

Review

Toll-like receptor-induced cytokines as immunotherapeutic targets in cancers and autoimmune diseases

Mahesh Chandra Patra¹, Masaud Shah¹, Sangdun Choi^{*}

Department of Molecular Science and Technology, Ajou University, Suwon, 16499, Republic of Korea

ARTICLE INFO

Keywords:
Autoimmune disease
Cancer
Cytokine
Innate immunity
Toll-like receptor

ABSTRACT

Immune cells of the myeloid and lymphoid lineages express Toll-like receptors (TLRs) to recognize pathogenic components or cellular debris and activate the immune system through the secretion of cytokines. Cytokines are signaling molecules that are structurally and functionally distinct from one another, although their secretion profiles and signaling cascades often overlap. This situation gives rise to pleiotropic cell-to-cell communication pathways essential for protection from infections as well as cancers. Nonetheless, deregulated signaling can have detrimental effects on the host, in the form of inflammatory or autoimmune diseases. Because cytokines are associated with numerous autoimmune and cancerous conditions, therapeutic strategies to modulate these molecules or their biological responses have been immensely beneficial over the years. There are still challenges in the regulation of cytokine function in patients, even in those who take approved biological therapeutics. In this review, our purpose is to discuss the differential expression patterns of TLR-regulated cytokines and their cell type specificity that is associated with cancers and immune-system-related diseases. In addition, we highlight key structural features and molecular recognition of cytokines by receptors; these data have facilitated the development and approval of several biologics for the treatment of autoimmune diseases and cancers.

1. The immune microenvironment and Toll-like receptors (TLRs)

The cells of lymphoid and myeloid origins offer a nonspecific, transient, yet robust initial defense against host-invading objects. These cells are equipped with the germline-encoded pattern recognition receptors that are expressed both in the cytosol and on the cell surface. The receptors possess an ability to spontaneously recognize exogenous microbial antigens as well as endogenous substances. The notion that the host immune system is crucial for controlling cancer has remained the topic of discussion for over a century. The prodigious amount of data collected from human patients and animal models proves the existence of a functional immuno-surveillance process [1]. In addition to its extrinsic tumor-suppressing function, the immune system can facilitate tumor progression by sculpting the immunogenic phenotype of developing tumors. The idea that the immune system plays pro- and antitumor roles during the complex tumor-immunity interaction has changed the concept of cancer immuno-surveillance into a new phenomenon called “cancer immunoediting.” The theory of immunoediting, also called the theory of three Es, explains the involvement of the immune system in a tumor microenvironment with respect to elimination, equilibrium, and escape phases [1]. During the

elimination phase, the immune system marks and destroys cancerous cells, whereas some sporadic genetic variants manage to survive and enter the equilibrium phase. At this stage, the immune system still controls the growth of tumor cells, establishing a dynamic equilibrium between the tumor and immune system. During the escape phase, the immunologically sculpted tumor cells progressively grow in a fully equipped immunosuppressive tumor microenvironment [2]. Tumor cells evade the host immune system through the senescence and lie dormant during the equilibrium phase, where they re-emerge at a suitable condition. Besides, tumors are capable of exploiting various immunological processes, including but not limited to, the function of regulatory T-cells (Tregs) and their secretion, tumor-specific antigen presentation, modification of tumor-suppressing mediators and immune tolerance [3].

The conception of immuno-surveillance [4,5] has been widely accepted and confirmed by the existence of a higher density of intratumoral immune cells in patients surviving cancer [6]. Nonetheless, specific tumor-infiltrating immune cells are capable of secreting pro-tumor chemokines, leading to a chronic inflammatory state in the host [7]. During inflammation, the constitutively activated signaling pathways of nuclear factor kappa-light-chain enhancer of B cells (NF-κB)

* Corresponding author.

E-mail address: sangdunchoi@ajou.ac.kr (S. Choi).

¹ Contributed equally.

and mitogen-activated protein kinase (MAPK) may initiate a protumorous condition [8]. Of note, NF- κ B has been found to facilitate tumor growth by stimulating the production of proinflammatory cytokines in hepatocellular carcinoma (HCC) and colitis-associated cancer [9]. This NF- κ B-mediated procancerous response could be attributed to the release of antiapoptotic molecules from the activated cells [10,11].

TLRs are the mammalian counterpart of *Drosophila* Toll protein, which regulates the dorsoventral polarity of the embryo and provides innate immune resistance for the fly [12]. These type I transmembrane (TM) glycoproteins contain an extracellular leucine-rich repeat-containing ligand-binding domain, a single-pass TM domain, and a cytoplasmic Toll/interleukin 1 receptor (TIR) homology domain. TLRs can be located in the cell membrane (e.g., TLRs 1, 2, 4, 5, 6, and 10) or in the endosomal membrane (e.g., TLRs 3, 7, 8, and 9) and recognize a broad spectrum of pathogen-associated molecular patterns (PAMPs) or endogenous damage-associated molecular patterns (DAMPs) [13]. Although TLR10 was once considered an orphan receptor, recent evidence indicates that it may recognize bacterial lipopeptides or viral capsid subunits; however, studies have pointed to its regulatory effect on other TLRs [14–16]. Structural reports have revealed that the extracellular domain (ECD) of TLRs exists as a preformed loose dimer that undergoes conformational alterations upon ligand binding, followed by homo- or heterodimerization of the TM and TIR domains. The structural scaffold formed by the dimerization of TIR domains drives adaptor recruitment to initiate a complex cascade of signal transduction for the production of proinflammatory cytokines [17]. Subtypes of TLRs and interleukin 1 receptor (IL-1R) share a common structural feature in the intracellular TIR domains and activate a common signal transduction cascade through NF- κ B. TLRs activate two distinct signaling pathways (Fig. 1): the canonical pathway via the myeloid differentiation primary response 88 (MyD88) protein and the noncanonical pathway through TIR domain-containing adapter-inducing interferon β (TRIF) [18,19].

Concurrent TLR stimulation often results in strong systemic inflammation, thereby recruiting a large quantity of immune cells into the tumor microenvironment [20]. TLRs can manifest either pro- or anti-tumor activities depending on the tumor-infiltrating immune cells and cancer type; however, controversies exist regarding some TLRs in experimental tumor models [4]. Several intrinsic regulatory molecules control the overactivation of TLR signaling through a mechanism called negative regulation [21]; however, genetic variations or polymorphisms in these regulatory genes constitute faulty or dysfunctional auto-downregulation [22]. This situation leads to sustained production of unwanted cytokines for a longer period, causing chronic inflammatory conditions via the adaptive immune response [22]. Therefore, a thorough understanding of the diversified nature of cytokines in the immune system is necessary to develop novel therapeutics to address several unmet medical needs. Considering a TLR as a double-edged sword in the immune system, we will review the role of TLR-regulated cytokines in cancer immunoediting and highlight the clinical use of TLR- and cytokine-modulating therapeutics for the treatment of cancer and autoimmune diseases [23].

2. Cell type specificity and the TLR expression profile

The expression and cellular localization of TLRs are crucial in terms of their ligand recognition and homeostasis. Although TLRs such as TLRs 1, 2, 4, 5, and 6 are trafficked to the cell surface, TLRs 3, 7, 8, and 9 are exclusively localized to the intracellular compartments. Many sophisticated techniques have been utilized to trace the subcellular localization of these receptors, including the tracking of the TLR chimeras and fluorescent labeling of ECDs or TIR domains and adapter proteins such as MyD88 [24–26]. All the TLRs are synthesized from their mRNAs into functional configurations in the endoplasmic reticulum (ER) and are translocated to the Golgi complex followed by trafficking to either the plasma membrane or endosomes [27,28]. A member of the ER-resident HSP90 protein family, gp96, serves as a

general chaperone for the surface-expressed TLRs 1, 2, 4, and 5 and intracellular TLR7 and TLR9 [29]. Unc-93 homolog B1 (UNC93B1), a multipass TM protein, controls endosomal trafficking of TLRs. Apparently, evidence has emerged that UNC93B1 promotes trafficking of glycosylated TLR3 to the plasma membrane [30]. However, endocytosis of the TLR3-ligand complex and the endosomal acidification is essential for an effective immune signaling [31]. Besides, UNC93B1 maintains the balance between TLR9 and TLR7; it biases the nucleic acid recognition in dendritic cells (DCs) by favoring the expression and trafficking of TLR9 instead of TLR7 [32]. Protein associated with Toll-like receptor 4 A (PRAT4 A), an ER-resident protein, also controls the trafficking of TLRs 1, 2, and 4 to the cell membrane and TLR7 and TLR9 to endosomes [33]. The expression profile of TLRs has been studied in various cell types in different pathological states. We discuss the expression pattern of TLRs and induction of the related cytokines in subsection 4.1 and outline this information in Fig. 2.

3. Role of TLR-mediated signaling in cancer progression and control

The pro- or anticancer effect of TLRs can be linked to the immune cells infiltrating a tumor microenvironment and the type of cancer. The TLR stimulation in an experimental tumor model has an antitumor effect by directing immune cells to a tumor site or reducing tumor progression by enhancing tumor cell apoptosis. On the contrary, TLRs expressed by tumor cells have been widely associated with tumor progression. The primary location where host immune cells and tumor cells fight each other is proved to be the tumor microenvironment. To overcome tumor growth, a subset of immune cells is recruited to the tumor site through the cytokine signaling cascades. Owing to their typical plastic nature, tumor-associated macrophages are considered the pivotal players in tumor tissues and can contribute to the modulation of different stages of cancer. Due to similar plasticity, neutrophils that are associated with a tumor are also increasingly recognized as the key players in a tumor microenvironment. Regarded as the major tumor-infiltrating immune cells, various immune cells, including macrophages, B cells, natural killer (NK) cells, NKT cells, T cells, basophils, DCs, and neutrophils are reported to influence cancer progression in terms of proliferation, angiogenesis, immune suppression, metastasis, and cancer-related inflammatory conditions [34,35]. Considering the key fact of the immune microenvironment in a tumor microenvironment, researchers have expressed keen interest to work on immune therapies by targeting immune cells and their associated cancer-driving molecules (cytokines) [34]. The involvement of immune cells in cancer, the type of immune cells, and their respective cytokines under the control of TLRs are summarized in Fig. 2.

Researchers and pharmaceutical companies are continuously in search of TLR- and cytokine-targeting drugs to curb the related autoimmune and cancerous scenarios [36,37]. Briefly, the ligands of TLR2, CBLB612, ISA-201 (Hespecta), and OPN-305 are used in cancer therapy and are being evaluated in phase 2 clinical trials. These drugs are being tested as adjuvant therapies as well as a monotherapy [38,39]. TLR3 has also been targeted via an adjuvant therapy in a variety of cancers. TLR4 has been extensively evaluated as a drug target in a variety of pathological conditions including immune-system-related complications and cancers. The use of high-mobility group box 1 (HMGB1) as a TLR4 activator has been evaluated in recent clinical trials to improve the efficacy of chemotherapeutics in cancer (NCT02044185) [40]. VTX-2337, a TLR8 agonist, has been employed to improve the anticancer effect of doxorubicin in a mouse model of ovarian cancer and a variety of other cancers, including non-small cell lung cancer (NSCLC), pancreatic cancer, colorectal cancer, melanoma, breast cancer, renal cell carcinoma, and other solid tumors [41]. In most of these cases, this drug served as adjuvant therapy or monotherapy. The TLR-targeting therapeutics and the relevant cancer types are listed in Table 1, whereas the implication of cytokines in immune-system-related diseases and

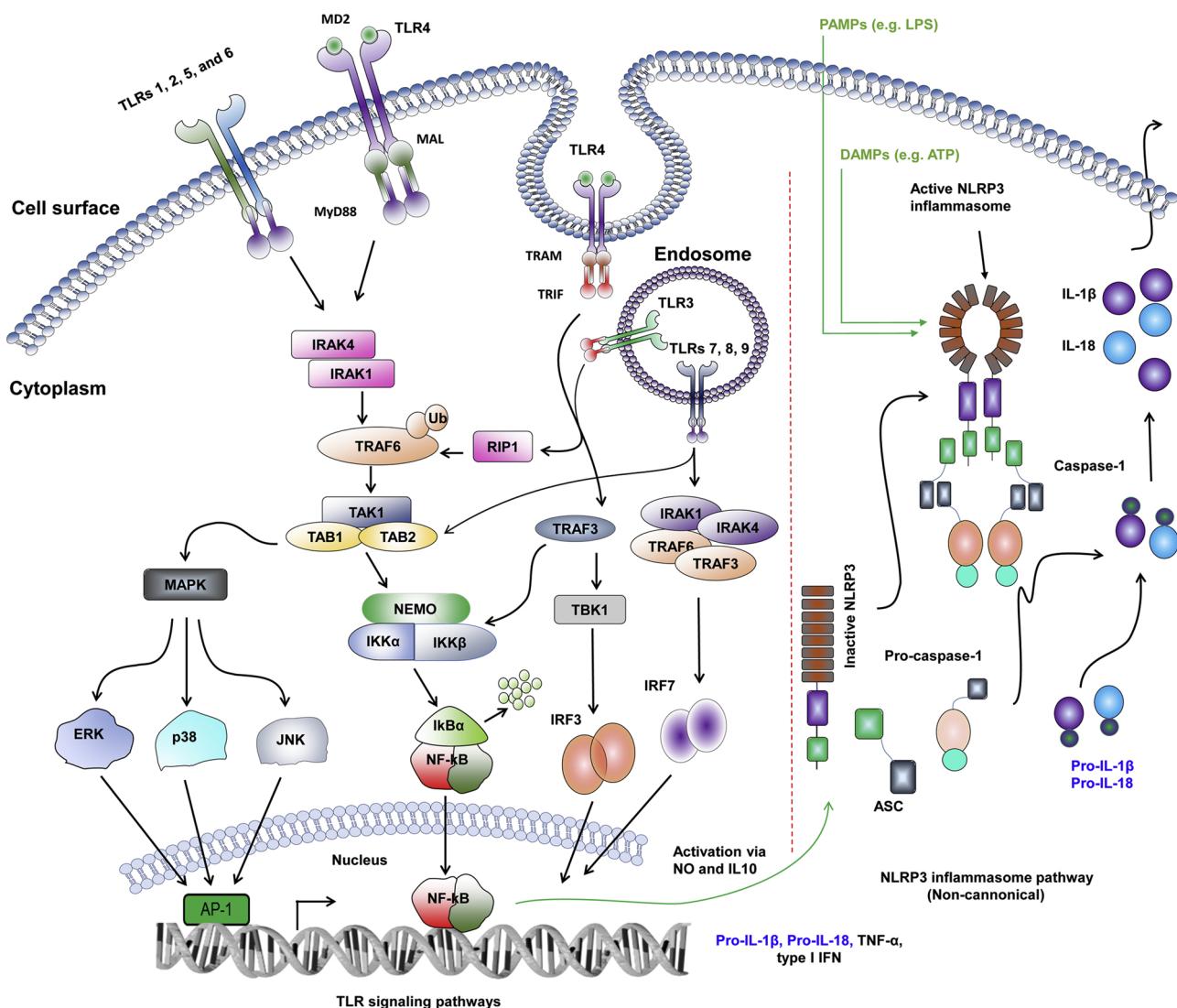


Fig. 1. Overall depiction of the TLR-mediated signaling pathways. In the myeloid differentiation primary response 88 (MyD88)-dependent pathway or the canonical pathway, TLRs (except TLR3) recruit MyD88 through a homotypic TIR-TIR interaction, bridged by TIR domain-containing adaptor protein [TIRAP, also called MyD88 adaptor-like (MAL)]. MyD88 recruits the serine/threonine kinase, known as IL-1R-associated kinase 4 (IRAK4), through the death domain interaction. IRAK4 activates IRAK1 or IRAK2 through phosphorylation at serine or threonine residues. The activated IRAK1 associates with tumor necrosis factor receptor-associated factor 6 (TRAF6), which interacts with a number of adaptors and kinases and ultimately activates the inhibitor of nuclear factor κB (IkB) kinase (IKK) complexes. The IKK complex phosphorylates IkB, leading to its ubiquitination and degradation. Afterwards, the p65/p50 subunits of NF-κB are translocated into the nucleus to assist with transcription of proinflammatory genes, such as tumor necrosis factor α (*TNFA*), interleukin 6 (*IL6*), interleukin 1 β (*IL1B*), and interleukin 18 (*IL18*). In the noncanonical or the TIR domain-containing adapter-inducing interferon β (TRIF)-dependent pathway, TLR3 and TLR4 recruit TRIF through a bridging adaptor, TRIF-related adaptor molecule (TRAM). TRIF stimulates IKK- ϵ (a counterpart of IKK in the canonical pathway) and TRAF family member-associated NF-κB activator (TANK) binding kinase 1 (TBK1). TBK1 activates a transcription factor, IFN-regulatory factor 3 (IRF3), which moves into the nucleus and facilitates transcription of proinflammatory genes, such as *TNFA*, *IL6*, and antiviral type I interferon (*IFN*). Activation of TLR signaling pathways, especially those of TLR4, is associated with the formation of a nucleotide-binding domain, leucine-rich repeat-containing family, pyrin domain-containing 3 (NLRP3) inflammasome. Caspase 1 of the inflammasome complex cleaves pro-IL-1 β and pro-IL-18 into biologically active forms, IL-1 β and IL-18.

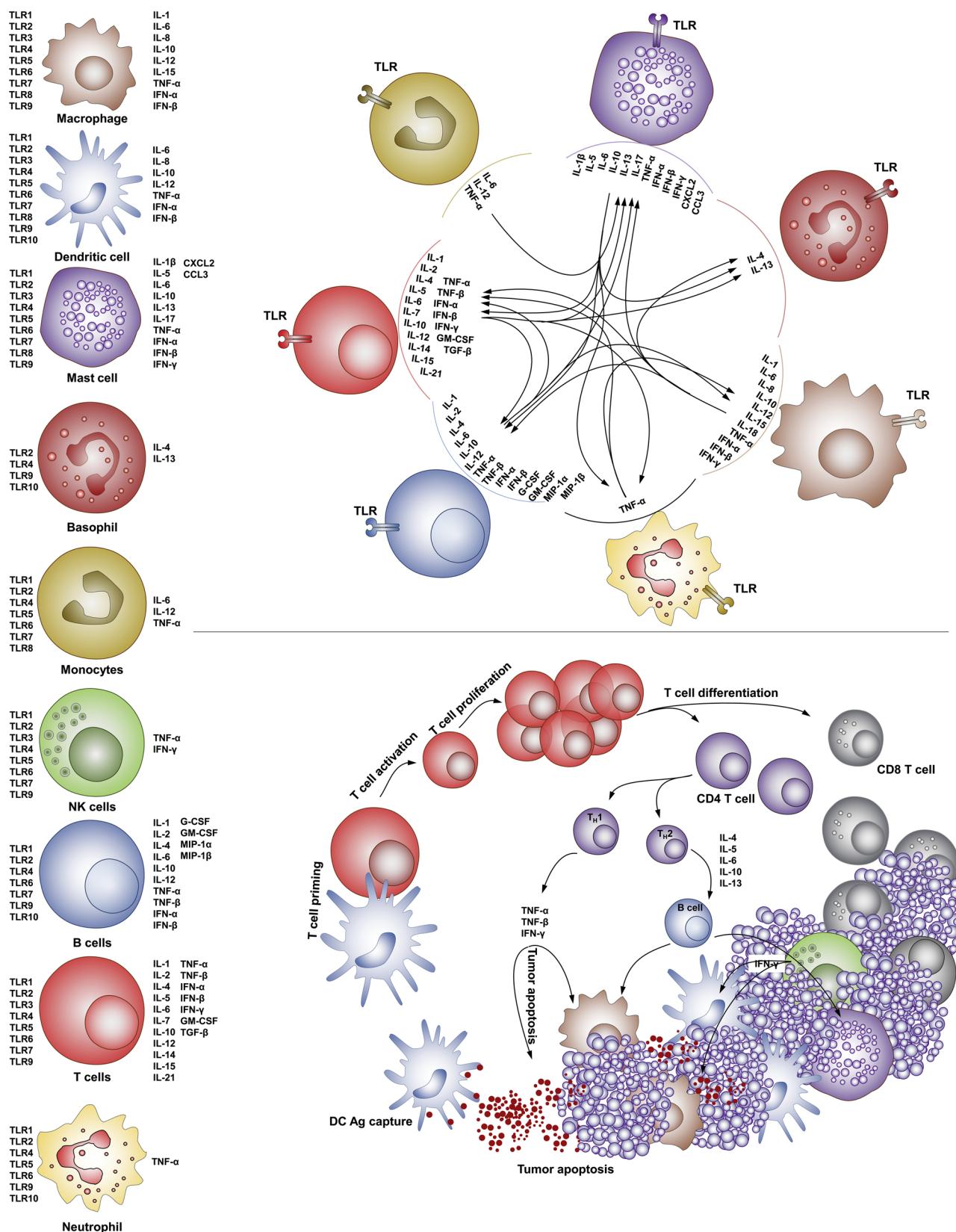
cancers and the relevant therapeutic interventions are thoroughly discussed in Section 5.

3.1. Anticancer role of TLRs

The antitumor capacity of the TLR1/TLR2 heterodimer has been studied in a mouse model of syngeneic Lewis lung carcinoma (LLC) cells. Tregs, which have the capacity for immunosuppression, are inhibited by a TLR1/TLR2 ligand, thereby increasing the activity of cytotoxic T lymphocytes (CTLs) [42]. A TLR2 knockout (TLR2-KO) in mice with colitis-induced colon cancer has also been found to increase tumor cell proliferation. The interferon γ (IFN- γ) level is reduced and IL-17A is upregulated in these mice. The tumor-facilitating effects in

these TLR2-KO mice are mainly attributed to the T helper (T_H) 1 to T_H17 switching [43]. The antitumor effect of TLR2 has also been evaluated in an HCC model, where the mouse survival rate dropped in TLR2-KO mice as compared to the wild type; besides, the number of CD8 $^{+}$ T cells increased and the IL-18 level dropped in the wild-type mice [44].

