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Review Article

# Cytokine Engineering: Revolutionizing Therapeutics and Immune Modulation

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## Abstract

Cytokines, intricate signaling molecules crucial for immune regulation, hold immense therapeutic promise for immune-related disorders. Despite their multifaceted roles spanning inflammation, immune cell differentiation, and tumorigenesis, clinical translation of cytokine-based therapies faces significant hurdles, including short half-lives and systemic side effects. Pleiotropism, intrinsic to cytokines, underscores both their therapeutic potential and the complexities in their clinical application. Cutting-edge cytokine engineering technologies have emerged, offering novel avenues for immunotherapeutics, particularly in advanced cancer treatment. However, challenges persist, prompting the development of tailored therapeutic strategies. Noteworthy advancements include recombinant IL-1 receptor antagonist Anakinra and engineered cytokine antagonists, addressing specificity and efficacy concerns. Precision medicine approaches herald a paradigm shift in cytokine-based therapy, facilitating targeted interventions tailored to individual patient profiles. This comprehensive review delineates existing challenges in cytokine-based therapies while elucidating innovative strategies poised to unlock their full clinical potential. By addressing limitations and leveraging the transformative power of cytokines, this review signals a new era of precision immunotherapy with profound implications for patient care and therapeutic efficacy.

**Keywords:** Adipokines; Chemokines; Cytokine Engineering; Cytokines; Cytokine Immunotherapy; Immuno Cytokines; Leptin; Modulation Strategies; Muteins; Neokines; Prodrug; Resistin

## Abbreviations

JAKs: Janus Kinases; STAT: Signal Transducers and Activators of Transcription; IL: Interleukin; TNF: Tumor Necrosis Factor; TGFβ: Transforming Growth Factor-β; GM-CSF: Granulocyte-Macrophage Colony-Stimulating Factor; IFNs: Interferons; TGFβ: Transforming Growth Factor-β; CCL2: CC-Chemokine Ligand 2; M-CSF: Macrophage Colony-Stimulating Factor

## Introduction

Cytokines, pivotal in immune system regulation, constitute a structurally diverse group of molecular families and individual proteins crucial for orchestrating cellular responses essential for host defence and homeostasis [1]. Originating predominantly from immune cells, cytokines facilitate communication during immune activation through direct cell-to-cell interaction or the release of biomolecules. Additionally, non-immune cells like skin epithelial and endothelial cells contribute to cytokine secretion, exemplified by IL-33 and IL-25 in the lungs, which play vital roles in allergic outcomes. Adipokines, such as leptin and resistin, secreted from adipose tissue, further underscore the multifaceted actions of cytokines [2].

Classified into distinct functional subclasses, cytokines delineate crucial roles in immune regulation, exhibiting remarkable diversity in their physiological functions. Pro-inflammatory cytokines like IL-1, IL-6, and TNF activate antimicrobial and immunostimulatory responses, while anti-inflammatory counterparts such as IL-10 and TGFβ resolve inflammation and promote wound healing. Chemokines like IL-8 and CCL2 direct immune cell migration, while interferons exhibit potent antiviral immunity. Additionally, colony-stimulating factors like GM-CSF, G-CSF, and M-CSF modulate immune responses and progenitor proliferation [3-5].

At the cellular level, cytokines act as intracellular messengers, mediating functions by binding to specific cell surface receptors and activating intracellular signaling cascades, thus regulating processes like proliferation, differentiation, migration, and apoptosis. They play pivotal roles in both innate and adaptive immune responses, particularly in inflammation modulation. Due to their diverse effects on immune responses and inflammation, cytokines emerge as promising targets for novel therapeutic approaches [6].

In the clinical context, cytokines exhibit dual roles, contributing to immune-mediated diseases like rheumatoid arthritis while also being crucial for beneficial responses to communicable agents. Engineered cytokines, such as IL-2, have been extensively studied for cancer therapy, highlighting their therapeutic potential [7]. However, challenges persist in cytokine-based therapeutics, including short circulation half-life and pleiotropic effects. Innovations in administration methods and targeted delivery systems aim to enhance efficacy and reduce toxicity [8].

Despite progress, challenges persist in developing cytokine-based therapeutics, necessitating further engineering efforts to overcome limitations. This review delves into current approaches and progress in cytokine research, addressing challenges and exploring the transformative potential of engineered cytokines in treating autoimmune diseases and other ailments [9].

### Families of cytokines

| Families | Examples |
|---|---|
| Interleukin-1 family | IL-1 alpha, IL-1 beta, IL-1Ra, IL-18, IL-33 |
| Hematopoietin (Class I cytokine) family | IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-12, IL-13, IL-15, IL-21, IL-23, GM-CSF, G-CSF, Growth hormone, Prolactin, and Hematopoietin |
| Interferon (Class II cytokine) family | IFN-alpha, IFN-beta, IFN-gamma, IL-19, IL-20, IL-22, and IL-24 |
| Chemokines | IL-8, CCL19, CCL21, CCL2, CCL3 |

**Table a**

(Source: 10).

