiles that might result problematic in the case of repeated administration by optimizing the biodegradability and biocompatibility of the systems.

Given the broaden area covered by this topic, in the following paragraphs we focused on a specific application of RNA-based nanomedicine that is IBD case.

5. RNA-based nanomedicine targeting TNF-α

The development of RNA drugs focuses on two approaches: enabling the expression of the transferred gene to replace defective proteins or produce antigens for vaccination or inhibiting the expression of target genes [97]. The success of RNA-based nanomedicine in ameliorating disease processes and clinical symptoms in animal models has made it an attractive approach in the treatment of human autoimmune and autoinflammatory diseases. The ideal treatment for an autoimmune disease should specifically target autoreactive cells, without the need for systemic immune suppression.

To provide some insights into how RNA-based nanomedicine could revolutionize the treatment of IMID, IBDs has been chosen as an example, as it provides strong case history to discuss the vast potential of this kind of therapeutics and to analyze the limitation and hurdles that still need to be solved in terms of drug delivery.

5.1. Inflammatory bowel diseases as a case of study

IBD, including CD and UC, is a complicated, chronic, relapsing, and heterogeneous disease induced by environmental, genomic, microbial, and immunological factors [110]. IBD therapy aims to induce and maintain clinical and endoscopic remission. Current treatments include amino salicylate drugs and antibiotics for mild-to-moderate forms, corticosteroids, immunosuppressants, and biologics for moderate-to-severe disease [111]. Because many patients with IBD are either refractory or intolerant to treatment with the classic agents, more specific therapeutic approaches need to be developed. In this context, previously described anti-TNF-α agents are currently parenterally administered as therapy for human IBD [112]. TNF-α is produced by immune and non-immune cells in the inflamed gut of IBD patients, such as macrophages, T cells, dendritic cells, fibroblasts, and fat cells . TNF-α has pleiotropic effects in the intestinal wall, as induction of neo-angiogenesis, activation of macrophages for the production of proinflammatory cytokines, stimulation of Paneth cell death, T-cells, and apoptosis of intestinal epithelial cells [113].

Although the pieces of evidence produced so far belong mainly to animal models of colitis, there is proof of the potential application of TNF-α-neutralizing antisense oligonucleotides or interfering RNAs in the clinic (Fig. 3) [114]. Table 2 lists some examples of how nanomedicines could improve RNA-based therapeutics in the treatment of IBDs.

ASOs are single-stranded nucleotides typically 10–50 nucleotides long, which can bind to complementary pre-mRNA or mRNA and alter splicing or induce degradation by endogenous RNase H [131]. In 1998 the first therapeutically used ASO was approved for the treatment of Cytomegalovirus (CMV) retinitis. Since then, many more oligonucleotide-based substances have been tested and have already entered clinical practice. Many ASOs for the treatment of IBD have reached advanced clinical stages, such as alicaforsen, mongersen, GATA3 DNAzyme, and cobitolimod [132]. The results obtained witnessed the excellent safety profile of ASOs suggesting that this treatment could be a valid option in the maintenance phase of the disease. However, the efficacy studies failed probably due to the inadequate selection of patients' subsets or technical issues during scale-up processes. Nanomedicines represent a valid alternative to deliver directly ASOs in inflamed tissues overcoming problems related to rapid clearance and degradation of these molecules. Myers et al. developed an antisense oligonucleotide (ISIS 25302) for the treatment of CD, specifically for murine TNF-α which reduced TNF-α mRNA *in vitro* and *in vivo*, resulting in improved pathologic scores of colitic mice [133]. In this study, ISIS 25302 was administered systemically, with off-target, systemic anti-inflammatory effects due to the lack of an efficient targeting delivery system. Zuo et al. used the same ASO to actively target macrophages by complexing it with low-molecular-weight galactosylated chitosan, which was previously shown to be able to target the macrophage galactose-type lectin (MGL) transmembrane receptor, which is over-expressed by activated macrophages under inflammatory conditions [115]. Indeed, the *in vitro* transfection efficiency obtained on activated macrophages was higher than that achieved on resting macrophages. Similarly, intracolonic administration of the complexes in mice with colitis showed their preferential accumulation in the colon over other organs and did not induce accumulation in healthy animals, indicating a good targeting strategy. This system has shown promising results in reducing the pathological score, with a reduction of TNF-α expression and inflammatory response. Sakisaka et al. developed an innovative complex formulated by the interactions between an ASO for TNF-α built

Table 2
Summary of nucleic acids drug delivery methods for the treatment of IBDs.

