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The role of γ_c cytokines in the immune system and cancer immunotherapy

Funkce γ_c cytokinů v imunitním systému a nádorové imunoterapii

Bachelor thesis

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Abstract

Cytokines are proteins produced mostly by cells of hematopoietic origin and transduce signals via engaging cell surface receptors on either the cytokine-producing cells (autocrine signaling) or other target cells (paracrine signaling). Common cytokine receptor subunit (γ_c) cytokines are small glycoproteins belonging to type I cytokines with pleiotropic activities in both the innate and adaptive immune systems. All γ_c cytokines share a γ_c receptor subunit in their complete receptors. The first part of this thesis aims to summarize information about the biology of γ_c cytokines, their receptors, and their role in the immune system and its functions. The second part discusses the use of γ_c cytokines in cancer immunotherapy, presenting examples of particular γ_c cytokine therapies, and describes the approaches to improve the pharmacological features of γ_c cytokines or efficiently combine them with other immunotherapies and anticancer treatments.

Keywords: γ_c cytokine, cytokine receptor, T cell, NK cell, cancer immunotherapy

Abstrakt

Cytokiny jsou proteiny produkované především buňkami hematopoetického původu, které poskytují signály zprostředkované specifickými receptory. Tyto receptory jsou exprimovány buďto na buňkách produkujících cytokiny (autokrinní signalizace), nebo jsou na povrchu jiných cílových buněk (parakrinní signalizace). Cytokiny sdílející obecnou cytokinovou receptorovou podjednotku (γ_c) jsou malé glykoproteiny patřící do skupiny cytokinů typu I, mají různorodé účinky jak na vrozený, tak i na adaptivní imunitní systém. Všechny γ_c cytokiny sdílejí ve svých kompletních receptorech podjednotku γ_c. První část této práce se zaměřuje na shrnutí základních poznatků o biologii těchto cytokinů, jejich receptorech a roli v imunitním systému. Druhá část práce se zabývá využitím γ_c cytokinů v nádorové imunoterapii, předkládá příklady konkrétních terapií, popisuje nové možnosti a přístupy ve zlepšení farmakologických vlastností γ_c cytokinů i případné možnosti kombinace γ_c cytokinové imunoterapie s dalšími imunoterapiemi či protinádorovou léčbou.

Klíčová slova: γ_c cytokin, cytokinový receptor, T lymfocyt, NK buňka, nádorová imunoterapie

List of abbreviations

AICD	activation-induced cell death
AP-1	activator protein-1
Bcl-2	B cell lymphoma-2
cAMP	cyclic adenosine monophosphate
CAR	chimeric antigen receptor
CD	cluster of differentiation
CHR	cytokine-binding homology region
CREM	cAMP-responsive element modulator
CTLA-4	cytotoxic T lymphocyte-associated protein-4
DC	dendritic cell
FLIP	FLICE-like inhibitor protein
Foxp3	forkhead box protein 3
γ_c	common cytokine receptor gamma chain
HD	high-dose
Ig	immunoglobulin
IL	interleukin
ILC	innate lymphoid cells
IU	international unit
i.v.	intravenous
JAK	Janus kinase
IL-2/JES6	complex of IL-2 and JES6-1A12 monoclonal antibody
IL-2/S4B6	complex of IL-2 and S4B6 monoclonal antibody
LAK	lymphokine-activated killer
mAb	monoclonal antibody
MAPK	mitogen-activated protein kinase
MHC	major histocompatibility complex
MS	microsphere
NF-AT	nuclear factor of activated T cells
NF-κB	nuclear factor κ-light-chain-enhancer of activated B cells
NK	natural killer
NKT	natural killer T cell
OCT-1	octamer transcription factor-1
PD-1	programmed cell death-1
PD-1L	PD-1-ligand
PEG	polyethylene glycol
PI3K	phosphatidylinositol 3-kinases
R	receptor
s.c.	subcutaneous
SCID	X-linked severe combined immunodeficiency
SOCS	suppressor of cytokine signaling
STAT	signal transducer and activator of transcription
TCR	T cell receptor
Tfh cell	T follicular helper cell
TGF-β	tumor growth factor-β
Th cell	T helper cell
TIL	tumor-infiltrating lymphocyte
TNF-α	tumor necrosis factor-α
Treg	T regulatory
VEGF	vascular endothelial growth
VLS	vascular leak syndrome
WT	wild type

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1. Introduction

Cytokines are small glycoproteins with pleiotropic effects on both the innate and adaptive immune systems. Interleukin-2 (IL-2), 4, 7, 9, 15, and 21 share the common cytokine receptor γ chain (γ_c), thus forming the γ_c cytokine family, which mediates a broad spectrum of roles in the immune system. They play a fundamental role in the development of T, B, and NK cells, particularly in the differentiation of B cells, the polarization of T helper lymphocyte subsets or homeostasis of naïve T cells, and shaping the activity of myeloid cells. They can also stimulate the proliferation and activation of various immune cell populations. Mutation of γ_c leads to X-linked severe combined immunodeficiency (SCID) and γ_c-deficient mice have diminished thymic development and nearly complete absence of T and B lymphocytes as well as natural killer cells (Kim et al., 2006).

The first member of the γ_c cytokine family used in tumor immunotherapy was IL-2. Furthermore, some γ_c cytokines are promising antitumor agents because of their ability to stimulate particularly T and NK cells. For this reason, this thesis aimed to summarize the current state of γ_c cytokines biology and their potential in cancer immunotherapy with an emphasis on IL-2 and IL-15 since these cytokines seem to possess the most promising antitumor activities.

2. Biology of γ_c cytokines

γ_c cytokines are four α-helical bundle type I cytokines that have a unique protein structure consisting of two pairs of antiparallel helices forming two layers. Most of their receptor subunits belong to the group of type I cytokine receptors. These receptors have a long N-terminal extracellular chain characterized by two fibronectin type III domains that form a cytokine-binding homology region (CHR). CHR is the recognition site for four α-helical bundle motifs of γ_c (and other) cytokines (Bazan, 1990a, 1990b; Sprang & Bazan, 1993; Spolski et al., 2017). However, there are two γ_c cytokine receptor subunits with different characteristics, IL-2 receptor subunit α (IL-2Rα, CD25) and IL-15Rα (CD215) bind their ligand via extracellular sushi domains, β-sandwich proteins consisting of 60-70 amino acid residues (Lorenzen et al., 2006; Wang et al., 2005). The composition of receptors for γ_c cytokine is depicted in Figure 1.

