

Averting inflammation by targeting the cytokine environment

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Abstract | Cytokines are key instigators and regulators of immune responses and therefore hold great potential as targets for new therapeutic strategies. However, the selection of which cytokines to target, and in particular the identification of which cytokines regulate the rate-limiting steps of disease pathways, is crucial to the success of such strategies. Moreover, balancing the need for ablating pathological inflammatory responses and simultaneously maintaining the ability to control infectious agents is a key consideration. Recent advances in our understanding of cytokine networks, as well as technical progress in blocking cytokines *in vivo*, are likely to be a source for new drugs that can control chronic inflammatory diseases.

In 1957, a soluble factor that protected cells from viral infection was discovered by Isaacs and Lindenmann and named interferon (IFN)^{1,2}. This landmark finding set the stage for cytokine research. Since then more than 90 cytokines and cytokine receptors have been identified, nine of which are the basis for current therapeutics on the market³. Given the fundamental roles of cytokines in the development and pathogenesis of many inflammatory diseases, there has been extensive worldwide research and development focused on blocking or enhancing cytokine activity.

There are numerous clinical applications for targeting cytokines and include inflammatory diseases, cancer immunotherapy, bone disorders, metabolic diseases, wound healing and antiviral therapy. Although not all potential cytokine targets can be covered in this Review, we will outline some of the most effective and promising cytokine targets that have been linked to inflammatory diseases in preclinical and clinical studies in recent years. This selection is based on an established overview of the literature and is shaped by a viewpoint of the probable success or failure of targeting these cytokine pathways. Finally, we will discuss some of the issues facing the development of cytokine-targeted drugs with respect to the type of therapeutics being developed and the inherent redundancies in inflammatory cytokine networks.

The early release of cytokines shapes the nature of inflammatory responses, and these responses can be beneficial — such as driving protective immunity — or detrimental — such as the induction of immunopathology. At the top of the inflammatory cytokine

cascade are molecules such as tumour necrosis factor- α (TNF α), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-1 (IL-1), IL-6, IL-12 and IL-23. They are secreted mainly by myeloid cells and they all have fundamental effects on multiple components of the immune system (FIG. 1). Together with the local environment (for example, other cytokines or the specific tissue) these cytokines lead to the differentiation of cell types, which will produce combinations of cytokines to aid the clearance of invading pathogens, or, in some cases, to induce inflammatory disorders. Thus, these early cytokines are at key rate-limiting steps of disease development and have been the focus of extensive research and development. Although some therapies targeting these cytokines — such as TNF α , discussed below — have been highly effective^{4–6}, there are still considerable numbers of individuals for whom this therapy is ineffective⁷. Primarily, this is consistent with the heterogeneous nature of most chronic inflammatory diseases; even key upstream cytokines may not be fundamental to the disease pathogenesis in all cases. Blocking multiple upstream cytokines holds some promise in the treatment of such individuals, but the trade-off of a severely compromised immune system may bring no further advantage to patients above that of current broad-spectrum immunosuppressive drugs.

As our understanding of cytokine networks improves, perhaps the next wave of cytokine-targeting therapeutics will abrogate single well-defined pathways of inflammation, which in combination could lead to treatment strategies with fewer side effects. Now, we discuss in more detail these early inflammatory cytokines that

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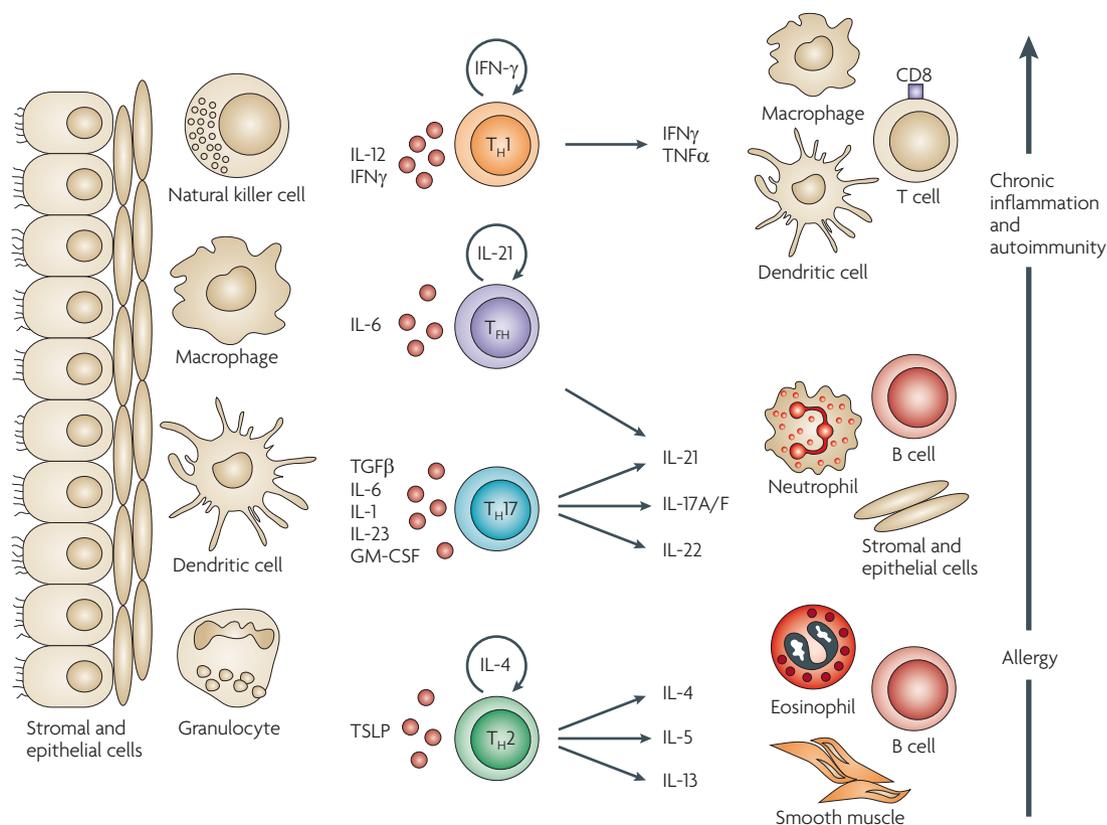


Figure 1 | Pro-inflammatory and effector cytokines involved in T_H1/T_H17 -type autoimmune inflammatory and T_H2 -type allergic diseases. Myeloid cells such as dendritic cells, monocytes and/or macrophages and granulocytes, together with stromal and epithelial cells secrete pro-inflammatory cytokines that promote the activation of naive $CD4^+$ T cells and their differentiation into diverse T helper (T_H) cell subsets with distinct effector functions. Autocrine production of interferon- γ (IFN γ), interleukin-4 (IL-4) and IL-21 secures differentiation of T_H1 , T_H2 and T follicular helper (T_{FH}) cells, respectively. However, T_H17 cell differentiation relies on factors (IL-6, IL-23, IL-1, transforming growth factor- β (TGF β)) produced by non-T cells. IFN γ and tumour necrosis factor- α (TNF α) secreted by T_H1 cells are essential for the antimicrobial activity of macrophages and dendritic cells by triggering production of reactive oxygen species and reactive nitrogen species, which also mediate collateral tissue damage. In addition, T_H1 memory cells are key for survival and function of memory $CD8^+$ T cells. IL-21 secreted by T_{FH} cells promotes germinal centre formation and B cell responses. IL-17A, IL-17F and IL-22 produced by T_H17 cells and $\gamma\delta$ T cells mediate an antibacterial activity at epithelial barriers by acting on neutrophils, macrophages and epithelial cells. T_H17 cells drive organ-related autoimmunity partially by the secretion of IL-17A. T_H2 cells protect against chronic nematode infection but are also responsible for allergy and asthma, and tissue fibrosis by the secretion of IL-4, IL-5 and IL-13. IL-5 is key for eosinophilic inflammation, whereas IL-4 and IL-13 promote B cell immunoglobulin E (IgE) production, goblet cell mucus secretion, and alternative activation of macrophages. GM-CSF, granulocyte–macrophage colony-stimulating factor; TSLP, thymic stromal lymphopoietin.

have been the focus of the pharmaceutical industry and academic institutions. This is followed by a discussion of the preclinical data from the latest cytokines that might hold promise for the future.