Numerous studies have confirmed the antitumor effects of TLR3 via direct activation of apoptosis in cancerous cells. PolyI:C, a well-known TLR3 agonist, has been widely used to test the antitumor effect of TLR3 in various cancerous cell lines and *in vivo* cancer models. PolyI:C stimulates transformed tumor cells, where the decreased expression of apoptosis inhibitors, survivin, X-linked inhibitor of apoptosis protein, FLICE (FADD-like IL-1 β -converting enzyme)-inhibitory protein, and B-



(caption on next page)

cell lymphoma extra-large (Bcl-XL) leads to the active apoptosis of tumor cells [45,46]. This kind of tumor stimulation has been confirmed in multiple cancerous cell lines when treated with polyI:C, for example, pharyngeal carcinoma cell lines [47], colon carcinoma cells [48],

human HCC cells [46], human melanoma cell lines [49], and human head and neck squamous cell carcinoma cells [4]. Similarly, the level of survivin decreases and that of proapoptotic caspases 8 and 3 increases in rat models of 2-acetylaminofluorene-induced HCC when treated

Fig. 2. Involvement of TLRs and TLR-regulated cytokines in cancer control and progression. TLRs are expressed by immune cells, such as macrophages, B cells, T cells, neutrophils, dendritic cells (DCs), basophils, natural killer (NK) cells, and mast cells in a cell state-dependent manner. Once activated, these cells release cytokines that coordinate the immune-system communication and control the immune cell proliferation and differentiation. The release of tumor necrosis factors (TNFs) from mast cells and recruitment of neutrophils and other immune cells are considered a crucial function of mast cells in the fight against infections. Secretion of TNF- α and TNF- β by T_H1 cells can inhibit neutrophil apoptosis, activate macrophages, and stimulate vascular endothelial cells, thereby enhancing the phagocytic activity via expression of adhesion molecules. Upon activation by interferon- α (IFN- α) and IFN- β , NK cells produce IFN- γ and TNF- α , which indirectly promote the initiation of a T-cell response through activation of macrophage-associated cytokines at the site of infection and in a tumor microenvironment. B- and T-cell interactions are tightly associated with the function of certain cytokines, such as interleukins (ILs) 1–6 and IL-10. IL-1 participates in the differentiation of B and T cells and regulates production of cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF). IL-12 also stimulates the activation and proliferation of B and T cells and facilitates the production of IFN- γ by T cells and NK cells. IL-4 plays a crucial part in the T_H2 differentiation and regulates B-cell activities by switching immunoglobulin types. IL-8, released by DCs and macrophages at a tumor or infection site, acts as a chemotactic factor to attract neutrophils, T cells, and basophils to the infection site. IL-7, which is known as the growth factor of immature B and T cells, is capable of inducing apoptosis in tumor cells. Aside from the induction of other cytokines like IL-2, IL-4, IL-6, and IL-11, IL-9 is capable of stimulating NK cells and cytotoxic T lymphocytes (CTLs). IL-15, the analogous partner of IL-2, possesses the capacity for enhancing the antitumor activity of CTLs and NK cells. IL-17 produced by CD4 $^{+}$ T cells is crucial for the regulation of multiple other cytokines, thus reinforcing the antibody-dependent tumor reduction. IL-21 produced by CD4 $^{+}$ T cells is crucial for the regulation of multiple other cytokines, thus reinforcing the antibody-dependent tumor reduction. IL-21 promotes the production of T cells, enhances the growth and maturation of NK cells, and expands the B-cell population.

with a TLR3 agonist, BM-06 [50]. The induction of IRF3-dependent caspases 3, 8, and 9 in human prostate cancer cells and in a mouse model is controlled by TLR3 activation [51]. In addition to the direct antitumor effect, TLR3 could set up a tumor-hostile environment by stimulating immune cells. The polyI:C injection in mouse models of HCC and colon carcinoma stimulates CD4 $^{+}$ T cells and recruits CD8 $^{+}$ T cells and NK cells to the tumor microenvironment [48]. M1 macrophages, which have tumoricidal abilities, are reported to increase in number when the protumor myeloid cells are converted into M1 macrophages. This TLR3-dependent M1 induction has been demonstrated to be effective in murine lung cancer models [52]. Besides, substantial antitumor clinical effects of TLR3 in patients with HCC have been found, where TLR3 is overexpressed [53]. Some of the widely used TLR3-activating ligands are listed in Table 1.

Just as TLR3, TLR4 has been extensively studied *in vitro* and *in vivo* with respect to its antitumor abilities. Here, we clarify the anticancer effectiveness of TLR4 as studied in mouse models of various cancerous states. A mixture of group A *Streptococcus pyogenes* strains called OK-432 (picibanil) has been proved to be effective in LLC-grafted mice in the presence of TLR4. The induction of anticancer IFN- γ is lost in the TLR4-deficient mice when they are treated with OK-432 [54]. OM-174, a lipid A analog and TLR4 agonist, can increase the number of CTLs and NK cells at a tumor site in a mouse model of melanoma [55]. HMGB1, released by dying tumor cells that activate TLRs including TLR4, is considered crucial for the adjuvant activity of anticancer chemotherapy and tumor restriction [56]. The use of TLR4-KO mice also supports the pivotal antitumor role of HMGB1 in glioblastoma, skin cancer, and mouse models of mammary tumors [57]. A reduction in IFN- γ levels and increased IL-8, IL-17, IL-23, macrophage inflammatory protein 2, and vascular endothelial growth factor (VEGF) levels are seen in TLR4-deficient mice with skin cancer as compared to wild-type mice [58]. TLR4-deficient mice in mammary tumor models are reported to show lower IFN- γ production and upregulation of VEGF in tumor cells [59]. The significant ability of TLR4 to reduce tumor proliferation has been described in HCC and breast cancer-grafted mouse models. Here, the absence of TLR4 leads to increased metastasis, increased tumor cell proliferation, and an impaired antitumor ability of CTLs [60].

The stimulation of TLR5 with flagellin in an immune microenvironment has been delineated by several studies. T cells with the ability to produce a constitutive TLR5-activating ligand have been reported to reduce tumor volume in a mouse model of melanoma [61]. Several other studies are suggestive of a similar tumor-shrinking effect of TLR5 in various cancerous conditions; they include B- and T-cell lymphoma, lung cancer, and colorectal cancer, where the tumor-limiting effect is derived from the neutrophil infiltration of the tumor microenvironment and tumor necrosis [4]. In a cohort study of patients with NSCLC, overexpression of TLR5 was linked with a better prognosis, perhaps owing to the tumor-limiting ability of TLR5 [62].

The stimulation of TLR7 with imiquimod may help to suppress

chronic lymphocytic leukemia (CLL) via production of proinflammatory cytokines and other stimulatory molecules that increase the tumor cells' sensitivity to CTLs [63]. This TLR7 ligand has yielded less modest results in CLL patients [64]. The activation of TLR7 in immune cells present in the tumor microenvironment has been demonstrated to be effective; this phenomenon has been confirmed in melanoma and a mouse model of breast cancer, where the injection of a TLR7 agonist reduces tumor size and activates intratumoral plasmacytoid DCs (pDCs) [65,66]. The antitumor effect of TLR7 in T-cell lymphoma could be attributed to its increased induction and recruitment of cytotoxic cells to the tumor site [67]. At a tumor site, the induction of type 1 IFN (produced by DCs) that is mediated by a TLR7/TLR8 ligand (gardi-quimod) can increase HCC cell lysis [67].

Owing to functional differences and cell-specific expression of TLR7 and TLR8, these TLRs can be differentially activated in cancerous and infected tissues. During an antiviral response, TLR7 agonists are more potent as compared to TLR8 agonists. This response has been evaluated in terms of IFNs, IFN-inducible T-cell α chemoattractant (I-TAC), and IFN-regulated cytokines in human peripheral blood mononuclear cells [68]. On the other hand, the proinflammatory response is effectively facilitated by the TLR8 agonism as compared to TLR7. VTX-2337, a TLR8 agonist, stimulates tumor necrosis factor α (TNF- α) and IL-12 production in human peripheral blood mononuclear cells, monocytes, and myeloid DCs (mDCs). In addition, this ligand has been found to enhance the antibody-dependent cytotoxic capacity of NK cells through IFN- γ induction [41]. In combination with doxorubicin, VTX-2337 improves its efficacy in ovarian-cancer models [69]. This TLR8 ligand has been widely evaluated in other cancers like melanoma and pancreatic, colorectal, and head and neck cancers; in NSCLCs and breast and renal cell carcinoma, VTX-2337 has been used in combination with other chemotherapeutic drugs but was evaluated as a stand-alone drug in lymphoma (Table 1) [41].

A TLR9 agonist can reduce tumor cell proliferation by enhancing apoptosis in a number of cancerous states, including neuroblastoma, B-cell CLL, and glioma [70–72]. TLR9, when stimulated in ovarian-cancer models, increases mouse survival [73]. Similarly, TLR9 stimulation in lung carcinoma has been found to increase the number of M1 macrophages and to decrease the number of M2 macrophages; besides, the CD8 $^{+}$ T-cell count increased, and that of Tregs dropped in cancer tissues [74]. In patients with renal cell carcinoma, the poor clinical outcomes are attributed to the reduced TLR9 expression in tumor cells and thus reduced immunosurveillance at the tumor site [75]. TLR9 agonists EMD 1201081 and GNKG168 have been evaluated in clinical trials of head and neck cancer and CLL in combination with chemotherapeutic agents and have been found to be ineffective in phase 2 and phase 1 trials, respectively [76,77].

Table 1

TLRs as therapeutic targets in various cancers.

TLR Type	Expressing cell type	Drug Name	Drug Type	NCT Number	Condition or disease
TLRs 1,2, & 6	mDC	CBLB612	Synthetic lipopeptide	NCT02778763	Breast cancer
	pDC		Phase 2		
	NK cell	LV305 (DCVex™)	RNA vaccines	NCT03450122	Cancer
	Neutrophil		Phase 1		
	Monocyte	ISA-201 (Hespecta)	Combination of peptide	NCT02821494	Head & neck tumor
	Eosinophil		Phase 2		
	Macrophage	OPN-305 (Tomaralimab)	Monoclonal antibody	NCT02363491	Myelodysplastic syndrome, pancreatic tumor
	Mast cell		Phase 2		
	Basophil				
	B cell				
	T cell				
TLR3	mDC	Poly-ICLC (Hiltonol)	Synthetic dsRNA	NCT01976585	Low-grade B cell lymphoma
	pDC		Phase 1 & 2		
	NK cell	Poly-ICLC + LV305	Synthetic dsRNA & drugs	NCT01079741	Melanoma
	Neutrophil		Phase 1 & 2		
	Monocyte	Poly-ICLC	Synthetic dsRNA	NCT02643303	Head & neck squamous cell carcinoma, sarcoma, Merkel cell carcinoma, cutaneous T cell lymphoma, melanoma, renal, bladder, breast & prostate cancer
	Eosinophil		Phase 1 & 2		
	Macrophage				
	Mast cell				
	Basophil	Poly-ICLC	Synthetic dsRNA	NCT02452775	Primary ovarian cancer, fallopian tube cancer, primary peritoneal cancer
	B cell				
	T cell	Poly-ICLC + Romidepsin	Synthetic dsRNA & Depsipeptide	NCT02061449	Cutaneous T cell lymphoma
		Poly-ICLC + Pembrolizumab	Synthetic dsRNA & Antibodies	NCT02834052	Metastatic colon cancer, solid tumor
			Phase 1 & 2		
TLR4	mDC	GLA-SE (Glucopyranosyl lipid adjuvant, G 100)	Glycolipid	NCT02320305	Stage IIA-IV skin melanoma
	pDC		Phase 1		
	NK cell	GLA-SE	Glycolipid	NCT02180698	Stage III/IV adult soft tissue sarcoma
	Neutrophil		Phase 1		
	Monocyte	GSK1795091	Glycolipid	NCT02798978	Cancer
	Eosinophil		Phase 1		
	Macrophage	GLA-SE	Glycolipid	NCT02501473	Follicular lymphoma (marginal zone allowed during dose escalation only)
	Mast cell		Phase 1 & 2		
	Basophil	CX-01 (ODSH, PGX-100)	Polysaccharide	NCT02995655	Refractory myelodysplastic syndrome and acute myeloid leukemia
	B cell		Phase 1		
	T cell	GLA-SE	Glycolipid	NCT02035657	Merkel cell carcinoma
		BGLP-40 (ONT-10)	Glycolipid	NCT02270372	Advanced breast and ovarian carcinoma
		BGLP-40 (ONT-10)	Glycolipid	NCT01556789	Solid tumors
		BGLP-40 (ONT-10)	Glycolipid	NCT01978964	Solid tumors
		G-305 (NY-ESO-1, CMB305)	Combination of protein & Small molecule	NCT02015416	Cancer
		GLA-SE + CMB-305	Glycolipid	NCT02609984	Sarcoma and its various forms
		GLA-SE + CMB-305	Glycolipid	NCT02387125	Sarcoma, melanoma, non-small cell lung carcinoma (NSCLC)
			Phase 2		
TLR5	mDC	Mobilan (M-VM3)	Recombinant protein	NCT02844699	Prostate cancer
	pDC		Phase 1 & 2		
	NK cell		NCT02654938		
	Neutrophil		Phase 1		
	Monocyte	Entolimod + radiation therapy)	Recombinant protein	NCT01527136	Unspecified adult solid tumor
	Eosinophil		Phase 1		
	Macrophage	Entolimod (radiation therapy)	Recombinant protein	NCT01728480	Mucositis, various types of squamous cell carcinoma
	Mast cell		Phase 1		
	T cell				
TLR7	mDC	Imiquimod (R837)	Small molecule	NCT01421017	Metastatic & recurrent breast cancer
	pDC		Phase 1 & 2		
	NK cell	Imiquimod	Small molecule	NCT00453050	Melanoma, metastatic cancer
	Neutrophil		Phase 1		
	Monocyte	Imiquimod	Small molecule	NCT00899574	Breast cancer, breast neoplasms
	Eosinophil		Phase 2		
	Macrophage	852 A (3M-852 A)	Small molecule	NCT00319748	Breast, ovarian, endometrial, & cervical cancer
	Mast cell		Phase 2		
	T cell	Imiquimod	Small molecule	NCT00941811	HPV (cervical cancer)
		Imiquimod	Small molecule	NCT01453179	Basal cell carcinoma approved

(continued on next page)

Table 1 (continued)

TLR Type	Expressing cell type	Drug Name	Drug Type	NCT Number	Condition or disease
TLRs 7, 8, & 9		Resiquimod + poly-ICLC	Small molecule, synthetic dsRNA	NCT01204684	Glioma, anaplastic astrocytoma, anaplastic astro-oligodendrogloma
		Poly-ICLC, Resiquimod,	Small molecule, synthetic dsRNA	NCT02126579	Melanoma & its metastatic mucosal variants
		MEDI9197 + Durvalumab	Small molecule + Anti-PD-L1 mab	NCT02556463	Solid tumors, cutaneous T cell lymphoma
		Resiquimod + NY-ESO-1	Small molecule	NCT00821652	Tumors
		IMO-8400 (Bazlitoran)	Oligonucleotide antagonist	NCT02252146	Diffuse large B cell lymphoma
TLR8	mDC pDC NK cell	VTX-2337 (Motolimod)	Small molecule	NCT01334177	A variety & different stages of metastatic squamous neck cancer with occult primary squamous cell carcinoma
	Neutrophil	VTX-2337	Small molecule	NCT02650635	For various types & stages of colorectal, pancreatic, breast, melanoma, NSCLC, pancreatic, renal cell carcinoma & solid neoplasm
	Monocyte			Phase 1	
	Eosinophil				
	Macrophage	VTX-2337	Small molecule	NCT01294293	Various types of ovarian cancers & fallopian tube carcinoma, recurrent ovarian carcinoma
	Mast cell			Phase 1	
	T cell	VTX-2337	Small molecule	NCT01666444	Epithelial ovarian cancer, fallopian tube cancer, primary peritoneal cancer
		VTX-2337 + radiotherapy	Small molecule	NCT01289210	Low-grade B cell lymphoma
		VTX-2337	Small molecule	NCT01836029	Carcinoma, squamous cell of head & neck
		VTX-2337 + Durvalumab	Small molecule + Anti-PD-L1 mab	NCT02431559	Ovarian cancer
TLR9	mDC pDC NK cell	MGN1703 (Lefotilimod) + Ipilimumab	DNA based molecule + Anti CTLA-4 mab	NCT02668770	Melanoma
	Neutrophil	SD-101 (SD101-Dynavax)	CpG-C class ODN	NCT02254772	Lymphoma and its various forms
	Monocyte	SD-101	CpG-C class ODN	NCT02927964	Grade 1/2/3 follicular lymphoma & recurrent & refractory follicular lymphoma
	Eosinophil			Phase 1 & 2	
	Macrophage	CpG vaccine (autologous tumor cell)	Oligonucleotide	NCT00780988	Colorectal neoplasms, anal, colon, & rectal cancers
	Mast cell			Phase 1	
	T cell	CYT003 (ARB 1598)	Oligonucleotide	NCT01673672	Moderate to severe allergic asthma
		DUK-CpG-001	Single-stranded synthetic DNA molecule	NCT02115126	Hodgkin lymphoma, non-Hodgkin lymphoma
		CpG-7909 (Agatolimod)	single-stranded synthetic DNA molecule	NCT00185965	Non-Hodgkin lymphoma
		GNKG168	CpG-C class ODN	NCT01743807	Relapsed acute lymphoblastic myelogenous leukemia

3.2. Procancer role of TLR signaling

Considering the double-edged sword property of TLRs in a tumor immune environment, TLRs have been proved to have cancer-promoting abilities as well [78]. A TLR2-KO or treatment of mice with anti-TLR2 antibodies has been reported to abrogate tumor proliferation in HCC and head and neck carcinoma [79,80]. In addition to the tumor expression of TLR2, host cells expressing TLR2 can facilitate tumor progression as observed in mouse models of a gastric tumor, where TLR2 was expressed by host tissues [81]. TLR2 deletion in intestinal and breast cancer models has been proved to reduce spontaneous formation of tumors, which otherwise are aggressive. Similarly, lymphomas in mouse models with or without TLR2 exhibit pro- or anti-tumor effects, respectively [82,83]. The activation of TLR3 as a negative regulator of cancer has been studied for a long time; however, few studies have revealed the cancer-promoting abilities of TLR3. The activation of TLR3 can promote activation of breast cancer stem cells (CSCs). These CSCs were activated through the TLR3-dependent β -catenin and NF- κ B signaling; simultaneous inhibition of these two pathways abrogated the CSC activation [84]. Another study has linked the poor clinical outcomes of prostate carcinoma with TLR3 activation

[85].

TLR4 has been largely associated with cancer progression as suggested by many *in vitro* and *in vivo* tumor models. Lipopolysaccharide (LPS) can increase the stimulation of IL-6 and IL-8 through tumor cell-expressed TLR4 and thus promote tumorigenesis [86]. Besides, TLR4 stimulation enhances tumor proliferation and promotes tumor cell resistance to apoptosis, as seen in models of lung, prostate, head and neck cancer, and HCC [87,88]. TLR4 has been associated with the increased induction of VEGF, IL-8, IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF) in tumor cells [89,90]. In colon cancer, TLR4 enhances the NO and IL-6 production by tumor cells, reduces NK-cell and T-cell proliferation, and enhances the resistance of a tumor to CTls [91]. Amphiregulin, an epidermal growth factor ligand, is involved in the production of cyclooxygenase 2 and prostaglandin 2 by the macrophages in lamina propria. These two substances have been linked to the TLR4-dependent formation of colitis-associated colorectal tumors [92]. In addition to these findings, overexpression of TLR4 has been associated with poor clinical outcomes in breast and colorectal cancers and in pancreatic ductal adenocarcinoma [93,94]. TLR5 correlates with tumor cell aggressiveness in gastric cancer and in adenocarcinoma of salivary glands. This effect is related to the increased induction of NF-

κ B and extracellular signal-regulated kinase (ERK) signaling [95]. Similarly, the TLR5-dependent increase in the induction of IL-6 has protumor effects in a mouse model of sarcoma [96].

There are several studies that indicate the involvement of TLR7 in tumor progression where tumor cells express TLR7. The increase in IL-6 production by TLR7 has been reported to increase tumor cell survival in human myeloma cell lines [97]. Both in human and murine models of pancreatic cancer, the expression of TLR7 has been associated with accelerated tumor progression [98]. Similarly, strong expression of TLR7 has been linked to the poor prognosis of patients with colorectal cancer [99]. Similar outcomes have been uncovered in an NSCLC prospective study of two cohorts, evaluated independently. Strong expression of TLR7 has been implicated in a poor prognosis and increased resistance to chemotherapy [100].

Furthermore, TLR9 has been positively associated with cancer progression and some poor prognoses in cancer patients. For example, overexpression of TLR9 by tumor cells in glioblastoma and prostate carcinoma has been associated with poor prognosis [101]. Besides, the overexpression of TLR9 has been associated with breast and ovarian cancer progression [102]. The tumor cells in myeloma and in prostate and esophageal cancers have been reported to be resistant to apoptosis and to undergo increased proliferation and migration owing to the TLR9 expression [103]. The tumor model of prostate carcinoma has also revealed increased inflammation due to TLR9 expression [104].

3.3. Controversial role of TLRs in cancer

Even though an enormous amount of data indicates case-dependent tumor-limiting or tumor-facilitating abilities of TLRs, some controversies do exist regarding the participation of TLRs in cancer. TLR2 has been confirmed in a number of studies to have both the anti- and protumor effect [50,80,91]. Nonetheless, these studies have been performed on different tumor models. Two independent studies on intestinal carcinoma have uncovered the controversial effects of TLR2. The first one points to effective tumor growth inhibition whereas TLR2-KO mice exhibit tumor progression and an increase in proinflammatory cytokine production in the tumor microenvironment [43]. On the other hand, the overexpression of TLR2 in human colon cancer is associated with poor clinical outcomes [83]. The antitumor effect of TLR3 has been well studied; however, poor clinical outcomes have also been reported where overexpression of TLR3 was maintained [85]. The dual role of TLR3 has been reported in pharynx metastatic cell line, where TLR3 stimulation leads to an enhanced cell migration and tumor progression by increasing the expression of tumor-promoting genes, urikinase-type plasminogen activator receptor (uPAR) and RAR-related orphan receptor B (RORB) [105]. The involvement of TLR4 in HCC is controversial as well. In a model of diethylnitrosamine (DEN)-induced HCC, TLR4 mediates the protumor effect in wild-type mice, whereas TLR4-deficient mice in the same model manifest enhanced growth of DEN-induced HCC. The difference could be due to the DEN concentration and the duration of treatment [106]. Similarly, the activation of TLR7 in CLL cells is known to make these cells sensitive to CTLs but was found to promote tumor proliferation in another study [63,107]. The role of TLR4 in cervical cancer remains debated. A higher expression level of TLR4 was observed in HPV-positive cells as compared to HPV-negative cells. This rise in TLR4 expression had facilitated cell proliferation and resistance to apoptosis in HPV-related cervical cancer [108]. TLR9 has been suggested to play a controversial part in lung carcinoma [74,109]. A systematic review was performed to link different cancers with TLRs; an enhanced cell survival and DNA-damage was reported in the absence of TLR2, while up-regulation was also associated with elevated tumorigenesis [110]. TLR4 and TLR9 are considered to have a positive relationship with tumor development, even though anti-tumorigenic effects have been associated with both receptors [110]. The TLR adaptor, MyD88, holds similar tumor-facilitating and tumor-limiting capacities [111]. Thus, care must be taken

while designing anti-cancer drugs targeting TLRs and their adopter molecules.

4. Cell type- and TLR-dependent cytokine expression

Cytokines are signaling molecules secreted by cells in response to external stimuli. They regulate cell differentiation, proliferation, angiogenesis, and inflammatory responses. Activated TLRs initiate downstream signaling for the production of various types of cytokines through multiple transcription factors including but not limited to NF- κ B, IRF3, and IRF7. The released cytokines may serve as autocrine, paracrine, and/or endocrine factors that control numerous cell type-specific responses by binding to their target receptors [112]. Cytokines can be (I) interferons, which perform antiviral and antiproliferative functions; (II) ILs, which exert proinflammatory and growth-inducing activities; (III) chemokines, which induce chemotaxis, recruit leukocytes, and promote inflammation; (IV) colony-stimulating factors (CSFs), which are important for the proliferation and differentiation of hematopoietic progenitor cells; or (V) TNF, which is proinflammatory in nature and activates CTLs. Cytokines perform diverse intercellular functions; therefore, their activity is greatly influenced by the presence of other cytokines in the environment [113]. These signaling molecules share limited intrafamily sequence identity but have a remarkable structural similarity. The conserved structural folds in these proteins enable them to carry signals through common intercellular communication pathways. The complex cytokine networks often overlap, initiating alternate or redundant pathways that create challenges for a clinical intervention into a specific cytokine response.