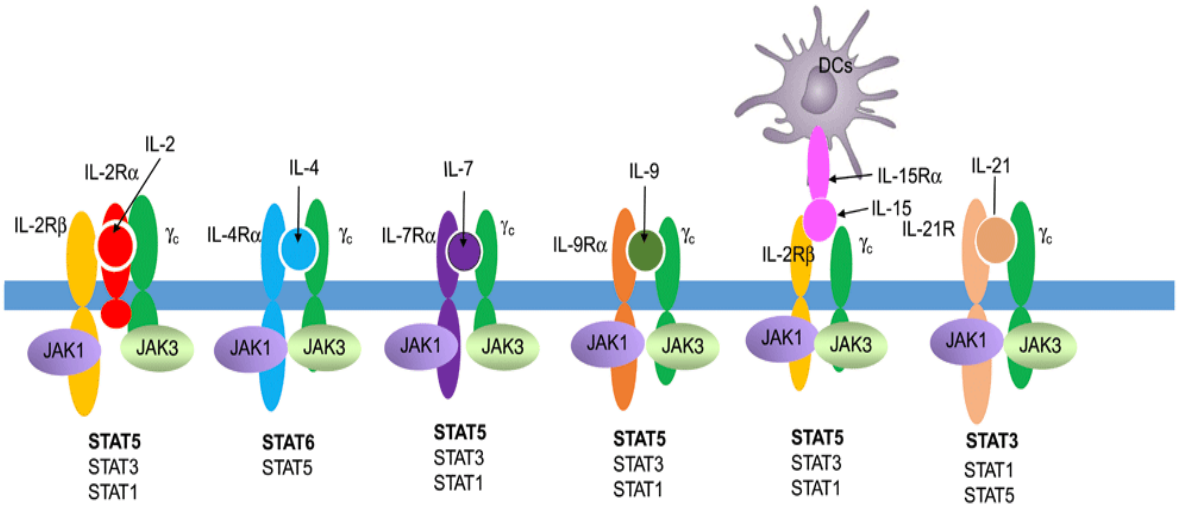

Figure 1. γ_c cytokines and their receptors interacting with Janus kinases (JAK) and signal transducer and activator of transcription (STAT) proteins (adapted from Spolski et al., 2017).

γ_c cytokines receptors do not have intrinsic enzymatic activity, instead, intracellular domains of γ_c cytokine receptors are constitutively associated with Janus tyrosine kinases (JAKs). The γ_c associates with JAK3, whereas IL-2Rβ (IL-15Rβ), IL-4Rα, IL-7Rα, IL-9Rα, and IL-21Rα associate with JAK1 (Figure 1 and Figure 3). The downstream signaling is initiated by cytokine binding to its receptor, leading to receptor dimerization and auto-activation of JAKs by transphosphorylation. Activated JAKs phosphorylate specific tyrosine residues on intracellular receptor tails and those phosphotyrosines provide docking sites for

STAT proteins. Receptor-localized STATs are then phosphorylated by JAKs, dissociate from the receptor, form dimers, and translocate to the nucleus, where they act as transcription factors (Figure 3). STATs promote the expression of cytokine-responsive genes (Morris et al., 2018; Spolski et al., 2017).

2.1. Interleukin-2

IL-2 is a glycoprotein consisting of a single polypeptide chain that shares the typical four α-helical structure and human IL-2 has a molecular weight of ~15.5 kDa, although it differs due to variable glycosylation (Gaffen & Liu, 2004; Spolski et al., 2017).

2.1.1. Production and function of IL-2

IL-2 was the first member of the γ_c cytokine family discovered in 1976 as an autocrine factor stimulating T lymphocyte proliferation and growth (i.e. T cell growth factor) in a culture with plant lectin phytohemagglutinin (Morgan et al., 1976). Later works revealed that the importance of IL-2 is not solely in stimulating the proliferation and effector response of T lymphocytes but it also plays a crucial role in the homeostasis and regulation of the immune system.

The main producers of IL-2 are efficiently primed T lymphocytes and to a lesser extent, dendritic cells (DC), NK cells, and mast cells (Gaffen & Liu, 2004; Granucci et al., 2001; Hershko et al., 2011; Setoguchi et al., 2005). Two crucial signals are necessary to initiate IL-2 production in T lymphocytes. The first signal arises when the T cell receptor (TCR) recognizes a peptide in the major histocompatibility complex I or II (MHC I or II) and the second signal is provided by the ligation of CD28 by co-stimulatory molecules CD80 or CD86 (Gaffen & Liu, 2004). These signals are usually delivered to T cells through interaction with antigen-presenting cells (typically matured DCs) since they express the required molecules (Figure 4).

IL-2 gene transcription is quick and transient depending on TCR and CD28 signals, with maximal IL-2 mRNA levels reached in a few hours post T cell activation, after which the mRNA is rapidly degraded and transcription is silenced (Jain et al., 1995; Powell et al., 1998). IL-2 gene transcription is mediated by several transcription factors including the nuclear factor of activated T cells (NF-AT), activator protein-1 (AP-1), nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB), and the octamer transcription factor (OCT-1). Negative regulation of IL-2 expression is mediated by inhibiting transcriptional factors like forkhead box protein 3 transcription factor (Foxp3) constitutively expressed in T regulatory (Treg) cells and cAMP-responsive element modulator (CREM) (Kim et al., 2006). The suppressor of cytokine signaling (SOCS) proteins provide another possibility to regulate JAK/STAT signaling (Inagaki-Ohara et al., 2013).

2.1.2. IL-2 receptor composition and downstream signaling

IL-2 signals through specific cell-surface receptors (Figure 2) and there are three IL-2R subunits, IL-2Rα, IL-2Rβ (CD122), and IL-2Rγ (γ_c, CD132). IL-2Rα binds IL-2 with low affinity ($K_d \sim 10^{-8}$ M) and it seems to have no contact with IL-2Rβ and γ_c subunits (Figure 2). IL-2Rα is not involved in intracellular signalization but induces a conformational change in IL-2, thereby increasing IL-2 affinity for the IL-2Rβ subunit (Malek, 2008; Wang et al., 2005).

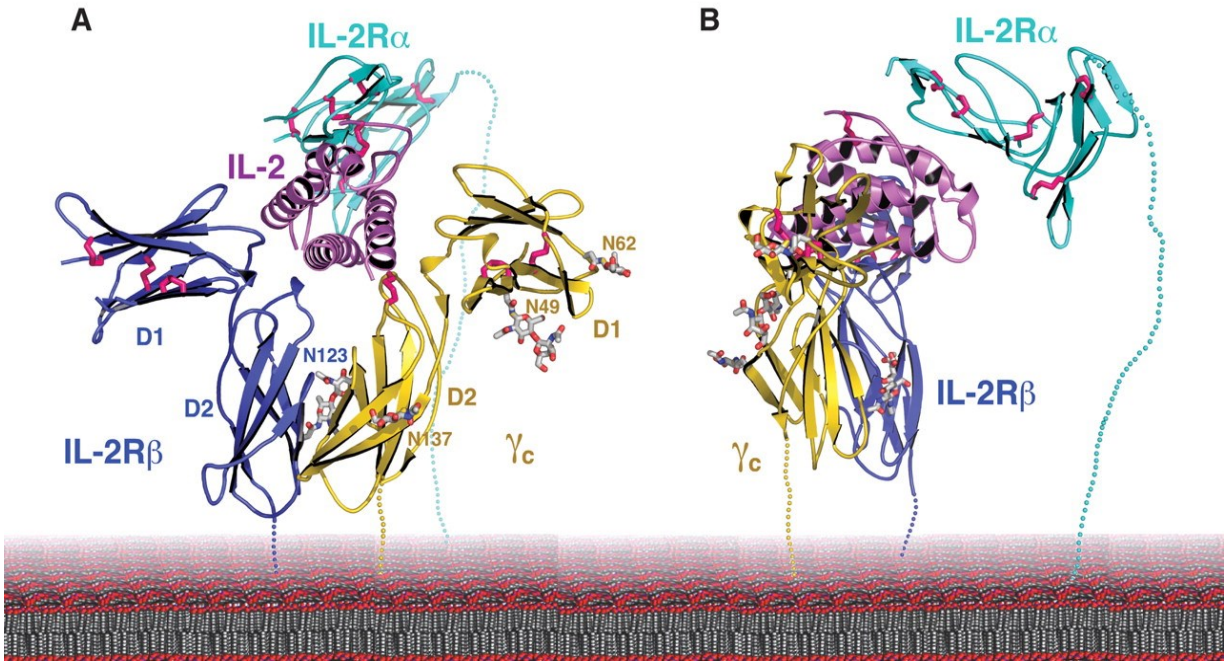

Figure 2. Structure of IL-2/IL-2R complex (adapted from Wang et al., 2005).