Administration route	Complex with nucleic acid	System composition	Type of nucleic acid	Target cell	Target approach	REF.
ASO						
Colonic instillation	Polymeric encapsulation	Galactosylated chitosan	TNF-α ASO	Macrophages	Active (MGL receptor)	[115]
Rectal	Polymeric encapsulation	Schizophyllan (β-(1–3) glucan family)	TNF-α ASO	Macrophages and DCs	Active (Dectin-1 receptor)	[116]
siRNA						
Rectal	Cationic lipid	Lipofectamine 2000	TNF-α siRNA	–	–	[117]
Rectal	Cationic lipid	Lipofectamine 2000	TNF-α siRNA	Macrophages	–	[118]
Rectal	Cationic moiety	Amphiphilic cationic cyclodextrins	TNF-α siRNA	Macrophages	–	[119]
Rectal	Calcium phosphate crystals	PLGA	TNF-α siRNA	Macrophages	–	[120]
Oral	PEI	1,3-d-glucan shells	*Map4k4* siRNA	Macrophages	Active (Glucan receptor)	[121]
Oral	Polymeric encapsulation	Galactosylatedtrimethyl chitosanecysteine (GTC) and TPP	*Map4k4* siRNA	Macrophages	Active (MGL receptor)	[122]
Oral	Polymeric encapsulation	Galactosylatedtrimethyl chitosanecysteine (GTC) and TPP	TNF-α siRNA	Macrophages	Active (MGL receptor)	[123]
–	Cationic moiety	PEI-PEG-Mannose	TNF-α siRNA	Macrophages	Active (Mannose receptor)	[124]
Oral	DOTAP	PPADT	TNF-α siRNA	–	Passive (ROS-mediated degradation)	[125]
Oral	PEI	PLA and PVA coating	TNF-α siRNA	Macrophages	–	[126]
Oral	PEI	PLA-PEG-Fab and PVA coating	TNF-α siRNA	Macrophages	Active (Fab portion of F4/80 Ab directed against mouse macrophages)	[127]
Oral	Polymeric encapsulation	PLGA coated with galactosylated chitosan	TNF-α siRNA	Macrophages	Active (MGL receptor)	[128]
Oral	Polymeric encapsulation	PLGA coated with PVA/chitosan followed by conjugation with galactose	TNF-α siRNA	Macrophages	Active (MGL receptor)	[129]
miRNA						
Colonic instillation	Polymeric encapsulation	Galactosylated chitosan	miR-16 precursors targeting TNF-α	Macrophages	Active (MGL receptor)	[130]

MGL = macrophage galactose-type lectin; DCs = dendritic cells; PLGA = poly(lactic-co-glycolic acid); PEI = polyethylenimine; Map4k4 = macrophage mitogen-activated protein kinase kinase kinase kinase 4; TPP = tripolyphosphate; PEG = polyethylene glycol; DOTAP = 1,2-dioleoyl-3-trimethylammonium propane; PPADT = poly-(1,4-phenyleneacetone dimethylene thioketal); ROS = reactive oxygen species; PLA = polylactic acid; PVA = polyvinyl alcohol; Ab = antibody.

on poly(dA) and schizophyllan (SPG), a polysaccharide belonging to the β-(1–3) glucan family [116]. SPG can efficiently bind to dectin-1, a type II C-type lectin receptor involved in the production of TNF-α and other pro-inflammatory cytokines, which is exposed on the surface of macrophages and DCs and overexpressed in the inflamed mucosa of DSS-induced colitis mouse models. The authors found how during the process of triple helix formation via hydrophobic and hydrogen bonding interactions, polynucleotides built on poly(dA) can form a stoichiometric complex with two single chains of SPG. The system is efficiently taken up by dectin-1-positive macrophages and DCs at the site of inflammation. Rectal administration of SPG/ASO at the inflammation site has been shown to be effective locally, being absorbed by macrophages and inhibiting TNF-α production, thus ameliorating intestinal inflammation.