4.1. Differential expression of TLRs and cytokines in endothelial cells (ECs)

Variation in the expression of TLRs on a particular cell type can affect the outcomes and pathogenesis of an inflammatory condition. For example, ERK5 serves as a key factor in the differential regulation and signaling of TLR2 in human monocytes and ECs [114]. Mitogen-activated protein kinase kinase 1 [MAP2K1; also known as MEK1 (MAP kinase/ERK kinase 1)], however, works counter to ERK5 in monocytes and ECs: it promotes TLR2 signaling in monocytes and negatively regulates it in ECs. Besides, TLR2 and TLR4 are strongly expressed on the surface of macrophages, neutrophils, NK cells, monocytes, and DCs, whereas ECs can express them intracellularly as well [114,115]. Multiple types of human ECs, including human umbilical cord vein ECs (HUVECs), human microvessel ECs (HMVECs) from the brain, HMVECs from lungs, liver HMVECs, and human coronary artery ECs lack TLR8 expression. Conversely, monocytes abundantly express TLR8 [116]. TLR2 is moderately expressed by the HMVECs in the brain but not in the liver. Besides, other TLRs such as TLRs 5, 6, 7, 9, and 10 are moderately expressed in ECs but highly expressed on monocytes [116].

In addition to the fact that TLRs have a cell type-specific expression pattern, they can exert divergent cytokine induction actions in the same manner. For example, TLR4 and TLR2, when activated in monocytes and macrophages with LPS and Pam₃Cys, respectively, robustly induce IL-6 and TNF- α secretion. By contrast, only a small amount of these two cytokines is released by HUVECs and lung HMVECs under similar conditions; instead, other factors, including CSF2, IL-8, IL-6, CSF3, and intracellular adhesion molecule 1 (ICAM-1) are abundantly released [117–119]. ECs secrete multiple CSFs, including GM-CSF and granulocyte colony-stimulating factor (G-CSF) and cytokines like IFN- β , IL-1 α , IL-6, IL-10, IL-28, and IL-29 after they are stimulated with TLR ligands [117,120]. On the other hand, leukocytes have been frequently reported to secrete many cytokines, including IL-1 α , IL-1 β , IL-2, IL-6, IL-9, IL-10, IL12, IL13, IL15, IFN- α , IFN- β , IFN- γ , G-CSF, transforming growth factor β 1 (TGF- β 1), and TNF- α , upon TLR stimulation [120].

4.2. Expression of TLR-dependent cytokines in NK cells

The expression pattern of TLRs and their underlying cytokine expression in NK cells also differ from those of macrophages and monocytes. Studies have revealed that compared to TLRs 2, 3, 5, and 6, TLR1 is highly expressed in isolated NK cells at the mRNA level [121]. Nonetheless, resting NK cells are confirmed to robustly express TLR2 mRNA as compared to TLR3 and TLR4, whereas TLR8 is not detectable [122]. TLR2 and TLR4 are expressed both extracellularly and intracellularly in human uterine NK cells [123]. At the protein level, TLRs 1, 2, 3, 4, and 9 are known to be expressed in adult human NK cells with the phenotype “cluster of differentiation (CD) 56^{bright} and CD56^{low}” [123,124].

Unlike immune cells such as neutrophils, macrophages, and monocytes, which secrete abundant cytokines upon TLR induction, NK cells require cytokines in addition to the TLR-activating ligands for their proper activation and cytokine induction. For example, NK cells are activated in the presence of TLR ligands (Pam₃CSK₄, CpG-DNA, and LPS) and a combination of accessory cytokines, such as IL-2–IL-18, IL-2–IL-12, or IL-18–IL-15; as a result, GM-CSF and IFN-γ are secreted [120,124]. As an indication of the TLR2 expression on NK cells, the induction of IFN-γ and expression of CD25 and CD69 have been reported, when NK cells are activated with lipoteichoic acid (LTA) [125]. A similar approach has been used to highlight the importance of TLR3 and TLR9 in freshly isolated human peripheral NK cells; a robust antitumor action is observed when IFN-γ and TNF-α are released after these cells are stimulated with TLR3 or TLR9 ligands [120]. A number of other studies have confirmed the use of TLR ligands as an effective NK-based immunotherapy in cancer settings; however, TLR-mediated NK-cell activation and the induction of IFN-γ in addition to the cytokine released by the surrounding immune cells have also been demonstrated to be crucial for immune-system-mediated inflammatory conditions like sepsis [126].

4.3. Expression of TLR-dependent cytokines in DCs

TLR-mediated regulation and function of DCs have a major impact on the pathogenesis of (and recovery from) microbial infections and inflammatory diseases. The regulation of DC activation by means of TLR agonists is known to be effective in animal models of hemorrhagic shock, where the symptoms of pneumonia are alleviated [127]. pDCs are known to express TLR7 and TLR9 and to mediate immune responses through MyD88; this activation requires the physical interaction of MyD88 with TRAF6 and IRF7 [128,129]. Human mDCs express all TLRs except TLR9, whereas human pDCs express a large amount of TLR9 and a normal amount of TLR10 but lack TLRs 2–6 and 8 [130]. DCs derived from monocytes abundantly express TLR4, a medium amount of other TLRs, and no TLR9 or TLR10 [130].

Depending on the TLR expression pattern, mDCs and monocyte-derived DCs are reported to produce IL-12 and IFNs, respectively, when stimulated with LPS [131]. DCs stimulated with TLR1/TLR2 or TLR2/TLR6 ligands can undergo maturation and secrete several cytokines, including IL-6, -8, -10, and -12 and TNF-α [130].

4.4. Expression of TLR-dependent cytokines in mast cells

Mast cells are well known for their crucial regulatory function in both the innate and adaptive immune system. The expression pattern of TLRs on human and mouse mast cells, including bone marrow mononuclear cells (BMMCs), connective-tissue mast cells, and fetal-skin-derived mast cells, and the underlying impact of TLR ligands has been studied in depth [132,133]. Multiple TLRs including TLRs 1, 3, and 5–10 are expressed in human mast cells at the mRNA level [134,135].

Human mast cells expressing TLR2 and TLR4 show different responses to LPS and lipoteichoic acid or peptidoglycan and respectively release ILs 5, 10, and 13 and TNF-α [136]. TLR3-expressing human

mast cells do not release IL-1β, IL-5, or TNF-α but release IFN-α and IFN-β after their activation with polyI:C or viruses [134]. When stimulated with ligands of TLRs 3, 7, or 9, human fetal-skin-derived mast cells release chemokine (C-X-C motif) ligand 2 (CXCL2), chemokine (C-C motif) ligand 3, IL-6, and TNF-α; however, these cytokines and chemokines are not observed when mouse BMMCs are stimulated by means of the same TLR ligands [133]. Mouse peritoneal-cell-derived mast cells in contrast to BMMCs release large amounts of pro- and/or anti-inflammatory cytokines, including IFN-γ, TNF-α, IL-1, IL-6, IL-10, and IL-17 when activated with TLR2 ligands, lipoteichoic acid, and macrophage-activating lipopeptide 2 [132].

4.5. Expression of TLR-dependent cytokines in T & B cells

Almost all TLRs found in human cells are expressed at the mRNA level in human peripheral blood T cells; however, TLRs 2–5 and 9 are reported to be expressed in these cells at the protein level [137,138]. TLR1 and TLR9 are known to be highly expressed in CD4⁺ T cells, whereas CD8⁺ T cells show abundant TLR3 and TLR4 expression [139]. This TLR expression level is probably cell state dependent because T cells extracted from an infected person have a different expression pattern. Studies on patients with recurrent tonsillitis and tonsillar hyperplasia suggest that CD4⁺ T cells have a lower TLR9-expression level, and higher TLR2 expression is seen in CD8⁺ T cells [139]. Human Tregs (CD25⁺ CD4⁺) express extracellular TLRs, including TLRs 2, 5, and 8 [140]. Depending on the mouse strain, TLR expression and the expression of underlying cytokines can vary in murine T cells. For example, naïve CD8⁺ T cells isolated from B6 mice express TLRs 1, 2, 6, and 9 at the mRNA level, but TLR4 mRNA is undetectable. CD4⁺ CD45RB^{high} cells from B6 mice express little or no TLRs 4, 5, and 9 at the mRNA level but highly express TLRs 1–3 and TLRs 6–8 [141,142]. CD8⁺ T cells isolated from BALB/c mice express only TLR2 at the protein level, while CD4⁺ T cells express TLRs 3, 4, and 9.

TLRs in B cells also have a differential expression pattern as compared to other TLR-expressing immune cells. The involvement of TLR ligands in B-cell induction and antibody production has a great practical value. TLR9 ligands have been employed as adjuvants in vaccination procedures, where they enhance the antibody response by activating B cells. Dozens of cytokines and chemokines have been demonstrated to be expressed by B cells upon activation with TLR ligands, including but not limited to macrophage chemotactic protein 1 (MCP1), GM-CSF, G-CSF, macrophage inflammatory proteins α & β, IL-1α & -1β, IL-6, IL-8, IL-10, and IL-13 [143,144]. TLR-dependent expression of cytokines and chemokines by T and B cells plays a vital role in the pathophysiology of immune-system-related diseases such as sepsis and rheumatoid arthritis (RA).

4.6. Expression of TLR-dependent cytokines in glial cells

Upon exposure to the PAMPs or DAMPs, microglia are known to increase own expression of TLRs 1–5 and TLRs 7–9; these TLRs are endogenously expressed in microglia [145,146]. Except for TLR4, which has been linked to some controversies regarding its expression in astrocytes, other TLRs, including TLRs 1–3, 5, and TLRs 7–9 are expressed by astrocytes [147–149]. Microglia and astrocytes are therefore located at the neuroimmune interface in the central nervous system, and this arrangement is of special concern when it comes to mechanical injuries and pathogen recognition and combating [150].

Not only human but also murine neuronal cells express TLR3, which induces the secretion of IFN-β, TNF-α, IL-6, CXCL10, and CCL5 when activated with dsRNA [151]. A similar response has been detected in microglia where TLR3 activation induces IL-6, IL-10, IL-12, CXCL10, TNF-α, and IFN-β. TLR4 induces a similar amount of CXCL10 and IL-10 in human microglia, but other cytokine responses are either minor or absent as compared to TLR3 activation. A TLR2-mediated response, however, is dominated by induction of IL-6 and IL-10 [152]. TLR3

activation in astrocytes can induce a cytokine expression profile similar to that observed in microglia via production of IL-6, CXCL10, and IFN- β . Another study has elucidated the TLR-dependent induction of multiple cytokines in microglia and astrocytes upon exposure to the peroxisome proliferator-activated receptor γ (PPAR- γ) agonists, which synergized with, inhibited, or had no effect on these cytokines. Upon activation with Pam₃CSK₄ (a TLR1/TLR2 ligand), the induction of IL-12, chemokine (C-X-C motif) ligand 2 (CCL2), and TNF- α is seen in microglia, and a similar pattern is observed in astrocytes as well. Other TLR ligands, including polyI:C (TLR3), LPS (TLR4), flagellin (TLR5), ssRNA (TLR7/TLR8), and oligodeoxynucleotide (ODN; for TLR9) were also evaluated in the same study, revealing secretion of the same cytokines as those induced by TLR2 [153].

5. Structural insights into the TLR-associated cytokines and their therapeutic value in cancer and autoimmune diseases

5.1. TNF- α

TNF- α is a proinflammatory cytokine that is mainly secreted by macrophages and DCs in response to trauma or infections [154]. It was first cloned in 1984 and described as a soluble factor that causes necrosis of tumor cells [155]. Subsequent discoveries have revealed an inflammatory potential of TNF- α in endotoxin-induced lethal septic shock [156]. Excessive production of TNF- α is involved in numerous pathological conditions, including inflammatory and autoimmune diseases and apoptosis or necroptosis of healthy cells. Even though TNF- α has been intrinsically endowed with antitumor ability, it has been implicated in a number of severe inflammatory diseases. Studies have revealed that TNF- α could transform normal cells into malignant ones through the release of proangiogenic factors, antiapoptotic proteins, and metastatic markers, thus promoting tumor survival [157]. Consequently, the efficacy of anti-TNF biologics has been widely studied in patients with various cancerous diseases [158].

TNF- α is expressed as a 233-amino acid TM polypeptide (in the form of a stable homotrimer) on the cell membrane. Proteolytic cleavage by TNF- α -converting enzyme (TACE) releases the trimer into the extracellular environment in soluble form. Triangular, pyramid-shaped soluble TNF- α is composed of two antiparallel eight-stranded β -sheets (one inner and one outer sheet) that have a “jellyroll” topology, a representative fold of the TNF family [159]. The inner sheet is formed by strands, B"-B-I-D-G, while the outer sheet is formed by strands, C"-C-H-E-F (Fig. 3A, B). TNF- α is recognized by two membrane-bound receptor subtypes, tumor necrosis factor receptor 1 (TNFR1) and TNFR2, which trigger distinct signaling pathways. Recognition of TNF- α by TNFR1 initiates proinflammatory and apoptotic cascades, whereas TNFR2 activates cell survival and proliferation pathways [160]. The crystal structure of the TNF-TNFR2 complex has revealed that the TNF- α -binding regions of TNFR1 and TNFR2 overlap [161]. Three TNFR molecules encircle the TNF trimer, where one receptor contacts two ligand monomers at once. The TNF-binding interface of the receptors can be divided into two regions, regions 3 and 4. Region 3 of TNFR2 consists of the A1 module of cysteine-rich domain 2 (CRD2), and region 4 contains the B2 module of CRDs 2 and 3. Although TNFR1 and TNFR2 have similar structures, TNF-binding residues differ in their electrostatic properties. Region 3 of TNFR2 is composed of acidic residues (D54, E57, and E70), forming a negatively charged patch, whereas region 4 contains basic residues (R77, K108, and R113) forming a positively charged patch. On the other hand, region 3 (residues H69, R53, and E56) and region 4 (residues R77, E79, and K78) of TNFR1 contain a combination of both positively charged and negatively charged residues (Fig. 3C).

To date, the United States Food and Drug Administration (FDA) has approved five TNF- α -targeting biologics to treat RA, inflammatory bowel disease, psoriasis, psoriatic arthritis, ankylosing spondylitis, and juvenile idiopathic arthritis [162]. Etanercept (Enbrel $^{\circledast}$) was the first

FDA-approved biologic sold on the market for the treatment of RA (Table 2). It is a recombinant fusion protein created by combining soluble TNFR2 with the Fc fragment of immunoglobulin G1 (IgG1) [163]. This 150 kDa protein binds to TNF- α with high affinity and is used to treat TNF- α -mediated pathological conditions, including RA, psoriasis, and spondylitis [164]. Infliximab (Remicade $^{\circledast}$) is a chimeric monoclonal antibody (mAb) composed of 75% of the complement-fixing human IgG1 constant region and 25% of the murine antigen-binding variable region [165]. This mAb neutralizes both soluble and membrane-bound TNF- α by preventing their interaction with cognate receptors. The FDA has approved infliximab for the treatment of autoimmune diseases, such as Crohn's disease [166], ulcerative colitis [167], spondylitis [168], psoriasis, and RA [169]. The crystal structure of the TNF- α -infliximab complex has revealed that the loop connecting strands E and F (EF loop) of TNF- α is crucial for the antibody binding. Nonetheless, the EF loop has a poor electron density in the crystal structure of the TNF-TNFR2 complex, suggesting that the flexible loop might be essential for the antibody affinity but not for the receptor binding [170]. Adalimumab (Humira $^{\circledast}$) is a TNF- α -targeting, FDA-approved human mAb that prevents the interaction of TNF- α with its receptor. It serves as a medication for the treatment of autoimmune diseases, including RA [171], psoriatic arthritis [172], spondylitis [168], and Crohn's disease [173]. Certolizumab (Cimzia $^{\circledast}$) and golimumab (Simponi $^{\circledast}$) are the most recent anti-TNF biologics approved by the FDA to treat RA, psoriasis, spondylitis, Crohn's disease, and ulcerative colitis. Certolizumab is a PEGylated Fab domain of a human anti-TNF mAb [174], whereas golimumab is a fully human mAb [175]. The anti-TNF biologics have shown modest therapeutic outcomes in cancer therapy as well. Particularly, infliximab and etanercept have prolonged the stabilization of renal cell carcinoma, breast cancer, and ovarian cancer in certain patients via downregulation of IL-6 and CCL2 [176].

5.2. IL-6

IL-6 is a pleiotropic, multifunctional cytokine that stimulates both hematopoietic cells and B and T lymphocytes. It belongs to a family of cytokines that share a common structural characteristic and receptor-binding mechanism. IL-6 has been found to exert both proinflammatory and anti-inflammatory (myokine) functions [177]. Growing evidence indicates an antitumor role of IL-6, which mobilizes tumor-limiting T cells in the tumor microenvironment. IL-6 can regulate the number of lymphocytes in tumor tissues by enhancing their survival and proliferation. Nonetheless, IL-6 is generally considered a malevolent actor in a tumor microenvironment, where it promotes tumor growth. The protumorigenic influence of chronic IL-6 upregulation has been confirmed in numerous mouse models. Intrinsically, IL-6 can support cancer cell proliferation, metastasis, and survival by the release of downstream effector molecules; while extrinsically, IL-6 can sustain a protumor environment by supporting angiogenesis and tumor evasion of immune surveillance [178].

The three-dimensional structure of IL-6 contains a four-helix bundle (denoted as A–D) connected by loops (AB, BC, and CD), as observed in other members of the family (Fig. 3D) [179]. The helices are packed together by an internal network of hydrophobic contacts spanning the entire bundle axis. IL-6 is structurally similar to G-CSF and human growth hormone with C α root mean square deviations of 1.1 and 1.4 Å, respectively. IL-6 receptor (IL-6R) consists of two subunits. The first subunit is an 80 kDa α chain (gp80), containing an Ig-like domain (D1), a TM domain, and a cytokine-binding homology region (CHR; D2 and D3). The second subunit is 130 kDa protein (gp130), consisting of an Ig-like domain (D1), CHR (D2 and D3), a TM region, and a cytosolic domain with box 1 and box 2 motifs [180]. The gp80 subunit forms an initial low-affinity heterodimer with soluble IL-6; afterwards, gp130 binds to and stabilizes the IL-6-gp80 complex (Fig. 3E). A hexameric assembly of IL-6:gp80:gp130 in 2:2:2 stoichiometry is required for effective signal transduction through downstream Janus kinase (JAK) or

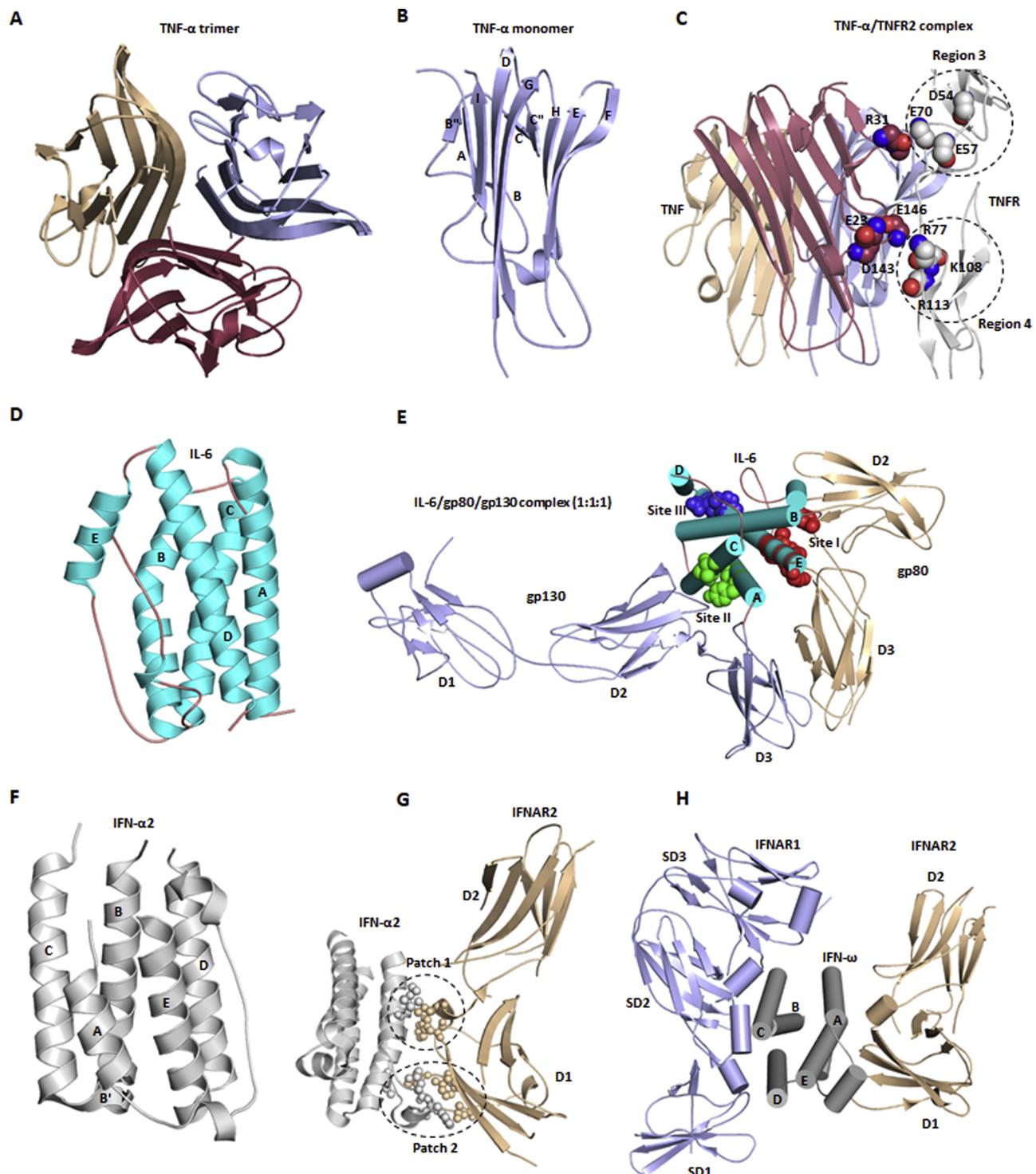


Fig. 3. Overall structures of tumor necrosis factor α (TNF- α), interleukin 6 (IL-6), and type I interferon (IFN) and their molecular recognition by receptors. (A) The TNF- α homotrimer. (B) A single TNF- α subunit. (C) Intermolecular interaction of TNF receptor 2 (TNFR2) with TNF- α . The structural coordinates were obtained from Protein Data Bank (PDB) under PDB ID of 3 ALQ. (D) The structure of IL-6 (PDB ID: 1 ALU). (E) Interaction of IL-6 with gp80 and gp130 in 1:1:1 stoichiometry (PDB ID: 1P9M). The receptor-binding sites (site I, site II, and site III) on IL-6 are colored red, green, and blue, respectively. Site III interacts with a second molecule of gp130 to complete the signaling complex. (F) The overall structure of a representative type I IFN subtype, IFN- α 2 (PDB ID: 3S9D). (G) Recognition of IFN- α 2 by interferon α/β receptor 2 (IFNAR2). The two hydrophobic patches (patch I and patch II) that are essential for the ligand–receptor interaction are highlighted in space fill models. (H) Recognition of IFN- ω by IFNAR1 and IFNAR2 (PDB ID: 3SE4). IFN- ω is shown as a grey cylindrical model at the center, IFNAR1 (light blue) is on the left, and IFNAR2 (light yellow) is to the right of IFN- ω .

tyrosine kinase 2, bound to the cytosolic domain of gp130 [181]. Site-directed mutagenesis of IL-6 has revealed multiple sites on IL-6 that are essential for receptor binding. Site I of IL-6 binds to D2 and D3 domains of gp80 and consists of amino acid residues R179, Q175, S176, R182,

and F74 from the C-terminal portion of helix D and loop AB. Site II includes residues from the middle of helices A and C (Y31, G35, S118, and V121) that interact with D2 and D3 domains of gp130 (chain A) [182]. Site III residues, W157, Q159, D160, and T162, from the N-

Table 2

Examples of some approved/investigational anti-cytokine biologics.