IL-2 signals either through the dimeric receptor composed of IL-2Rβ and γ_c or through the IL-2R$\alpha\beta\gamma_c$ trimeric receptor; these receptors have different affinities for IL-2. The intermediate-affinity dimeric receptor ($K_d \sim 10^{-9}$ M) can be found on resting or memory $CD8^+$ T lymphocytes or non-stimulated NK cells (Malek, 2008; Wang et al., 2005). The high-affinity trimeric IL-2 receptor ($K_d \sim 10^{-11}$ M) is constitutively expressed on Treg cells and its transient expression is also induced on activated T lymphocytes and a subset of NK cells (Figure 4). Thus, IL-2Rα increases the affinity of dimeric IL-2R by ~100 times. The binding of IL-2 to the IL-2 high-affinity receptor leads to rapid internalization and lysosomal degradation of IL-2 and receptor proteins, however, IL-2Rα recycles to the cell surface (Arima et al., 1992; Hatakeyama et al., 1986; Malek, 2008; Siegel et al., 1987; Wang et al., 2005). The ability of Treg cells to maintain constitutive high expression of trimeric IL-2R is enabled by their high intracellular abundance of receptor subunits, whereas other cell types synthesize and immediately transport the subunits on the cell surface (Hémar et al., 1995; Smith et al., 2017).

The binding of IL-2 to IL-2Rβ and γ_c initiates downstream signaling. These receptor subunits dimerize, and associated JAK1 and JAK3 kinases come into proximity and trans-phosphorylate, with the phosphorylation of specific tyrosine residues of IL-2Rβ.

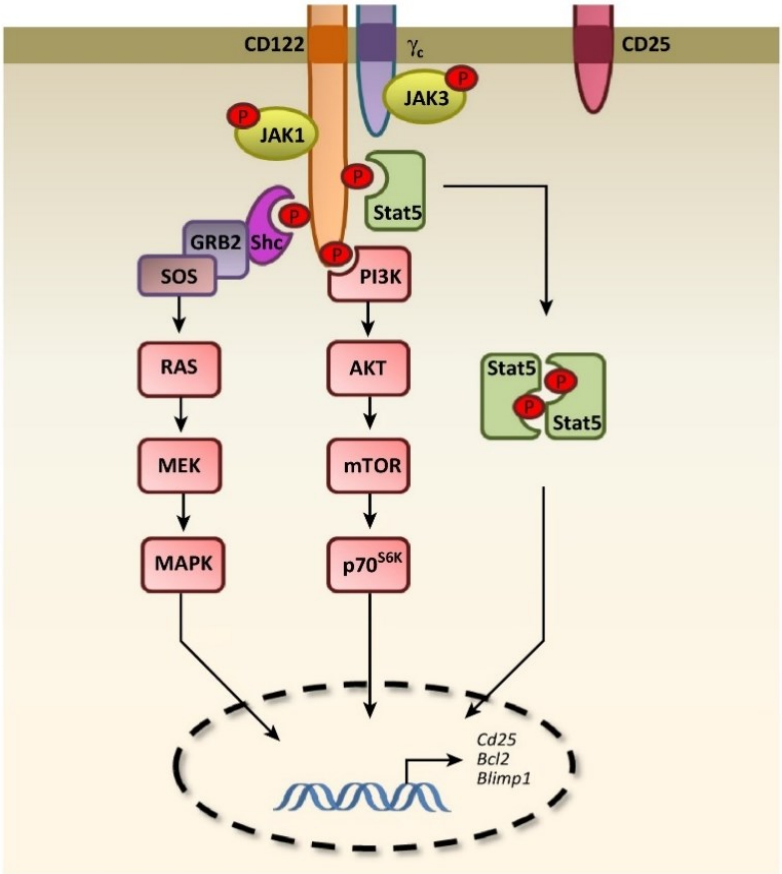

Figure 3. Interleukin-2 signaling pathways (adapted from Arenas-Ramirez et al., 2015).

The phosphorylation IL-2Rβ enables interaction with adaptor Shc protein and STAT1, STAT3, or more importantly, STAT5A and STAT5B. STAT5 regulates the expression of fundamental genes for T cell effector functions and growth. The Shc adaptor protein provides a platform for the other two signaling pathways shown in Figure 3, mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 3-kinase (PI3K) pathways (Lin & Leonard, 2000; Lin et al., 2012; Morris et al., 2018; Spolski et al., 2017).

2.1.3. Role of IL-2 in the immune system

IL-2 is a pleiotropic cytokine with several important roles in the immune system. It regulates the proliferation and differentiation of antigen-primed naïve T cells into effector or memory phenotype cells and can enhance the expression of perforin, granzyme B and IFN-γ, thus, IL-2 is essential for the cytolytic activity of $CD8^+$ T cells, which is further increased by the presence of IL-12. It also stimulates the proliferation and cytotoxic activity of NK cells (Curtsinger & Mescher, 2010; Pipkin et al., 2010; Shi et al., 2008; Siegel et al., 1987; Williams et al., 2006). IL-2 promotes clonal expansion of $CD4^+$ T cells before/within their polarization into T helper (Th) lymphocytes Th1, Th2, Th9, Th17, or T follicular helper (Tfh) subsets. IL-2 augments the responsiveness to IL-12 that drives the differentiation of Th1 cells. IL-4 promotes Th2 differentiation and the combination of IL-4 and tumor growth factor-β (TGF-β) induces Th9 differentiation. IL-2 signaling decreases Th17 differentiation driven by IL-6 and TGF-β as well as decreasing Tfh differentiation promoted by IL-6 and IL-21 (Liao et al., 2013). In contrast, IL-2 together with TGF-β induces Treg cells in the periphery (Horwitz et al., 2003).

IL-2 is also an important factor for the development, homeostasis, and maintenance of suppressive activity of Treg cells, which are primarily responsible for maintaining peripheral immunological tolerance. Treg cells constitutively express high-affinity trimeric IL-2 receptors, which enables them to utilize low background levels of IL-2. They are also highly responsive to IL-2 signaling required for their survival and Foxp3 expression. Of note, Treg cells do not produce IL-2 and the deficiency of IL-2 or IL-2 receptor subunits leads to the absence of Treg cells and severe autoimmunity (Fontenot et al., 2005; Setoguchi et al., 2005; Yu et al., 2015). Hershko et al. (2011) showed the importance of IL-2 for the suppression of chronic allergic dermatitis.