MicroRNAs (miRNAs) are small, non-coding RNA strands of endogenous origin, typically of 20–25 nucleotides, involved in several cellular and gene regulatory processes, modulating the expression of multiple mRNAs by blocking translation or promoting degradation of target mRNAs [134]. To date, there are no miRNA-based drugs on the market, but some promising candidates are currently in clinical trials, such as MRG-106 for the treatment of blood cancer [135]. Huang et al. reported the formulation of miR-16, which can bind to the AU-rich region at the 3′-untranslated region (3′UTR) of TNF-α thus inducing TNF-α mRNA degradation [130]. This miRNA can also target a similar binding site on IL-12p40, which activates mucosal inflammation in CD patients, allowing a dual mechanism of action. The system has been formulated to specifically target activated colonic macrophages, as miR-16 can regulate several genes and interfere with normal macrophagic physiological functions. For this purpose, they used a recurring strategy that we have already highlighted in this manuscript, namely, the complexation of miRNA with galactosylated low-molecular-weight chitosan that shows selectivity for activated macrophages. The complexes were administered via colonic instillation into TNBS-induced colitic mice where a 50% reduction in mRNAs and protein levels of both TNF-α and IL-12p40 were reported.

RNA interference mediated by siRNAs is a powerful tool for post-transcriptionally silencing of gene expression and has been recognized as an efficient approach for downregulating TNF-α in immune cells. siRNAs are typically 15–25 nucleotides long and can induce mRNA degradation by binding to endogenous RNA-induced silencing complexes. The success of RNAi depends largely on the administration route and the carrier and delivery method used [136]. In 2006, Zhang et al. hypothesized that controlling gene expression in the rectal mucosa could serve as a therapeutic target in IBD [117]. Indeed, although rectal administration can cover a restricted area of the lower intestine, it allows for bypassing many of the hurdles typical of intestinal delivery [110]. They employed Lipofectamine™ 2000, a commonly used and commercially available transfecting agent based on cationic lipids, to complex TNF-α siRNA through electrostatic interactions and form stable NPs. Comparing the administration of the nucleic acid alone and that of its lipid-based formulation, the authors proved the system's efficacy in decreasing TNF-α levels locally at the site of administration in mouse models of acute colitis. Indeed, numerous studies have shown an increase in TNF-α levels not only in the serum but also in the mucosa [137], highlighting the potential of this strategy. Following the same hypothesis, Ocampo et al. produced different modified 3′-End siRNA to further improve the stability and silencing efficiency of nucleic acid, prior to complexation with Lipofectamine™ 2000 [118]. In mouse