### Functions of cytokines

Cytokines play a multifaceted role in modulating inflammation, orchestrating a complex interplay of inflammatory mediators and immune responses. They elicit pro-inflammatory effects by upregulating the expression of inflammatory genes and promoting immune responses. Conversely, cytokines also possess anti-inflammatory properties, exerting downregulatory effects on inflammatory genes and mitigating cytokine-induced lethality. Furthermore, cytokines regulate various physiological processes, including cellular emigration and activation, hematopoiesis, proliferation, and differentiation. They also play a crucial role in

wound healing, facilitating tissue repair mechanisms. Additionally, cytokines contribute to the regulation of cell replication and apoptosis, crucial for maintaining tissue homeostasis. Notably, cytokines exhibit anti-tumor activity, underscoring their diverse and intricate roles in immune regulation and disease pathology [11].

## Signal transduction of cytokines

The Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway is renowned as a fundamental paradigm in cellular signaling, esteemed for its efficiency and pivotal role in governing diverse cellular processes. Constituting a receptor, kinase, and transcription factor, this cascade underscores its indispensable contribution to cellular physiology and therapeutic potential [12]. Upon cytokine binding, the intracellular domains of receptors intricately interact with members of the Janus Kinase (JAK) family, instigating essential downstream signaling events driven by specific cytokines. Initially quiescent, JAKs undergo auto-mobilization via transphosphorylation upon cytokine-receptor engagement, resulting in the phosphorylation of specific tyrosine residues within the receptor's intracellular tails. These phosphorylated residues serve as precise docking sites for Signal Transducers and Activators of Transcription (STAT) family transcription factors, which, upon phosphorylation by JAKs, dissociate from the receptor complex and translocate into the nucleus (Figure 1) [13]. Within the nucleus, activated STATs orchestrate the transcription of cytokine-responsive genes crucial for processes such as proliferation and differentiation, essential for maintaining tissue homeostasis and regulating immune responses. To uphold fidelity in signaling, an intricate network of regulatory proteins modulates cytokine signaling along the pathway [14]. Notably, the suppressors of cytokine signaling (SOCS) family assume a pivotal role by furnishing negative feedback inhibition within the cascade. Despite its apparent simplicity, cytokine signaling adheres to a meticulously regulated principle, wherein each cytokine engages a specific receptor, activating designated JAKs and STATs, culminating in signaling termination by distinct SOCS proteins. While preserving its fundamental architecture, the pathway has evolved with an augmented abundance of each component over time [15].

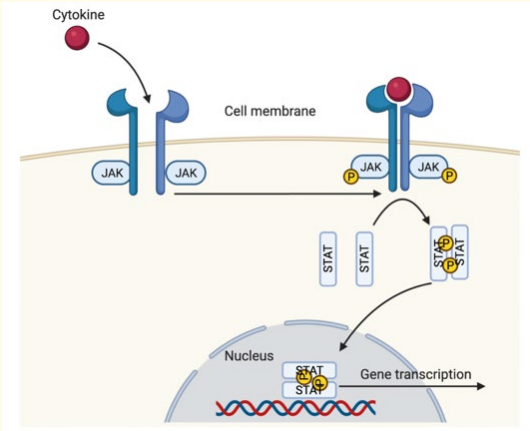


**Figure 1:** Cytokine Signal Transduction Pathways.

(Source: 13).

## Mechanisms of negative regulation in cytokine signaling

Negative regulation mechanisms play a pivotal role in modulating cytokine signaling pathways to prevent uncontrolled growth or autoimmunity. These regulatory mechanisms operate at various stages, including transcription, translation, post-translational modification, and protein stability. Tyrosine phosphatases, notably SHP1 and SHP2, are key players in cytokine signaling modulation. While SHP1 exerts suppressive effects, SHP2 stimulates signaling by dephosphorylating repressive tyrosine residues. Additionally, the CISH/SOCS family of SH2-domain-containing proteins serves as significant negative regulators, dampening JAK-STAT signaling [16]. Another group of negative regulators includes the protein inhibitors of activated STAT proteins (PIAS family proteins), with PIAS1 and PIAS3 negatively regulating the actions of STAT1 and STAT3, respectively, and PIASx and PIASy inhibiting STAT4 and STAT1, respectively. These PIAS proteins act as E3 ligases facilitating the sumoylation of target proteins [17].

Activation of the JAK-Signal Transducer and Activator of Transcription (JAK-STAT), MAPK, and PI3K-AKT signaling pathways are indispensable for orchestrating various cellular responses. The elucidation of interleukin-2 (IL-2) signaling offers a paradigmatic insight, showcasing the phosphorylation of specific tyrosine residues on IL-2Rβ, a mechanism widely applicable to diverse type I cytokines. This phosphorylation event leads to the formation