IL-2 may also promote so-called activation-induced cell death (AICD), which is apoptotic cell death of effector T cells. Long-lasting stimulation by high levels of IL-2 induces strong expression of Fas-ligand (CD-95L) and inhibits the expression of FLICE-like inhibitor protein (FLIP) in T cells. Interaction of Fas (CD95) with Fas-ligand on the cell surface activates caspases and leads to apoptosis, indicating the feedback regulation function of IL-2 (Lenardo, 1991; Refaeli et al., 1998).

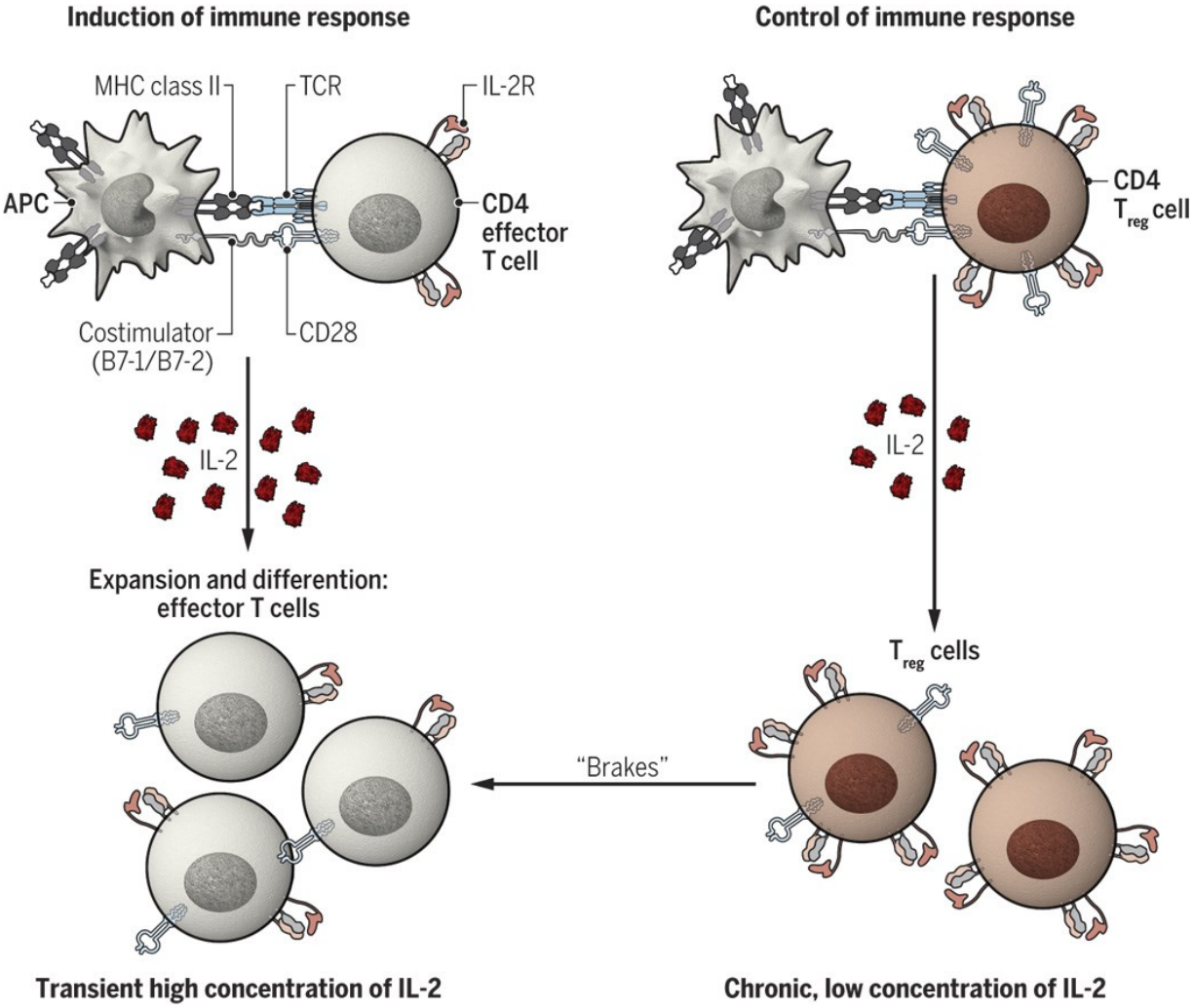

Figure 4. Dual role of IL-2 in T cell proliferation and effector functions (adapted from Abbas et al., 2018).

2.2. Interleukin-4

IL-4 is a ~15 kDa cytokine described in 1982 as a factor stimulating the proliferation of B lymphocytes and inducing an immunoglobulin isotype switch (Howard et al., 1982; Isakson et al., 1982). A wide range of cells express the IL-4 receptor which is formed by a high-affinity IL-4Rα chain (CD124) that dimerizes with the γ_c chain. Alternatively, IL-4Rα may associate with the IL-13 receptor α chain (IL-13Rα, CD213), mainly on non-hematopoietic cells (Aman et al., 1996; Lowenthal et al., 1988; Ohara & Paul, 1987).

IL-4Rα associates with JAK1 and γ_c with JAK3. Downstream signaling is mediated via STAT6 and STAT5, the latter to a lesser extent. IL-4 signaling in cooperation with the TCR signal facilitates IL-4 expression creating a positive feedback loop and enhancing Th2 differentiation (Dardalhon et al., 2008; Junttila, 2018). Consequently, this leads to the suppression of Th1 polarization since IL-4 inhibits IL-12 production in myeloid cells (Levings & Schrader, 1999). As mentioned above, IL-4 together with TGF-β is also a Th9 differentiation factor. The main producers of IL-4 are Th2-polarized $CD4^+$ T lymphocytes but mast cells, basophils, and eosinophils or natural killer T (NKT) cells produce IL-4 to a certain extent (Dardalhon et al., 2008; Junttila, 2018; Liao et al., 2013).

The presence of IL-4 promotes alternative activation of macrophages into M2 cells and inhibits classical activation of macrophages into M1 cells. These M2 tissue macrophages play an important role in chronic inflammation and wound repair (Ito et al., 2017). However, IL-4 overproduction is associated with allergies since it induces IgE production (Corry et al., 1996).

2.3. Interleukin-7

IL-7 is a glycoprotein of molecular weight ~25 kDa and was discovered in the late 1980s as a stromal cell-derived factor stimulating the growth of pre-B lymphocytes (Goodwin et al., 1989; Namen et al., 1988). IL-7 is a non-hematopoietic cell-derived cytokine produced in the bone marrow and thymus as well as in other epithelial cells and stromal cells in secondary lymphoid organs (Chen et al., 2021).

The IL-7 receptor is a heterodimeric complex consisting of the α-chain (IL-7Rα, CD127) and γ_c. IL-7Rα is expressed on B lymphocyte progenitors, innate lymphoid cells (ILCs), and naïve T lymphocytes (Sinclair et al., 2011). Principles of signal transduction are analogous to previously described pathways. The ligation of the IL-7 receptor induces dimerization of the IL-7R subunits, transphorylation of associated kinases JAK1 and JAK3, then activated JAK

kinases mainly phosphorylate STAT5, followed by STAT5 dimerization and translocation into the nucleus (Chen et al., 2021).