Innate inflammatory cytokines

TNF α . One of the most avidly studied and clinically targeted cytokines is TNF α . TNF α is a pleiotropic cytokine that has fundamental roles in lymphoid organogenesis, inflammation, antitumour activity and host defence against intracellular pathogens. It is expressed by several cell types including macrophages, monocytes, neutrophils, T lymphocytes and natural killer (NK) cells. Its receptors, TNF receptor 1 (TNFR1; also known as p60) and TNFR2 (also known as p75), are ubiquitously expressed. TNF α is primarily produced as a biologically

active membrane-bound pro-form arranged as a homotrimer, which is cleaved by the metalloproteinase TNF α -converting enzyme (TACE; also known as ADAM17) to release soluble TNF α . Deregulated TNF α production can be detrimental and has been associated with sepsis and several other inflammatory and autoimmune diseases, including colitis and rheumatoid arthritis (RA). Indeed, a pathogenic role of TNF α has been confirmed in most mouse models of inflammatory bowel disease (IBD) and organ-related autoimmunity^{8–13} except myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE), which is exacerbated in TNF α -deficient mice¹⁴. A potential anti-inflammatory role of TNF α in neural inflammation is supported by the finding that TNF α treatment reduces the severity of MOG-induced disease¹⁴.

Most preclinical data showing efficacy of cytokine blockade during chronic inflammatory diseases has yet to be translated into clinical practice. TNF α inhibitors are, however, leaders in this field and have shown efficacy in patients with RA^{4–6}, IBD¹⁵ and psoriasis¹⁶. By contrast, TNF α blockade was reported to worsen disease in patients with multiple sclerosis¹⁷.

So far, five TNF α -blocking biologicals have shown efficacy in the clinic. Infliximab (Remicade; Centocor Ortho Biotech) is an immunoglobulin G1 (IgG1) mouse–human chimeric antibody, whereas adalimumab (Humira; Abbott) and golimumab (Simponi; Centocor Ortho Biotech) are fully human antibodies. Notably, besides TNF α neutralization, these drugs may also act by apoptotic lysis of cells that express TNF α on their surface¹⁸. Etanercept (Enbrel; Amgen/Pfizer) is a TNFR2–crystallizable fragment (Fc) IgG1 chimera molecule that has shown efficacy in treating patients with RA but not IBD, and does not seem to lead to the death of cells expressing surface TNF α . Certolizumab pegol (Cimzia; UCB Celltech) is a new, polyethylene glycol-conjugated, humanized, antigen-binding fragment (Fab') of an TNF α -specific monoclonal antibody (mAb) that does not mediate apoptosis. TNF α -blocking drugs are typically administered with the immunosuppressant drug methotrexate, which improves their efficacy⁴ and probably also impairs host responses against the therapeutics themselves.

Unfortunately, not all patients respond equally to each medication, highlighting the often heterogeneous nature of RA and IBD. A patient may be unresponsive to several medications, either alone or in combination, and then respond to another medication. For this reason, there is room in the therapeutic armoury for several effective blockers of the same target.

TNF α was initially identified on the basis of its tumour-killing activity¹⁹ and was described by Coley more than 100 years ago²⁰. Indeed, TNF α can induce haemorrhagic necrosis of certain tumours, in particular in combination with chemotherapeutic drugs. TNF α possibly induces destruction of the tumour-associated vasculature, together with a direct or free-radical-induced apoptotic effect on tumour cells²¹. A crucial role of TNF α for CD8⁺ T cell-mediated elimination of A9 Lewis lung carcinoma has been established in mice²². Using the same model antigen²³, Calzascia and colleagues concluded that TNF α has a major role in antitumour responses, but was redundant for antiviral immunity, presumably owing to the presence of additional inflammatory factors secreted in response to viral-specific factors such as Toll-like receptor ligands.

Accordingly, the consequences of TNF α blockade on the short-term and the long-term development of cancer remain a debated concern. Indeed, meta-analyses of clinical trials indicated the possibility of a markedly increased risk of several types of cancer occurring within months of the initiation of treatment with TNF α blockers^{24,25}. However, in a large observational study there was no increase in the short-term or the mid-term development of malignant tumours in 6,366 patients with RA (during a combined 25,693 human years) receiving therapy

with TNF α blockers (that is, adalimumab, infliximab or etanercept) compared with patients who received no medication or standard methotrexate therapy²⁶.

Notably, some inflammatory autoimmune diseases such as RA, systemic lupus erythematosus (SLE), Sjögren's syndrome, Hashimoto thyroiditis and coeliac disease are associated with an increased risk of developing malignant lymphoma that may range from twofold in RA to 18-fold in Sjögren's syndrome²⁷. According to a large observational study TNF α blockers did not further increase this risk in patients with RA²⁸. However, investigations by the US Food and Drug Administration led to the conclusion that TNF α blockers enhance cancer risk, in particular the risk of developing lymphoma in children and in adolescents. Although the evidence is not strong, this new safety information led to the issuing of a black box warning to the label of these products.

Nevertheless, many tumours have been shown to produce TNF α themselves and there is strong evidence to suggest that TNF α can promote tumour growth directly or indirectly by the induction of other pro-inflammatory cytokines and angiogenic factors involved in cancer development²⁹. Chemical carcinogen-induced skin tumour development and proliferation of oval cells during the preneoplastic phase of liver carcinogenesis is strikingly reduced in TNF α and TNFR1 knockouts, respectively^{30–32}. Moreover, TNF α has been shown to contribute to the development of hepatocellular carcinoma^{33,34}. Thus, TNF α blockers may actually reduce cancer risk depending on the type of tumour. The improved characterization of tumours using proteomics and genomics will help to identify such tumours in the future.

Given the concerns of increasing susceptibility to infections by the systemic blockade of soluble and membrane-bound TNF α using neutralizing antibodies such as infliximab^{24,35,36}, the selective targeting of soluble TNF α using the Fc–TNFR2 fusion protein (etanercept) or active vaccination with virus-like particles linked to a peptide from the amino terminus of TNF α seems to lower the risk of bacterial infection and are enough to protect against RA^{37,38}. Future approaches encompass tissue-specific or cell-specific targeting of TNF α and its receptors using small interfering RNA (siRNA)-mediated knockdown and may hold therapeutic potential. The intra-articular injection of TNF α -specific siRNA and the targeting of intraperitoneal macrophages by chitosan nanoparticles containing TNF α siRNA have been reported to reduce joint inflammation in models of murine collagen-induced arthritis (CIA)^{39,40}. TNF α and its receptors TNFR1 and TNFR2 are cleaved from the cell membrane by a proteolytic process referred to as ectodomain shedding, which is mediated by TACE. Blockade of TACE is thought to inhibit the shedding of TNF α , which is supported by the observation that mice lacking TACE exclusively on myeloid cells are protected from endotoxin-induced lethality⁴¹. Notably, TACE is responsible for the shedding of several (>30) transmembrane proteins including cytokine receptors (for example, TNFR1 and IL-6 receptor (IL-6R)), growth factors (for example, transforming growth factor- α (TGF α)) and

Systemic lupus erythematosus

(SLE). SLE is a chronic autoimmune disease that can affect any tissue and is associated with the induction of various autoantibodies, such as anti-double-stranded DNA and antihistones.

Sjögren's syndrome

Sjögren's syndrome is a disease in which the glands that produce tears and saliva are destroyed by an inflammatory autoimmune response. Sjögren's syndrome can be associated with other autoimmune diseases such as rheumatoid arthritis.

Hashimoto thyroiditis

Hashimoto thyroiditis is an autoimmune disease in which the thyroid gland is destroyed. The disease is characterized by infiltrating T cells and antibodies against thyroid peroxidase and/or thyroglobulin.

Coeliac disease

Coeliac disease is an autoimmune disorder of the small intestine that is caused by a reaction to gliadin, a prolamin (gluten protein) found in wheat. Although multiple mechanisms may be involved, genetic analysis has linked certain major histocompatibility complex II haplotypes and deficiency in immunoglobulin A with the disease.

Black box warning

The most serious safety warning required on a pharmaceutical label, indicative of a considerable risk of a serious or even life-threatening adverse drug reaction.

Chitosan

A linear polysaccharide that is the structural element in the exoskeleton of crustaceans.

other epidermal growth factor receptor ligands and adhesion molecules⁴², which make it a potent target in inflammation and cancer therapy. Thus, preclinical studies indicated that inhibition of TACE might be beneficial for patients with RA. However, clinical trials failed owing to a lack of efficacy or even hepatotoxicity^{42,43}. Given the malignancies observed in complete TACE-deficient mice (early lethality and eye, hair, skin and lung defects)⁴⁴ and the diversity of TACE substrates, selective and cell-specific and tissue-specific, TACE-targeting approaches seem mandatory to prevent potential adverse effects.

In summary, TNF α is a proven target to ameliorate inflammatory conditions in patients. However, as with most other pro-inflammatory cytokines, it is a double-edged sword with both beneficial and detrimental effects. Harnessing the good and destroying the bad is the goal for future therapies, which may be achieved by new approaches using siRNA-based strategies to block TNF α in a cell-specific and/or in a tissue-specific manner.