Cytokine	Drug Name/Type	Company	NCT Number	Condition or disease
Anti-TNF- α	Etanercept (Enbrel®) <i>Fusion protein</i>	Amgen, Inc.	NCT02486302 (Phase N/A) NCT01313208 (Phase 4)	Rheumatoid arthritis, psoriatic arthritis, axial spondyloarthritis, plaque psoriasis Rheumatoid arthritis
	Infliximab (Remicade®) <i>Monoclonal antibody (mAb)</i>	Janssen Biotech, Inc.	NCT00207688 (Phase N/A) NCT00207701 (Phase 3) NCT00051623 (Phase 3)	Ulcerative colitis Ankylosing spondylitis Psoriatic arthritis
	Adalimumab (Humira®) <i>Human mAb</i>	AbbVie pharmaceuticals, Inc.	NCT00409617 (Phase 3) NCT00195676 (Phase 3) NCT00408629 (Phase 3)	Crohn's disease Psoriasis Ulcerative colitis
	Certolizumab (Cimzia®) <i>PEGylated Fab fragment</i>	Union Chimique Belge (UCB), SA	NCT00291668 (Phase 2) NCT00717236 (Phase 3) NCT01087762 (Phase 3)	Crohn's disease Rheumatoid arthritis Spondyloarthropathies
	Golimumab (Simponi®) <i>Human mAb</i>	Janssen Biotech, Inc.	NCT00299546 (Phase 3) NCT01313858 (Phase N/A)	Rheumatoid arthritis Rheumatoid arthritis, psoriatic arthritis, spondylitis ankylosing
Anti-IL-6	Olokizumab (CDP-6038) <i>Humanized mAb</i>	R-Pharm, JSC (licensed by UCB, SA)	NCT01242488 (Phase 2) NCT02760433 (Phase 3)	Rheumatoid arthritis Rheumatoid arthritis
	Sirukumab (CNTO-136) <i>Humanized mAb</i>	Janssen Biotech, Inc.	NCT01606761 (Phase 3) NCT01604343 (Phase 3)	Rheumatoid arthritis Rheumatoid arthritis
	Tocilizumab (Atuzumab®) <i>Anti-IL-6R mAb</i>	Hoffmann-La Roche, AG and Chugai, Co., Ltd.	NCT01119859 (Phase 4) NCT00109408 (Phase 3)	Rheumatoid arthritis Rheumatoid arthritis
	Siltuximab (Sylvant®) <i>Chimeric mAb</i>	Janssen Research & Development, LLC	NCT01484275 (Phase 2) NCT01400503 (Phase 2) NCT00841191 (Phase 1 & 2)	High-risk ameliorating multiple myeloma Multicentric Castleman's disease
	Sarilumab (Kevzara®) <i>Humanized mAb</i>	Regeneron Pharmaceuticals, Inc. and Sanofi, SA	NCT01146652 (Phase 3)	Ovarian neoplasms, pancreatic neoplasms, colorectal neoplasms, head and neck neoplasms, lung neoplasms Rheumatoid arthritis
Anti-IFN (type I)	Sifalimumab (MEDI-545) <i>Human mAb</i>	MedImmune, LLC	NCT01283139 (Phase 2)	Systemic lupus erythematosus
	Rontalizumab (rhuMAb IFN- α) <i>Humanized mAb</i>	Genentech, Inc.	NCT00962832 (Phase 2)	Systemic lupus erythematosus
	Anifrolumab (MEDI-546) <i>mAb</i>	MedImmune, LLC	NCT02446899 (Phase 3)	Active systemic lupus erythematosus
Anti-IL-1 β	Anakinra (Kineret®) <i>Recombinant IL-1Ra</i>	Swedish Orphan Biovitrum, AB	NCT02236481 (Phase N/A) NCT01441076 (Phase 1 & 2)	Diabetes mellitus (type 2), Rheumatoid arthritis Autoimmune connective tissue disorder, immune system diseases
	Rilonacept (Arcalyst®) <i>Fusion protein</i>	Regeneron Pharmaceuticals, Inc.	NCT00288704 (Phase N/A)	Familial cold autoinflammatory syndrome, familial cold urticaria, muckle-wells syndrome, inborn genetic diseases
	Canakinumab (Ilaris®) <i>Human mAb</i>	Novartis, AG	NCT00991146 (Phase 3) NCT02059291 (Phase 3)	Cryopyrin-associated periodic syndromes, familial cold autoinflammatory syndrome, Muckle-Wells syndrome, neonatal onset multisystem inflammatory disease Hereditary periodic fevers
Anti-IL-18	GSK1070806 <i>Humanized mAb</i>	GlaxoSmithKline, PLC	NCT01648153 (Phase 2)	Diabetes mellitus
	IL-18BP (tadekinig alfa) <i>Recombinant protein</i>	AB2 Bio, Ltd.	NCT02398435 (Phase 2)	adult-onset Still's disease

(continued on next page)

Table 2 (continued)

Cytokine	Drug Name/Type	Company	NCT Number	Condition or disease
Anti-IL-8	HuMax-IL-8® (BMS-986253) <i>Human mAb</i>	Bristol-Myers Squibb Co.	NCT02536469 (Phase 1)	Solid tumor
			NCT03400332 (Phase 1 & 2)	Cancer
			NCT03689699 (Phase 1 & 2)	Prostate cancer, adenocarcinoma of the prostate
ABX-IL-8 <i>Humanized mAb</i>	Abgenix, Inc.		NCT00035828 (Phase 2)	Chronic bronchitis and chronic obstructive pulmonary disease

terminal portion of the AB loop, the C-terminal portion of the CD loop, and the N-terminal portion of the helix D bind to D1 of gp130 (chain B). The cytosolic domain of gp80 does not contribute to IL-6 signaling, but that of gp130 is essential for adaptor binding. Based on the structural and mutagenesis data, a model for the sequential binding of IL-6 with IL-6 receptor has been proposed. First, soluble IL-6 binds to site I of gp80, forming a loose heterodimer. Next, IL-6–gp80 complex binds to gp130 through site II on IL-6. Finally, this 1:1:1 heterotrimer is associated with an analogous complex at the cell surface mediated by the interaction between site III on IL-6 and gp130, thus constituting a hexameric signaling-competent molecular assembly.

The structural properties of IL-6 and its receptor-binding mechanism have been utilized to develop several therapeutic antibodies to treat autoimmune diseases [183]. Olokizumab is an anti-IL-6 humanized mAb that targets IL-6 and shows efficiency in the treatment of patients with moderate to severe RA (NCT01242488) [184]. The antibody is currently being developed by a Russian company, R-Pharm JSC, under the license from Union Chimique Belge, SA (Table 2). The crystal structure of the Fab portion of the olokizumab–IL-6 complex has revealed that olokizumab interacts with an epitope at the gp130-binding surface of IL-6. Several direct and water-mediated hydrogen bonds (H-bonds) stabilize the complex at the antibody–IL-6 interface. W157 of IL-6, which is crucial for the interaction with D1 of gp130, forms extensive hydrophobic contacts with the tryptophan and phenylalanine residues of the olokizumab heavy chain [185]. Sirukumab (CNTO-136) is an anti-IL-6 human mAb currently being developed by Janssen Biotech, Inc., for the treatment of RA (NCT01604343). The results of a phase 3 clinical trial indicate that sirukumab alleviates the signs and symptoms of RA and is safe during the treatment course of 52 weeks [186]. Tocilizumab (i.e., atlizumab, Actemra®) is an anti-IL-6R antibody that was first approved for the treatment of large-cell lung carcinoma [187] but currently is prescribed against RA [188]. It has affinity for both soluble and membrane-bound IL-6R, preventing IL-6 from inducing agonistic signaling. Siltuximab (Sylvant®) is a human/mouse chimeric mAb directed against IL-6 [189]. It is approved for the treatment of multicentric Castleman's disease [190]. Siltuximab is under active investigation for the treatment of several diseases, including metastatic renal cell cancer [191] and prostate cancer [192]. Sarilumab (Kevzara®) is the most recently approved human mAb against IL-6 that has been developed to treat RA [193]. In addition, Blanchetot et al. have designed two Fabs derived from conventional camelid antibodies that antagonize the interaction between IL-6 and its receptor [194]. Apart from biologics, synthetic molecules have shown promising anti-IL-6 effects in preclinical studies. A SOMAmer is a nucleotide aptamer designed to inhibit the biological activity of IL-6. It contains side chains of modified amino acids attached to flexible nucleotide bases, extending outwards to form strong intermolecular interactions with IL-6 ($K_d = 0.20 \text{ nM}$). An X-ray crystallography study suggests that the SOMAmer consists of a G-quartet domain and a stem-loop domain that interact with IL-6 with high shape complementarity. The aptamer targets both gp80- and gp130-binding sites on the IL-6 surface [195].

5.3. Type I IFNs

IFNs were first described in 1957 as a factor that inhibits propagation of viruses by interfering with their replication process. Besides antiviral activities, IFNs have been found to exert immunomodulatory and antiproliferative effects on cells. Owing to their diverse biological roles, IFNs are directly used to treat numerous diseases, such as hepatitis C, multiple sclerosis, and some types of cancer [196,197]. IFNs are grouped into three distinct families—type I, type II, and type III—based on the receptors they target [198]. Type I IFNs are produced by all nucleated cells that are exposed to viruses or viral dsRNA; TLRs specifically trigger production of IFN- α and IFN- β [199,200]. These two cytokines are by far the best-defined, broadly expressed molecules that confer antiviral defense onto infected cells via transcription of genes whose products interfere with viral replication [201]. On the other hand, production of type I IFN by uninfected cells can have detrimental effects on the host [202].

All type I IFNs bind to a single cell surface IFN- α receptor (IFNAR), which is composed of two chains: IFNAR1 and IFNAR2. The ECD of IFNAR2 consists of fibronectin III (FNIII)-like domains (D1 and D2), whereas that of IFNAR1 comprises four FNIII subdomains (SD1–SD4) in tandem order. The cytosolic domain of IFNAR1 is associated with tyrosine kinase 2, whereas that of IFNAR2 is bound to the JAK1 kinase. Activated IFNAR initiates a signaling cascade through the signal transducer and activator of transcription (STAT) protein [203]. To date, the X-ray and nuclear magnetic resonance (NMR) structures of IFN- α 2b [204], IFN- α 2a [205], IFN- β [206], and IFN- ω [207] have been reported, showing a common tertiary fold. Human IFN- α 2b consists of five α -helices (denoted as A–E) connected by one long loop (AB) and three short loops (BC, CD, and DE). The molecule has a classical up-up-down-down four-helix bundle topology, consisting of helices A, B, C, and E (Fig. 3F). IFN- α 2b shares a considerable sequence similarity with other members of the family, thus indicating a common architecture. The average length of the five α -helices ranges from 13 to 24 residues, which are packed against each other by a conserved network of hydrogen bonds and hydrophobic interactions [204]. The ternary complex of IFN- ω and IFNAR has uncovered an essentially identical tertiary architecture and receptor-binding mechanism as those of IFN- α 2 [207]. The similar structural features of the type I IFN family cytokines explain their recognition by the same cell surface receptor complex. Meanwhile, the distinct biological activities of each cytokine–receptor module are associated with ligand discrimination by receptors [208]. The difference in ligand–receptor binding chemistry and energetics that is contributed by amino acid substitutions at key positions creates a mechanistically distinct orientation of the intracellular signaling complex.

The IFNAR1–IFNAR2 receptor complex recognizes both IFN- α 2 and IFN- ω in an identical mode, where the receptor subunits bind to the opposite sides of the ligand molecule [207]. The $\text{C}\alpha$ root mean square deviation between two receptor–IFN complexes has been found to be 0.9 Å. Helices A and E and the AB loop of the IFN ligand interact with the D1 domain and the loop between strands 13 and 14 in the D2 domain of IFNAR2 [209]. The IFN molecules form major contacts with the

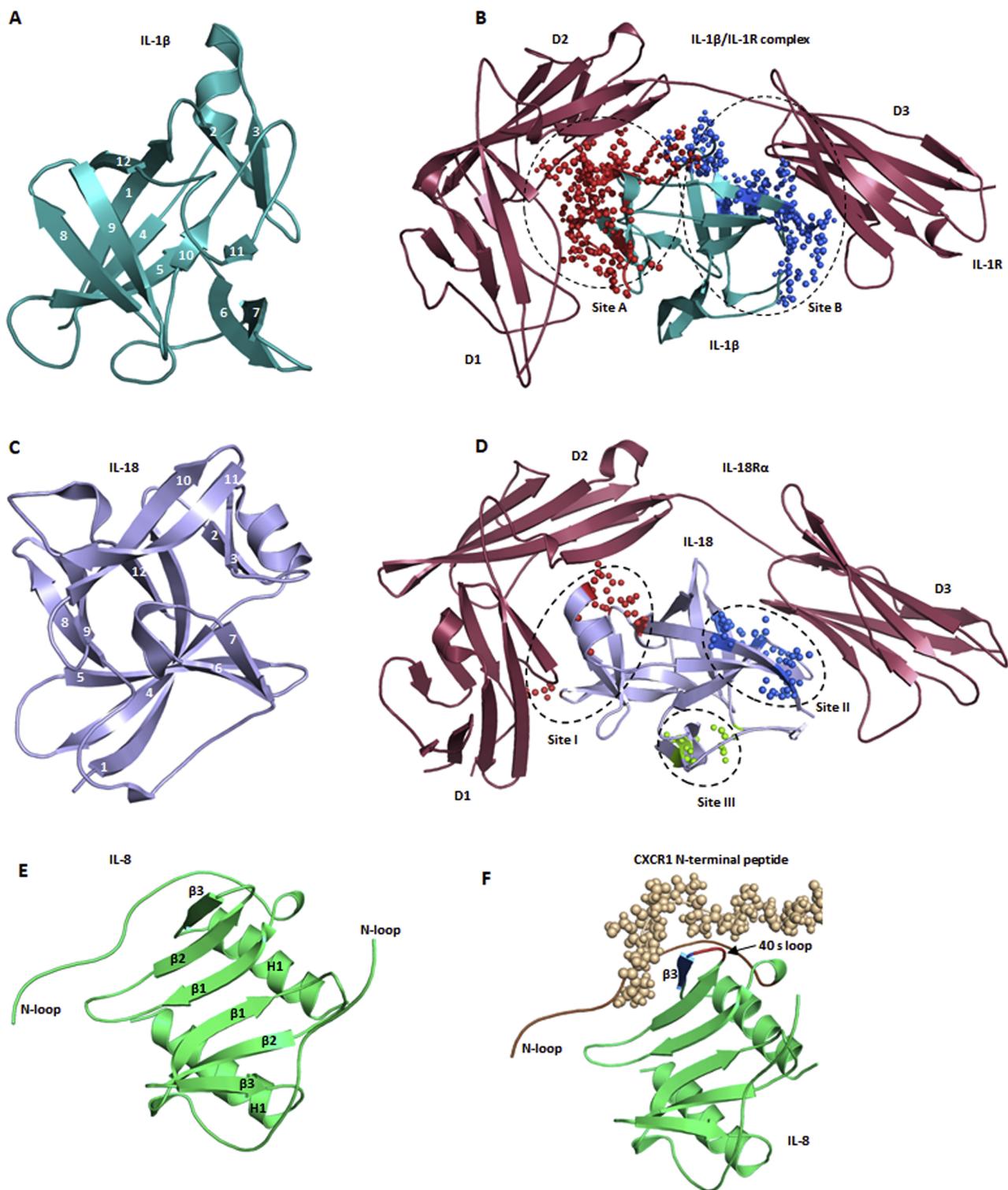


Fig. 4. Overall structures of interleukin 1 β (IL-1 β), IL-18, and IL-8 and their molecular recognition by receptors. (A) The overall structure of IL-1 β (PDB ID: 3O4O). (B) Molecular recognition of IL-1 β by IL-1 receptor (PDB ID: 1ITB). The two receptor-interacting regions of IL-1 β are red for site A (consisting of residues R11, S13-Q15, M20-G22, K27, L29-M36, Q38, Q126-P131, T147, and Q149) and blue for site B (residues A1-R4, L6, F46, Q48, E51, N53, D54, I56, K92-K94, K103, E105, I106, N108, K109, F150, and S152). (C) The overall structural fold of IL-18 (PDB ID: 3WO3). (D) Interaction between IL-18 and IL-18 receptor α (IL-18R α). Site I (red) and site II (blue) are located on the same surface of IL-18 and are recognized by IL-18R α , whereas site III (green) is recognized by IL-1R β . (E) The structure of the IL-8 homodimer (PDB ID: 1ILQ). Each monomer contains three β -strands (β 1– β 3), a single α -helix, and a long N-terminal loop (N-loop). (F) The structure of IL-8 bound to the extracellular N-terminal loop of CXCR1 (IL-8 receptor; PDB ID: 1ILP). The 16-residue N-terminal peptide of the receptor is shown as light-yellow beads that interact with the N-loop (brown), 40 s loop (red; the loop between β 2 and β 3, as shown in panel E), and β 3 (blue) of IL-8.

D1 domain of IFNAR2. The receptor-binding mechanism of the type I IFNs is highly conserved because most of the residues forming the interface between IFN α 2 and IFNAR2 are also found to form the IFN ω -IFNAR2 interface. R35 of IFN- α 2 (R33 in IFN- ω) is known to be the most important residue for the interaction with the receptor. It forms an extensive network of H-bonds with the main chain or side chain groups of I45, E50, and T44 of IFNAR2. Two hydrophobic clusters exist at the ligand-receptor interfaces: the first patch includes residues L15 and M16 of IFN and W100 and I103 of IFNAR2; the second one comprises L26, F27, L30, and V142 of IFN and M46, L52, V80, and T44 of IFNAR2 (Fig. 3G). The residues corresponding to W100, I103, M46, V80, and T44 of IFNAR2 and those corresponding to M148, F27, L30, and V142 of IFN- α 2 are conserved in the IFN- ω -IFNAR2 interface. On the other hand, the IFNAR1-IFN interaction surface is defined by subdomains SD1, SD2, and SD3 of the receptor and helices B, C, and D of the ligand (Fig. 3H). IFN molecules mainly interact with the hinge between SD2 and SD3, while the SD1 domain caps the top of the IFN molecule. The binding mode of IFN- ω is essentially identical to that of IFN- α 2. The side chains of Y70, F96, and L131 of the IFNAR1 SD2 domain form a hydrophobic patch that packs against the ligand. R123 in helix D of IFN- ω forms an H-bond with S182 of the SD2 domain and makes a salt bridge with D132 of IFNAR1. The IFN- ω -IFNAR1 interaction surface is also stabilized by van der Waals and hydrophobic contacts involving L134 (SD2) and F238 (SD3) of IFNAR1 and F67 (F54 in IFN- α 2) in helix B of IFN- ω .

Dysregulation of a type I IFN, particularly IFN- α , is directly linked with the pathogenesis of systemic lupus erythematosus (SLE), and anti-IFN biologics have shown promise for alleviation of this disease. Sifalimumab (MEDI-545) is a fully human IgG1 κ mAb that exerts neutralizing action against multiple IFN- α subtypes by suppressing IFN- α -induced genes (Table 2). Phase 1 clinical trials have validated its safety profile in patients with SLE, thereby encouraging advanced trials to examine the therapeutic effect on the clinical indications associated with IFN- α [210]. In a phase 2b trial, sifalimumab showed consistently better safety and efficacy parameters across various clinical endpoints in adults with moderate to severe active SLE [211]. An X-ray crystallographic study has shown that sifalimumab specifically targets the IFNAR1-interacting surface of IFN- α 2a but does not prevent the binding of IFN- α 2a to IFNAR2 [212]. Likewise, rontalizumab (rhuMAb IFN- α) is a human anti-IFN- α mAb that binds to twelve IFN- α subtypes and neutralizes their agonistic activity via IFNARs. In a phase 1 clinical trial, rontalizumab showed reasonable safety and adequate pharmacodynamic effects in patients with mild SLE [213]. In a phase 2 trial (named ROSE: Rontalizumab in Systemic Lupus Erythematosus), rontalizumab treatment reduced disease severity with a low IFN signature matrix (ISM) score [214]. The X-ray structure of the complex between IFN- α 2 and the Fab portion of rontalizumab has revealed that the antibody targets the IFNAR2-binding region on IFN- α 2 and neutralizes the biological activity of the cytokine [215]. Besides, therapeutic mAbs targeting IFNAR have been developed. A phase 2b clinical trial has been conducted to assess the efficacy and safety of an anti-IFNAR mAb called anifrolumab. The result of the study indicated that anifrolumab significantly reduces the disease severity across multiple clinical endpoints in patients with moderate-to-severe SLE [216]. Recently, a preclinical IgG antibody AIFNa1bIgG01 has been described that specifically recognizes IFN- α 1b with high affinity ($K_d = 0.747$ nM). The molecule was first discovered as a single-chain antibody (AIFNa1bScFv01) in a synthetic human antibody phage display library and later was converted into a full human form (AIFNa1bIgG01). The antibody has turned out to neutralize IFN- α 1b and to downregulate the expression of genes—interferon-stimulated gene 15 (ISG15) and interferon-induced protein with tetratricopeptide repeats 1 (IFIT1)—thus reducing IgG and IgM levels in lupus-like mouse models as well as in the serum of patients with SLE [185]. The crystal structure of the IFN α -AIFNa1bIgG01 complex has revealed that the AB loop (residues L30, D32, and D35) and helix E (R150) of IFN- α 1b are critical for AIFNa1bScFv01

recognition. The IFN α -binding region on AIFNa1bIgG01 is somewhat similar to that of IFNAR2 but has comparatively greater affinity for the cytokine [185].

5.4. IL-1 β

IL-1 β is a key participant in TLR-mediated proinflammatory processes and is involved in numerous autoimmune diseases. It is mainly produced by myeloid cells and triggers broad-spectrum inflammatory responses in target cells [217]. IL-1 β is synthesized as an inactive precursor (pro-IL-1 β), which is cleaved by caspase 1 (a crucial component of the inflammasome complex) into the biologically active form [218]. It stimulates differentiation of T cells [219], proliferation of B cells [220], and expression of inflammatory genes, such as cyclooxygenase 2, nitric oxide synthetase, and platelet-activating factor [221]. Under inflammatory conditions, caspase 1-mediated activation of IL-1 β increases along with an elevated level of circulating monocytes [222]. The secreted IL-1 β folds into a β -trefoil architecture, which is a 12-stranded β -barrel structure (Fig. 4A). The other two IL-1 family members, IL-1 α and IL-1 receptor antagonist (IL-1Ra), also have an identical tertiary fold.