IL-7 signaling leads to the expression of the B cell lymphoma-2 (Bcl-2) gene family that has anti-apoptotic functions (e.g., Bcl-2 and Mcl-1 proteins), therefore, IL-7 is a crucial survival factor for IL-7R expressing cell populations (Chen et al., 2021). IL-7 is also a hematopoietic growth factor essential for T and B lymphocyte development in mice (Peschon et al., 1994), homeostasis of T and B lymphocytes and NK cells through inducing the expression of the prosurvival anti-apoptotic genes (Opferman et al., 2003; Rathmell et al., 2001). IL-7 promotes the proliferation and effector functions of T lymphocytes and supports the maintenance of memory phenotype $CD8^+$ T cells (Geiselhart et al., 2001; Kieper et al., 2002).

2.4. Interleukin-9

IL-9 was described in 1988 as a Th lymphocyte and mast cell growth factor, originally named P40. It is a glycoprotein with a molecular weight of 32-39 kDa (Hültner et al., 1990; Uyttenhove et al., 1988). IL-9 is mainly produced by $CD4^+$ Th cells, although other cell types like ILCs also produce certain amounts of IL-9 (Wilhelm et al., 2011). IL-9 was initially considered to be a Th2 cytokine because it is involved in some typical Th2 responses but is now regarded as a signature cytokine of the Th9 response. Differentiation from naïve $CD4^+$ T lymphocytes into Th9 is enhanced by the presence of IL-4, TGF-β, and IL-2. Naturally, Th9 cells can produce large amounts of IL-9 (Dardalhon et al., 2008).

The IL-9 receptor is a dimer consisting of γ_c and a specific IL-9Rα chain (CD129) which cannot transduce any signal alone, although it binds IL-9 with high affinity (Demoulin & Renauld, 1998; Renauld et al., 1992). The IL-9 receptor is expressed on effector T lymphocytes, macrophages, ILCs, and mast cells or nonhematopoietic cells, e.g. bronchial epithelial cells (Druez et al., 1990; Longphre et al., 1999; Turner et al., 2013). Signal transduction is mediated mainly via STAT5 and leads to proliferation and inhibition of apoptosis in T lymphocytes (Demoulin et al., 1996).

IL-9 stimulates proliferation and prolongs survival in mast cells. It also affects the proliferation and function of B cells as it enhances IgE and IgG production (Dugas et al., 1993). IL-9 may be an autocrine anti-apoptotic factor for type 2 ILCs (Turner et al., 2013) and is involved in the regulation of hematopoiesis (Donahue et al., 1990). It also plays an important role in the protection against extracellular parasites and in allergic and inflammatory reactions (Temann et al., 2002; Townsend et al., 2000).

2.5. Interleukin-15

IL-15 is a ~15 kDa glycoprotein that was identified as a factor stimulating T lymphocyte growth with significant function similarities to IL-2. IL-15 is mainly produced by monocytes, DCs, and macrophages, although it is constitutively expressed by various cell types including endothelial cells, keratinocytes, fibroblasts, etc. (Bamford et al., 1996; Blauvelt et al., 1996; Grabstein et al., 1994).

The IL-15 receptor consists of IL-15Rβ (CD122) and γ_c subunits that are shared with IL-2. There is a unique high-affinity ($K_d \sim 5 \cdot 10^{-11}$ M) IL-15Rα subunit, which is expressed by a broad spectrum of hematopoietic as well as non-hematopoietic cells (Anderson et al., 1995; Dubois et al., 2002; Giri et al., 1995). IL-15 signal transduction is mediated by JAK3 associated with γ_c and JAK1 associated with IL-15Rβ. These kinases primarily activate STAT5a and STAT5b (Spolski et al., 2017). IL-15 is mainly a membrane-bound cytokine and is presented on the cell surface of IL-15-producing cells in complex with IL-15Rα. This complex is *trans*-presented to the cells expressing IL-15Rβ and γ_c receptor subunits (Figure 1) and possesses higher biological activity than soluble IL-15 alone (Dubois et al., 2002; Grabstein et al., 1994; Kennedy & Park, 1996; Rubinstein et al., 2006).

As mentioned above, IL-2 and IL-15 share many features including structure, the shared CD122 and γ_c receptor subunits, and similar functions, although they may exert unique functions under some conditions. Both IL-2 and IL-15 stimulate activated T and B lymphocytes as well as NK cell proliferation and enhance immunoglobulin production (Spolski et al., 2017).

Furthermore, IL-15 and IL-15Rα are a fundamental factor for the development of NK cells and NKT cells as well as some intraepithelial lymphocytes due to the requirement for *trans*-presentation of IL-15. The same applies for the maintenance of memory-phenotype $CD8^+$ T lymphocytes. IL-15 supports the survival of memory $CD8^+$ T lymphocytes and has an anti-apoptotic function in contrast to activation-induced cell death caused by IL-2 (Lodolce et al., 1998; Marks-Konczalik et al., 2000; Waldmann et al., 2001; Waldmann & Tagaya, 1999). Monocytes, macrophages, and neutrophils stimulated by IL-15 show increased phagocytosis and production of pro-inflammatory cytokines and they are protected from apoptosis. DCs stimulated by IL-15 express increased levels of co-stimulatory molecules and MHC II glycoproteins. IL-15 also affects non-hematopoietic cells, e.g., myocytes and endothelial cells (Girard et al., 1996; Mattei et al., 2001; Quinn et al., 1995; Vázquez et al., 1998).

2.6. Interleukin-21

IL-21 is the most recently discovered member of the γ_c cytokine family described in 2000. It is a ~15 kDa glycoprotein produced mainly by activated $CD4^+$ T lymphocytes, NKT cells, and Tfh cells. The IL-21 receptor is formed by the γ_c subunit and IL-21Rα (CD360). IL-21R is expressed mainly on B lymphocytes and to a lesser extent on NK cells, T lymphocytes, macrophages, DCs and fibroblasts, keratinocytes, and intestinal epithelial cells. IL-21R ligation activates JAK1 and JAK3, which are responsible for phosphorylation of STAT3. Weak activation of STAT1 and STAT5 pathways was also described upon ligation of IL-21R (Leonard et al., 2008; Ozaki et al., 2000; Parrish-Novak et al., 2000).

IL-21 enhances B lymphocyte differentiation to plasma cells and induces an immunoglobulin class switch to IgG1, while it concomitantly suppresses IgE production (Ozaki et al., 2004). It also affects the proliferation and effector functions of NK cells (Brady et al., 2004). IL-21 and IL-15 act synergistically on $CD8^+$ T cell proliferation and IL-21R signaling increases the cytotoxic activity of $CD8^+$ T cells (Zeng et al., 2005). Furthermore, IL-21 and IL-6 are crucial for Tfh differentiation (Liao et al., 2013).

3. IL-2 is a key γ_c cytokine in cancer immunotherapy

The first intravenous (i.v.) application of multiple cycles of IL-2 therapy (600 000 or 720 000 IU/kg, every 8 hours) was performed in the 1980s on patients with metastatic melanoma and renal cell carcinoma with promising results, as regression of tumors occurred in a significant number of patients. Therefore, IL-2 immunotherapy was later approved by the U.S. Food and Drug Administration for the treatment of these cancers (Atkins et al., 1999; Rosenberg et al., 1994).