IL-6. IL-6 is produced by various haematopoietic and non-haematopoietic cells in response to infection and tissue damage. It is a central mediator of the immune system inducing the liver acute-phase response and optimal B cell and T cell effector responses to pathogens⁴⁵. IL-6-deficient mice are highly susceptible to infection with *Listeria monocytogenes*^{46,47}, *Mycobacterium tuberculosis*⁴⁸, *Toxoplasma gondii*⁴⁹, *Candida albicans*⁵⁰, vaccinia virus and vesicular stomatitis virus⁴⁶. IL-6 also regulates fever through the hypothalamic–pituitary–adrenal axis. IL-6 also has a deleterious role in the development of experimental autoimmune inflammatory diseases including EAE^{51,52}, CIA^{53,54} and experimental autoimmune myocarditis (EAM)⁵⁵, which has been explained by its pivotal role in the induction of pathogenic T helper type 17 (T_H17) cells (FIG. 1).

Although arthritis development in IL-6-deficient mice is completely inhibited, up to 20% of TNF α -deficient mice still develop the disease, indicating that the targeting of IL-6 holds therapeutic potential⁵⁶. Similar to TNF α , IL-6 has a central role in several models of IBD including T cell transfer colitis in severe combined immunodeficient mice, IL-10-deficient colitis and trinitrobenzene sulphuric acid-induced colitis. The severity of disease in these models is strikingly reduced in IL-6-deficient mice⁵⁷ or by the blockade of the IL-6R⁵⁸. Moreover, combined treatment with mAbs against TNF α and IL-6R led to a stronger suppression of colitis activity than did treatment with an antibody against TNF α alone, indicating a potential synergism of the two cytokines. Elevated levels of IL-6 or soluble IL-6R are seen in several inflammatory diseases including RA⁵⁹, psoriasis⁶⁰ and colitis^{61,62}. Furthermore, several neoplastic diseases are associated with increased levels of IL-6 and acute-phase proteins, which in many studies have been correlated to disease severity and outcome⁶³. Taken together, these data highlight IL-6 as an attractive target for therapies.

Clinical investigations with neutralizing IL-6 mAbs in the treatment of lymphoproliferative diseases and, in particular, multiple myeloma began in the early 1990s.

Although the use of mouse human IL-6-specific mAbs resulted in the generation of human mouse-specific antibodies and the concomitant elimination of the IL-6 mAb, treatment of patients with multiple myeloma who were resistant to second-line chemotherapy with a chimeric mouse–human IL-6 antibody (CNTO 328) resulted in disease stabilization but no remission^{64,65}. Endogenous IL-6 production levels immediately decreased on the induction of therapy in most patients, probably through the inhibition of a positive feedback loop. Concerns that IL-6–IL-6-specific mAb complexes may accumulate at high levels in the circulation and act as a depot releasing IL-6 at a high off-rate led to the development of an IL-6R-specific antibody, which has been successfully applied in patients with Castleman's disease⁶⁶.

An important consideration for the design of therapies that modulate the activity of IL-6 is that the IL-6R occurs in a membrane-bound and a soluble form, and requires the accessory molecule glycoprotein 130 (gp130; also known as IL-6R β and CD130) for signal transduction (FIG. 2). Expression of membrane-bound IL-6R α is restricted mainly to cells of the immune system and to hepatocytes, whereas gp130 is ubiquitously expressed. Besides classical signalling involving membrane-bound IL-6R α and gp130, IL-6 bound to soluble IL-6R α can induce trans-signalling by associating with gp130 in a multitude of cell types that do not express membrane-bound IL-6R α (FIG. 2). The use of antibodies directed against the IL-6R α chain allows for the targeting of both membrane-bound and soluble forms of the receptor. Tocilizumab (Actemra/RoActemra; Genetech/Chugai/Roche) is such an IL-6R α -specific humanized antibody that has efficacy when combined with methotrexate in patients with RA, including those who are refractory to TNF α blockers^{67–69}. Notably, a recent study showed that tocilizumab monotherapy was superior to methotrexate in the treatment of RA⁶⁹. Furthermore, tocilizumab was efficacious in the treatment of systemic-onset juvenile idiopathic arthritis⁷⁰, a devastating systemic inflammatory disease affecting growing children. A soluble gp130–Fc fusion protein has been generated to specifically target the soluble IL-6R–IL-6 complex and blocks trans-signalling but not classical membrane IL-6R signalling⁷¹, and it was sufficient to inhibit experimental colitis and experimental arthritis^{58,72} (FIG. 2). This approach may turn out to be therapeutically favourable and lower the risk of susceptibility to bacterial and viral infections observed in the complete absence of IL-6.

However, caution is warranted given that IL-6 has several beneficial effects in addition to protection from infection, and it remains unclear as to which of these activities are mediated by trans-signalling. There is increasing evidence to show that IL-6 has a protective role during neural and liver injury. Pituitary adenylate cyclase-activating polypeptide decreases ischaemic neuronal cell death by the induction of IL-6 (REF. 73) and IL-6R antibody injection increased brain infarct volume after ischaemia⁷⁴. Furthermore, IL-6 promotes liver regeneration⁷⁵ and protects against a multitude of liver-damaging influences including alcohol⁷⁶, concanavalin A⁷⁷ and the environmental toxin carbon tetrachloride⁷⁸. Interestingly,

Castleman's disease

A non-cancerous (benign) disorder of one or many lymph nodes characterized by non-clonal hyperproliferation of B cells as a result from hypersecretion of interleukin-6.

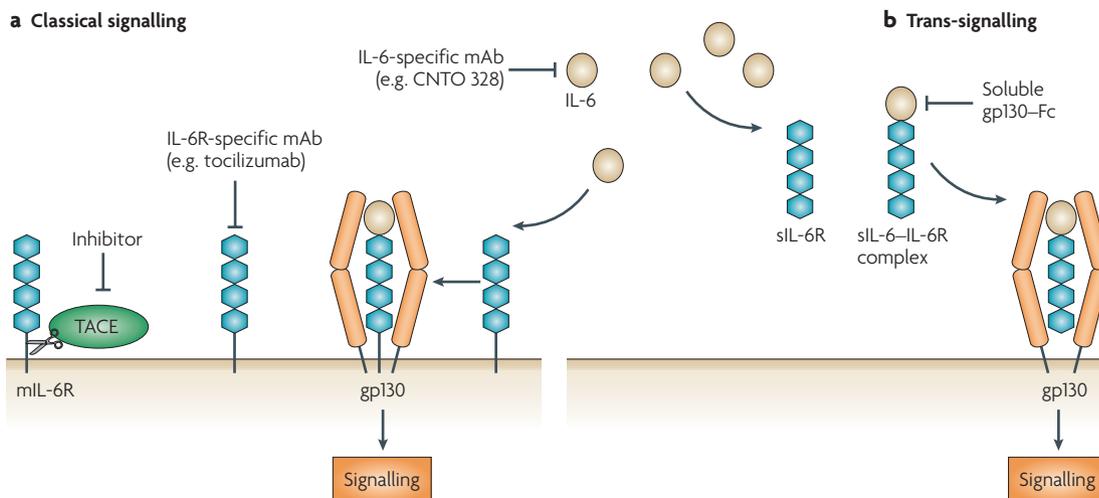


Figure 2 | The IL-6-IL-6R complex. Interleukin-6 (IL-6) signals through a ligand-binding IL-6 receptor (IL-6R; also known as CD126) chain and a common signal-transducing chain glycoprotein 130 (gp130; also known as CD130), which is also engaged by receptors specific for IL-11, IL-27, leukaemia inhibitory factor, oncostatin M (OSM), ciliary neurotrophic factor (CNTF) and cardiotrophin 1 (CT1). Although gp130 is found ubiquitously on almost every cell in the body, expression of membrane-bound IL-6R (mIL-6R) is restricted mainly to haematopoietic cells and hepatocytes. A soluble form of the IL-6R (sIL-6R) can be generated by proteolytic cleavage of mIL-6R by the metalloproteinases TNF α -converting enzyme (TACE; also known as ADAM17) and ADAM10 or alternatively spliced mRNA. **a** | IL-6 responses can be induced classically in cells expressing mIL-6R through a high-affinity tetrameric complex consisting of IL-6, IL-6R and two gp130 molecules (or a hexameric complex consisting of two IL-6, two mIL-6R and two gp130 molecules). **b** | Alternatively, a sIL-6-IL-6R complex directly binds to and signals through gp130 in cells lacking mIL-6R in a process that has been termed trans-signalling. High levels of IL-6 and sIL-6R have been reported in several chronic inflammatory diseases and in cancer. Several drugs that target different components of the IL-6 and IL-6R system have been described, including IL-6-specific monoclonal antibodies (mAbs) (for example, CNTO 328), IL-6R-specific mAbs (for example, tocilizumab) and soluble gp130-Fc, an antagonist of IL-6R trans-signalling. Development of compounds targeting TACE has been discouraging and often discontinued owing to toxicity, lack of specificity and efficacy⁴².

IL-6-specific autoantibodies have been detected in normal human serum⁷⁹ and are associated with the increased mortality of patients with alcoholic cirrhosis⁸⁰, which are consistent with a role of IL-6 in liver protection.