At the early stage of signal transduction, IL-1 β binds to IL-1 receptor (IL-1R) with 1:1 stoichiometry, facilitated by the IL-1R accessory protein (IL-1RAcP) [223]. IL-1R consists of three Ig-like domains that wrap around IL-1 β in a distinct manner not seen in other cytokine-receptor interactions (Fig. 4B) [224]. Site-directed mutagenesis data suggest that IL-1 β contains two binding sites for the receptor; the first site binds simultaneously to domains 1 and 2, while the second site specifically targets domain 3 of IL-1R. The first IL-1R-binding site (site A) consists of residues R11, S13-Q15, M20-G22, K27, L29-M36, Q38, Q126-P131, T147, and Q149. Among these, the most critical residues have turned out to be R11 and Q15, which interact with domain 2 of IL-1R, while H30 and Q32 bind to the junction between domains 1 and 2 of the receptor. Site B makes contacts exclusively with domain 3 of the receptor and comprises residues A1-R4, L6, F46, Q48, E51, N53, D54, I56, K92-K94, K103, E105, I106, N108, K109, F150, and S152. The affinity between site A and IL-1R is mainly determined by van der Waals interactions, whereas the binding between site B and IL-1R is governed by both hydrophilic and hydrophobic interactions.

IL-1-targeting biologics are a subject of active pharmaceutical research for the treatment of a broad range of diseases. Currently, anakinra, rilonacept, and canakinumab are three IL-1-neutralizing agents approved by the FDA (Table 2). Anakinra (Kineret $^{\circledast}$) is a recombinant version of naturally occurring IL-1Ra, which blocks the agonistic effects of both IL-1 α and IL-1 β . In 2001, the FDA approved its use for the treatment of RA. In a phase 2 clinical trial, anakinra showed a moderate effect by reducing the growth of myeloma cells in patients at a risk of progressive active myeloma (NCT00635154) [225]. In addition, anakinra has manifested acceptable safety and pharmacokinetic profiles during the treatment of Behcet's disease [226]. Rilonacept (Arcalyst $^{\circledast}$) is a soluble decoy receptor that contains the ECD of human IL-1R1 and the Fc portion of human IgG1 with the ability to block both IL-1 α and IL-1 β . It was approved by the FDA in 2008 for the treatment of cryopyrin-associated periodic syndromes (CAPS) [227]. Apart from CAPS, clinical trials have been initiated to test the effect of rilonacept on several medical conditions, including diabetes (NCT00962026), atherosclerosis (NCT00417417), hepatitis (NCT01903798), and chronic kidney disease (NCT01663103). Canakinumab (Ilaris $^{\circledast}$) is a human mAb that specifically targets IL-1 β with high affinity and prevents it from binding to the cognate receptor [228]. Canakinumab was approved by the FDA in 2009 for the treatment of CAPS [229]. Additional clinical trials have been conducted to examine its efficacy in the treatment of osteoarthritis (NCT01160822) [230], chronic obstructive pulmonary disease (NCT00581945), type 2 diabetes (NCT00605475), atherosclerosis (NCT00995930), and RA (NCT00504595 and NCT00424346). Recently, the FDA approved it for the treatment of TNFR-associated

periodic syndrome (TRAPS) caused by mutations in the TNFR superfamily 1 A gene, leading to hypersecretion of IL-1 β and several other proinflammatory cytokines [231].

5.5. IL-18

IL-18, previously known as IFN- γ -inducing factor, is a pleiotropic immunoregulatory cytokine that causes cells to produce IFN- γ , induces Fas-mediated apoptosis, and regulates the development of T helper cells (T_H1 and T_H2) [232]. IL-18 plays a significant role in the immune response against a pathogenic infection; however, the dysregulation of IL-18 activity is implicated in several inflammatory and autoimmune diseases, such as RA, SLE, and neurological disorders [232]. Therefore, downregulation of IL-18 bioactivity holds great promise for the treatment of rheumatoid and neurological diseases.

As an inactive 23 kDa precursor, IL-18 undergoes proteolytic cleavage (by caspase 1) and forms an 18 kDa mature protein. The secreted IL-18 initiates the signaling cascade by binding to IL-18R α and IL-18R β [233]. The solution structure of IL-18 has provided key insights into the mechanism of receptor activation. The overall structure of IL-18 consists of 12 β -strands, which fold together into β -trefoil, resembling IL-1 β (Fig. 4C). The whole β -trefoil consists of three four-stranded β -sheets packed against each other [234]. Site-directed mutagenesis has uncovered three distinct sites on IL-18 that are essential for the interaction with the receptor. The mode of receptor interaction bears a striking resemblance to that of IL-1 β . The first two sites bind to IL-18R α , whereas the third site may interact with IL-18R β [235]. Although IL-18 shares 17% sequence identity with IL-1 β , the α deviation of 1.6 Å points to great structural similarity. Mutagenesis research on the ability of mutant IL-18 to induce IFN- γ production has revealed that residues Arg13, Asp17, Met33, Asp35, and Asp132 form site I, and residues Lys4, Leu5, Lys8, Arg58, Met60, and Arg104 form site II and are essential for IL-18R α binding. Sites I and II are located on the same surface of β -trefoil structure. A third site (site III)—consisting of residues Lys79, Lys84, and Asp98 present on the opposite surface toward sites I and II—is thought to be important for the binding to IL-18R β (Fig. 4D) [234].

The potential therapies that block the interaction of IL-18 with IL-8R α include antibodies or recombinant proteins [236,237], which have gone through initial phases of clinical trials to investigate their safety and efficacy in patients (NCT01035645 and NCT01648153) [238]. An IL-18-blocking peptide (IL-18BP) is a recombinant form of a naturally occurring peptide with high affinity for IL-18 (Table 2). Studies suggest that IL-18BP has a remarkable therapeutic effect on inflammatory skin diseases and LPS-induced liver injury [239]. The primary advantage of the IL-18-neutralizing antibody over the blocking peptide is longer elimination half-life, which reduces the frequency of dose administration (NCT01035645 and NCT01648153). Recently, GlaxoSmithKline (GSK) developed an anti-IL-18 humanized IgG1/ κ antibody (GSK1070806), which has been tested in a phase 2 clinical trial to examine its pharmacodynamics and efficacy in the treatment of type 2 diabetes mellitus (NCT01648153) [240]. Nevertheless, biologics neutralizing both IL-1 β and IL-18, which are cleaved by a common upstream caspase, would be highly effective in treating the inflammation-associated systemic and rheumatic diseases, such as RA, SLE, ankylosing spondylitis, and sepsis [241].

5.6. IL-8

Activated TLRs also induce the expression of an 8.4 kDa proinflammatory chemokine, IL-8 (also known as CXCL8), secreted mostly by macrophages in response to a proinflammatory stimulus [242]. IL-8 belongs to the CXC chemokine family, characterized by two N-terminal cysteines separated by an amino acid residue, and is one of the key components of the complex cytokine network [243]. In humans, the CXCL8 gene initially encodes a precursor IL-8 protein of 99 amino acid

residues, which undergoes proteolytic cleavage to form several isoforms of varying length. The 72-amino acid peptide is the major isoform secreted by macrophages and forms a stable homodimer at higher concentrations (Fig. 4E) [244]. IL-8 is a chemoattractant that triggers the migration of T cells, neutrophils, and granulocytes to the site of infection, thereby playing a key role in an immune response, wound healing, and angiogenesis [245]. IL-8 is implicated in the onset of several inflammatory diseases (e.g., psoriasis), promotes colorectal cancer by acting as an autocrine growth factor, and causes cystic fibrosis by guiding neutrophils to the lung epithelium, thereby damaging lung tissues through inflammation [181].

IL-8 is recognized by membrane-bound G protein-coupled receptors—CXC chemokine receptor 1 (CXCR1) and CXCR2—to varying degrees of specificity [246]. IL-8 interacts with two contiguous binding sites on CXCR1. Site I is located within the extracellular region of the N-terminal loop, whereas site II is formed by extracellular loop 2 (residues R119 and R203) and extracellular loop 3 (residue D265). Synthetic peptides derived from these two loops have high binding affinity for IL-8, with K_D of 0.5 μ M. Binding site 1 of CXCR1 and that of CXCR2 show great sequence diversity, indicating a difference in ligand-binding specificity. Isolated N-terminal sequences of CXCR have strong binding affinity for IL-8, where residues P21, P22, D24, E25, D26, and P29 have been found to be crucial. The IL-8 residues that are involved in the receptor interaction belong to the N-loop, the 40 s loop, and the third β -strand (Fig. 4F). Residues Y13, K15, and F21 of the N-loop distinguish the specificity of IL-8 between two receptors [247].

An increased concentration of IL-8 in blood is associated with aberrant activation of neutrophils, thereby causing chronic inflammatory conditions that can lead to such diseases as RA, inflammatory bowel disease, and psoriasis [248]. Therefore, IL-8 is considered a crucial target for such therapeutic agents as antibodies, which neutralize the biological action of IL-8 in a disease (Table 2). HuMab 10F8 is a novel fully human mAb that binds to the IL-8 epitope overlapping with the binding site for the receptor. In a clinical study, HuMab 10F8 proved effective at preventing IL-8-mediated neutrophil activation in patients with palmoplantar pustulosis: a chronic inflammatory skin disease [249]. In a recent phase 1 clinical trial, HuMax-IL-8 $^{\circ}$ (BMS-986253; previously known as HuMab 10F8) was tested for safety and pharmacokinetic properties in patients with a metastatic or unresectable solid tumor (NCT02536469). Earlier, IL-8-neutralizing antibodies were shown to inhibit neutrophil activation in animal disease models [250]. In addition, a humanized anti-IL-8 mAb, ABX-IL-8, reduces tumor growth and angiogenesis in xenograft models of bladder cancer [251]. Furthermore, mouse IL-8-targeting antibodies have potential neutralizing effects in a number of animal models [252]. This recent progress in the therapeutic targeting of IL-8 indicates that antibody-based neutralization of IL-8 holds great promise for the treatment of various inflammatory disorders.

6. Implications of cytokine-targeted therapy

Although cytokines perform a key function in the human immune system, they are implicated in the pathogenesis of numerous inflammatory diseases, autoimmune disorders, and cancers. For instance, TNF is a potent inducer of programmed cell death of tumor cells, but its malfunction causes inflammation-induced cancer [253]. The TNF-mediated activation of NF- κ B or secretion of tumor-promoting cytokines (e.g., IL-6) enables the growth and survival of malignant cells. This crosstalk between inflammatory processes and cancer progression makes cytokines an attractive drug target via the application of therapeutic biologics, with the most notable example being TNF- α inhibitors for the treatment of severe RA [247]. Nevertheless, cytokine blockers frequently face treatment failure in the clinic, thus raising safety concerns. Furthermore, there remains uncertainty regarding the immunogenicity of these biologics [254]. For instance, TNF-blocking agents often cause severe immune suppression, increasing the risk of

new (or reactivation of) latent bacterial or viral infections [255]. This notion is evident in a study where patients with RA receiving anti-TNF therapy with infliximab and adalimumab developed reactivated tuberculosis and hepatitis B virus infection, malignant tumors, and other diseases, though etanercept posed a lower risk [256]. Some biologics possess shorter biological half-life; for example, anakinra (an IL-1 blocker) has a half-life of 6 h. This property requires administration of frequent subcutaneous injections, and the treatment is withdrawn after a diagnosis of severe infections, such as pneumonia or pulmonary tuberculosis [257]. Likewise, the IL-1 trap (rilonacept) and the anti-IL-1 β antibody (canakinumab), used for the treatment of CAPS, are withdrawn if patients are at a risk of serious infections [258]. On the other hand, withdrawal of treatment is unlikely to eliminate the adverse effects immediately in the case of the biologics with longer half-life, for example, canakinumab having a mean terminal half-life of 26 days [259]. Similarly, therapeutic concerns have been raised about siltuximab too, which is a human–mouse chimeric anti-IL-6 antibody approved for the treatment of multicentric Castleman's disease [260]. Although siltuximab has beneficial effects in the treatment of multiple myeloma and prostate, ovarian, and lung cancers, it increases the risk of severe respiratory tract infection [261].

7. Conclusions and future direction

Cytokines constitute a key component both of innate- and adaptive-immune system, performing pleiotropic functions in different cell types through multiple overlapping signaling cascades. Therefore, all anti-cytokine biologics, including recombinant proteins, decoy receptors, and antibodies pose a risk of moderate-to-life-threatening infections in the host. Despite numerous clinical complications, the cytokine-blocking agents have shown favorable effects in the majority of patients with autoimmune diseases for which no alternative treatment options are available. Thus, consistent research efforts are essential for developing highly effective biologics with preferable safety and pharmacokinetic parameters in the future.

The innate immune signaling associated with the activation of TLRs can trigger a multitude of cellular responses in a cell-type or tissue-dependent manner. The differential effects of TLR signaling is largely dependent on the cognate ligands that stimulate distinct signal transduction cascades for the expression of specific cytokines in different cell-types. The involvement of TLRs in the activation of DCs, B- and T-cells responses, production of IFNs and other cytokines regulate the innate and adaptive immune reactions to ouster various pathogens from the host and thus maintain cellular/tissue homeostasis. There is ample evidence to support the use of TLR ligands as potent vaccine adjuvants or as monotherapy for the treatment of malignant tumors. However, unwarranted activation of TLRs on the surface of tumor-prone cells is attributed to the growth of several mild-to-severe cancers. Based on the concurrent data, it is clear that TLR agonists and the secreted cytokines have dual effects on the host-immunity as well as tumor progression. Thus, the TLR biology needs to be studied in a more comprehensive manner in order to clarify the functional role of each TLR in a given tumorigenic condition via extending the traditional research beyond the NF- κ B or IFN-regulatory factor 3 activation. Further, multiple factors should be considered while studying the influence of different TLR ligands on tumor cells, such as TLR expression level, tumor-originating cell-type, and the dynamics of the tumor microenvironment as a whole. Moreover, obtaining a systems-level knowledge about the differential role of TLRs in malignant cells is required to open a new avenue for the development of novel, TLR-specific antitumor pharmaceutical agents with improved bioactivity.

Conflict of interest statement

The authors state that there is no conflict of interest.

Acknowledgements

This work was supported by the Commercializations Promotion Agency for R&D Outcomes funded by the Ministry of Science and ICT (2018K000369) and the National Research Foundation of Korea (NRF-2019R1H1A2039674).

References

- [1] G.P. Dunn, L.J. Old, R.D. Schreiber, The three Es of cancer immunoediting, *Annu. Rev. Immunol.* 22 (2004) 329–360.
- [2] D. Mittal, M.M. Gubin, R.D. Schreiber, M.J. Smyth, New insights into cancer immunoediting and its three component phases—elimination, equilibrium and escape, *Curr. Opin. Immunol.* 27 (2014) 16–25.
- [3] D.S. Vinay, E.P. Ryan, G. Pawelec, W.H. Talib, J. Stagg, E. Elkord, T. Lichtor, W.K. Decker, R.L. Whelan, H. Kumara, E. Signori, K. Honoki, A.G. Georgakilas, A. Amin, W.G. Helferich, C.S. Boosani, G. Guha, M.R. Ciriolo, S. Chen, S.I. Mohammed, A.S. Azmi, W.N. Keith, A. Bilsland, D. Bhakta, D. Halicka, H. Fujii, K. Aquilano, S.S. Ashraf, S. Nowsheen, X. Yang, B.K. Choi, B.S. Kwon, Immune evasion in cancer: mechanistic basis and therapeutic strategies, *Semin. Cancer Biol.* 35 (Suppl) (2015) S185–S198.
- [4] M. Dajon, K. Iribarren, I. Cremer, Toll-like receptor stimulation in cancer: a pro- and anti-tumor double-edged sword, *Immunobiology* 222 (1) (2017) 89–100.
- [5] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, *Cell* 144 (5) (2011) 646–674.
- [6] Q. Yu, X.M. Lou, Y. He, Prediction of local recurrence in cervical cancer by a Cox model comprised of lymph node status, lymph-vascular space invasion, and intratumoral Th17 cell-infiltration, *Med. Oncol.* 31 (1) (2014) 795.
- [7] W.H. Fridman, F. Pages, C. Sautes-Fridman, J. Galon, The immune contexture in human tumours: impact on clinical outcome, *Nat. Rev. Cancer* 12 (4) (2012) 298–306.
- [8] F. Balkwill, L.M. Coussens, Cancer: an inflammatory link, *Nature* 431 (7007) (2004) 405–406.
- [9] E. Pikarsky, R.M. Porat, I. Stein, R. Abramovitch, S. Amit, S. Kasem, E. Gutkovich-Pyest, S. Urieli-Shoval, E. Galun, Y. Ben-Neriah, NF- κ B functions as a tumour promoter in inflammation-associated cancer, *Nature* 431 (7007) (2004) 461–466.
- [10] L.M. Coussens, Z. Werb, Inflammation and cancer, *Nature* 420 (6917) (2002) 860–867.
- [11] M. Karin, Nuclear factor- κ B in cancer development and progression, *Nature* 441 (7092) (2006) 431–436.
- [12] S. Valanne, J.H. Wang, M. Ramet, The Drosophila Toll signaling pathway, *J. Immunol.* 186 (2) (2011) 649–656.
- [13] S. Akira, K. Takeda, Toll-like receptor signalling, *Nat. Rev. Immunol.* 4 (7) (2004) 499–511.
- [14] S.M. Lee, K.H. Kok, M. Jaume, T.K. Cheung, T.F. Yip, J.C. Lai, Y. Guan, R.G. Webster, D.Y. Jin, J.S. Peiris, Toll-like receptor 10 is involved in induction of innate immune responses to influenza virus infection, *Proc. Natl. Acad. Sci. U. S. A.* 111 (10) (2014) 3793–3798.
- [15] S. Jiang, X. Li, N.J. Hess, Y. Guan, R.I. Tapping, TLR10 is a negative regulator of both MyD88-dependent and -independent TLR signaling, *J. Immunol.* 196 (9) (2016) 3834–3841.
- [16] M. Oosting, S.C. Cheng, J.M. Bolscher, R. Vesterling-Stenger, T.S. Plantinga, I.C. Verschueren, P. Arts, A. Garritsen, H. van Eenennaam, P. Sturm, B.J. Kullberg, A. Hoischen, G.J. Adema, J.W. van der Meer, M.G. Netea, L.A. Joosten, Human TLR10 is an anti-inflammatory pattern-recognition receptor, *Proc. Natl. Acad. Sci. U. S. A.* 111 (42) (2014) E4478–84.
- [17] N.J. Gay, M.F. Symmons, M. Gangloff, C.E. Bryant, Assembly and localization of Toll-like receptor signalling complexes, *Nat. Rev. Immunol.* 14 (8) (2014) 546–558.
- [18] T. Kawai, S. Akira, The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors, *Nat. Immunol.* 11 (5) (2010) 373–384.
- [19] J. Krishnan, K. Selvarajoo, M. Tsuchiya, G. Lee, S. Choi, Toll-like receptor signal transduction, *Exp. Mol. Med.* 39 (4) (2007) 421–438.
- [20] R. Chen, A.B. Alvero, D.A. Silasi, G. Mor, Inflammation, cancer and chemoresistance: taking advantage of the toll-like receptor signaling pathway, *Am. J. Reprod. Immunol.* 57 (2) (2007) 93–107.
- [21] M.A. Anwar, S. Basith, S. Choi, Negative regulatory approaches to the attenuation of Toll-like receptor signalling, *Exp. Mol. Med.* 45 (2013) e11.
- [22] T. Kondo, T. Kawai, S. Akira, Dissecting negative regulation of Toll-like receptor signalling, *Trends Immunol.* 33 (9) (2012) 449–458.
- [23] S. Basith, B. Manavalan, G. Lee, S.G. Kim, S. Choi, Toll-like receptor modulators: a patent review (2006–2010), *Expert Opin. Ther. Pat.* 21 (6) (2011) 927–944.
- [24] E. Latz, A. Visintin, E. Lien, K.A. Fitzgerald, B.G. Monks, E.A. Kurt-Jones, D.T. Golenbock, T. Espesvik, Lipopolysaccharide rapidly traffics to and from the Golgi apparatus with the toll-like receptor 4-MD-2-CD14 complex in a process that is distinct from the initiation of signal transduction, *J. Biol. Chem.* 277 (49) (2002) 47834–47843.
- [25] E. Latz, A. Schoenemeyer, A. Visintin, K.A. Fitzgerald, B.G. Monks, C.F. Knetter, E. Lien, N.J. Nilsen, T. Espesvik, D.T. Golenbock, TLR9 signals after translocating from the ER to CpG DNA in the lysosome, *Nat. Immunol.* 5 (2) (2004) 190–198.
- [26] T. Nishiya, A.L. DeFranco, Ligand-regulated chimeric receptor approach reveals distinctive subcellular localization and signaling properties of the Toll-like receptors, *J. Biol. Chem.* 279 (18) (2004) 19008–19017.