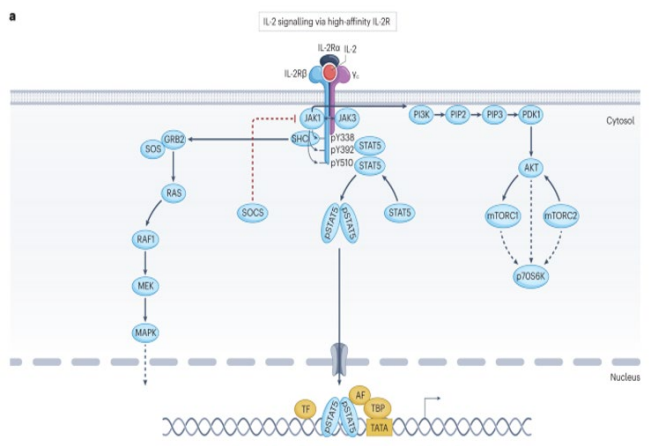


**Figure 2:** Negative regulation of cytokine signaling. (Source: 16).

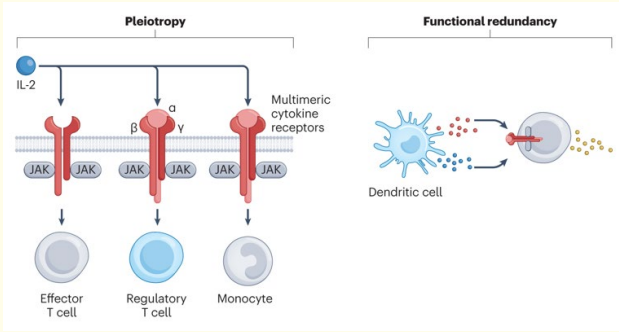


**Figure 3:** Cytokines regulating immune response. (Source: 1).

of phosphorylated STAT5 (pSTAT5) dimers, which translocate into the nucleus, thereby regulating the transcription of target genes. Adding to the complexity of cytokine signaling regulation, suppressors of cytokine signaling (SOCS) proteins emerge as crucial players, wielding their influence as SH2-domain-containing proteins adept at binding to and inhibiting Janus kinases (JAKs) (Figure 2) [16].

## The challenges of cytokines as therapeutic targets

Cytokines face several challenges as therapeutic targets. Their short blood half-lives, pleiotropism, redundancy, and unfavorable tissue distribution collectively contribute to a narrow therapeutic range. The cytokine network functions as a highly regulated and balanced system, where any alteration can lead to an impaired immune response. Furthermore, the production of cytokines requires sterile conditions and multiple stages of purification to ensure their safety and efficacy.

## Pleiotropic property

Cytokines exhibit the ability to activate many phenotypic traits, a phenomenon known as pleiotropy. In this context, a specific cytokine receptor can be located on different cell types, leading to a diverse array of biological outcomes. Many individual cytokines demonstrate pleiotropy, exerting multiple actions; *in vitro*, several cytokines have overlapping effects. Various mechanistic approaches can explain the pleiotropic and overlapping actions among different cytokines. Pleiotropic actions may arise from the presence of receptors for a cytokine on several cell lineages, or a cytokine may activate multiple signaling pathways, with each pathway contributing differentially to distinct functions. Overlapping actions between different cytokines could be attributed to similarities in the cellular distribution of key receptors for different cytokines, as well as the sharing of signaling pathways. This sharing often occurs when different receptors possess similar motifs that facilitate coupling to the same pathways (Figure 3) [18].

## Redundancy

Cytokines have the ability to exhibit partial functional redundancy, meaning that more than one cytokine can perform identical biological functions. For instance, both IL-2 and IL-15 can induce T cell proliferation, while IL-1α and TNF can activate endothelial cells. This redundancy enhances the robustness of the immune system. However, it also complicates therapeutic immunomodulation and the development of cytokine-based immunotherapeutics (Figure 3) [19].

## Strategies to modulate cytokine actions

### Prolonging cytokine half-life through pegylation

The kinetics of cytokine circulation profoundly influence the viability of cytokine-based therapeutics *in vivo*. PEGylation, a prominent strategy, involves conjugating proteins with polyethylene glycol (PEG) to extend their half-life in circulation [20]. This modification enhances protein molecular weight, fostering a hydrophilic environment that reduces renal clearance and minimizes interactions with plasma constituents, thereby

mitigating immunogenicity. While successful with colony-stimulating factors and interferons, PEGylation has limitations in addressing IL-2 toxicity, despite enabling intermittent dosing regimens by altering pharmacokinetics.

Beyond prolonging half-life, PEGylation offers versatile applications. Consider interleukin-10 (IL-10), known for its anti-inflammatory prowess, now under clinical investigation for inflammatory disease therapy. At high concentrations, IL-10 can activate cytotoxic T cells, altering its cancer immunotherapy potential. To enhance local IL-10 concentrations while curbing side effects, re-engineering was imperative. This led to the development of an IL-10 PEG-conjugate with improved pharmacokinetics, currently undergoing trials for various cancer conditions. This innovation represents a shift from traditional PEGylation, enhancing receptor binding alongside extending half-life. Additionally, an engineered IL-15-PEG variant (NKTR-255) aims to alleviate toxicity associated with conventional IL-15 therapies for malignancies [21].

However, a potential challenge arises with the emergence of anti-PEG antibodies in patients, which could neutralize cytokines or induce inflammatory responses. Although not previously observed in PEGylated cytokine studies, recent events, like reactions to mRNA COVID-19 vaccines containing PEG, highlight concerns. Hence, monitoring anti-PEG antibody evolution is crucial amidst the growing use of PEGylated therapeutics [22].