Thus, the potential beneficial effects of long-term IL-6 neutralization for patients suffering from chronic inflammatory diseases may be outweighed in some cases by adverse effects.

IL-1. IL-1 α and IL-1 β (collectively known as IL-1) have important roles in inflammation and host response to infection, often by acting in concert with IL-6 and TNF α . Both IL-1 α and IL-1 β bind to the IL-1R type I (IL-1RI) expressed by a wide range of cells. Binding induces the formation of a high-affinity complex with the IL-1R accessory protein (IL-1RAcP) and the recruitment of the intracellular adaptor protein myeloid differentiation factor 88 (MYD88) and IL-1R-associated kinase 1 (IRAK), which are the proximal mediators of IL-1 signalling (FIG. 3). Given that this signal mediates profound effects in virtually every organ system of the body, it is not surprising that IL-1 activity is physiologically tightly controlled and deregulated in many disease processes. IL-1R antagonist (IL-1Ra) is an endogenously secreted inhibitor that competes with IL-1 α and IL-1 β for binding to IL-1RI without transducing a signal. A second endogenous inhibitor is the nonfunctional IL-1R type II (IL-1RII), a so-called decoy receptor, with a high affinity

for IL-1 β , but only low affinity for IL-1 α and IL-1Ra. A soluble form of the IL-1RII is generated by the proteolytic cleavage of the membrane form⁸¹ (FIG. 3).

Although IL-1 α and IL-1 β induce similar signals by binding to the same receptor, their activity is compartmentalized *in vivo*. They both exist as an intracellular inactive pro-form that is activated by cleavage. Pro-IL-1 α is cleaved by calpain into mature IL-1 α , which is thought to act mainly bound to the cell surface. Pro-IL-1 β is cleaved into mature IL-1 β by the cysteine protease caspase 1 following interaction with one of multiple types of activated inflammasomes. The NALP3-containing inflammasome is implicated in several human diseases. Various gain-of-function mutations of NALP3 that result in enhanced caspase 1 activity and overproduction of IL-1 β have been associated with a group of inflammatory disorders (summarized as cryopyrin-associated periodic syndromes) including Muckle-Wells syndrome, familial cold urticaria, and chronic infantile neurological, cutaneous and articular syndrome (also known as neonatal-onset multisystem inflammatory disease)^{82,83}. Different IL-1 blockers have proved beneficial in patients with these rare genetic diseases including anakinra (Kineret; Biogen), a recombinant human IL-1Ra⁸⁴⁻⁸⁶; canakinumab (Ilaris; Novartis), a fully human IL-1 β -specific mAb⁸⁷; and rilonacept (Arcalyst; Regeneron)^{88,89} (FIG. 4). Rilonacept (also known as IL-1 Trap) is a long-acting IL-1 blocker comprising a dimeric fusion protein of the

NALP3-containing inflammasome

The NALP3-containing inflammasome is a multiprotein complex that functions to activate caspase 1 leading to the cleavage of pro-interleukin-18 (IL-18) and pro-IL-1 β into their active subunits. Most inflammatory diseases associated with IL-1 involve activation of the inflammasome complex.

ligand-binding domains of IL-1RI and IL-1RAcP fused to human IgG1. Inhibition of IL-1 may also be a promising therapy in patients with gout, a disease triggered by excess serum levels of uric acid^{90,91}. The rationale of this approach comes from studies showing that uric acid is an endogenous danger signal released by dying cells and that uric acid crystals induce inflammation by activation of NALP3 and IL-1 β secretion⁹²⁻⁹⁴.

IL-1 activity is crucial for the severity of autoimmune inflammatory disease in animal models. Mice lacking IL-1RI or both IL-1 α and IL-1 β are protected from the development of EAE⁹⁵, EAM⁹⁶ and CIA⁹⁷. Moreover, IL-1Ra-deficient BALB/c mice spontaneously develop chronic inflammatory arthropathy resembling human RA, and overexpression of IL-1Ra protected mice from CIA⁹⁸. These results indicate that IL-1 may be an

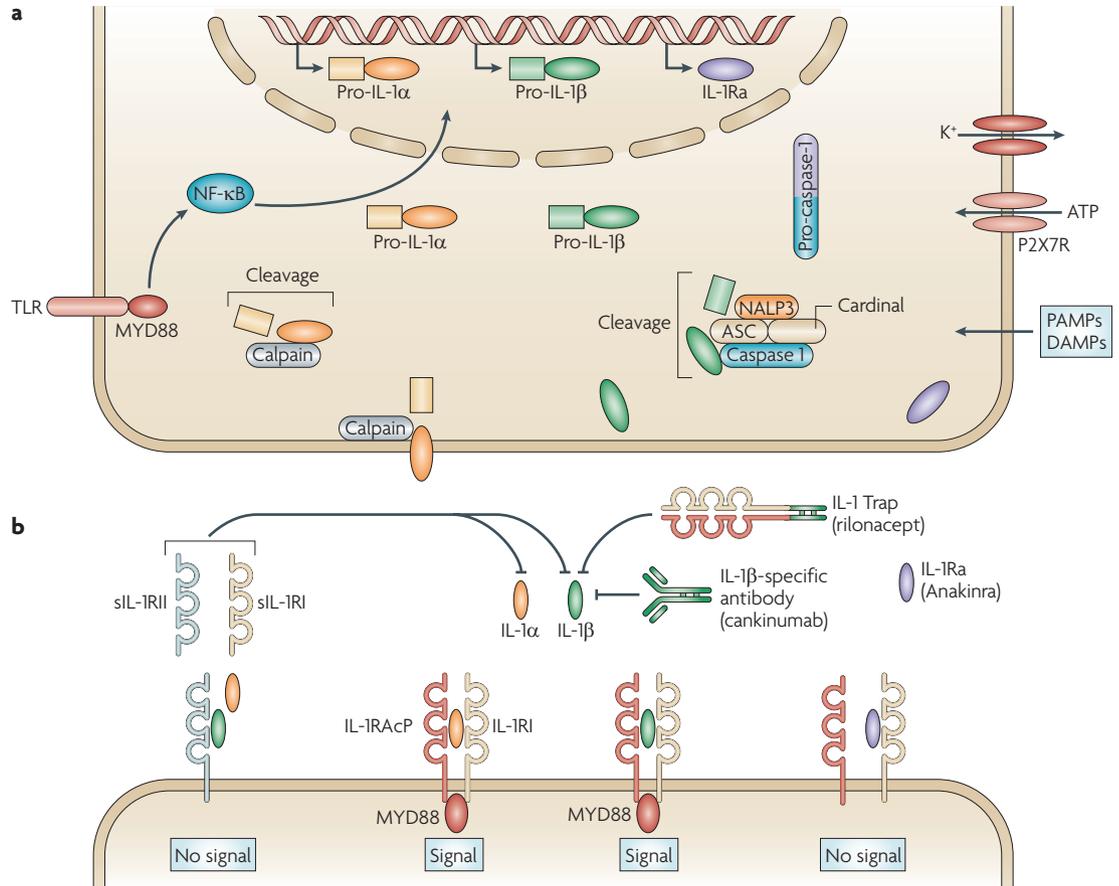


Figure 3 | IL-1 production, binding and signalling. a Toll-like receptor (TLR) ligands induce expression and synthesis of inactive cytosolic interleukin-1 α (IL-1 α) and IL-1 β precursors (known as pro-IL1 α and pro-IL-1 β , respectively). Release of biologically active IL-1 α and IL-1 β requires proteolytic cleavage of the precursors by calpain and caspase 1, respectively. Activation of caspase 1 is also strictly controlled, as it is produced as an inactive precursor (pro-caspase 1), which is cleaved and activated on assembly and oligomerization of protein complexes termed inflammasomes. The NALP3 inflammasome core consists of NALP3, the adaptors ASC and CARDINAL, and pro-caspase 1. Various pathogen-associated molecular patterns (PAMPs) and endogenous danger-associated molecular patterns (DAMPs) such as uric acid crystals, cholesterol crystals or ATP induce assembly and oligomerization of the inflammasome, probably by changes of ion fluxes. For example, ATP activates the P2X7 purinergic ion receptor allowing potassium efflux and the lowering of intracellular potassium concentrations, which triggers caspase 1 and IL-1 β processing and secretion. **b** Binding of IL-1 α or IL-1 β to the IL-1 receptor type I (IL-1RI) recruits IL-1R accessory protein (IL-1RAcP), which results in the association of myeloid differentiation factor 88 (MYD88), IL-1R-associated kinase 1 (IRAK), TNF receptor-associated factor 6 (TRAF6) and nuclear factor- κ B (NF- κ B) activation. A naturally occurring IL-1R antagonist (IL-1Ra) competes with IL-1R signalling by binding with similar affinity to IL-1RI without recruitment of IL-1RAcP. The IL-1RII acts as a sink (or decoy receptor). Although this protein binds tightly to IL-1 β (but not to IL-1 α nor IL-1Ra), it does not signal owing to the absence of a cytoplasmic domain. Proteolytic cleavage (or shedding) of IL-1RI and IL-1RII results in soluble forms (sIL-1RI and sIL-1RII, respectively), which are potent negative regulators of IL-1 activity. A soluble form of the IL-1RAcP arising from alternate mRNA splicing increases the affinity of binding of human IL-1 α and IL-1 β to sIL-1RII by 100-fold and leaves the low binding affinity of IL-1Ra unaltered. Therapeutic approaches aimed at targeting both IL-1 α and IL-1 β include using recombinant IL-1Ra (anakinra) or an engineered dimeric fusion protein consisting of the ligand-binding domains of the extracellular portions of the human IL-1RI and IL-1RAcP linked to the Fc portion of human immunoglobulin G1 (IgG1) (riloncept, also known as IL-1 Trap). Alternatively, monoclonal antibodies have proved efficient in neutralization of IL-1 β (for example, canakinumab).