- [27] K. Tabeta, K. Hoebe, E.M. Janssen, X. Du, P. Georgel, K. Crozat, S. Mudd, N. Mann, S. Sovath, J. Goode, L. Shamel, A.A. Herskovits, D.A. Portnoy, M. Cooke, L.M. Tarantino, T. Wiltshire, B.E. Steinberg, S. Grinstein, B. Beutler, The Unc93B1 mutation 3d disrupts exogenous antigen presentation and signaling via Toll-like receptors 3, 7 and 9, *Nat. Immunol.* 7 (2) (2006) 156–164.
- [28] Y.M. Kim, M.M. Brinkmann, M.E. Paquet, H.L. Ploegh, UNC93B1 delivers nucleotide-sensing toll-like receptors to endolysosomes, *Nature* 452 (7184) (2008) 234–238.
- [29] Y. Yang, B. Liu, J. Dai, P.K. Srivastava, D.J. Zammit, L. Lefrancois, Z. Li, Heat shock protein gp96 is a master chaperone for toll-like receptors and is important in the innate function of macrophages, *Immunity* 26 (2) (2007) 215–226.
- [30] J. Pohar, N. Pirher, M. Bencina, M. Mancek-Keber, R. Jerala, The role of UNC93B1 protein in surface localization of TLR3 receptor and in cell priming to nucleic acid agonists, *J. Biol. Chem.* 288 (1) (2013) 442–454.
- [31] J. Pohar, N. Pirher, M. Bencina, M. Mancek-Keber, R. Jerala, The ectodomain of TLR3 receptor is required for its plasma membrane translocation, *PLoS One* 9 (3) (2014) e92391.
- [32] R. Fukui, S. Saitoh, F. Matsumoto, H. Kozuka-Hata, M. Oyama, K. Tabeta, B. Beutler, K. Miyake, Unc93B1 biases Toll-like receptor responses to nucleic acid in dendritic cells toward DNA- but against RNA-sensing, *J. Exp. Med.* 206 (6) (2009) 1339–1350.
- [33] K. Takahashi, T. Shibata, S. Akashi-Takamura, T. Kiyokawa, Y. Wakabayashi, N. Tanimura, T. Kobayashi, F. Matsumoto, R. Fukui, T. Kouro, Y. Nagai, K. Takatsu, S. Saitoh, K. Miyake, A protein associated with Toll-like receptor (TLR) 4 (PRAT4A) is required for TLR-dependent immune responses, *J. Exp. Med.* 204 (12) (2007) 2963–2976.
- [34] S.K. Biswas, Metabolic reprogramming of immune cells in cancer progression, *Immunity* 43 (3) (2015) 435–449.
- [35] D. Hanahan, L.M. Coussens, Accessories to the crime: functions of cells recruited to the tumor microenvironment, *Cancer Cell* 21 (3) (2012) 309–322.
- [36] A. Achek, D. Yesudhas, S. Choi, Toll-like receptors: promising therapeutic targets for inflammatory diseases, *Arch. Pharm. Res.* 39 (8) (2016) 1032–1049.
- [37] M.A. Anwar, M. Shah, J. Kim, S. Choi, Recent clinical trends in Toll-like receptor targeting therapeutics, *Med. Res. Rev.* 39 (2018) 1053–1090.
- [38] M. Reilly, R.M. Miller, M.H. Thomson, V. Patris, P. Ryle, L. McLoughlin, P. Mutch, P. Gilboy, C. Miller, M. Broekema, B. Keogh, W. McCormack, J. van de Wetering de Rooij, Randomized, double-blind, placebo-controlled, dose-escalating phase I, healthy subjects study of intravenous OPN-305, a humanized anti-TLR2 antibody, *Clin. Pharmacol. Ther.* 94 (5) (2013) 593–600.
- [39] P. Dar, R. Kalaiyanan, N. Sied, B. Mamo, S. Kishore, V.V. Suryanarayana, G. Kondabattula, Montanide ISA 201 adjuvanted FMD vaccine induces improved immune responses and protection in cattle, *Vaccine* 31 (33) (2013) 3327–3332.
- [40] R. Kang, Q. Zhang, H.J. Zeh 3rd, M.T. Lotze, D. Tang, HMGB1 in cancer: good, bad, or both? *Clin. Cancer Res.* 19 (15) (2013) 4046–4057.
- [41] H. Lu, G.N. Dietsch, M.A. Matthews, Y. Yang, S. Ghanebar, M. Inokuma, M. Suni, V.C. Maino, K.E. Henderson, J.J. Howbert, M.L. Disis, R.M. Hershberg, VTX-2337 is a novel TLR8 agonist that activates NK cells and augments ADCC, *Clin. Cancer Res.* 18 (2) (2012) 499–509.
- [42] Y. Zhang, F. Luo, Y. Cai, N. Liu, L. Wang, D. Xu, Y. Chu, TLR1/TLR2 agonist induces tumor regression by reciprocal modulation of effector and regulatory T cells, *J. Immunol.* 186 (4) (2011) 1963–1969.
- [43] E.L. Lowe, T.R. Crother, S. Rabizadeh, B. Hu, H. Wang, S. Chen, K. Shimada, M.H. Wong, K.S. Michelsen, M. Ardit, Toll-like receptor 2 signaling protects mice from tumor development in a mouse model of colitis-induced cancer, *PLoS One* 5 (9) (2010) e13027.
- [44] S. Li, R. Sun, Y. Chen, H. Wei, Z. Tian, TLR2 limits development of hepatocellular carcinoma by reducing IL18-mediated immunosuppression, *Cancer Res.* 75 (6) (2015) 986–995.
- [45] N. Nomi, S. Kodama, M. Suzuki, Toll-like receptor 3 signaling induces apoptosis in human head and neck cancer via survivin associated pathway, *Oncol. Rep.* 24 (1) (2010) 225–231.
- [46] K. Yoneda, K. Sugimoto, K. Shiraki, J. Tanaka, T. Beppu, H. Fuke, N. Yamamoto, M. Masuya, R. Horie, K. Uchida, Y. Takei, Dual topology of functional Toll-like receptor 3 expression in human hepatocellular carcinoma: differential signaling mechanisms of TLR3-induced NF- κ B activation and apoptosis, *Int. J. Oncol.* 33 (5) (2008) 929–936.
- [47] T. Matijevic, M. Marjanovic, J. Pavelic, Functionally active toll-like receptor 3 on human primary and metastatic cancer cells, *Scand. J. Immunol.* 70 (1) (2009) 18–24.
- [48] R. Takemura, H. Takaki, S. Okada, H. Shime, T. Akazawa, H. Oshiumi, M. Matsumoto, T. Teshima, T. Seya, PolyI:C-induced, TLR3/RIP3-dependent necrosis backs up immune effector-mediated tumor elimination in vivo, *Cancer Immunol. Res.* 3 (8) (2015) 902–914.
- [49] B. Salau, S. Lebecque, S. Matikainen, D. Rimoldi, P. Romero, Toll-like receptor 3 expressed by melanoma cells as a target for therapy? *Clin. Cancer Res.* 13 (15 Pt 1) (2007) 4565–4574.
- [50] H. Lin, J. Yan, Z. Wang, F. Hua, J. Yu, W. Sun, K. Li, H. Liu, H. Yang, Q. Lv, J. Xue, Z.W. Hu, Loss of immunity-supported senescence enhances susceptibility to hepatocellular carcinogenesis and progression in Toll-like receptor 2-deficient mice, *Hepatology* 57 (1) (2013) 171–182.
- [51] G. Gambari, M. Desideri, A. Stoppacciaro, F. Padula, P. De Cesaris, D. Starace, A. Tubaro, D. Del Bufalo, A. Filippini, E. Ziparo, A. Riccioli, TLR3 engagement induces IRF-3-dependent apoptosis in androgen-sensitive prostate cancer cells and inhibits tumour growth in vivo, *J. Cell. Mol. Med.* 19 (2) (2015) 327–339.
- [52] H. Shime, M. Matsumoto, H. Oshiumi, S. Tanaka, A. Nakane, Y. Iwakura, H. Tahara, N. Inoue, T. Seya, Toll-like receptor 3 signaling converts tumor-supporting myeloid cells to tumoricidal effectors, *Proc. Natl. Acad. Sci. U. S. A.* 109 (6) (2012) 2066–2071.
- [53] V. Chew, C. Tow, C. Huang, E. Bard-Chapeau, N.G. Copeland, N.A. Jenkins, A. Weber, K.H. Lim, H.C. Toh, M. Heikenwalder, I.O. Ng, A. Nardin, J.P. Abastado, Toll-like receptor 3 expressing tumor parenchyma and infiltrating natural killer cells in hepatocellular carcinoma patients, *J. Natl. Cancer Inst.* 104 (23) (2012) 1796–1807.
- [54] M. Okamoto, T. Oshikawa, T. Tano, G. Ohe, S. Furuchi, H. Nishikawa, S.U. Ahmed, S. Akashi, K. Miyake, O. Takeuchi, S. Akira, Y. Moriya, S. Matsubara, Y. Ryoma, M. Saito, M. Sato, Involvement of Toll-like receptor 4 signaling in interferon-gamma production and antitumor effect by streptococcal agent OK-432, *J. Natl. Cancer Inst.* 95 (4) (2003) 316–326.
- [55] C. D'Agostini, F. Pica, G. Febbraro, S. Grelli, C. Chiavaroli, E. Garaci, Antitumour effect of OM-174 and cyclophosphamide on murine B16 melanoma in different experimental conditions, *Int. Immunopharmacol.* 5 (7–8) (2005) 1205–1212.
- [56] L. Apetoh, F. Ghiringhelli, A. Tesniere, M. Obeid, C. Ortiz, A. Criollo, G. Mignot, M.C. Maiuri, E. Ulrich, P. Saulnier, H. Yang, S. Amigorena, B. Ryffel, F.J. Barrat, P. Saftig, F. Levi, R. Lidereau, C. Nogues, J.P. Mira, A. Chompret, V. Joulin, F. Clavel-Chapelon, J. Bourhis, R. Andre, S. Delaloye, T. Tursz, G. Kroemer, L. Zitvogel, Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy, *Nat. Med.* 13 (9) (2007) 1050–1059.
- [57] M.R. Chicoine, M. Zahner, E.K. Won, R.R. Kalra, T. Kitamura, A. Perry, R. Higashikubo, The in vivo antitumoral effects of lipopolysaccharide against glioblastoma multiforme are mediated in part by Toll-like receptor 4, *Neurosurgery* 60 (2) (2007) 372–380 discussion 381.
- [58] N. Yusuf, T.H. Nasti, J.A. Long, M. Naseemuddin, A.P. Lucas, H. Xu, C.A. Elmets, Protective role of Toll-like receptor 4 during the initiation stage of cutaneous chemical carcinogenesis, *Cancer Res.* 68 (2) (2008) 615–622.
- [59] M. Naseemuddin, A. Iqbal, T.H. Nasti, J.L. Ghandhi, A.D. Kapadia, N. Yusuf, Cell mediated immune responses through TLR4 prevents DMBA-induced mammary carcinogenesis in mice, *Int. J. Cancer* 130 (4) (2012) 765–774.
- [60] A. Ahmed, J.H. Wang, H.P. Redmond, Silencing of TLR4 increases tumor progression and lung metastasis in a murine model of breast cancer, *Ann. Surg. Oncol.* 20 (Suppl 3) (2013) S389–96.
- [61] D. Geng, S. Kaczanowska, A. Tsai, K. Younger, A. Ochoa, A.P. Rapoport, S. Ostrand-Rosenberg, E. Davila, TLR5 ligand-secreting T cells reshape the tumor microenvironment and enhance antitumor activity, *Cancer Res.* 75 (10) (2015) 1959–1971.
- [62] H. Zhou, J.H. Chen, J. Hu, Y.Z. Luo, F. Li, L. Xiao, M.Z. Zhong, High expression of Toll-like receptor 5 correlates with better prognosis in non-small-cell lung cancer: an anti-tumor effect of TLR5 signaling in non-small cell lung cancer, *J. Cancer Res. Clin. Oncol.* 140 (4) (2014) 633–643.
- [63] D.E. Spaner, Y. Shi, D. White, J. Mena, C. Hammond, J. Tomic, L. He, M.A. Tomai, R.L. Miller, J. Booth, L. Radvanyi, Immunomodulatory effects of Toll-like receptor-7 activation on chronic lymphocytic leukemia cells, *Leukemia* 20 (2) (2006) 286–295.
- [64] D.E. Spaner, Y. Shi, D. White, S. Shah, L. He, A. Masellis, K. Wong, R. Gorczynski, A phase I/II trial of TLR-7 agonist immunotherapy in chronic lymphocytic leukemia, *Leukemia* 24 (1) (2010) 222–226.
- [65] C. Aspord, L. Tramcourt, C. Leloup, J.P. Molens, M.T. Leccia, J. Charles, J. Plumas, Imiquimod inhibits melanoma development by promoting pDC cytotoxic functions and impeding tumor vascularization, *J. Invest. Dermatol.* 134 (10) (2014) 2551–2561.
- [66] I. Le Mercier, D. Poujol, A. Sanlaville, V. Sisirak, M. Gobert, I. Durand, B. Dubois, I. Treilleux, J. Marvel, J. VLach, J.Y. Blay, N. Bendriss-Vermaire, C. Caux, I. Puiseux, N. Goutagny, Tumor promotion by intratumoral plasmacytoid dendritic cells is reversed by TLR7 ligand treatment, *Cancer Res.* 73 (15) (2013) 4629–4640.
- [67] J. Zhu, S. He, J. Du, Z. Wang, W. Li, X. Chen, W. Jiang, D. Zheng, G. Jin, Local administration of a novel Toll-like receptor 7 agonist in combination with doxorubicin induces durable tumouricidal effects in a murine model of T cell lymphoma, *J. Hematol. Oncol.* 8 (2015) 21.
- [68] K.B. Gorden, K.S. Gorski, S.J. Gibson, R.M. Kedl, W.C. Kieper, X. Qiu, M.A. Tomai, S.S. Alkan, J.P. Vasiliakos, Synthetic TLR agonists reveal functional differences between human TLR7 and TLR8, *J. Immunol.* 174 (3) (2005) 1259–1268.
- [69] B.J. Monk, A. Facciabene, W.E. Brady, C.A. Aghajanian, P.M. Fracasso, J.L. Walker, H.A. Lankes, K.L. Manjarrez, G.H. Danet-Desnoyers, K.M. Bell-McGuinn, C.K. McCourt, A. Malikhin, R.M. Hershberg, G. Coukos, Integrative development of a TLR8 agonist for ovarian cancer chemoimmunotherapy, *Clin. Cancer Res.* 23 (8) (2017) 1955–1966.
- [70] C. Brignole, D. Marimpietri, D. Di Paolo, P. Perri, F. Morandi, F. Pastorino, A. Zorzoli, G. Pagnan, M. Loi, I. Caffa, G. Erminio, R. Haupt, C. Gambini, V. Pistoia, M. Ponzone, Therapeutic targeting of TLR9 inhibits cell growth and induces apoptosis in neuroblastoma, *Cancer Res.* 70 (23) (2010) 9816–9826.
- [71] A. El Andaloussi, A.M. Sonabend, Y. Han, M.S. Lesniak, Stimulation of TLR9 with CpG ODN enhances apoptosis of glioma and prolongs the survival of mice with experimental brain tumors, *Glia* 54 (6) (2006) 526–535.
- [72] B. Jahrsdorfer, J.E. Wooldridge, S.E. Blackwell, C.M. Taylor, T.S. Griffith, B.K. Link, G.J. Weiner, Immunostimulatory oligodeoxynucleotides induce apoptosis of B cell chronic lymphocytic leukemia cells, *J. Leukoc. Biol.* 77 (3) (2005) 378–387.
- [73] M. De Cesare, C. Calcaterra, G. Pratesi, L. Gatti, F. Zunino, S. Menard, A. Balsari, Eradication of ovarian tumor xenografts by locoregional administration of targeted immunotherapy, *Clin. Cancer Res.* 14 (17) (2008) 5512–5518.
- [74] T. Sato, T. Shimosato, A. Ueda, Y. Ishigatsubo, D.M. Klinman, Intrapulmonary delivery of CpG microparticles eliminates lung tumors, *Mol. Cancer Ther.* 14 (10)

- (2015) 2198–2205.
- [75] H. Ronkainen, P. Hirvikoski, S. Kauppila, K.S. Vuopala, T.K. Paavonen, K.S. Selander, M.H. Vaarala, Absent Toll-like receptor-9 expression predicts poor prognosis in renal cell carcinoma, *J. Exp. Clin. Cancer Res.* 30 (2011) 84.
- [76] A. Ruzsa, M. Sen, M. Evans, L.W. Lee, K. Hideghety, S. Rottey, P. Klimak, P. Holeckova, J. Fayette, T. Csoszi, J. Erfan, U. Forssmann, T. Goddemeier, A. Bexon, C. Nutting, N.E.S. Group, Phase 2, open-label, 1:1 randomized controlled trial exploring the efficacy of EMD 1201081 in combination with cetuximab in second-line cetuximab-naïve patients with recurrent or metastatic squamous cell carcinoma of the head and neck (R/M SCCHN), *Invest. New Drugs* 32 (6) (2014) 1278–1284.
- [77] N. Muthusamy, H. Breidenbach, L. Andritsos, J. Flynn, J. Jones, A. Ramanunni, X. Mo, D. Jarjoura, J.C. Byrd, N.A. Heerema, Enhanced detection of chromosomal abnormalities in chronic lymphocytic leukemia by conventional cytogenetics using CpG oligonucleotide in combination with pokeweed mitogen and phorbol myristate acetate, *Cancer Genet.* 204 (2) (2011) 77–83.
- [78] S. Basith, B. Manavalan, T.H. Yoo, S.G. Kim, S. Choi, Roles of toll-like receptors in cancer: a double-edged sword for defense and offense, *Arch. Pharm. Res.* 35 (8) (2012) 1297–1316.
- [79] B. Huang, J. Zhao, S. Shen, H. Li, K.L. He, G.X. Shen, L. Mayer, J. Unkeless, D. Li, Y. Yuan, G.M. Zhang, H. Xiong, Z.H. Feng, Listeria monocytogenes promotes tumor growth via tumor cell toll-like receptor 2 signaling, *Cancer Res.* 67 (9) (2007) 4346–4352.
- [80] W. Shi, L. Su, Q. Li, L. Sun, J. Lv, J. Li, B. Cheng, Suppression of toll-like receptor 2 expression inhibits the bioactivity of human hepatocellular carcinoma, *Tumour Biol.* 35 (10) (2014) 9627–9637.
- [81] H. Tye, C.L. Kennedy, M. Najdovska, L. McLeod, W. McCormack, N. Hughes, A. Dev, W. Sievert, C.H. Ooi, T.O. Ishikawa, H. Oshima, P.S. Bhathal, A.E. Parker, M. Oshima, P. Tan, B.J. Jenkins, STAT3-driven upregulation of TLR2 promotes gastric tumorigenesis independent of tumor inflammation, *Cancer Cell* 22 (4) (2012) 466–478.
- [82] A. Maruyama, H. Shime, Y. Takeda, M. Azuma, M. Matsumoto, T. Seya, Pam2 lipopeptides systemically increase myeloid-derived suppressor cells through TLR2 signaling, *Biochem. Biophys. Res. Commun.* 457 (3) (2015) 445–450.
- [83] F.A. Scheeren, A.H. Kuo, L.J. van Weele, S. Cai, I. Glykofridis, S.S. Sikandar, M. Zabala, D. Qian, J.S. Lam, D. Johnston, J.P. Volkmer, D. Sahoo, M. van de Rijn, F.M. Dirbas, G. Somlo, T. Kalisky, M.E. Rothenberg, S.R. Quake, M.F. Clarke, A cell-intrinsic role for TLR2-MYD88 in intestinal and breast epithelia and oncogenesis, *Nat. Cell Biol.* 16 (12) (2014) 1238–1248.
- [84] D. Jia, L. Wang, The other face of TLR3: a driving force of breast cancer stem cells, *Mol. Cell. Oncol.* 2 (4) (2015) e981443.
- [85] S. Gonzalez-Reyes, J.M. Fernandez, L.O. Gonzalez, A. Aguirre, A. Suarez, J.M. Gonzalez, S. Escaff, F.J. Vizoso, Study of TLR3, TLR4, and TLR9 in prostate carcinomas and their association with biochemical recurrence, *Cancer Immunol. Immunother.* 60 (2) (2011) 217–226.
- [86] H. Yang, H. Zhou, P. Feng, X. Zhou, H. Wen, X. Xie, H. Shen, X. Zhu, Reduced expression of Toll-like receptor 4 inhibits human breast cancer cells proliferation and inflammatory cytokines secretion, *J. Exp. Clin. Cancer Res.* 29 (2010) 92.
- [87] C.C. Hsiao, P.H. Chen, C.I. Cheng, M.S. Tsai, C.Y. Chang, S.C. Lu, M.C. Hsieh, Y.C. Lin, P.H. Lee, Y.H. Kao, Toll-like receptor-4 is a target for suppression of proliferation and chemoresistance in HepG2 hepatoblastoma cells, *Cancer Lett.* 368 (1) (2015) 144–152.
- [88] S. Jain, S. Suklabaitya, B. Das, S.K. Raghav, S.K. Batra, S. Senapati, TLR4 activation by lipopolysaccharide confers survival advantage to growth factor deprived prostate cancer cells, *Prostate* 75 (10) (2015) 1020–1033.
- [89] W. He, Q. Liu, L. Wang, W. Chen, N. Li, X. Cao, TLR4 signaling promotes immune escape of human lung cancer cells by inducing immunosuppressive cytokines and apoptosis resistance, *Mol. Immunol.* 44 (11) (2007) 2850–2859.
- [90] M.J. Szczepanski, M. Czystowska, M. Szajnik, M. Harasymczuk, M. Bojiadzis, A. Kruk-Zagajewska, W. Szyfter, J. Zeromski, T.L. Whiteside, Triggering of Toll-like receptor 4 expressed on human head and neck squamous cell carcinoma promotes tumor development and protects the tumor from immune attack, *Cancer Res.* 69 (7) (2009) 3105–3113.
- [91] B. Huang, J. Zhao, H. Li, K.L. He, Y. Chen, S.H. Chen, L. Mayer, J.C. Unkeless, H. Xiong, Toll-like receptors on tumor cells facilitate evasion of immune surveillance, *Cancer Res.* 65 (12) (2005) 5009–5014.
- [92] M. Fukata, A. Chen, A.S. Vamadevan, J. Cohen, K. Breglio, S. Krishnareddy, D. Hsu, R. Xu, N. Harpaz, A.J. Dannenberg, K. Subbaramaiah, H.S. Cooper, S.H. Itzkowitz, M.T. Abreu, Toll-like receptor-4 promotes the development of colitis-associated colorectal tumors, *Gastroenterology* 133 (6) (2007) 1869–1881.
- [93] J.J. Zhang, H.S. Wu, L. Wang, Y. Tian, J.H. Zhang, H.L. Wu, Expression and significance of TLR4 and HIF-1 α in pancreatic ductal adenocarcinoma, *World J. Gastroenterol.* 16 (23) (2010) 2881–2888.
- [94] D. Yesudhas, V. Gosu, M.A. Anwar, S. Choi, Multiple roles of toll-like receptor 4 in colorectal cancer, *Front. Immunol.* 5 (2014) 334.
- [95] J.H. Park, H.E. Yoon, D.J. Kim, S.A. Kim, S.G. Ahn, J.H. Yoon, Toll-like receptor 5 activation promotes migration and invasion of salivary gland adenocarcinoma, *J. Oral Pathol. Med.* 40 (2) (2011) 187–193.
- [96] M.R. Rutkowski, T.L. Stephen, N. Svoronos, M.J. Allegrezza, A.J. Tesone, A. Perales-Puchalt, E. Brencicova, X. Escovar-Fadul, J.M. Nguyen, M.G. Cadungog, R. Zhang, M. Salatinio, J. Tchou, G.A. Rabinovich, J.R. Conejo-Garcia, Microbially driven TLR5-dependent signaling governs distal malignant progression through tumor-promoting inflammation, *Cancer Cell* 27 (1) (2015) 27–40.
- [97] G. Jego, R. Bataille, A. Geffroy-Luseau, G. Descamps, C. Pellat-Deceunynck, Pathogen-associated molecular patterns are growth and survival factors for human myeloma cells through Toll-like receptors, *Leukemia* 20 (6) (2006) 1130–1137.
- [98] T. Grimmig, N. Matthes, K. Hoeland, S. Tripathi, A. Chandraker, M. Grimm, R. Moench, E.M. Moll, H. Friess, I. Tsaur, R.A. Blaheta, C.T. Germer, A.M. Waaga-Gasser, M. Gasser, TLR7 and TLR8 expression increases tumor cell proliferation and promotes chemoresistance in human pancreatic cancer, *Int. J. Oncol.* 47 (3) (2015) 857–866.
- [99] M. Grimm, M. Kim, A. Rosenwald, U. Heemann, C.T. Germer, A.M. Waaga-Gasser, M. Gasser, Toll-like receptor (TLR) 7 and TLR8 expression on CD133+ cells in colorectal cancer points to a specific role for inflammation-induced TLRs in tumorigenesis and tumour progression, *Eur. J. Cancer* 46 (15) (2010) 2849–2857.
- [100] S. Chatterjee, L. Crozet, D. Damotte, K. Iribarren, C. Schramm, M. Alifano, A. Lupo, J. Cherfils-Vicini, J. Goc, S. Katsahan, M. Younes, M.C. Dieu-Nosjean, W.H. Fridman, C. Sautes-Fridman, I. Cremer, TLR7 promotes tumor progression, chemotherapy resistance, and poor clinical outcomes in non-small cell lung cancer, *Cancer Res.* 74 (18) (2014) 5008–5018.
- [101] Y. Luo, Q.W. Jiang, J.Y. Wu, J.G. Qiu, W.J. Zhang, X.L. Mei, Z. Shi, J.M. Di, Regulation of migration and invasion by Toll-like receptor-9 signaling network in prostate cancer, *Oncotarget* 6 (26) (2015) 22564–22574.
- [102] R. Berger, H. Fieg, G. Goebel, P. Obexer, M. Ausserlechner, W. Doppler, C. Hauser-Kronberger, R. Reitsamer, D. Eggle, D. Reimer, E. Muller-Holzner, A. Jones, M. Widschwendter, Toll-like receptor 9 expression in breast and ovarian cancer is associated with poorly differentiated tumors, *Cancer Sci.* 101 (4) (2010) 1059–1066.
- [103] Y. Zhang, Q. Wang, A. Ma, Y. Li, R. Li, Y. Wang, Functional expression of TLR9 in esophageal cancer, *Oncol. Rep.* 31 (5) (2014) 2298–2304.
- [104] D. Moreira, Q. Zhang, D.M. Hossain, S. Nechaev, H. Li, C.M. Kowollik, M. D'Apuzzo, S. Forman, J. Jones, S.K. Pal, M. Kortylewski, TLR9 signaling through NF-kappaB/RELA and STAT3 promotes tumor-propagating potential of prostate cancer cells, *Oncotarget* 6 (19) (2015) 17302–17313.
- [105] T. Matijevic, J. Pavelic, The dual role of TLR3 in metastatic cell line, *Clin. Exp. Metastasis* 28 (7) (2011) 701–712.
- [106] D.H. Dapito, A. Mencin, G.Y. Gwak, J.P. Pradere, M.K. Jang, I. Mederacke, J.M. Caviglia, H. Khiabanian, A. Adeyemi, R. Bataller, J.H. Lefkowitch, M. Bower, R. Friedman, R.B. Sartor, R. Rabidan, R.F. Schwabe, Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4, *Cancer Cell* 21 (4) (2012) 504–516.
- [107] Y. Li, Y. Shi, L. McCaw, Y.J. Li, F. Zhu, R. Gorczynski, G.S. Duncan, B. Yang, Y. Ben-David, D.E. Spaner, Microenvironmental interleukin-6 suppresses toll-like receptor signaling in human leukemia cells through miR-17/19A, *Blood* 126 (6) (2015) 766–778.
- [108] N. Jiang, F. Xie, Q. Guo, M.Q. Li, J. Xiao, L. Sui, Toll-like receptor 4 promotes proliferation and apoptosis resistance in human papillomavirus-related cervical cancer cells through the Toll-like receptor 4/nuclear factor-kappaB pathway, *Tumour Biol.* 39 (6) (2017) 1010428317710586.
- [109] L. Belmont, N. Rabbe, M. Antoine, D. Cathelin, C. Guignabert, J. Kurie, J. Cadran, M. Wislez, Expression of TLR9 in tumor-infiltrating mononuclear cells enhances angiogenesis and is associated with a worse survival in lung cancer, *Int. J. Cancer* 134 (4) (2014) 765–777.
- [110] J.A. Lopes, M. Borges-Canha, P. Pimentel-Nunes, Innate immunity and hepatocarcinoma can toll-like receptors open the door to oncogenesis? *World J. Hepatol.* 8 (3) (2016) 162–182.
- [111] L. Wang, K. Yu, X. Zhang, S. Yu, Dual functional roles of the MyD88 signaling in colorectal cancer development, *Biomed. Pharmacother.* 107 (2018) 177–184.
- [112] J.J. O'Shea, P.J. Murray, Cytokine signaling modules in inflammatory responses, *Immunity* 28 (4) (2008) 477–487.
- [113] P. Lacy, J.L. Stow, Cytokine release from innate immune cells: association with diverse membrane trafficking pathways, *Blood* 118 (1) (2011) 9–18.
- [114] K. Wilhelmsen, K.R. Mesa, J. Lucero, F. Xu, J. Hellman, ERK5 protein promotes, whereas MEK1 protein differentially regulates, the Toll-like receptor 2 protein-dependent activation of human endothelial cells and monocytes, *J. Biol. Chem.* 287 (32) (2012) 26478–26494.
- [115] S. Dunzendorfer, H.K. Lee, K. Soldau, P.S. Tobias, Toll-like receptor 4 functions intracellularly in human coronary artery endothelial cells: roles of LBP and sCD14 in mediating LPS responses, *FASEB J.* 18 (10) (2004) 1117–1119.
- [116] S. Khakpour, K. Wilhelmsen, J. Hellman, Vascular endothelial cell Toll-like receptor pathways in sepsis, *Innate Immun.* 21 (8) (2015) 827–846.
- [117] K. Wilhelmsen, K.R. Mesa, A. Prakash, F. Xu, J. Hellman, Activation of endothelial TLR2 by bacterial lipoprotein upregulates proteins specific for the neutrophil response, *Innate Immun.* 18 (4) (2012) 602–616.
- [118] T. Imaizumi, H. Itaya, K. Fujita, D. Kudoh, S. Kudoh, K. Mori, K. Fujimoto, T. Matsumiya, H. Yoshida, K. Satoh, Expression of tumor necrosis factor-alpha in cultured human endothelial cells stimulated with lipopolysaccharide or interleukin-1 α , *Arterioscler. Thromb. Vasc. Biol.* 20 (2) (2000) 410–415.
- [119] V. Ranta, A. Orpana, O. Carpen, U. Turpeinen, O. Ylikorkala, L. Viinikka, Human vascular endothelial cells produce tumor necrosis factor-alpha in response to proinflammatory cytokine stimulation, *Crit. Care Med.* 27 (10) (1999) 2184–2187.
- [120] K. Vijay, Toll-like receptors in immunity and inflammatory diseases: past, present, and future, *Int. Immunopharmacol.* 59 (2018) 391–412.
- [121] K. Popko, E. Gorska, The role of natural killer cells in pathogenesis of autoimmune diseases, *Cent. J. Immunol.* 40 (4) (2015) 470–476.
- [122] K.U. Saikh, J.S. Lee, T.L. Kissner, B. Dyas, R.G. Ulrich, Toll-like receptor and cytokine expression patterns of CD56+ T cells are similar to natural killer cells in response to infection with Venezuelan equine encephalitis virus replicons, *J. Infect. Dis.* 188 (10) (2003) 1562–1570.
- [123] F. Souza-Fonseca-Guimaraes, M. Parlato, C. Fitting, J.M. Cavailon, M. Adib-Conquy, NK cell tolerance to TLR agonists mediated by regulatory T cells after polymicrobial sepsis, *J. Immunol.* 188 (12) (2012) 5850–5858.