models of colitis, the system showed a reduction of the inflammation score at the macro- and microscopic levels, in agreement with the silencing ability demonstrated both *in vitro* and *in vivo*. Other systems consisting of cyclodextrins [119] and of inorganic/polymeric mixture [121] have also been explored for the mucosal treatment of IBD by encapsulating TNF-α siRNA showing encouraging results. Although topical and local administration can offer several advantages, treatable areas limited to the lower colon and rectum have prompted the development of other administration routes, focusing mainly on orally administered systems. Oral formulations offer advantages such as ease of administration, patient compliance, and the ability to have a localized delivery in the inflamed intestine while minimizing systemic side effects. Some particles encapsulating siRNA are exploiting the ability of NPs to passively accumulate at the site of inflammation through the enhanced permeation and retention effect (EPR effect), while others have been tailored to actively respond to local stimuli, such as the presence of ROS or specific enzymes [138]. In terms of delivering the NPs to appropriate target cells, receptor-mediated gene delivery is a promising approach to achieve target specificity and avoid nonspecific interactions [139]. One of the first siRNA-based oral formulations for the treatment of IBD has been developed by Aouadi et al. [121]. The siRNA was complexed with PEI and further encapsulated in 1,3-d-glucan shells of baker's obtained *by* solvent extraction. The target was the *macrophage mitogen-activated protein kinase kinase kinase kinase 4* (*Map4k4*) for TNF-α suppression. *Map4k4* has been demonstrated to be a key mediator upstream of TNF-α action. Macrophages in the intestinal Payer's patches can phagocytize the systems via the beta 1,3-d-glucan receptor pathway. The particles' ability to knockdown *Map4k4* mRNA, as well as that to reduce the amount of TNF-α, was assessed *in vitro* on activated macrophages, and *in vivo* in a murine model of lipopolysaccharide-induced death. Taking advantage of a similar approach, Zhang et al. targeted Map4k4 for TNF-α suppression as a siRNA-based strategy for the treatment of UC [122]. For macrophage targeting, the authors selected the MGL transmembrane receptor by exploiting galactosylated low-molecular-weight chitosan. Then, it was used to form NPs by electrostatically interacting with the siRNA, together with tripolyphosphate (TPP) to create a more compact structure that could withstand the harsh gastrointestinal environment. These complexes showed robust efficacy in suppressing *Map4k4* and TNF-α mRNA expression as well as TNF-α production in activated macrophages. They also showed efficacy in mice with DSS-induced UC. He et al. used the same strategy for the encapsulation of antiTNF-α siRNA, providing evidence for the creation of a siRNA platform for the treatment of autoimmune diseases [123]. Other systems prepared using different encapsulation methods that actively target MGL have been successfully produced and listed in Table 2. Xiao et al. suggested the mannose receptor expressed on the surface of macrophages as target for siRNA-loaded NPs, which can then be rapidly internalized and induce transfection for IBD therapy [124]. To this aim they formulated a bioreducible PEI derivative functionalized with PEG and mannose groups, with a tuned structure that allows high TNF-α siRNA condensation capacity. In addition, good biostability together with the proton-sponge effect promoting endosomal/lysosomal escape, and limited cytotoxicity are important features of this system. These NPs were efficiently engulfed *in vitro* by macrophages and favored incorporation of high levels of RNAi, resulting in decreased TNF-α expression, and conferring anti-inflammatory properties on Raw 264.7 activated macrophages. *Ex vivo* treatment of excised colon tissue from animals with induced colitis exhibited a marked reduction in TNF-a expression in macrophages consequently demonstrating the system's ability to tare target cells.

6. TNF-α blockade: Limits and future roles of mRNA-based therapeutics

As extensively commented, TNF-α inhibitors are remarkable in the treatment of several autoimmune and autoinflammatory conditions, such as IBDs, RA, psoriasis, and asthma. Due to the widespread use of TNF-α-blockers, there is now a better understanding of the adverse effects and long-term tolerability of these therapies [141]. Lin et al. described the onset of a demyelinating neurological disorder similar to multiple sclerosis (MS) often reported as a side effect upon beginning of anti-TNF therapy [140]. Other case reports have described the occurrence of other neurological adverse reactions, such as serious viral infections and central and peripheral demyelination disorders (optic neuropathy and Guillain-Barre syndrome) [142]. Although neurological adverse effects rarely occur with the use of TNF-α inhibitors, along with better investigation of risk factors, innovative alternatives, and effective therapeutic strategies need to be developed.

Since the development of COVID-19 vaccines, the scientific community has shown increasing interest in messenger RNA (mRNA), which only after 30 years of research receives its first approval. The two mRNA vaccines of Pfizer-BioNTech and Moderna have demonstrated that lipid nanoparticles (LNPs) can deliver mRNA into dendritic cells (DCs) to induce immunization against SARS-CoV-2. Apart from DCs, other immune cells are promising targets for mRNA therapy.