3.1. High-dose IL-2 immunotherapy

High-dose (HD) IL-2 therapy was combined with other antitumor approaches including lymphokine activated killer (LAK) cells, tumor-infiltrating lymphocytes (TILs), chemotherapy, etc. The combination of IL-2 with TILs showed an increased response rate in clinical trials (Rosenberg et al., 1988). However, HD IL-2 therapy is limited by severe side effects like diarrhea, renal dysfunction, and particularly, vascular leak syndrome (VLS) leading to interstitial fluid accumulation in various organs including the lungs resulting in pulmonary edema; VLS may be a consequence of the interaction between IL-2 and IL-2R-expressed on lung endothelial cells (Kammula et al., 1998; Krieg et al., 2010). Besides these HD IL-2 therapy-related toxicities, the expansion and increased suppressive activity of Treg cells is another undesirable side effect hampering efficient tumor treatment.

As stated above, Treg cells express the high-affinity trimeric IL-2 receptor, thus, even low-dose IL-2 immunotherapy can efficiently expand them and increase their capacity to suppress T-cell-mediated immune responses. In contrast to HD IL-2 therapy, this type of immunotherapy has significant potential in the treatment of chronic graft-versus-host reactions or autoimmune diseases such as systematic lupus erythematosus (Klatzmann & Abbas, 2015; Koreth et al., 2011).

3.2. Complexes of IL-2 and anti-IL-2 mAbs

Dramatically increased biological activity and prolonged *in vivo* half-life of IL-2 from minutes to hours can be achieved if the IL-2 is associated with anti-IL-2 monoclonal antibodies (mAb). The complexes of IL-2 and anti-IL-2 mAb in a mouse model are distinguished into two types according to the mAb clone used. The complex of IL-2 and JES6-1A12 mAb (IL-2/JES6) highly selectively stimulates IL-2Rα^{high} cell populations (Treg and activated T cells).

The complex of IL-2 and S4B6 mAb (IL-2/S4B6) preferentially stimulates IL-2Rβ^{high} cell populations (memory CD8$^+$ T and NK cells). This functional split enables at least partial separation of unwanted IL-2 effects on Treg cells from stimulatory effects on effector CD8$^+$ T lymphocytes. IL-2/JES6 could be beneficial in the treatment of autoimmune diseases or in transplantology, while IL-2/S4B6 is a promising cancer immunotherapy (Boyman et al., 2006; Spangler et al., 2015; Tomala et al., 2009).

The difference in the selective stimulatory activities of IL-2/JES6 and IL-2/S4B6 is caused by binding to distinct epitopes in the IL-2 molecule thus blocking the binding sites of different IL-2R subunits. JES6-1A12 mAb almost completely blocks the binding site for IL-2Rβ and γ_c in IL-2, whereas the S4B6 mAb effectively hampers the binding of IL-2 to IL-2Rα (Spangler et al., 2015).

Tomala et al. showed the ability of IL-2/anti-IL-2 mAb complexes to strongly stimulate the proliferation of primed CD8$^+$ T lymphocytes and the ability of IL-2/S4B6 to potently expand memory CD8$^+$ T cells and NK cells. The *in vivo* antitumor activity of IL-2/S4B6 seems to be superior to IL-2 alone with IL-2/S4B6 (15 000 IU) showing similar antitumor efficacy to the 13-fold higher dose of IL-2 (200 000 IU) in the mouse melanoma model.

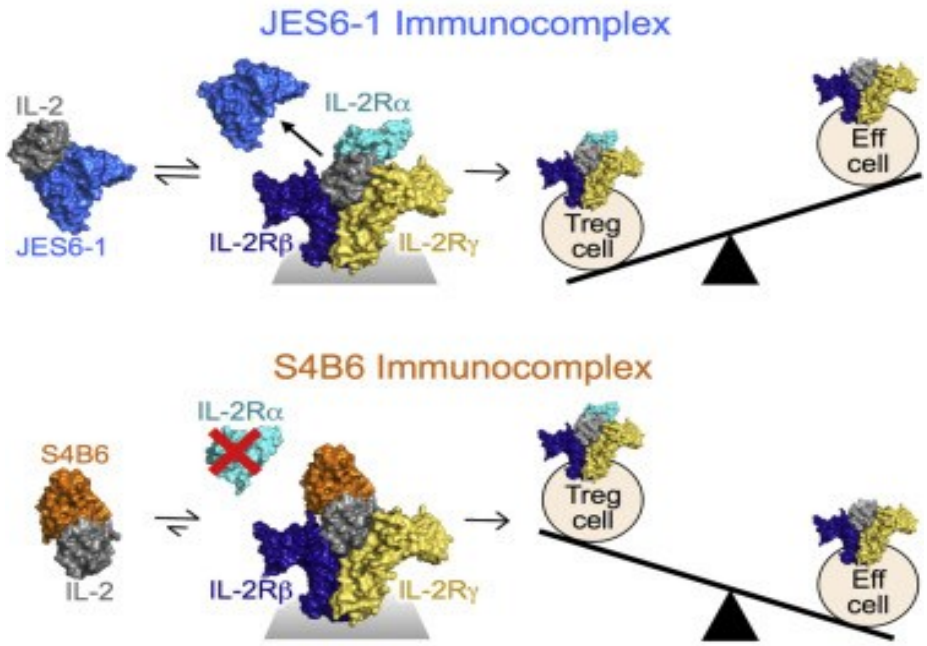

Figure 5. Structure of mIL-2/S4B6 and mIL-2/JES6 complexes and depiction of their prevailing stimulatory activity (adapted from Spangler et al., 2015).

Moreover, administration of these complexes efficiently mediates tumor control without causing severe pulmonary edema, whereas the HD IL-2 treatment required for a comparable antitumor effect caused increased the pulmonary wet weight. It has been also demonstrated that IL-2/S4B6 therapy leads to the significant proliferation of effector immune cells without severe toxicities. Importantly, the partial selectivity of IL-2/S4B6 for $CD122^{high}$ cells lowers the amount of the complex utilized by Treg cells (Boyman et al., 2006; Krieg et al., 2010; Létourneau et al., 2010; Tomala et al., 2009).

3.3. Modifications of IL-2 with improved pharmacological features

The first modification of IL-2 to improve its pharmacological features was the conjugation of IL-2 and polyethylene glycol (PEG). The conjugate was i.v. administered to a mouse sarcoma model and showed increased half-life in circulation as well as superior antitumor effects in comparison to IL-2 alone (Katre et al., 1987). Nevertheless, clinical studies with melanoma or renal cell cancer patients did not show significant differences in antitumor activity between PEGylated IL-2 and IL-2 alone (Yang et al., 1995).

A more recent modification is NKTR-214 (Bempegaldesleukin), which consists of six releasable PEG chains that are bound to IL-2. NKTR-214 is an inactive prodrug activated by the slow release of PEG chains. Since PEG chains are slightly more numerous in the IL-2Rα binding site of IL-2, NKTR-214 preferentially stimulates dimeric IL-2R-expressing cells similar to IL-2/S4B6. It shows higher efficacy in comparison to free IL-2 without causing VLS. Synergistic results were achieved with the combination of NKTR-214 and the checkpoint inhibitor anti-CTLA-4 mAb (Charych et al., 2016). The combination of NKTR-214 and the checkpoint inhibitor anti-programmed cell death-1 mAb (anti-PD-1, Nivolumab) has significant antitumor activity (60% objective response rate) and increased T-cell infiltration within the tumor (Diab et al., 2020).