- [124] F. Souza-Fonseca-Guimaraes, M. Parlato, F. Philipart, B. Misset, J.M. Cavaillon, M. Adib-Conquy, g. Captain study, Toll-like receptors expression and interferon-gamma production by NK cells in human sepsis, *Crit. Care* 16 (5) (2012) R206.
- [125] K.C. Newman, E.M. Riley, Whatever turns you on: accessory-cell-dependent activation of NK cells by pathogens, *Nat. Rev. Immunol.* 7 (4) (2007) 279–291.
- [126] D. Andaluz-Ojeda, V. Iglesias, F. Bobillo, R. Almansa, L. Rico, F. Gandia, A.M. Loma, C. Nieto, R. Diego, E. Ramos, M. Nocito, S. Resino, J.M. Eiros, E. Tamayo, R.O. de Lejarazu, J.F. Bermejo-Martin, Early natural killer cell counts in blood predict mortality in severe sepsis, *Crit. Care* 15 (5) (2011) R243.
- [127] A. Roquilly, A. Broquet, C. Jacqueline, L. Gautreau, J.P. Seguin, P. de Coppet, J. Caillou, F. Altare, R. Josien, K. Aschneroune, Toll-like receptor-4 agonist in post-haemorrhage pneumonia: role of dendritic and natural killer cells, *Eur. Respir. J.* 42 (5) (2013) 1365–1378.
- [128] K. Honda, H. Yanai, T. Mizutani, H. Negishi, N. Shimada, N. Suzuki, Y. Ohba, A. Takaoka, W.C. Yeh, T. Taniguchi, Role of a transducentional-transcriptional processor complex involving MyD88 and IRF-7 in Toll-like receptor signaling, *Proc. Natl. Acad. Sci. U. S. A.* 101 (43) (2004) 15416–15421.
- [129] M. Fuchsberger, H. Hochrein, M. O'Keefe, Activation of plasmacytoid dendritic cells, *Immunol. Cell Biol.* 83 (5) (2005) 571–577.
- [130] H. Hochrein, M. O'Keefe, H. Wagner, Human and mouse plasmacytoid dendritic cells, *Hum. Immunol.* 63 (12) (2002) 1103–1110.
- [131] P.Y. Perera, T.N. Mayadas, O. Takeuchi, S. Akira, M. Zaks-Zilberman, S.M. Goyert, S.N. Vogel, CD11b/CD18 acts in concert with CD14 and Toll-like receptor (TLR) 4 to elicit full lipopolysaccharide and taxol-inducible gene expression, *J. Immunol.* 166 (1) (2001) 574–581.
- [132] S. Mrabet-Dahbi, M. Metz, A. Dudeck, T. Zuberbier, M. Maurer, Murine mast cells secrete a unique profile of cytokines and prostaglandins in response to distinct TLR2 ligands, *Exp. Dermatol.* 18 (5) (2009) 437–444.
- [133] H. Matsushima, N. Yamada, H. Matsue, S. Shimada, TLR3-, TLR7-, and TLR9-mediated production of proinflammatory cytokines and chemokines from murine connective tissue type skin-derived mast cells but not from bone marrow-derived mast cells, *J. Immunol.* 173 (1) (2004) 531–541.
- [134] M. Kulka, L. Alexopoulou, R.A. Flavell, D.D. Metcalfe, Activation of mast cells by double-stranded RNA: evidence for activation through Toll-like receptor 3, *J. Allergy Clin. Immunol.* 114 (1) (2004) 174–182.
- [135] H. Sandig, S. Bulfone-Paus, TLR signaling in mast cells: common and unique features, *Front. Immunol.* 3 (2012) 185.
- [136] S. Varadarajalu, F. Feiger, N. Thieblemont, N.B. Hamouda, J.M. Pleau, M. Dy, M. Arock, Toll-like receptor 2 (TLR2) and TLR4 differentially activate human mast cells, *Eur. J. Immunol.* 33 (4) (2003) 899–906.
- [137] V. Hornung, S. Rothenfusser, S. Britsch, A. Krug, B. Jahrdsdorfer, T. Giese, S. Endres, G. Hartmann, Quantitative expression of toll-like receptor 1–10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides, *J. Immunol.* 168 (9) (2002) 4531–4537.
- [138] K.A. Zaremba, P.J. Godowski, Tissue expression of human Toll-like receptors and differential regulation of Toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines, *J. Immunol.* 168 (2) (2002) 554–561.
- [139] A. Mansson, M. Adner, L.O. Cardell, Toll-like receptors in cellular subsets of human tonsil T cells: altered expression during recurrent tonsillitis, *Respir. Res.* 7 (2006) 36.
- [140] N.K. Crellin, R.V. Garcia, O. Hadisfar, S.E. Allan, T.S. Steiner, M.K. Levings, Human CD4+ T cells express TLR5 and its ligand flagellin enhances the suppressive capacity and expression of FOXP3 in CD4+ CD25+ T regulatory cells, *J. Immunol.* 175 (12) (2005) 8051–8059.
- [141] I. Caramalho, T. Lopes-Carvalho, D. Ostler, S. Zelenay, M. Haury, J. Demengeot, Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide, *J. Exp. Med.* 197 (4) (2003) 403–411.
- [142] T. Tomita, T. Kanai, T. Fujii, Y. Nemoto, R. Okamoto, K. Tsuchiya, T. Totsuka, N. Sakamoto, S. Akira, M. Watanabe, MyD88-dependent pathway in T cells directly modulates the expansion of colitogenic CD4+ T cells in chronic colitis, *J. Immunol.* 180 (8) (2008) 5291–5299.
- [143] S. Agrawal, S. Gupta, TLR1/2, TLR7, and TLR9 signals directly activate human peripheral blood naive and memory B cell subsets to produce cytokines, chemokines, and hematopoietic growth factors, *J. Clin. Immunol.* 31 (1) (2011) 89–98.
- [144] J. Booth, H. Wilson, S. Jimbo, G. Mutwiri, Modulation of B cell responses by Toll-like receptors, *Cell Tissue Res.* 343 (1) (2011) 131–140.
- [145] M. Bsibsi, R. Ravid, D. Gveric, J.M. van Noort, Broad expression of Toll-like receptors in the human central nervous system, *J. Neuropathol. Exp. Neurol.* 61 (11) (2002) 1013–1021.
- [146] J.K. Olson, S.D. Miller, Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs, *J. Immunol.* 173 (6) (2004) 3916–3924.
- [147] C. Farina, F. Aloisi, E. Meinl, Astrocytes are active players in cerebral innate immunity, *Trends Immunol.* 28 (3) (2007) 138–145.
- [148] P.J. Crack, P.J. Bray, Toll-like receptors in the brain and their potential roles in neuropathology, *Immunol. Cell Biol.* 85 (6) (2007) 476–480.
- [149] M.L. Hanke, T. Kielian, Toll-like receptors in health and disease in the brain: mechanisms and therapeutic potential, *Clin. Sci. (Lond.)* 121 (9) (2011) 367–387.
- [150] M. Shah, S. Choi, Toll-like receptor-dependent negative effects of opioids: a battle between analgesia and hyperalgesia, *Front. Immunol.* 8 (2017) 642.
- [151] J.S. Cameron, L. Alexopoulou, J.A. Sloane, A.B. DiBernardo, Y. Ma, B. Kosaras, R. Flavell, S.M. Strittmatter, J. Volpe, R. Sidman, T. Vartanian, Toll-like receptor 3 is a potent negative regulator of axonal growth in mammals, *J. Neurosci.* 27 (47) (2007) 13033–13041.
- [152] C.S. Jack, N. Arbour, J. Manusow, V. Montgrain, M. Blain, E. McCrea, A. Shapiro, J.P. Antel, TLR signaling tailors innate immune responses in human microglia and astrocytes, *J. Immunol.* 175 (7) (2005) 4320–4330.
- [153] C. Gurley, J. Nichols, S. Liu, N.K. Phulwani, N. Esen, T. Kielian, Microglia and astrocyte activation by toll-like receptor ligands: modulation by PPAR-gamma agonists, *PPAR Res.* 2008 (2008) 453120.
- [154] G.D. Kalliolias, L.B. Ivashkiv, TNF biology, pathogenic mechanisms and emerging therapeutic strategies, *Nat. Rev. Rheumatol.* 12 (1) (2016) 49–62.
- [155] B.B. Aggarwal, S.C. Gupta, J.H. Kim, Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey, *Blood* 119 (3) (2012) 651–665.
- [156] B. Beutler, I.W. Milsark, A.C. Cerami, Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin, *Science* 229 (4716) (1985) 869–871.
- [157] I. Nenu, D. Tudor, A.G. Filip, I. Baldea, Current position of TNF-alpha in melanogenesis, *Tumour Biol.* 36 (9) (2015) 6589–6602.
- [158] R. van Horssen, T.L. Ten Hagen, A.M. Eggertmann, TNF-alpha in cancer treatment: molecular insights, antitumor effects, and clinical utility, *Oncologist* 11 (4) (2006) 397–408.
- [159] M.J. Eck, S.R. Sprang, The structure of tumor necrosis factor-alpha at 2.6 Å resolution. Implications for receptor binding, *J. Biol. Chem.* 264 (29) (1989) 17595–17605.
- [160] E.S. Vanamee, D.L. Faustman, Structural principles of tumor necrosis factor superfamiliy signaling, *Sci. Signal.* 11 (511) (2018).
- [161] Y. Mukai, T. Nakamura, M. Yoshikawa, Y. Yoshioka, S. Tsunoda, S. Nakagawa, Y. Yamagata, Y. Tsutsumi, Solution of the structure of the TNF-TNFR2 complex, *Sci. Signal.* 3 (148) (2010) ra83.
- [162] C. Monaco, J. Nanchahal, P. Taylor, M. Feldmann, Anti-TNF therapy: past, present and future, *Int. Immunopharmacol.* 27 (1) (2015) 55–62.
- [163] K. Peppel, D. Crawford, B. Beutler, A tumor necrosis factor (TNF) receptor-IgG heavy chain chimeric protein as a bivalent antagonist of TNF activity, *J. Exp. Med.* 174 (6) (1991) 1483–1489.
- [164] J. Braun, N. McHugh, A. Singh, J.S. Wajdula, R. Sato, Improvement in patient-reported outcomes for patients with ankylosing spondylitis treated with etanercept 50 mg once-weekly and 25 mg twice-weekly, *Rheumatology (Oxford)* 46 (6) (2007) 1999–2004.
- [165] D.M. Knight, H. Trinh, J. Le, S. Siegel, D. Shealy, M. McDonough, B. Scallon, M.A. Moore, J. Vilcek, P. Daddona, et al., Construction and initial characterization of a mouse-human chimeric anti-TNF antibody, *Mol. Immunol.* 30 (16) (1993) 1443–1453.
- [166] M.C. Dubinsky, P.P. Fleshner, Treatment of Crohn's disease of inflammatory, stenotic, and fistulizing phenotypes, *Curr. Treat. Opt. Gastroenterol.* 6 (3) (2003) 183–200.
- [167] P. Rutgeerts, W.J. Sandborn, B.G. Feagan, W. Reinisch, A. Olson, J. Johanns, S. Travers, D. Rachmilevitz, S.B. Hanauer, G.R. Lichtenstein, W.J. de Villiers, D. Present, B.E. Sands, J.F. Colombel, Infliximab for induction and maintenance therapy for ulcerative colitis, *N. Engl. J. Med.* 353 (23) (2005) 2462–2476.
- [168] L.J. Maxwell, J. Zochling, A. Boonen, J.A. Singh, M.M. Veras, E. Tanjong Ghogomu, M. Benkhalti Jandu, P. Tugwell, G.A. Wells, TNF-alpha inhibitors for ankylosing spondylitis, *Cochrane Database Syst. Rev.* 4 (2015) CD005468.
- [169] A.F. Kavanaugh, C.T. Ritchlin, G.T.G. Committee, Systematic review of treatments for psoriatic arthritis: an evidence based approach and basis for treatment guidelines, *J. Rheumatol.* 33 (7) (2006) 1417–1421.
- [170] S. Liang, J. Dai, S. Hou, L. Su, D. Zhang, H. Guo, S. Hu, H. Wang, Z. Rao, Y. Guo, Z. Lou, Structural basis for treating tumor necrosis factor alpha (TNFalpha)-associated diseases with the therapeutic antibody infliximab, *J. Biol. Chem.* 288 (19) (2013) 13799–13807.
- [171] F. Navarro-Sarabia, R. Ariza-Ariza, B. Hernandez-Cruz, I. Villanueva, Adalimumab for treating rheumatoid arthritis, *Cochrane Database Syst. Rev.* 3 (2005) CD005113.
- [172] N. Scheinfeld, Adalimumab (HUMIRA): a review, *J. Drugs Dermatol.* 2 (4) (2003) 375–377.
- [173] D.K. Podolsky, Inflammatory bowel disease, *N. Engl. J. Med.* 347 (6) (2002) 417–429.
- [174] N. Goel, S. Stephens, Certolizumab pegol, *MAbs* 2 (2) (2010) 137–147.
- [175] S. Mazumdar, D. Greenland, Golimumab, *MAbs* 1 (5) (2009) 422–431.
- [176] K.V. Korneev, K.N. Atretkhan, M.S. Drutskaya, S.I. Grivennikov, D.V. Kuprash, S.A. Nedospasov, TLR-signaling and proinflammatory cytokines as drivers of tumorigenesis, *Cytokine* 89 (2017) 127–135.
- [177] A. Boe, M. Baiocchi, M. Carbonatto, R. Papoian, O. Serlupi-Crescenzi, Interleukin 6 knock-out mice are resistant to antigen-induced experimental arthritis, *Cytokine* 11 (12) (1999) 1057–1064.
- [178] D.T. Fisher, M.M. Appenheimer, S.S. Evans, The two faces of IL-6 in the tumor microenvironment, *Semin. Immunol.* 26 (1) (2014) 38–47.
- [179] W. Somers, M. Stahl, J.S. Seehra, 1.9 Å crystal structure of interleukin 6: implications for a novel mode of receptor dimerization and signaling, *EMBO J.* 16 (5) (1997) 989–997.
- [180] A. Usacheva, R. Sandoval, P. Domanski, S.V. Kotenko, K. Nelms, M.A. Goldsmith, O.R. Colamonti, Contribution of the Box 1 and Box 2 motifs of cytokine receptors to Jak1 association and activation, *J. Biol. Chem.* 277 (50) (2002) 48220–48226.
- [181] M.D. Turner, B. Nedjai, T. Hurst, D.J. Pennington, Cytokines and chemokines: at the crossroads of cell signalling and inflammatory disease, *Biochim. Biophys. Acta* 1843 (11) (2014) 2563–2582.
- [182] M.J. Boulanger, D.C. Chow, E.E. Brevnova, K.C. Garcia, Hexameric structure and assembly of the interleukin-6/IL-6 alpha-receptor/gp130 complex, *Science* 300 (5628) (2003) 2101–2104.
- [183] J.F. Rossi, Z.Y. Lu, M. Jourdan, B. Klein, Interleukin-6 as a therapeutic target, *Clin. Cancer Res.* 21 (6) (2015) 1248–1257.