## Targeting cytokines with fusion proteins

Fusing a cytokine to distinct proteins offers a grand design to revamp the cytokine's biodistribution profile and enhance its therapeutic window. This method enables the targeting of cytokines to specific sites or cell types. Cytokine fusion designs facilitate tumor localization and address the lower pharmacokinetic applications and unfavorable biodistribution profiles associated with cytokine-based drugs.

## Immunocytokines

Immunocytokines, a fusion of cytokines and antibodies, represent a promising class of therapeutics designed to harness the specificity of monoclonal antibodies and the potent

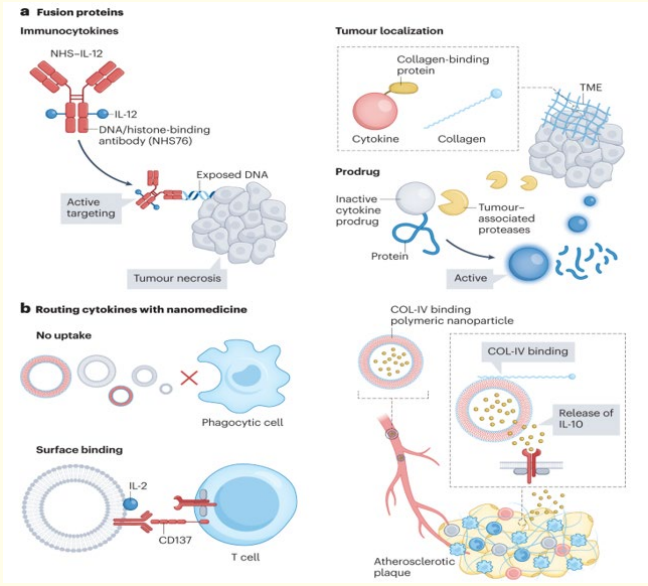


**Figure 4:** (a) Cytokine fusion proteins and their roles in redirecting cytokines to a key site (b) Overwhelm cytokines with nanomedicine.

(Source: 1).

immunomodulatory properties of cytokines for targeted cancer therapy [23]. By conjugating cytokines to monoclonal antibodies specific to tumor antigens, immunocytokines can precisely deliver cytokines to tumor sites, minimizing off-target effects and enhancing therapeutic efficacy [23]. One such example is the fusion of interleukin-2 (IL-2) with antibodies targeting tumor-associated antigens, such as CD25 or HER2, to selectively stimulate immune responses against cancer cells [24]. Additionally, immunocytokines can be engineered to include mutations that reduce cytokine toxicity while preserving their immunostimulatory functions, thus improving their therapeutic index [25]. Despite their potential, challenges remain in optimizing the pharmacokinetics, biodistribution, and immunogenicity of immunocytokines for clinical applications. Nevertheless, ongoing research efforts continue to explore novel strategies to enhance the efficacy and safety of immunocytokine-based cancer therapies, offering new hope for precision oncology [26].

The fate of cytokine-based therapeutics hinges significantly on the dynamics of cytokine circulation within the body. PEGylation,

a cornerstone approach, involves the conjugation of proteins with polyethylene glycol (PEG) to extend their half-life in circulation [20]. This sophisticated modification enhances the molecular weight of proteins, fostering a hydrophilic environment that reduces renal clearance and minimizes interactions with plasma constituents, thus mitigating immunogenicity. While PEGylation has demonstrated success with colony-stimulating factors and interferons, it encounters challenges in addressing IL-2 toxicity, despite enabling intermittent dosing regimens through alterations in pharmacokinetics.

The utility of PEGylation transcends mere half-life extension, offering versatile applications in therapeutic optimization. Take interleukin-10 (IL-10), revered for its anti-inflammatory prowess, currently under clinical investigation for inflammatory disease therapy. However, at elevated concentrations, IL-10 can paradoxically activate cytotoxic T cells, necessitating a delicate balance for effective cancer immunotherapy. To harness the therapeutic potential of IL-10 while minimizing adverse effects, a strategic re-engineering approach was imperative. This led to the development of an IL-10 PEG-conjugate boasting improved pharmacokinetics, currently undergoing trials for various cancer conditions. This transformative innovation heralds a departure from conventional PEGylation strategies by enhancing receptor binding alongside extending half-life. Moreover, the advent of an engineered IL-15-PEG variant (NKTR-255) holds promise in mitigating the toxicity associated with conventional IL-15 therapies for malignancies [21].

Nevertheless, the emergence of anti-PEG antibodies in patients poses a formidable challenge, potentially neutralizing cytokines or inciting inflammatory responses. While such occurrences have not been prevalent in PEGylated cytokine studies, recent incidents, such as reactions to mRNA COVID-19 vaccines containing PEG, underscore the importance of vigilant monitoring. As the utilization of PEGylated therapeutics continues to surge, it becomes imperative to closely track the evolution of anti-PEG antibodies to ensure the safety and efficacy of these innovative therapeutic modalities [22].