attractive target to inhibit organ-related autoimmune inflammatory diseases. Disappointingly, however, anakinra treatment was only modestly efficacious in patients with RA and was inferior to TNF α blockers, according to the recent Cochrane systematic review of five recent trials involving 2,876 patients⁹⁹. In addition, the occurrence of serious upper respiratory tract infections has been concerning. Notably, IL-1 inhibition improved vascular and left ventricular function in patients with RA¹⁰⁰, which is consistent with a role of IL-1 in arterial inflammation and cardiac hypertrophy¹⁰¹. IL-1 mediates autoimmunity by promoting dendritic cell maturation and by the induction and expansion of T_H17 cells, which is mediated, at least partially, through impaired T_H17 cell development^{96,102} (FIG. 1). This would suggest that IL-1 has a role at the onset rather than at the effector stage of disease, which may explain why IL-1 blockade during an ongoing autoimmune response led to moderate amelioration of RA. In contrast to RA, IL-1 blockade by anakinra potentially inhibited the clinical symptoms associated with systemic-onset juvenile idiopathic arthritis¹⁰³.

Interestingly, IL-1 seems to also have an important role in lipid metabolism through the suppression of insulin secretion. IL-1Ra-deficient mice showed reduced body fat accumulation with both a normal and a high-fat diet, which were associated with reduced insulin serum levels¹⁰⁴, suggesting that inhibition of IL-1 may improve β -cell function in the pancreas. Indeed, anakinra treatment of patients with type 2 diabetes over 3 months improved glycaemic indexes and insulin secretion¹⁰⁵.

Recently, blocking IL-1 has also been tested in the treatment of cancer. IL-1 β by induction of IL-6 has been suggested to drive progression from a premalignant state of multiple myeloma (smouldering myeloma) to active disease. Indeed, promising results in the arrest of disease progression have been obtained in a study with 47 patients treated with anakinra¹⁰⁶.

GM-CSF. GM-CSF was first characterized by its ability to cause the differentiation of myeloid cells into granulocytic and macrophage and dendritic cell colonies *in vitro*. GM-CSF has subsequently been associated with pro-inflammatory cytokine networks and thus the progression of certain inflammatory diseases such as RA. It has been speculated that GM-CSF may have a role in perpetuating inflammatory disease cycles by increasing macrophage and granulocyte survival and differentiation, leading to increased inflammatory responses and indeed increased production of GM-CSF itself^{107,108}. Surprisingly, GM-CSF-deficient mice do not exhibit dramatic defects in myeloid cell development. However, these mice do have impaired alveolar macrophage function and consequently develop pulmonary alveolar proteinosis.

It has been reported that GM-CSF promotes T_H17 cell development and survival through the induction of IL-6 and IL-23 (REF. 109) (FIG. 1). GM-CSF-deficient mice are protected against the development of CIA¹¹⁰, EAE¹¹¹ and EAM¹⁰⁹. In line with these data, experiments in mice have shown that neutralizing antibodies against GM-CSF similarly ameliorate RA and EAE,

whereas administration of GM-CSF exacerbates disease. By contrast, GM-CSF has recently been shown to protect mice against dextran sodium sulphate-induced colitis¹¹² and to provide considerable benefit to patients with Crohn's disease^{113,114}. Moreover, GM-CSF is used to increase myeloid cell expansion and differentiation after chemotherapy-induced myelosuppression¹¹⁵. Thus, although GM-CSF is an attractive target for the modulation of inflammatory diseases, concern about abrogating its beneficial effects needs to be considered. Clinical trials have already commenced for GM-CSF-specific and GM-CSF receptor-specific antibodies in patients with RA (TABLE 1) (ClinicalTrials.gov identifiers NCT00995449, NCT01050998 and NCT01023256), whereas recombinant GM-CSF is being used in the clinic or is being tested in clinical trials for efficacy as an adjuvant with cancer therapy and vaccines¹⁰⁷.

IL-23 and IL-12. IL-23 is a heterodimeric cytokine composed of the p19 and the p40 subunits. The p40 subunit can also link up with the IL-12p35 subunit to form biologically active IL-12 (that is, IL-12p70). IL-23-deficient mice are resistant to the development of several autoimmune inflammatory diseases including EAE¹¹⁶, CIA¹¹⁷ and T cell-mediated colitis¹¹⁸. Notably, this protection was associated with a decreased number of T_H17 cells, whereas there was no difference in T_H1 cells¹¹⁹. By contrast, mice lacking IL-12 or IFN γ show exacerbated autoimmunity^{120–122}. Initially IL-23 was thought to be a key differentiation factor for T_H17 cells; however, it now seems that its primary role is to maintain populations of potentially pathogenic T_H17 cells (BOX 1; FIG. 1).

The role of IL-23 and T_H17 cells in disease has been the content of many reviews^{123–125} recently and will not be discussed in more detail here. Suffice to say that although T_H17 cells have been associated with autoimmune diseases, the phenotype of IL-23-deficient mice is more pronounced compared with mice lacking IL-17A, IL-17F, IL-22 or IL-21 (REFS 126–129). This suggests that the role of IL-23 goes beyond T_H17 differentiation or the existence of a yet unidentified pathogenic T_H17 cell effector cytokine. Support for a crucial role of IL-23 in human autoimmune inflammatory disease comes from genome-wide association studies that link IL-23R polymorphisms with psoriasis¹³⁰, ankylosing spondylitis¹³¹, Crohn's disease and ulcerative colitis^{132,133}. Notably, the requirement of IL-23 and T_H17 cells for protection against pathogens seems to be limited to some respiratory and intestinal bacteria, whereas the IL-12–T_H1-mediated pathway is essential to ward off various bacterial, viral, protozoan and fungal pathogens.

Ustekinumab (also known as CNTO 1275) and briakinumab (also known as ABT-874) are therapeutic mAbs targeting the p40 subunit of both IL-12 and IL-23 (FIG. 4; TABLE 1). In line with results from preclinical mouse studies, targeting the IL-12 and the IL-23 inflammatory pathways was effective in Phase III clinical trials for the treatment of psoriasis^{134,135} and psoriatic arthritis¹³⁶. Ustekinumab also showed benefits in the treatment of moderate to severe Crohn's colitis, especially in patients who did not previously respond to infliximab¹³⁷. However,

Pulmonary alveolar proteinosis

This is a disease in which abnormal accumulation of surfactant occurs in the alveoli and consequently impairs gas exchange. The mechanisms underlying the disease still need to be discovered; however, autoantibodies against the cytokine granulocyte–macrophage colony-stimulating factor can cause the disease.