Future perspectives for the treatment of these pathologies are focused on the development of nanomedicines to target either a specific cell population or cell-derived soluble factors as well as in solving safety concerns of mRNA-based treatments. Krienke et al. are now repurposing the mRNA technology to reduce disease activity in mice with Experimental Autoimmune Encephalomyelitis (EAE), the most widely used animal model resembling MS [91]. BioNTech had previously developed liposomal formulations of mRNA vaccines (mRNA-LPX) optimized for the systemic delivery of mRNA-encoded antigens for the targeted delivery to DCs in lymphoid compartments by tailoring the lipid composition and controlling complexes formation [106]. They revised the properties of the mRNA to avoid strong T-helper1 responses by activation of toll-like receptors (TLRs), desired in the case of COVID-19 vaccination, by replacing the uracil with 1-methyl pseudouridine (m1Ψ), thus avoiding the binding to TLRs. m1Ψ mRNA-LPX did not induce inflammatory cytokines or activate immune cells and allowed for higher and prolonged antigen expression. The system was tested in the EAE mouse model, and the results demonstrated the ability of the formulation in ameliorating disease establishment and progression in a preventive and therapeutic setting, respectively. The treatment induced *de novo* $FOXP3^+$ Treg cells and enhanced the expression of exhaustion markers such as PD1 and CTLA4 on antigen-specific $CD4^+$ T cells [91]. In August 2021, Moderna initiated a phase I clinical trial for its autoimmune mRNA candidate, mRNA-6231, which encodes for a mutated form of mutein human interleukin-2 (IL-2) and is adapted to enhance the selectivity of regulatory T cells selectivity [143]. Multiple IL-2 molecules are in clinical development for a variety of autoimmune conditions, such as IBDs, psoriasis, SLE, graft versus host disease, and autoimmune hepatitis. IL-2, through selective expansion of Tregs, has a crucial role in immune homeostasis restoration. To systemically deliver the nucleic acid, Moderna uses LNPs technology, which already has proven its efficacy in the Spikevax vaccine developed by the company during the SARS-CoV-2 pandemic [108].

Van Hoecke et al. recently reviewed a different approach for using mRNA as a response to solve the issues related to antibody production, purification, and immunogenicity by providing the genetic information of the antibody itself [144]. Transient gene transfer aims at administering the nucleotide sequences encoding the antibody to patients in the form of mRNA, enabling an *in-situ* production, potentially for a long period of time. mRNA as a protein replacement therapy requires targeted expression and repeated, often systemic, administration, which implies a high safety threshold and makes this a challenge. Pardi et al. published in 2017 the first feasibility trial of using antibodies-encoding mRNA for passive vaccination [145]. m1ψ-containing mRNAs encoding both the light and heavy chains of a broadly neutralizing antibody against HIV-1 were formulated in LNPs and delivered systemically, not only resulting efficient in an HIV-1 prophylactic mouse model but also

outperforming the purified recombinant protein. So far, the very few pre-clinical studies on mRNA-encoding antibodies only cover the fields of oncology and infectious diseases [143–147], but it could be an interesting approach in the treatment of autoimmune and auto-inflammatory diseases.

7. Conclusions

The arsenal of possible therapies to treat autoimmune and auto-inflammatory diseases is rapidly growing as a result of improved understanding of molecular mechanisms on the one hand, and the rapid development of efficient formulation approaches on the other. Biologic agents, and in particular TNF-α inhibitors, have revolutionized treatment strategies in the field of autoimmune-based disorders holding the highest anti-inflammatory potential. Despite the significant benefits, there are still some substantial limitations to consider, such as the need for parenteral administration with systemic effects which are beneficial for those diseases, but at the same time can lead to immunogenicity and serious adverse events. Therefore, it is speculated that the introduction of RNA-based therapies may revolutionize the way autoimmunity is managed. The obstacles limiting the development of RNA-based drug delivery systems, now appears to be a constraint largely circumvented by the recent progresses of the various delivery platforms. As outlined in this review, the use of RNA-based nanotechnology to address autoimmunity is very promising. We are witnessing a revolution in the field of RNA-based therapies and several diseases that have no cure available today are expected to be greatly impacted soon.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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