## Navigating Tumor Terrain: Approaches to localization

Utilizing collagen as a targeting moiety for cytokine delivery represents a promising approach to enhance tumor localization within the complex tumor microenvironment, addressing limitations associated with traditional protein antigen targeting strategies. Fusion constructs, such as those incorporating IL-2 or IL-12 with collagen-binding domains, have been engineered to anchor cytokines to collagen fibers within the tumor microenvironment, facilitating localized and targeted delivery for improved therapeutic efficacy [27]. This strategy not only enhances cytokine retention at the tumor site but also reduces systemic toxicity, while concurrently augmenting cellular antitumor immunity.

Furthermore, fusion of IL-12 with a phosphorylated aluminum hydroxide (alum) binding peptide tag demonstrates prolonged retention within the tumor over weeks, illustrating the potential for sustained therapeutic impact [28]. Similarly, fusing IL-12 to the A3 collagen-binding domain of Von Willebrand factor offers a promising avenue for specifically targeting cytokine delivery to the tumor microenvironment, potentially enhancing therapeutic outcomes in cancer treatment. The versatility of this approach is evident in its potential application to IL-2, highlighting its capacity to improve cytokine accumulation within tumors [29].

## Prodrug strategies

Prodrug constructs offer a compelling strategy to mitigate off-target effects by rendering the cytokine inactive through conjugation with a peptide or protein. Subsequent cleavage of the inactivating unit by disease-site overexpressed proteases facilitates cytokine reactivation, enhancing therapeutic precision and efficacy, particularly in cancer treatment, where several tumor-specific proteases have been identified. The integration of re-engineered cytokines into cytokine prodrug designs is a prevalent strategy, exemplified by ProIL2. Serving as an inert precursor to the IL-2 superkine, ProIL2 undergoes activation upon cleavage by tumor-associated proteases, underscoring the potential of tailored cytokine delivery systems for targeted cancer therapies [30].

Another prodrug, employing a mechanism akin to IL-2Rα targeting but with a distinct target, is currently undergoing clinical trials for the treatment of advanced solid tumors, demonstrating the versatility and potential applicability of prodrug approaches in cancer therapy. Furthermore, researchers are focused on designing continuous slow-release prodrugs to enhance IL-2Rβγ bias. This strategy aims to mitigate sudden spikes in IL-2 concentrations while prolonging the cytokine's blood half-life, potentially improving therapeutic outcomes in cancer treatment [31].

## Cytokine muteins

The generation of cytokine muteins stands as a longstanding strategy aimed at producing molecules with modified activity, be it enhanced, reduced, or qualitatively altered [32]. By employing mutagenesis techniques, it becomes possible to adjust the affinity of a cytokine, tailoring its interaction with specific receptor chains [33]. This selective targeting of receptor interactions holds the potential to induce differential effects, particularly in cases where cytokines engage with multiple receptor chains [34].

## Mutations Affecting the IL-2Rα or IL-2Rβ interface

Over 30 years ago, the development of IL-2 muteins marked a significant advancement in cytokine engineering. Early investigations focused on identifying specific residues of IL-2 crucial for interaction with its receptor components, particularly IL-2Rβ and IL-2Rα, including residues K35, R38, F42, and K43 [35]. Subsequent studies using mutant mouse IL-2 proteins further elucidated the regions and residues mediating interactions with individual receptor components [36]. Notably, muteins lacking interaction sites with IL-2Rα, termed 'no-α' mutants, retained biological activity by signaling through IL-2Rβ and the common gamma chain (γc) [37]. One notable mutein, featuring the N88R substitution within Proleukin, exhibited enhanced selectivity for T cells over natural killer (NK) cells in vitro and demonstrated potent anti-tumor activity [38].

Furthermore, the engineering of human IL-2 muteins with amino acid alterations at key residues, such as R38, F42, Y45, and E62, at the IL-2Rα interface, resulted in muteins capable of stimulating the proliferation of NK cells and CD8+ T cells while attenuating the proliferation of regulatory T (Treg) cells [39]. Conversely, efforts to amplify IL-2 binding to IL-2Rβ led to the creation of "super-IL-2" molecules, exploiting a unique property of IL-2 in interacting first with IL-2Rα, followed by a conformational change enabling efficient interaction with IL-2Rβ, and subsequent recruitment of γc to form the high-affinity receptor [40]. Notably, one such super-IL-2 variant, referred to as H9, selectively expanded effector T (Teff) cells without preferentially targeting Treg cells [41]. This strategy was extended to IL-4, yielding super IL-4 variants with enhanced binding either to γc or IL-13Rα1, thereby selectively impacting type I or type II IL-4 receptor signaling, respectively [42]. Additionally, the H9 super-IL-2 molecule served as a template for generating muteins with modifications at the interaction site with γc, offering further versatility in cytokine engineering (Figure 5a and b) [43].