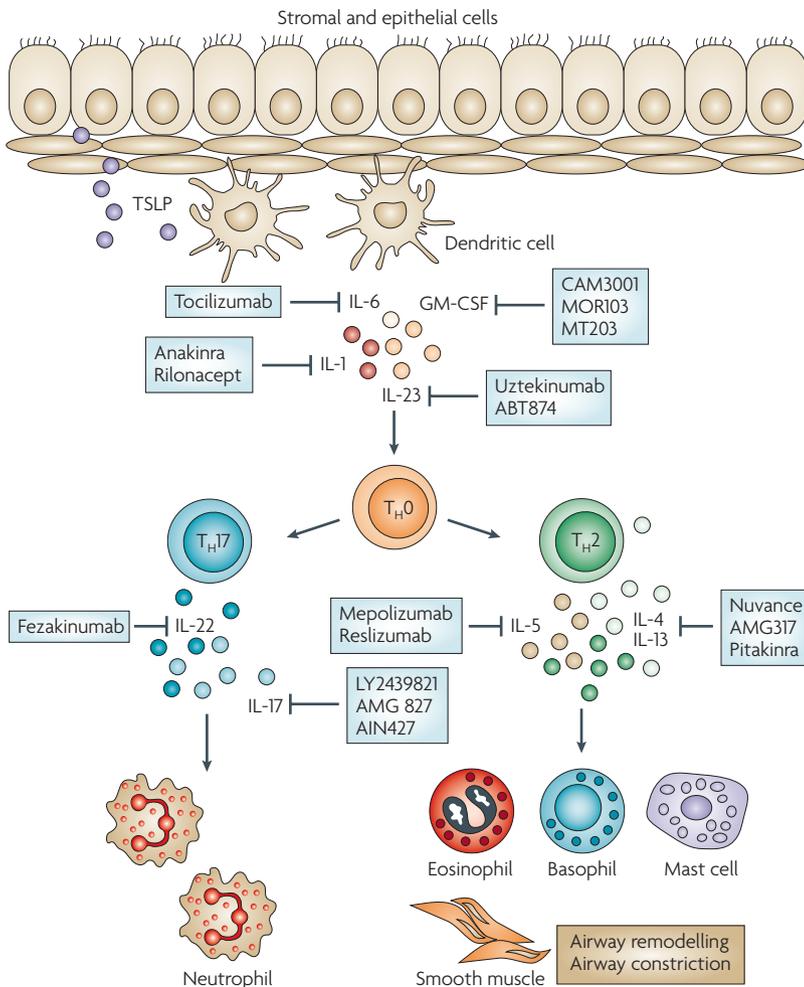


Figure 4 | Cytokine-based drugs. This figure shows some of the drugs listed in TABLE 1 and their targets. GM-CSF, granulocyte–macrophage colony-stimulating factor; IL, interleukin; T_H, T helper cell; TSLP, thymic stromal lymphopoietin.

ustekinumab failed to prevent inflammation in patients with multiple sclerosis¹³⁸. The impressive clinical efficacy together with a good safety profile of ustekinumab led to its recent licensing approval by the European Medicines Agency and the US Food and Drug Administration.

Targeting cytokine subunits that are shared between multiple cytokines is an enticing strategy to consider, in particular when these cytokines are involved early in the inflammatory cascade. However, given the superior role of the IL-12–T_H1-mediated pathway over the IL-23–T_H17-mediated pathway in the defence against mycobacteria and fungal infections^{139–142}, the targeting of IL-23 specifically is probably the safer strategy.

Effector cytokines driving autoimmunity

IFN γ . IFN γ is produced mainly by T_H1 cells but also by CD8⁺ T cells, $\gamma\delta$ T cells, NK T cells and NK cells. It has a crucial role in host defence against bacteria, protozoa, fungi and viruses by inducing a general defence alert in macrophages and dendritic cells (that is, nitric oxide production, enhanced antigen presentation and co-stimulation).

IFN γ -producing T_H1 cells are typically associated with inflammatory diseases in humans and in experimental mouse models. Indeed, T_H1 cells have been linked to the induction and the progression of many autoimmune diseases¹⁴³. In a small clinical trial dating back almost 10 years, beneficial effects of IFN γ -specific antibodies were reported in seven out of ten patients with active RA¹⁴⁴. Clinical activity of a humanized IFN γ -specific antibody (fontolizumab) was also observed in patients with Crohn’s disease¹⁴⁵. Although well tolerated in these studies, targeting IFN γ may have its own pitfalls considering data from animal experiments suggests that IFN γ actually inhibits organ-related autoimmune disease. Thus, unexpectedly, mice lacking either IFN γ or IFN γ receptor (IFN γ R) developed exacerbated inflammation in experimental models of encephalitis (EAE)¹²², myocarditis (EAM)¹²¹, arthritis (CIA)¹⁴⁶ and hapten-induced colitis¹⁴⁷. These data and the identification of pro-inflammatory IL-17A-producing T_H17 cells that can mediate autoimmunity suggest that T_H1 cells might not be the primary driving force in organ-related autoimmune disease. However, a more balanced review of recent data argues that this view may have been formed too quickly¹⁴⁸.

Of note, a potential suppressive role of IFN γ in autoimmune inflammation and a predisposition to severe infection with poorly pathogenic mycobacteria in patients with inherited disorders in the IFN γ –IFN γ R pathway¹⁴⁹ raises serious concerns about the risks associated with the long-term blockade of IFN γ .

IL-17A. IL-17A (formerly termed IL-17) is a pro-inflammatory cytokine that has been the focus of intense research in recent years. It is the founding member of a family of six homologous cytokines termed IL-17A to IL-17F, which typically form homodimers (reviewed in REF. 150). In addition, an IL-17A–IL-17F heterodimer has been described¹⁵¹. IL-17A (and IL-17F) induces the expression of several cytokines and chemokines such as IL-1, IL-6, TNF α , C-X-C motif chemokine 1 (CXCL1) and CXCL2 (REF. 150). The expression of IL-17A has been linked to autoimmune diseases such as RA, myocarditis, multiple sclerosis, in addition to IBD, psoriasis and asthma^{123,152}. Indeed, selective ablation of IL-17A leads to decreased disease severity in mouse models of these disorders. IL-17A does not seem to regulate T cell function directly, but rather it acts on other cell types (for example, macrophages, fibroblasts, and epithelial and endothelial cells) to induce the release of pro-inflammatory factors, particularly leading to the recruitment of neutrophils (FIG. 1). Although IL-17A probably developed to aid host defence against extracellular bacteria and some other pathogens located in particular at mucosal epithelial barriers¹⁵³, the primary focus of IL-17A has so far been linked with its role in mediating the pathogenesis of autoimmune and inflammatory disorders, as it is the key cytokine produced by T_H17 cells. However, as noted above, the relative contribution of IL-17A to the pathology mediated by CD4⁺ T cells compared with IL-6, IL-1 and IL-23 remains unclear and depends on the organ

and the type of autoimmune disease. IL-17F seems to be dispensable in delayed-type hypersensitivities and contact hypersensitivities, as well as in EAE and CIA¹⁵⁴.

Given the quick succession in which data on IL-17A and IL-17F are being reported, it remains too early to definitively state what parameters govern their expression

in mice and in humans. Moreover, it is important not to suddenly disregard data, which, before the discovery of IL-17A and IL-17F, implicated T_H1 cells and the production of IFN γ and TNF α in chronic inflammatory diseases. Recent studies have directly addressed the respective roles of IFN γ -producing T_H1 cells and

Table 1 | **Drugs that target cytokines**

Compound	Mechanism of action	Indication	Phase
TNFα			
Infliximab	Mouse–human chimeric TNF α -specific antibody	RA, psoriatic arthritis, psoriasis, ALS, ulcerative colitis, Crohn's disease	Approved
Adalimumab	Fully human TNF α -specific antibody	RA, psoriatic arthritis, psoriasis, ALS, juvenile RA, Crohn's disease	Approved
Golimumab	Fully human TNF α -specific antibody	RA, psoriatic arthritis, ALS	Approved
Etanercept	Fusion protein of the Fc portion of a human antibody and the ligand-binding portion of the human TNF receptor	RA, psoriatic arthritis, psoriasis, ALS, juvenile RA	Approved
Certolizumab	PEG-conjugated, humanized, antigen-binding fragment (Fab') of a TNF α -specific antibody	RA, Crohn's disease	Approved
ANO128	Small-molecule TNF α inhibitor	Acne	I
		Peridontitis	II
ART621	TNF α -specific domain antibody comprising the smallest antigen binding unit of the antibody	RA, psoriasis, sciatica	II
TNF α nanobody	TNF α -specific nanobody; antibody-derived therapeutic proteins that contain the unique structural and functional properties of naturally occurring heavy-chain antibodies	RA	I
IL-6			
Tocilizumab	Humanized IL-6 receptor-specific mAb	RA, juvenile RA, Crohn's disease, Castleman's disease	Approved
CNTO 136	Fully human IL-6-specific mAb	RA	II
CNTO 328	Chimeric IL-6-specific mAb	Multiple myeloma, prostate cancer	II
ALD 518	Humanized IL-6-specific mAb	RA	II
C326	Novel avimer protein therapeutic, smaller than most therapeutic proteins and antibodies	Crohn's disease	I
CDP6038	IL-6-specific mAb	Autoimmune disorders	I
REGN 88	Fully human mAb to the IL-6 receptor	RA	I
CR5/18	Soluble gp130–Fc fusion protein	Inflammation	Preclinical
IL-1			
Anakinra	Recombinant nonglycosylated human IL-1 receptor antagonist	RA	Approved
Canakinumab	Human IL-1 β -specific mAb	Gout, juvenile RA, COPD	II
		Cryopyrin-associated periodic syndrome	Approved
XOMA 052	Human IL-1 β -specific mAb	RA, type 2 diabetes	II
		Cryopyrin-associated periodic syndrome	Approved
		Gout	III
		Coronary atherosclerosis	II
Riloncept (also called IL-1 Trap)	Dimeric fusion protein of the ligand-binding domains of IL-1 receptor type I and IL-1 receptor accessory protein fused to human IgG1	RA, anaemia	I
CYT013-IL1bQb	Vaccine	Type 2 diabetes	I/II