- [184] M.C. Genovese, R. Fleischmann, D. Furst, N. Janssen, J. Carter, B. Dasgupta, J. Bryson, B. Duncan, W. Zhu, C. Pitzalis, P. Durez, K. Kretos, Efficacy and safety of olokizumab in patients with rheumatoid arthritis with an inadequate response to TNF inhibitor therapy: outcomes of a randomised Phase IIb study, *Ann. Rheum. Dis.* 73 (9) (2014) 1607–1615.
- [185] S. Ouyang, B. Gong, J.Z. Li, L.X. Zhao, W. Wu, F.S. Zhang, L. Sun, S.J. Wang, M. Pan, C. Li, W. Liang, N. Shaw, J. Zheng, G.P. Zhao, Y. Wang, Z.J. Liu, M. Liang, Structural insights into a human anti-IFN antibody exerting therapeutic potential for systemic lupus erythematosus, *J. Mol. Med. (Berl.)* 90 (7) (2012) 837–846.
- [186] T. Takeuchi, C. Thorne, G. Karpouzas, S. Sheng, W. Xu, R. Rao, K. Fei, B. Hsu, P.P. Tak, Sirukumab for rheumatoid arthritis: the phase III SIRROUND-D study, *Ann. Rheum. Dis.* 76 (12) (2017) 2001–2008.
- [187] M.M. Schoels, D. van der Heijde, F.C. Breedveld, G.R. Burmester, M. Dougados, P. Emery, G. Ferraccioli, C. Gabay, A. Gibofsky, J.J. Gomez-Reino, G. Jones, T.K. Kvien, M. Murakami, N. Nishimoto, J.S. Smolen, Blocking the effects of interleukin-6 in rheumatoid arthritis and other inflammatory rheumatic diseases: systematic literature review and meta-analysis informing a consensus statement, *Ann. Rheum. Dis.* 72 (4) (2013) 583–589.
- [188] A. Venkateswaran, Tocilizumab, *Mabs* 1 (5) (2009) 432–438.
- [189] S.A. Jones, J. Scheller, S. Rose-John, Therapeutic strategies for the clinical blockade of IL-6/gp130 signaling, *J. Clin. Invest.* 121 (9) (2011) 3375–3383.
- [190] S.C. Williams, First IL-6-blocking drug nears approval for rare blood disorder, *Nat. Med.* 19 (10) (2013) 1193.
- [191] J.F. Rossi, S. Negrier, N.D. James, I. Kocak, R. Hawkins, H. Davis, U. Prabhakar, X. Qin, P. Mulders, B. Berns, A phase I/II study of siltuximab (CINTO 328), an anti-interleukin-6 monoclonal antibody, in metastatic renal cell cancer, *Br. J. Cancer* 103 (8) (2010) 1154–1162.
- [192] J. Karkera, H. Steiner, W. Li, V. Skradski, P.L. Moser, S. Riethdorf, M. Reddy, T. Puchalski, K. Safer, U. Prabhakar, K. Pantel, M. Qi, Z. Culig, The anti-interleukin-6 antibody siltuximab down-regulates genes implicated in tumorigenesis in prostate cancer patients from a phase I study, *Prostate* 71 (13) (2011) 1455–1465.
- [193] G.R. Burmester, Y. Lin, R. Patel, J. van Adelsberg, E.K. Mangan, N.M. Graham, H. van Hoogstraten, D. Bauer, J. Ignacio Vargas, E.B. Lee, Efficacy and safety of sarilumab monotherapy versus adalimumab monotherapy for the treatment of patients with active rheumatoid arthritis (MONARCH): a randomised, double-blind, parallel-group phase III trial, *Ann. Rheum. Dis.* 76 (5) (2017) 840–847.
- [194] C. Blanchetot, N. De Jonge, A. Desmyter, N. Ongena, E. Hofman, A. Klarenbeek, A. Sadi, A. Hultberg, A. Kretz-Rommel, S. Spinelli, R. Loris, C. Cambillau, H. de Haard, Structural mimicry of receptor interaction by antagonistic interleukin-6 (IL-6) antibodies, *J. Biol. Chem.* 291 (26) (2016) 13846–13854.
- [195] A.D. Gelinas, D.R. Davies, T.E. Edwards, J.C. Rohloff, J.D. Carter, C. Zhang, S. Gupta, Y. Ishikawa, M. Hirota, Y. Nakaishi, T.C. Jarvis, N. Janjic, Crystal structure of interleukin-6 in complex with a modified nucleic acid ligand, *J. Biol. Chem.* 289 (12) (2014) 8720–8734.
- [196] E.C. Borden, G.C. Sen, G. Uze, R.H. Silverman, R.M. Ransohoff, G.R. Foster, G.R. Stark, Interferons at age 50: past, current and future impact on biomedicine, *Nat. Rev. Drug Discov.* 6 (12) (2007) 975–990.
- [197] P. Berraondo, M.F. Sanmamed, M.C. Ochoa, I. Etxeberria, M.A. Aznar, J.L. Perez-Gracia, M.E. Rodriguez-Ruiz, M. Ponz-Sarvise, E. Castanon, I. Melero, Cytokines in clinical cancer immunotherapy, *Br. J. Cancer* 120 (2018) 6–15.
- [198] G. Uze, G. Schreiber, J. Piehler, S. Pellegrini, The receptor of the type I interferon family, *Curr. Top. Microbiol. Immunol.* 316 (2007) 71–95.
- [199] M.K. Crow, M. Olfertiev, K.A. Kirou, Type I interferons in autoimmune disease, *Annu. Rev. Pathol.* (2018).
- [200] S. Uematsu, S. Akira, Toll-like receptors and type I interferons, *J. Biol. Chem.* 282 (21) (2007) 15319–15323.
- [201] N. Yan, Z.J. Chen, Intrinsic antiviral immunity, *Nat. Immunol.* 13 (3) (2012) 214–222.
- [202] F. McNab, K. Mayer-Barber, A. Sher, A. Wack, A. O'Garra, Type I interferons in infectious disease, *Nat. Rev. Immunol.* 15 (2) (2015) 87–103.
- [203] L.B. Ivashkin, L.T. Donlin, Regulation of type I interferon responses, *Nat. Rev. Immunol.* 14 (1) (2014) 36–49.
- [204] R. Radhakrishnan, L.J. Walter, A. Hruza, P. Reichert, P.P. Trotta, T.L. Nagabushan, M.R. Walter, Zinc mediated dimer of human interferon-alpha 2b revealed by X-ray crystallography, *Structure* 4 (12) (1996) 1453–1463.
- [205] W. Klaus, B. Gsell, A.M. Labhardt, B. Wipf, H. Senn, The three-dimensional high resolution structure of human interferon alpha-2a determined by heteronuclear NMR spectroscopy in solution, *J. Mol. Biol.* 274 (4) (1997) 661–675.
- [206] M. Karpusas, M. Nolte, C.B. Benton, W. Meier, W.N. Lipscomb, S. Goetz, The crystal structure of human interferon beta at 2.2-A resolution, *Proc. Natl. Acad. Sci. U. S. A.* 94 (22) (1997) 11813–11818.
- [207] C. Thomas, I. Moraga, D. Levin, P.O. Krutzik, Y. Podoplelova, A. Trejo, C. Lee, G. Yarden, S.E. Vleck, J.S. Glenn, G.P. Nolan, J. Piehler, G. Schreiber, K.C. Garcia, Structural linkage between ligand discrimination and receptor activation by type I interferons, *Cell* 146 (4) (2011) 621–632.
- [208] N.A. de Weerd, A.Y. Matthews, P.R. Pattie, N.M. Bourke, S.S. Lim, J.P. Vivian, J. Rossjohn, P.J. Hertzog, A hot spot on interferon alpha/beta receptor subunit 1 (IFNAR1) underpins its interaction with interferon-beta and dictates signaling, *J. Biol. Chem.* 292 (18) (2017) 7554–7565.
- [209] S.R. Quadri-Akabayov, J.H. Chill, R. Levy, N. Kessler, J. Anglister, Determination of the human type I interferon receptor binding site on human interferon-alpha2 by cross saturation and an NMR-based model of the complex, *Protein Sci.* 15 (11) (2006) 2656–2668.
- [210] M. Petri, D.J. Wallace, A. Spindler, V. Chindalore, K. Kalunian, E. Mysler, C.M. Neuwelt, G. Robbie, W.I. White, B.W. Higgs, Y. Yao, L. Wang, D. Ethgen, W. Greth, Sifalimumab, a human anti-interferon-alpha monoclonal antibody, in systemic lupus erythematosus: a phase I randomized, controlled, dose-escalation study, *Arthritis Rheum.* 65 (4) (2012) 1011–1021.
- [211] M. Khamashta, J.T. Merrill, V.P. Werth, R. Furie, K. Kalunian, G.G. Illei, J. Drappa, L. Wang, W. Greth, C.D.S. investigators, Sifalimumab, an anti-interferon-alpha monoclonal antibody, in moderate to severe systemic lupus erythematosus: a randomised, double-blind, placebo-controlled study, *Ann. Rheum. Dis.* 75 (11) (2016) 1909–1916.
- [212] V. Oganesyan, L. Peng, R.M. Woods, H. Wu, W.F. Dall'Acqua, Structural insights into the neutralization properties of the fully human, anti-interferon monoclonal antibody sifalimumab, *J. Biol. Chem.* 290 (24) (2015) 14979–14985.
- [213] J.M. McBride, J. Jiang, A.R. Abbas, A. Morimoto, J. Li, R. Maciuba, M. Townsend, D.J. Wallace, W.P. Kennedy, J. Drappa, Safety and pharmacodynamics of rontalizumab in patients with systemic lupus erythematosus: results of a phase I, placebo-controlled, double-blind, dose-escalation study, *Arthritis Rheum.* 64 (11) (2012) 3666–3676.
- [214] K.C. Kalunian, J.T. Merrill, R. Maciuba, J.M. McBride, M.J. Townsend, X. Wei, J.C. Davis Jr., W.P. Kennedy, A phase II study of the efficacy and safety of rontalizumab (rhuMAb interferon-alpha) in patients with systemic lupus erythematosus (ROSE), *Ann. Rheum. Dis.* 75 (1) (2016) 196–202.
- [215] B. Maurer, I. Bosanac, S. Shia, M. Kwong, R. Corpuz, R. Vandlen, K. Schmidt, C. Eigenbrot, Structural basis of the broadly neutralizing anti-interferon-alpha antibody rontalizumab, *Protein Sci.* 24 (9) (2015) 1440–1450.
- [216] R. Furie, M. Khamashta, J.T. Merrill, V.P. Werth, K. Kalunian, P. Brohawn, G.G. Illei, J. Drappa, L. Wang, S. Yoo, C.D.S. Investigators, Anifrolumab, an anti-interferon-alpha receptor monoclonal antibody, in moderate-to-severe systemic lupus erythematosus, *Arthritis Rheumatol.* 69 (2) (2017) 376–386.
- [217] D. Wyllie, K.C. Sogaard, K. Holland, X. Yaobo, M. Bregu, A.V. Hill, E. Kiss-Toth, Identification of 34 novel proinflammatory proteins in a genome-wide macrophage functional screen, *PLoS One* 7 (7) (2012) e42388.
- [218] C. Garlanda, C.A. Dinarello, A. Mantovani, The interleukin-1 family: back to the future, *Immunity* 39 (6) (2013) 1003–1018.
- [219] D. Burger, N. Molnarfi, L. Gruaz, J.M. Dayer, Differential induction of IL-1 β and TNF by CD40 ligand or cellular contact with stimulated T cells depends on the maturation stage of human monocytes, *J. Immunol.* 173 (2) (2004) 1292–1297.
- [220] A.S. Freedman, G. Freeman, J. Whitman, J. Segil, J. Daley, L.M. Nadler, Pre-exposure of human B cells to recombinant IL-1 enhances subsequent proliferation, *J. Immunol.* 141 (10) (1988) 3398–3404.
- [221] C.A. Dinarello, Immunological and inflammatory functions of the interleukin-1 family, *Annu. Rev. Immunol.* 27 (2009) 519–550.
- [222] M.G. Netea, C.A. Nold-Petry, M.F. Nold, L.A. Joosten, B. Opitz, J.H. van der Meer, F.L. van de Veerdonk, G. Ferwerda, B. Heinrichs, I. Devesa, C.J. Funk, R.J. Mason, B.J. Kullberg, A. Rubartelli, J.W. van der Meer, C.A. Dinarello, Differential requirement for the activation of the inflammasome for processing and release of IL-1 β in monocytes and macrophages, *Blood* 113 (10) (2009) 2324–2335.
- [223] D. Wang, S. Zhang, L. Li, X. Liu, K. Mei, X. Wang, Structural insights into the assembly and activation of IL-1 β with its receptors, *Nat. Immunol.* 11 (10) (2010) 905–911.
- [224] G.P. Vigers, L.J. Anderson, P. Caffes, B.J. Brandhuber, Crystal structure of the type-I interleukin-1 receptor complexed with interleukin-1 β , *Nature* 386 (6621) (1997) 190–194.
- [225] J.A. Lust, M.Q. Lacy, S.R. Zeldenrust, A. Dispensieri, M.A. Gertz, T.E. Witzig, S. Kumar, S.R. Hayman, S.J. Russell, F.K. Buadi, S.M. Geyer, M.E. Campbell, R.A. Kyle, S.V. Rajkumar, P.R. Greipp, M.P. Kline, Y. Xiong, L.L. Moon-Tasson, K.A. Donovan, Induction of a chronic disease state in patients with smoldering or indolent multiple myeloma by targeting interleukin 1 β -induced interleukin 6 production and the myeloma proliferative component, *Mayo Clin. Proc.* 84 (2) (2009) 114–122.
- [226] P.C. Grayson, Y. Yazici, M. Merideth, H.N. Sen, M. Davis, E. Novakovich, E. Joyal, R. Goldbach-Mansky, C.H. Sibley, Treatment of mucocutaneous manifestations in Behcet's disease with anakinra: a pilot open-label study, *Arthritis Res. Ther.* 19 (1) (2017) 69.
- [227] R. Terkeltaub, J.S. Sundy, H.R. Schumacher, F. Murphy, S. Bookbinder, S. Biedermann, R. Wu, S. Mellis, A. Radin, The interleukin 1 inhibitor rilonacept in treatment of chronic gouty arthritis: results of a placebo-controlled, mono-sequence crossover, non-randomised, single-blind pilot study, *Ann. Rheum. Dis.* 68 (10) (2009) 1613–1617.
- [228] H. Gram, Preclinical characterization and clinical development of ILARIS(R) (canakinumab) for the treatment of autoinflammatory diseases, *Curr. Opin. Chem. Biol.* 32 (2016) 1–9.
- [229] J.B. Kuemmerle-Deschner, I. Haug, Canakinumab in patients with cryopyrin-associated periodic syndrome: an update for clinicians, *Ther. Adv. Musculoskeletal Dis.* 5 (6) (2013) 315–329.
- [230] S.X. Wang, S.B. Abramson, M. Attur, M.A. Karsdal, R.A. Preston, C.J. Lozada, M.P. Kosloski, F. Hong, P. Jiang, M.J. Saltarelli, B.A. Hendrickson, J.K. Medema, Safety, tolerability, and pharmacodynamics of an anti-interleukin-1alpha/beta dual variable domain immunoglobulin in patients with osteoarthritis of the knee: a randomized phase 1 study, *Osteoarthr. Cartil.* 25 (12) (2017) 1952–1961.
- [231] F. La Torre, M.C. Caparello, R. Cimaz, Canakinumab for the treatment of TNF-receptor associated periodic syndrome, *Expert Rev. Clin. Immunol.* 13 (6) (2017) 513–523.
- [232] G. Kaplanski, Interleukin-18: biological properties and role in disease pathogenesis, *Immunol. Rev.* 281 (1) (2018) 138–153.
- [233] I.S. Afonina, C. Muller, S.J. Martin, R. Beyaert, Proteolytic processing of interleukin-1 family cytokines: variations on a common theme, *Immunity* 42 (6) (2015) 991–1004.
- [234] Z. Kato, J. Jee, H. Shikano, M. Mishima, I. Ohki, H. Ohnishi, A. Li, K. Hashimoto,

- E. Matsukuma, K. Omoya, Y. Yamamoto, T. Yoneda, T. Hara, N. Kondo, M. Shirakawa, The structure and binding mode of interleukin-18, *Nat. Struct. Biol.* 10 (11) (2003) 966–971.
- [235] N. Tsutsumi, T. Kimura, K. Arita, M. Ariyoshi, H. Ohnishi, T. Yamamoto, X. Zuo, K. Maenaka, E.Y. Park, N. Kondo, M. Shirakawa, H. Tochio, Z. Kato, The structural basis for receptor recognition of human interleukin-18, *Nat. Commun.* 5 (2014) 5340.
- [236] C.A. Dinarello, D. Novick, S. Kim, G. Kaplanski, Interleukin-18 and IL-18 binding protein, *Front. Immunol.* 4 (2013) 289.
- [237] S.L. Doyle, E. Ozaki, K. Brennan, M.M. Humphries, K. Mulfaul, J. Keaney, P.F. Kenna, A. Maminishkis, A.S. Kiang, S.P. Saunders, E. Hams, E.C. Lavelle, C. Gardiner, P.G. Fallon, P. Adamson, P. Humphries, M. Campbell, IL-18 attenuates experimental choroidal neovascularization as a potential therapy for wet age-related macular degeneration, *Sci. Transl. Med.* 6 (230) (2014) 230ra44.
- [238] P.P. Tak, M. Bacchi, M. Bertolino, Pharmacokinetics of IL-18 binding protein in healthy volunteers and subjects with rheumatoid arthritis or plaque psoriasis, *Eur. J. Drug Metab. Pharmacokinet.* 31 (2) (2006) 109–116.
- [239] R. Faggioni, R.C. Cattley, J. Guo, S. Flores, H. Brown, M. Qi, S. Yin, D. Hill, S. Scully, C. Chen, D. Brankow, J. Lewis, C. Baikalov, H. Yamane, T. Meng, F. Martin, S. Hu, T. Boone, G. Senaldi, IL-18-binding protein protects against lipopolysaccharide-induced lethality and prevents the development of Fas/Fas ligand-mediated models of liver disease in mice, *J. Immunol.* 167 (10) (2001) 5913–5920.
- [240] E.A. McKie, J.L. Reid, P.C. Mistry, S.L. DeWall, L. Abberley, P.D. Ambery, B. Gil-Extremera, A study to investigate the efficacy and safety of an anti-interleukin-18 monoclonal antibody in the treatment of type 2 diabetes mellitus, *PLoS One* 11 (3) (2016) e0150018.
- [241] T. Vanden Berghe, D. Demon, P. Bogaert, B. Vandendriessche, A. Goethals, B. Depuydt, M. Vuylsteke, R. Roelandt, E. Van Wontghem, J. Vandenbroecke, S.M. Choi, E. Meyer, S. Krautwald, W. Declercq, N. Takahashi, A. Cauwels, P. Vandeneebele, Simultaneous targeting of IL-1 and IL-18 is required for protection against inflammatory and septic shock, *Am. J. Respir. Crit. Care Med.* 189 (3) (2014) 282–291.
- [242] J. Sarmiento, C. Shumate, K. Suetomi, A. Ravindran, L. Villegas, K. Rajarathnam, J. Navarro, Diverging mechanisms of activation of chemokine receptors revealed by novel chemokine agonists, *PLoS One* 6 (12) (2011) e27967.
- [243] S.T. Das, L. Rajagopalan, A. Guerrero-Plata, J. Sai, A. Richmond, R.P. Garofalo, K. Rajarathnam, Monomeric and dimeric CXCL8 are both essential for in vivo neutrophil recruitment, *PLoS One* 5 (7) (2010) e11754.
- [244] A. Ravindran, P.R. Joseph, K. Rajarathnam, Structural basis for differential binding of the interleukin-8 monomer and dimer to the CXCR1 N-domain: role of coupled interactions and dynamics, *Biochemistry* 48 (37) (2009) 8795–8805.
- [245] J. Heidemann, H. Ogawa, M.B. Dwinell, P. Rafiee, C. Maaser, H.R. Gockel, M.F. Otterman, D.M. Ota, N. Lugerling, W. Domischke, D.G. Binion, Angiogenic effects of interleukin 8 (CXCL8) in human intestinal microvascular endothelial cells are mediated by CXCR2, *J. Biol. Chem.* 278 (10) (2003) 8508–8515.
- [246] S.H. Park, S. Berkamp, J. Radoicic, A.A. De Angelis, S.J. Opella, Interaction of monomeric interleukin-8 with CXCR1 mapped by proton-detected fast MAS solid-state NMR, *Biophys. J.* 113 (12) (2017) 2695–2705.
- [247] S. Berkamp, S.H. Park, A.A. De Angelis, F.M. Marassi, S.J. Opella, Structure of monomeric interleukin-8 and its interactions with the N-terminal binding site-I of CXCR1 by solution NMR spectroscopy, *J. Biomol. NMR* 69 (3) (2017) 111–121.
- [248] B.S. Qazi, K. Tang, A. Qazi, Recent advances in underlying pathologies provide insight into interleukin-8 expression-mediated inflammation and angiogenesis, *Int. J. Inflamm.* 2011 (2011) 908468.
- [249] L. Skov, F.J. Beurskens, C.O. Zachariae, S. Reitamo, J. Teeling, D. Satijn, K.M. Knudsen, E.P. Boot, D. Hudson, O. Baadsgaard, P.W. Parren, J.G. van de Winkel, IL-8 as antibody therapeutic target in inflammatory diseases: reduction of clinical activity in palmoplantar pustulosis, *J. Immunol.* 181 (1) (2008) 669–679.
- [250] V.C. Broadbudd, A.M. Boylan, J.M. Hoeffel, K.J. Kim, M. Sadick, A. Chunthrapai, C.A. Hebert, Neutralization of IL-8 inhibits neutrophil influx in a rabbit model of endotoxin-induced pleurisy, *J. Immunol.* 152 (6) (1994) 2960–2967.
- [251] B.M. Mian, C.P. Dinney, C.E. Bermejo, P. Sweeney, C. Tellez, X.D. Yang, J.M. Gudas, D.J. McConkey, M. Bar-Eli, Fully human anti-interleukin 8 antibody inhibits tumor growth in orthotopic bladder cancer xenografts via down-regulation of matrix metalloproteases and nuclear factor-kappaB, *Clin. Cancer Res.* 9 (8) (2003) 3167–3175.
- [252] R.A. Dumont, B.D. Car, N.N. Voitenok, U. Junker, B. Moser, O. Zak, T. O'Reilly, Systemic neutralization of interleukin-8 markedly reduces neutrophilic pleocytosis during experimental lipopolysaccharide-induced meningitis in rabbits, *Infect. Immun.* 68 (10) (2000) 5756–5763.
- [253] E. Elinav, R. Nowarski, C.A. Thaiss, B. Hu, C. Jin, R.A. Flavell, Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms, *Nat. Rev. Cancer* 13 (11) (2013) 759–771.
- [254] D. Wendling, F. Verhoeven, X. Guillot, C. Prati, Immunogenicity of TNF alpha inhibitors in rheumatology: many questions, enough answers? *Expert Opin. Drug Saf.* 16 (1) (2017) 1–3.
- [255] R.S. Wallis, Reactivation of latent tuberculosis by TNF blockade: the role of interferon gamma, *J. Invest. Dermatol. Symp. Proc.* 12 (1) (2007) 16–21.
- [256] F. Tubach, D. Salmon, P. Ravaud, Y. Allanore, P. Goupille, M. Breban, B. Pallot-Prades, S. Pouplin, A. Sacchi, R.M. Chicheamian, S. Bretagne, D. Emilie, M. Lemann, O. Lortholary, X. Mariette, G. Research Axed on Tolerance of Biotherapies, Risk of tuberculosis is higher with anti-tumor necrosis factor monoclonal antibody therapy than with soluble tumor necrosis factor receptor therapy: the three-year prospective French Research Axed on Tolerance of Biotherapies registry, *Arthritis Rheum.* 60 (7) (2009) 1884–1894.
- [257] L.D. Settas, G. Tsimirikas, G. Vosvotekas, E. Triantafyllidou, P. Nicolaides, Reactivation of pulmonary tuberculosis in a patient with rheumatoid arthritis during treatment with IL-1 receptor antagonists (anakinra), *J. Clin. Rheumatol.* 13 (4) (2007) 219–220.
- [258] H.M. Hoffman, M.L. Throne, N.J. Amar, M. Sebai, A.J. Kivitz, A. Kavanaugh, S.P. Weinstein, P. Belomestnov, G.D. Yancopoulos, N. Stahl, S.J. Mellis, Efficacy and safety of rilonacept (interleukin-1 Trap) in patients with cryopyrin-associated periodic syndromes: results from two sequential placebo-controlled studies, *Arthritis Rheum.* 58 (8) (2008) 2443–2452.
- [259] E. Dhimolea, Canakinumab, *Mabs* 2 (1) (2010) 3–13.
- [260] S. Sarosiek, R. Shah, N.C. Munshi, Review of siltuximab in the treatment of multicentric Castleman's disease, *Ther. Adv. Hematol.* 7 (6) (2016) 360–366.
- [261] M. Arruebo, N. Vilaboa, B. Saez-Gutierrez, J. Lambea, A. Tres, M. Valladares, A. Gonzalez-Fernandez, Assessment of the evolution of cancer treatment therapies, *Cancers (Basel)* 3 (3) (2011) 3279–3330.