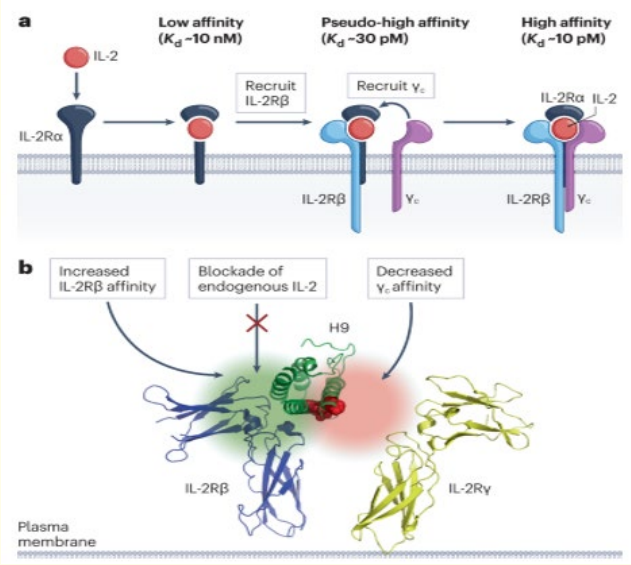


**Figure 5:** (a) Assembly of the high-affinity IL-2 receptor. IL-2 initially binds to IL-2Rα (low-affinity receptor), resulting in a conformational change in IL-2, allowing its efficient binding to IL-2Rβ to form the pseudo-high-affinity receptor. Common γ-chain (γc) is then recruited to the IL-2–IL-2Rβ interface, forming the high-affinity receptor. (b) Super-IL-2 (H9) was selected by phage display and contains five amino acid changes that enhance its binding to IL-2Rβ.

(Source: 44).

## Neokines

A novel technique known as neokines is employed to modify molecules for medical applications. Instead of precisely mimicking natural cytokines, these molecules are engineered to possess a more condensed structure. One exemplary instance is NL-201, which differs in shape from natural cytokines like IL-2 or IL-15. NL-201 has exhibited promising outcomes in stimulating cell growth and has proven effective in combating colon cancer and melanoma in murine models, with minimal side effects. Unlike natural cytokines, NL-201 does not engage with a specific protein known as IL-2Rα. This strategy can be extended to develop analogous

molecules for other cytokines, and researchers can fine-tune these molecules to enhance their efficacy or tailor them to target specific cells or pathways. Neokines exhibit resistance against heat and stability. However, due to their considerable deviation from natural proteins, there is a concern that they might elicit immune reactions that could be harmful for patients during therapeutic applications [45].

### Surrogate cytokine agonists

Researchers have developed a novel strategy to produce surrogate cytokine agonists, molecules that mimic the function of natural cytokines, by combining various antibody fragments [46]. These compounds exhibit selective binding to specific cell receptors, such as IL-2Rβ and γc, offering promising opportunities for targeted therapeutic interventions in immune-related disorders. Upon binding to receptors, these molecules initiate cell signaling pathways, replicating the actions of endogenous cytokines like IL-2 or IL-15 [47]. One approach involves utilizing a distinct class of antibodies obtained from immunized rats to construct these surrogate cytokine agonists. By fusing different antibody segments, researchers generated molecules with varying levels of activity, primarily affecting T cells and natural killer (NK) cells.Similarly, another group employed a comparable strategy but with a broader range of antibody fragments, establishing a platform for producing molecules targeting not only IL-2 but also other receptors involved in immune responses. This versatile approach enabled the creation of a diverse array of molecules with distinct functional effects.

A notable observation arose when a molecule initially designed to inhibit T cell proliferation was repurposed to stimulate proliferation when combined with modified receptor molecules. This discovery highlights the potential for innovative applications of these molecules in modulating immune responses. In essence, this approach offers a method to design molecules with precise immune-modulating effects by targeting receptor interactions [48].

### Nanomediated cytokine routing

Exploring nanomedicine strategies to precisely modulate the pharmacokinetic and pharmacodynamic profiles of cytokines represents a promising avenue for improving therapeutic outcomes in immune-related disorders. Nanocarriers offer a versatile platform for targeted cytokine delivery, with crucial considerations for evading immune cell uptake while promoting efficient surface interactions (Figure 4 a and 4 b).

One primary strategy involves presenting cytokines on the surface of nanoparticles to directly engage with their specific receptors, exemplified by surface-functionalized PEGylated liposomes. These liposomes, equipped with interleukin-2 (IL-2) and anti-CD137 antibodies, enable targeted delivery to effector T cells, enhancing the efficacy of immunotherapy strategies. Upon intravenous administration, they preferentially accumulate in tumors, leading to CD8+ T cell expansion and eliciting an anti-tumor response in murine models [49].

In contrast, a non-cellular targeting strategy harnesses nanomedicines to interact with extracellular matrix components like collagen, facilitating localized cytokine delivery. Type-IV collagen binding polymeric nanoparticles serve as carriers for anti-inflammatory interleukin-10 (IL-10) directed to atherosclerotic plaques. These particles, undergoing controlled polymer degradation, gradually release IL-10, which interacts with inflammatory cells, reducing plaque formation and promoting stabilization in atherosclerosis conditions. Administration of this regimen effectively mitigates plaque formation over a four-week period [50]. Overall, these nanomedicine approaches offer tailored solutions for enhancing the therapeutic efficacy of cytokines in immune-related disorders, demonstrating the potential of precision medicine in optimizing treatment outcomes.