3.4. IL-2 muteins

Another approach to improve the pharmacological features of IL-2 is to create mutated recombinant variants which possess changed affinities for different IL-2R subunits. They can be generally divided into CD25-biased and CD122-biased IL-2 muteins with partially selective stimulatory activity for immune cells expressing trimeric and dimeric IL-2R, respectively.

An example of the IL-2 mutein is a CD25-independent protein called Super-2 that has substantially increased binding affinity to IL-2Rβ. Super-2 induces stronger expansion of $CD8^+$ T lymphocytes than wild-type (WT) IL-2 and showed superior antitumor activity to WT IL-2

in mouse models of B16F10 melanoma, MC38 colon carcinoma, and LL2 lung carcinoma (Levin et al., 2012). IL-2 muteins may also restrict $CD8^+$ T lymphocytes exhaustion through decreased expression of exhaustion markers (e.g., PD-1), enhance $CD8^+$ T cell stemness, change metabolism and signaling (Mo et al., 2021) or show increased stability and thus also biological activity in the acidic tumor microenvironment with reduced toxicity (Gaggero et al., 2022). These effects may lead to the enhanced antitumor activity of immune cells expanded with those IL-2 muteins. Interesting results were achieved by the engineering of the orthogonal murine IL-2/IL-2Rβ high-affinity pair to provide an opportunity to strongly stimulate chimeric antigen receptor (CAR) T cells expressing ortho-IL-2Rβ by ortho-IL-2 and enhance CAR T-cell adoptive therapy with near to absolute specificity (Zhang et al., 2021).

4. IL-15 is another promising γ_c cytokine

The biological activities of IL-15 are very similar to IL-2. The most dominant effect is the stimulation of proliferation and survival of $CD8^+$ T lymphocytes and NK cells. In contrast, IL-15 has a more pronounced anti-apoptotic function and exerts less severe side effects, so IL-15 is a promising cancer immunotherapy drug.

Most IL-15-based immunotherapies under development are based on IL-15/IL15-Rα sushi-domain fusion thus employing the original discovery that IL-15 complexed with IL15Rα has higher biological activity in comparison to IL-15 alone both *in vitro* and *in vivo* (Rubinstein et al., 2006).

4.1. IL-15-based cancer immunotherapy

Conlon et al. described the results of their first human clinical trial of recombinant IL-15 in patients with metastatic malignant melanoma and renal cancer (bolus infusions of 3; 1 and 0.3 μg/kg per day, for 12 days) leading to redistribution and extensive proliferation of NK cells as well as memory $CD8^+$ T cells; γδ T and NKT lymphocytes also proliferated but to a lesser extent. The major side effects of recombinant IL-15 therapy are thrombocytopenia, hypotension, and toxicities mediated via increased production of inflammatory cytokines (Conlon et al., 2014). It has been suggested that these toxicities could be reduced through subcutaneous (s.c.) administration or slower and continuous i.v. administration of IL-15, the latter being the most prospective for further research (Waldmann et al., 2020).

The antitumor potential of IL-15 therapy can be augmented by simultaneous blockage of PD-1 and CTLA-4 molecules. Yu et al. (2012) demonstrated reduced tumor growth and prolonged survival rate as well as increased tumor-specific $CD8^+$ T cells in a transgenic adenocarcinoma of mouse prostate C2. Recent work by Xu et al. (2021a) reported promising results of IL-15 mutein fused to anti-PD-1 mAb. The mutein does not bind to IL-15Rα and PD-1 delivers IL-15 particularly to intratumoral $CD8^+$ T lymphocytes to induce their proliferation as well as enhance their cytotoxicity. Overall, IL-15 fused to anti-PD-1 mAb is an efficient antitumor therapy in mice tumor models without severe side effects compared to the super-agonist IL-15 (discussed later in the thesis) combined with PD-1 blockage.

Another interesting approach to IL-15 immunotherapy is to covalently attach IL-15 to hydrogel microspheres (MSs) by a releasable linker. The MSs enable the slow release of IL-15 dramatically prolonging its biological activity. S.c. administration of IL-15 MSs increases stimulation activity and maintains the IL-15 level above the threshold concentration. Furthermore, IL-15 MS increases the proliferative effect of IL-15 toward NK, γδ, and $CD8^+$ T cells and shows antitumor activity (Hangasky et al., 2022).

4.2. Complexes of IL-15 and IL-15Rα

The biological activity and the half-life of IL-15 are significantly increased when using IL-15/IL-15Rα complexes also called IL-15 super-agonists. Rubinstein et al. (2006) described the enhanced stimulatory effect of IL-15/IL-15Rα-Fc complex on memory-phenotype $CD8^+$ T lymphocytes and NK cells ($CD122^{high}$ populations) in comparison to IL-15 alone. The assembly of the complex leads to a conformational change of IL-15 having increased affinity to IL-15Rβ. IL-15Rα also seems to have a chaperone-like protective role. Bergamaschi et al. administrated IL-15/IL-15Rα complexes via s.c. route into rhesus macaques (*Macaca mulatta*), resulting in the expansion of the IL-15-responding cell pool. However, animals treated with HD (50 μg/kg/dose) therapy suffered from fever or renal dysfunction (Bergamaschi et al., 2018; Chertova et al., 2013).

ALT-803, a recombinant fusion of the IL-15 mutein with increased affinity for CD122 and fused to the sushi domain of IL-15Rα linked to the IgG1 Fc part showed promising results. ALT-803 was i.v. (1, 3, 6, and 10 μg/kg) and s.c. administered to patients (6 and 10 μg/kg) without severe side effects. The therapy was clinically beneficial and significantly increased the number of NK cells as well as $CD8^+$ T lymphocytes (Romee et al., 2018). Wrangle et al. describe promising results of ALT-803 and anti-PD-1 mAb combination therapy in lung cancer patients (Wrangle et al., 2018). Furthermore, Xu et al. (2021b) claim that increased efficiency of IL-15/IL-15Rα therapy could be reached by using full-length IL-15Rα, not only the sushi domain.

5. Other γ_c cytokines in cancer immunotherapy

5.1. Contradictory effects of IL-4 therapy in cancer

IL-4 exhibits contradictory roles in cancer progression and therapy, promoting cancer growth as well as tumor suppressive effects depending on the tumor type and nature of the tumor microenvironment. Initial works showed considerable antitumor activity of IL-4 but IL-4 drives Th2 differentiation and suppresses the Th1 response. Thus, IL-4 may indirectly diminish the antitumor activity of $CD8^+$ T lymphocytes and NK cells, possibly even contributing to tumor growth.

The clinical trials of IL-4 cancer immunotherapy did not prove significant therapeutical activity. For instance, Gilleece et al. (1992) administered s.c. recombinant IL-4 (daily doses of 0.5, 1, or 5 μg/kg) to patients bearing solid tumors of the gastrointestinal tract (e.g., adenocarcinoma of pancreas or caecum) or multiple myeloma without any evidence of significant antitumor effects (Gilleece et al., 1992). However, IL-4 seems to be a promising drug for the treatment of hematological malignancies, as IL-4 treatment (intraperitoneal, 60 μg/kg/day) induces apoptosis of leukemic cells in a mouse model of acute myeloid leukemia via induced expression of transcriptional factor p53 and activation of caspases (Qian et al., 2022).