IL-17-producing T_H17 cells in driving autoimmunity¹⁴⁸. The overall conclusion is that both cell types can drive disease development, which is dependent upon the experimental model, but the nature of the immunopathology is distinct. Extrapolating these preclinical data to a human disease setting is no doubt going to be similarly complex and inconclusive. Blocking IL-17A

may well show efficacy in some patients or in some diseases, but alone it is unlikely to be the magic bullet against chronic inflammatory diseases. Two humanized mAbs neutralizing IL-17 (AIN457 and LY2439821) were recently reported to be safe and to reduce disease activity in patients with RA taking other disease-modifying drugs^{155,156} (FIG. 4). However, the response

Table 1 (cont.) | **Drugs that target cytokines**

Compound	Mechanism of action	Indication	Phase
IL-23			
Ustekinumab	Fully human IL-12/23-specific mAb targeting the p40 subunit	Psoriasis Psoriatic arthritis, Crohn's disease	Approved II
ABT874	Fully human IL-12/23-specific mAb targeting the p40 subunit	Psoriasis, Crohn's disease	III, II
Apilimod	Orally administered, small molecule inhibits the production of IL-12 and IL-23	RA, Crohn's disease	II
IFNγ			
AMG 811	Fully human IFN γ -specific mAb	SLE	I
IL-17			
AIN457	Fully human IL-17A-specific mAb	RA, Crohn's disease, psoriasis, psoriatic arthritis Uveitis	II III
LY2439821	Humanized IL-17-specific mAb	RA	II
AMG 827	Fully human IL-17 receptor-specific mAb	RA, psoriasis	II
RG4934	IL-17-specific mAb	Psoriatic arthritis	I
IL-22			
Fezakinumab	IL-22-specific mAb	RA Psoriasis	II I
IL-4 and IL-13			
AMG317	IL-4 receptor- α -specific mAb (inhibits IL-4 and IL-13 signalling)	Asthma	II
Pitrakinra	Recombinant human IL-4 variant that is an inhibitor of IL-4 and IL-13 receptors	Asthma	II
Nuvance	Genetically engineered soluble human IL-4 receptor (inhibits IL-4 and IL-13 signalling)	Asthma	II
AIR645	2'-O-methoxyethyl antisense drug targeting the IL-4 receptor- α mRNA	Asthma	I
IL-13			
Anrukizumab	Humanized IL-13-specific mAb	Asthma	II
Lebrikizumab	Humanized IL-13-specific mAb	Asthma	II
CAT-354	Fully human IL-13-specific mAb	Asthma	II
IMA-026	IL-13-specific mAb	Asthma	II
IL-5			
Mepolizumab	Humanized IL-5-specific mAb	Hypereosinophilic syndrome Asthma, eosophagitis	III II
MEDI-563	Humanized IL-5-specific receptor mAb	Asthma	II
Reslizumab	Humanized IL-5-specific mAb	Eosophagitis Asthma	III II

ALS, amyotrophic lateral sclerosis; COPD, chronic obstructive pulmonary disease; Fc, crystallizable fragment; GM-CSF, granulocyte-macrophage colony-stimulating factor; Ig, immunoglobulin; IL, interleukin; mAb, monoclonal antibody; PEG, polyethylene glycol; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; TNF, tumour necrosis factor.

Box 1 | **T_H17 cells**

T helper type 17 (T_H17) cells are an inflammatory and potentially pathogenic CD4⁺ T cell subset distinct from T_H1 and T_H2 cells (for reviews see REFS 123–125, 152). Originally characterized by and named according to production of the key effector cytokine interleukin-17A (IL-17A), T_H17 cells are now known to produce IL-17F, IL-21, IL-22, tumour necrosis factor- α and granulocyte-macrophage colony-stimulating factor in various combinations together with IL-17A. T_H17 cell differentiation and maintenance are promoted by the transcription factor retinoid-related orphan receptor- γ t and the cytokines IL-23, IL-6, IL-1 and transforming growth factor- β , whereas IL-4, IL-25, interferon- γ and IL-12 are negative regulators of T_H17 cell development (FIG. 1). Although human and mouse T_H17 cell polarization show many similarities, an essential role of TGF β in human T_H17 cell differentiation remains controversial. Similarly, controversy surrounds the importance of IL-21 in T_H17 cell differentiation as it was recently shown that it had no role in T_H17-mediated autoimmune models *in vivo*^{128,129}.

rates were lower compared with biologicals targeting TNF α or IL-6 and results did not reach statistical significance for the primary end point in one of the studies¹⁵⁶. Perhaps cytokines upstream of IL-17, such as IL-23 and IL-6, may hold more potential by targeting a range of cytokines secreted by T_H17 cells.

IL-22. IL-22 is a member of the IL-10 family of cytokines and is predominantly produced by T_H17 cells (FIG. 1). However, IL-22 production has also been reported for T_H1 cells, CD8⁺ T cells and $\gamma\delta$ T cells¹⁵⁷. Although IL-17 and IL-22 are co-expressed by T_H17 cells, their production exhibits differential requirements for IL-6 and IL-23. Specifically, IL-22 production can be induced by IL-23 and/or IL-6 alone and it does not require TGF β . The receptor for IL-22 (IL-22R) is expressed primarily on epithelial cells and keratinocytes, and expression has not been reported for cells of haematopoietic origin. An extensive array of data have shown that IL-22 has widespread effects mediated through its ability to activate epithelial cells to produce pro-inflammatory molecules, antimicrobial peptides and tissue-repair gene-expression cascades¹⁵⁸. IL-22 expression has been linked with psoriasis and preclinical models utilizing IL-22-deficient mice have confirmed its role in disease pathogenesis¹⁵⁷.

Interestingly, a subset of intestinal NK cells expressing NKp46, IL-7R α (also known as CD127) and the transcription factor retinoid-related orphan receptor γ t (ROR γ t) that produces IL-22 (but not IFN γ and IL-17) driven by the commensal flora has recently been described in mice^{159,160}. A human equivalent of this IL-22-producing population of NK cells expressing NKp44 and CD56 has also been identified¹⁶¹. This cell population together with IL-22-producing CD4⁺ T cells protects mice against IBD in the T cell transfer and the dextran sodium sulphate model¹⁶². In line with this, Sugimoto and colleagues described a new means of *IL22* gene delivery by pressurized local microinjection of a vector and cationic lipid directly into the colonic mucosa in a model of ulcerative colitis, which acted to ameliorate disease development¹⁶³. A potential role of IL-22 in IBD may be derived from the identification of an ulcerative colitis susceptibility locus including *IL22* and *IL26* genes in a whole genome-wide association study, as well as polymorphisms in the genes

encoding for the *IL10R2* gene — which encodes a common subunit shared by IL-10R, IL-22R and IL-26R — in patients with early-onset colitis^{133,164}. IL-22 also protects the liver in an acute concanavalin A-induced hepatitis model by supporting hepatocyte survival and growth¹⁶⁵. As mentioned previously, IL-22 can increase the production of antimicrobial peptides and pro-inflammatory cytokines and, indeed, IL-22 has central roles in the protection against *Klebsiella pneumoniae*¹⁶⁶ and *Citrobacter rodentium*¹⁶⁷, but not *L. monocytogenes*, infections¹⁶⁵. Thus, depending on the inflammatory context and on the tissue, IL-22 could conceivably have both positive and detrimental influences on disease pathogenesis, raising a question mark over its suitability as a target for therapeutic intervention.

IL-21. IL-21 is the most recently described member of a subfamily of the type I cytokines characterized by specific receptors (that is, IL-2R, IL-4R, IL-7R, IL-9R and IL-15R) that all share the common gamma chain (γ c) and signal through the JAK-STAT pathway. IL-21 and its relatives exhibit pleiotropic effects on the immune response^{168,169}. The main source of IL-21 is activated CD4⁺ T cells, although numerous other cell types express the receptor¹⁷⁰. To date, *in vitro* evidence suggests that IL-21 regulates T_H cell subset differentiation, with an important role of IL-21 in the generation of T_H17 cells^{171–173}. *In vitro*, IL-21 together with TGF β induced T_H17 cell differentiation by the activation of ROR γ t and STAT3 independently of IL-6. At the same time IL-21 inhibited the development of peripherally induced T_{Reg} cells. An autocrine mechanism for IL-21-mediated T_H17 cell differentiation was proposed owing to the predominant expression of IL-21 in T_H17 cells as compared with T_H1, T_H2 and T_{Reg} cells¹⁷³. However, these data remain controversial as the differentiation of T_H17 cells by IL-21 is much weaker than that driven by IL-6 and T_H17 cell differentiation, and T_H17-associated autoimmune diseases were unaffected in IL-21R-deficient and IL-21-deficient mice^{128,129}.