### Strategies to inhibit cytokine actions

Cytokine inhibitors, also referred to as anti-cytokines, encompass a diverse range of drugs designed to diminish cytokine synthesis, reduce their concentration in their active free form, impede their interaction with their respective receptors, and disrupt cytokine receptor signaling. Their application has swiftly gained traction in clinical settings across many disease states, such as myeloproliferative neoplasms, rheumatoid arthritis, and other immune-mediated arthropathies, as well as numerous inflammatory dermatological disorders and inflammatory bowel disease [51].

### Inhibitors of cytokine synthesis

All drugs that decrease the number of producing cells inherently also inhibit cytokine synthesis. Cytokine synthesis can be inhibited

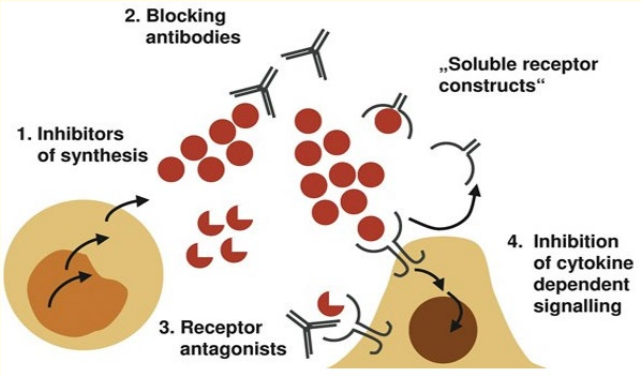


**Figure 6:** Strategies to inhibit cytokine actions. (Source: 51).

without compromising the viability of cells. Glucocorticoids exemplify this mode of action; their anti-inflammatory or immunosuppressive effects were clinically observed long before it was recognized that inhibiting the synthesis of cytokines such as interleukin-1, tumor necrosis factor, or interleukin-2 constitutes their primary mechanism of action. Glucocorticoids inhibit the synthesis of numerous cytokines involved in immune responses and inflammation. Moreover, some modern immunosuppressive drugs primarily target the T lymphocyte growth factor interleukin-2, thereby preventing the expansion of these cells, which is essential for an effective cellular immune reaction. In certain instances, cytokines exhibit antagonistic behavior towards the effects of others. For instance, interleukin-4 and interleukin-10 exert potent anti-inflammatory actions (Figure 6).

## Blockers of cytokines in free active form

A highly specific approach to blocking cytokines involves generating monoclonal antibodies that bind to the cytokine, thereby reducing its companionship with their respective receptors. While this approach has been explored for numerous cytokines, only one has thus far demonstrated sufficient clinical efficacy: infliximab, a humanized monoclonal antibody aiming tumor necrosis factor. Cytokines produce their biological actions by binding to membrane receptors on target cells. As a physiological regulatory mechanism, the extracellular cytokine-binding portions can be released, thereby attenuating the biological actions of the respective cytokines. This principle, known as “soluble receptors,” has been effectively harnessed. To enhance effectiveness and prolong in vivo half-life, soluble receptors moiety has been jointed to non-antigen-binding portions of the IgG molecule. One such construct is etanercept, which contains the soluble receptor for tumor necrosis factor. Both infliximab and etanercept exhibit potent anti-inflammatory efficacy towards the lots of immune-mediated as well as other inflammatory diseases.

## Cytokine receptor blockers

Similar to cytokine inhibitors, monoclonal antibodies could efficiently target cytokine receptors, thereby impeding cytokine function. Basiliximab and daclizumab, for instance, are humanized monoclonal antibodies intend against the interleukin-2 receptor. By obstructing this receptor for the pivotal T lymphocyte growth factor, they suppress cellular immune reactions. Interleukin-1 as well as tumor necrosis factor, is a highly potent pro-inflammatory cytokine. In nature, an antagonist has been naturally created, which, when secreted, dampens the action of interleukin-1. With the potential of modern molecular biological techniques, cytokines can be modified to alter their biological properties. For example, a mutated protein (mutein) of interleukin-4 has been developed, acting as an IL-4 receptor antagonist. This mutein is presently undergoing clinical trials to evaluate its effectiveness in treating allergic diseases, predominantly asthma as well as other allergic ailments. Similarly, chemokines are mutated to chemokine receptor antagonists are being assessed in HIV infections [52].

## Inhibiting JAKs and STATs

Inhibiting Janus Kinases (JAKs) and Signal Transducers and Activators of Transcription (STATs) represents a fundamental approach in modulating cytokine activity. JAKs play a crucial role in transmitting extracellular signals to the nucleus by phosphorylating and activating STAT proteins, which subsequently regulate gene expression. Targeting JAKs with selective inhibitors prevents the activation of downstream signaling pathways, thereby attenuating the effects of cytokines. Similarly, inhibiting STAT proteins can disrupt the transcriptional activity induced by cytokine stimulation, offering a means to modulate cellular responses. This approach holds promise for therapeutic intervention in various diseases characterized by dysregulated cytokine signaling pathways, including autoimmune disorders and certain cancers.