The tumor-promoting activity of IL-4 was summarized by Shurin et al. (1999) who described a significant imbalance in the Th1/Th2 cytokine ratio and a higher number of Th2 cytokine-producing $CD4^+$ T cells in patients with lung, breast, or bladder cancer, as well as renal cell and prostate cancer. Furthermore, higher IL-4 levels were observed in patients with a variety of cancer types (Shurin et al., 1999). Consistent with these observations, Ito et al. (2017) showed the antitumor activity of neutralizing anti-IL-4 mAb. Such anti-IL-4 mAb treatment of mice bearing CT26 colon carcinoma or 4T1 breast tumor led to significantly delayed tumor growth and higher survival rate as well as more TILs. IL-4 also seems to play an important role in metastatic formation in lung cancer (Rodriguez-Tirado et al., 2022).

Another connection between IL-4 and tumor promotion is STAT6 which mediates the downstream signaling from IL-4R. Expression of STAT6 is significantly enhanced in lung, thyroid, or breast carcinomas but its deficiency inhibits tumor growth and increases the ratio of the tumor-suppressive M1 macrophages to M2 macrophages as differentiation of M2 macrophages is IL-4 dependent (Fu et al., 2019). Thus, IL-4 seems to be the only γ_c cytokine whose neutralization may have antitumor effects.

5.2. IL-7 in cancer immunotherapy

IL-7 shows notable antitumor activity as it has mitogenic and pro-survival effects in T cells. One of the original ideas to apply IL-7 in cancer immunotherapy was to benefit from its ability to achieve rapid repopulation of T lymphocytes after chemotherapy (Morrissey et al., 1991). Treatment of metastatic melanoma and sarcoma with IL-7 (every 3 days; 8 doses of 3, 10, 30, or 60 μg/kg) leads to dose-dependent increases of $CD4^+$ and $CD8^+$ T cells in peripheral blood with limited toxicity. In contrast to IL-2 immunotherapy, the minimal stimulation of Treg cells suggested the idea of using IL-7 as a lymphopoietic factor without the undesirable effect of stimulating Treg cells (Rosenberg et al., 2006).

IL-7 seems to impair the immune-suppressive effect of TGF-β on activated $CD8^+$ T cells, maintaining their homeostasis and survival. Administration of IL-7 also promotes higher levels of tumor-infiltrating T, NK, and NKT cells as well as improved cytotoxic activity of $CD8^+$ T cells by stimulating granzyme B expression (Pellegrini et al., 2009).

5.3. IL-9 has a low potential to treat cancer

IL-9 seems to play only a minor role in cancer immunotherapy and may affect tumor growth by altering the cytokine environment. IL-9 treatment of mice with SGC-7901 gastric cancer inhibited tumor growth and decreased levels of IL-4, IL-10, vascular endothelial growth factor (VEGF), and TGF-β. In this model, IL-9 also seems to reduce tumor cell-induced angiogenesis and metastasis (Cai et al., 2019). Purwar et al. (2012) showed antitumor activity of both recombinant IL-9 and IL-9-producing Th9 cells in an OT-II mouse model of B16F10-OVA melanoma (the cancer cells express ovalbumin). IL-9 may also exert a pro-tumor activity in hematological tumors, for instance, it has stimulatory and anti-apoptotic effects on thymic lymphoma cell lines (Renauld et al., 1995).

5.4. IL-21 shows promising anticancer activity

IL-21 has anticancer potential particularly through its ability to stimulate proliferation and cytotoxic activity of NK cells and $CD8^+$ T cells (Frederiksen et al., 2008; Moroz et al., 2004).

Moroz et al. compared the antitumor activities of IL-21, IL-15, and IL-2 using the E.G7 thymoma mouse model. The mice were treated with IL-2 (2000 IU/day), IL-15 (5 μg/day), and IL-21 (20 μg/day) on days 2, 4, 6, 8, 10, and 12, with IL-21 having the most potent antitumor activity. Nevertheless, the efficiency of IL-21 cancer immunotherapy seems to be

highly dependent on its timing, with the highest IL-21 antitumor activity achieved several days after tumor challenge (Moroz et al., 2004).

Clinical trials of IL-21 cancer immunotherapy showed promising antitumor effects. IL-21 was i.v. administered (two 5-day cycles, 3 to 100 μg/kg/dose) to patients with metastatic melanoma or renal cell carcinoma and was well tolerated up to doses of 30 μg/kg and the overall response rate was 67% (Thompson et al., 2008). Frederiksen et al. described the stimulatory effect of IL-21 therapy on the expansion and effector functions of NK as well as $CD8^+$ T cells, with IL-21 being an important factor for T cell motility (Frederiksen et al., 2008). Consistent with this observation, T-cell migration is significantly reduced in IL-21R knockout mice. Furthermore, IL-21R deficiency impairs the memory responses to tumor rechallenge (Zheng et al., 2018).

IL-21 antitumor activities can be combined with some other treatment effects to achieve additive or synergistic therapeutic effects. For example, the strong anticancer activity of the IL-21 and anti-CD25 mAb was observed in a mammary carcinoma mouse model, anti-CD25 mAb depletes Treg cells causing tumor-related immune suppression (Comes et al., 2006) and IL-21 acts synergistically with soluble PD-1. The PD-1 molecule on the surface of T cells thus cannot bind to the PD-1-ligand (PD-1L) on tumor cells saturated by soluble PD-1, thereby inhibiting cytotoxic immune responses similar to the administration of anti-PD-1L mAb. The combination therapy of IL-21 and soluble PD-1 showed potent antitumor activity in an H22 hepatocellular carcinoma mouse model (Pan et al., 2013).

6. Conclusion

Two γ_c cytokines, namely IL-2 and IL-15, possess remarkable antitumor activity through the potent stimulation of proliferation, survival, and induction of effector functions of $CD8^+$ T and NK cells thus showing the capacity to control tumor progression.

IL-2 was the first γ_c cytokine to be tested in clinical trials for cancer treatment but the associated severe side effects, short half-life, AICD and simultaneous stimulation of Treg cells have limited its application. One of the most interesting approaches to overcoming the disadvantages of IL-2 therapy is complexes of IL-2 and anti-IL-2 mAbs that possess dramatically increased biological activity *in vivo* and exert selective stimulatory activity for either $CD122^{high}$ immune cell subsets or $CD25^+$ cells. This feature is governed by the anti-IL-2 mAb clone used to form the complexes thus hindering different epitopes in IL-2. Another suitable approach is the covalent modification of IL-2, such as PEGylation, to prolong the half-life and thus increase the *in vivo* biological activity. They may also show some selectivity depending on the site of attachment of the PEG chains. Finally, the construction of recombinant IL-2 muteins with desired pharmacological features can be achieved via increasing/decreasing IL-2 affinity for a particular IL-2R subunit (CD122- or CD25-biased muteins).

IL-15 has strong immunostimulatory effects, particularly for memory $CD8^+$ T and NK cells, and importantly, has less severe side effects and does not cause AICD in comparison to IL-2. Furthermore, the enhanced biological activity of IL-15 is achieved by using IL-15/IL-15Rα complexes or recombinant fusion proteins mimicking these complexes both structurally and functionally. The efficiency of IL-15-based immunotherapy can be further increased by the simultaneous immune-checkpoint blockage.

7. References

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