### JAK inhibitors

Inhibiting Janus kinases (JAKs) has emerged as a widely utilized therapeutic approach. JAK inhibitors are typically small-molecule inhibitors. In this context, Tofacitinib was developed as a selective JAK inhibitor and has achieved approval for treating rheumatoid arthritis and other diseases. Over time, tofacitinib has expanded its applications across various conditions. JAK inhibitors have been evolved for a spectrum of therapies, including ankylosingspondyloarthritis, psoriatic arthritis, juvenile arthritis, plaque psoriasis, ulcerative colitis, atopic dermatitis, alopecia areata, myeloproliferative neoplasms, vitiligo, and coronavirus disease 2019 (COVID-19). While there has been a notable increase in the number of investigated and approved molecules, adverse events have been observed, leading to black box warnings for tofacitinib, baricitinib, and upadacitinib [53].

### STAT inhibitors

STAT proteins play a pivotal role in mediating the actions of cytokines and selective inhibition of a specific STAT can alter the balance towards another phenotype. Gain-of-function mutations of STAT3 and STAT5B have been identified in patients with both hematopoietic and solid malignancies. A variety of STAT3 inhibitors have been developed, ranging from small-molecule inhibitors to cyclic STAT3 oligonucleotides, with STAT5 inhibitors also undergoing evaluation [54]. In this, one of the most well-known molecules is STATTIC (STAT three inhibitory compound), and additional inhibitors targeting STAT3 have also been identified [55].

### Selective protein degradation strategies

Extracellular proteins, including cytokines, growth factors, and chemokines, are pivotal in driving pathological signaling pathways implicated in diseases such as inflammation and cancer [56]. However, the development of small molecule inhibitors targeting these extracellular proteins is challenging due to the complexity of their interactions and the need for precise specificity and efficacy [57].

A novel strategy to address this challenge involves ENDosomeTArgeting Chimeras (ENDTACs), heterodimeric molecules designed to selectively target extracellular proteins for lysosomal degradation [58]. ENDTACs comprise two essential components: a small molecule agonist that binds to a plasma membrane receptor, typically a G protein-coupled receptor (GPCR), and a moiety attached via a linker that recruits the extracellular protein of interest (POI), such as a cytokine, for degradation [56].

Upon binding of the ENDTAC to the GPCR, the complex undergoes internalization through receptor-mediated endocytosis, leading to the internalization of the attached extracellular protein [57]. Subsequently, the protein is routed to the lysosome, where it is degraded [58].

This innovative approach demonstrates considerable potential for selectively targeting extracellular proteins involved in disease pathogenesis, offering a promising avenue for therapeutic development [56]. By exploiting the intricate interplay between small molecule agonists, plasma membrane receptors, and extracellular proteins, ENDTACs represent a significant advancement in the quest for effective therapies targeting pathological signaling pathways (Figure 7A, B and C).

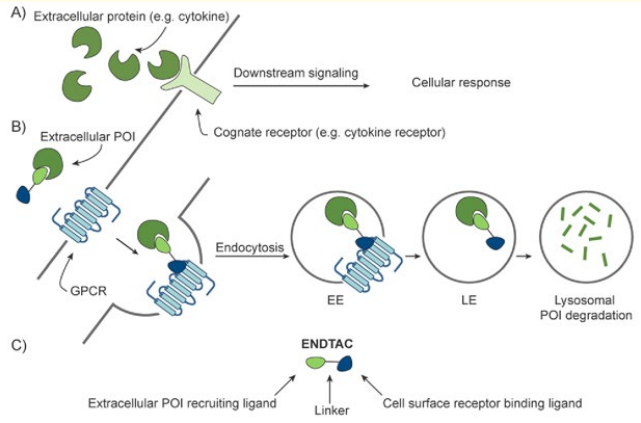


**Figure 7:** ENDTAC technology.

(Source: 59).

## Conclusions

Cytokines play a crucial role in orchestrating cellular differentiation, growth, and immune regulation, making them indispensable mediators of both physiological equilibrium and pathological responses. As our understanding of cytokine biology deepens, there is a growing effort to harness their therapeutic potential. Cytokine and cytokine receptor engineering has emerged as a promising approach, offering the ability to judiciously

modulate cytokine activity in therapeutic contexts. Notably, the meticulous refinement of cytokines like IL-2 has led to the development of variants that can differentially influence regulatory T cells for autoimmune conditions and effector T cells for cancer immunotherapy. This engineering extends to other cytokines, such as IL-10, interferons, and IL-1 family cytokines, leveraging their diverse immunosuppressive, antiviral, and anticancer attributes. Various strategies have been employed to shape cytokine actions, including nuanced alterations in receptor affinities, extension of cytokine half-lives in vivo, and precise fine-tuning of their functional profiles. While progress has been made, the journey towards optimizing therapeutic outcomes across a wide range of diseases is ongoing. The dynamic landscape of cytokine engineering necessitates continuous exploration and innovation, holding the promise of revolutionizing the therapeutic paradigm for numerous ailments.

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## Conflict of Interest

None.

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