Although the therapeutic value of targeting IL-21 for modulating T_H17-mediated autoimmune inflammatory diseases remains questionable, blocking IL-21 in T_H2-mediated allergic disorders and in antibody-mediated diseases may hold promise. IL-21R-deficient mice develop substantially reduced T_H2-driven allergic airway inflammation in a model of asthma. Similarly, key features of T_H2-type inflammation such as intestinal type 2 granuloma triggered by nematode parasites were impaired in IL-21R knockouts¹⁷⁴. Moreover, IL-21 links CD4⁺ T cell help and B cell responses, as it drives the development of T follicular helper (T_{FH}) cells (BOX 2) and acts directly on activated B cells to induce antibody responses and germinal centre development^{175–178}. T_{FH} cells are also principal producers of IL-21 and so IL-21 could therefore be an interesting target for conditions in which dysregulated antibody responses underlie disease pathogenesis such as SLE. Notably, IL-21 polymorphisms have been identified in human SLE¹⁷⁹ and a pathogenic role of IL-21 has also been described in a mouse model of SLE^{180,181}.

JAK-STAT pathway

(Janus kinase–signal transducers and activators of transcription pathway). JAK-STAT signalling is involved in the signalling from many cytokine receptors. On ligation of a receptor by its ligand, JAK phosphorylates tyrosine residues on the receptor, which allows STAT dimers to form and ultimately leads to the transcription of their target genes.

T_{Reg} cells

(Regulatory T cells). T_{Reg} cells are present in the periphery and in the thymus. They develop in the thymus and are defined by the expression of the forkhead family transcription factor FOXP3, which is required for their development and function. T_{Reg} cells in the periphery are thought to be potent suppressors of inflammation.

Box 2 | **T_{FH} cells**

T follicular helper (T_{FH}) cells are a recently described subset of CD4⁺ T cells, which regulate antigen-specific B cell responses¹⁸¹. They have been characterized by expression of inducible co-stimulator (ICOS), the transcription factor B cell lymphoma 6 (BCL-6) and the chemokine (C-X-C motif) receptor 5 (CXCR5). Naive CD4⁺ T cells express the chemokine receptor type 7 (CCR7) allowing extrafollicular localization in the T cell zone rich in chemokine (C-C) ligand 19 (CCL19) and CCL21. On encounter with antigen-presenting dendritic cells, a proportion of the primed CD4⁺ T cells downregulate CCR7 and upregulate CXCR5 allowing recruitment to chemokine (C-X-C motif) ligand 13 (CXCL13)-rich areas, in which they provide help to B cells that express the ICOS ligand and CXCR5. T_{FH} cells produce substantial amounts of IL-21, which promote germinal centre development and immunoglobulin G1 (IgG1) antibody responses by acting directly on B cells. T_{FH} cells have been implicated in the development of systemic autoimmune diseases such as systemic lupus erythematosus²¹¹. The question of whether T_{FH} cells are a discrete lineage and the relationship of T_{FH} cells with other T_H cell subsets, in particular T_H17 and T_H2 cells, is still a matter of debate.

Effector cytokines involved in asthma

IL-4, IL-5 and IL-13. IL-4, IL-5 and IL-13 are the classical T_H2-type cytokines that are seen as key regulators of T_H2 cell differentiation and IgE antibody isotype switching (IL-4); eosinophil maturation and recruitment (IL-5); and mucus production and airway hyperresponsiveness (IL-13)^{198,199} (FIG. 1). As such, they are the target for the development of therapeutics that have progressed through clinical trials. The most successful one being a soluble IL-4R (TABLE 1), which improved pulmonary function, decreased airway hyperresponsiveness and decreased symptom scores in patients with asthma^{200–202}.

Eosinophils are thought to have a key role in the pathogenesis of chronic asthma and as such several IL-5 neutralizing antibodies have been studied in the clinic. Humanized mAbs (reslizumab and mepolizumab) were effective in reducing circulating eosinophil numbers. However, no statistically significant effect on the late asthmatic response or airways hyperresponsiveness was identified^{203–205}. Clinical benefit could only be shown in a small subset of patients with asthma who had sputum eosinophilia²⁰⁶. Thus, further development of drugs targeting IL-5 are questionable. It is unlikely that targeting IL-4, IL-5 or IL-13 individually will bring substantial therapeutic benefit to patients. Rather, cytokine receptors that bind to more than one cytokine (for example, the soluble complex of the IL-4R α -IL-13R1) or the combined administration of multiple therapeutics may provide the key to treating allergies.

IL-25. IL-25 (also known as IL-17E) is associated with the development of T_H2-type immune responses. When first identified, recombinant IL-25 was administered to mice and discovered to induce the production of the T_H2 cytokines IL-4, IL-5 and IL-13, which consequently resulted in IgE antibody production, eosinophilia and mucus production¹⁸². Later studies showed that normally susceptible mice could be protected against infection with *Trichuris muris* by the administration of IL-25. This study showed that IL-25 acted to enhance T_H2-mediated immunity, and in line with these data, IL-25-deficient mice were unable to clear infection caused by *Nippostrongylus*

brasiliensis. Notably, blocking T_H1-type cytokines in IL-25-deficient mice was sufficient to restore T_H2-type cytokine production and render mice resistant against *T. muris*, indicating that IL-25 might function to inhibit T_H1 cell differentiation^{183,184}.

The biological activities of IL-25 are mediated through IL-17RB and IL-17RA^{185,186}. Neutralization of IL-25 by soluble IL-17RB-Fc or IL-25 mAb in a mouse model of airway inflammation decreased lung T_H2 cell and eosinophil recruitment^{187,188}, which highlight IL-25 as a potential therapeutic target for the treatment of T_H2-mediated allergic diseases. However, a caveat to such treatment was highlighted by the findings that IL-25 could act directly to suppress T_H17 cell function, and IL-25-deficient mice were highly susceptible to the development of EAE¹⁸⁹. Such cross regulation between cytokines families (for example, T_H1 versus T_H2 versus T_H17) is a consistent concern, which perhaps will be outweighed by the benefit of blocking the primary disease, or perhaps it will provide the impetus to develop therapeutics in which activity could be restricted to specific organs.

Thymic stromal lymphopoietin. Thymic stromal lymphopoietin (TSLP), the IL-7-like epithelial cell-derived cytokine is highly expressed in keratinocytes and in airway epithelial cells of atopic individuals¹⁹⁰. TSLP conditions dendritic cells to drive differentiation of T_H2 cells through OX40L (CD252)–OX40 (CD134) interactions and recruits T_H2 cells and eosinophils to sites of inflammation^{191,192}. In addition, TSLP can directly act on CD4⁺ T cells to induce their differentiation into classical T_H2 cells that produce IL-4, IL-5 and IL-13 (REF 212) (FIG. 4).

Conditional overexpression of TSLP in keratinocytes leads to a strong T_H2-mediated immune response and to immunopathology reminiscent of atopic dermatitis¹⁹³. Patients with atopic dermatitis expressed high levels of TSLP in skin lesions¹⁹⁴. Moreover, forced expression of TSLP in the lung drives a strong allergic asthmatic response and TSLP-receptor-deficient mice fail to develop robust allergic airway inflammation following a deliberate challenge with an allergen^{191,195}. Further data indicate that TSLP can act in synergy with IL-1 and TNF α to directly activate mast cells to produce high levels of IL-5 and IL-13 (REF 196). Recent reports now link TSLP with the suppression of IL-12 production¹⁹⁷, indicating that its mechanism of action may not be the direct induction of T_H2-cell-mediated immune responses but rather the suppression of T_H1 cells. Overall, TSLP seems to be a key pro-allergic molecule that acts upstream of the classical T_H2-type cytokines to enhance inflammatory responses. This, combined with the finding that TSLP can act on both the innate and the adaptive immune systems, makes TSLP a compelling target for therapeutic intervention.

Blocking cytokines: the power of biologicals

There are numerous possibilities to block cytokines. The most established are mAbs, soluble receptors or receptor–Fc fusion molecules and cytokine antagonists. In general, only biologicals (that is, large molecules) seem to be able to block cytokine–receptor interactions efficiently, as small molecules have proved inefficient

because they are too small to interfere with the large surface interactions that are present at the cytokine–receptor interface. Although it may be possible to block cytokine receptor signalling using small molecules, for example, by inhibiting JAK–STAT activation, the development of small-molecule inhibitors for kinases and alike has proved difficult. This is mostly because of the poor specificity of either the drug or the drug target as many cytokine and growth hormone receptors share similar signal transduction pathways. Thus, biologicals will probably remain the most effective means of blocking cytokines in the near term to midterm.

Active immunization — rather than passive administration — against cytokines is an approach growing in popularity, with vaccines usually administered at doses of several hundred micrograms at a frequency of months to years. This is in contrast to mAbs that are injected frequently at doses of >10 mg. Vaccines therefore have a roughly 10,000-fold increased efficiency compared with mAbs, provided they are able to induce clinically relevant amounts of antibodies. In addition, vaccines induce polyclonal antibody responses, and induction of neutralizing anti-idiotypic antibodies are neither expected nor observed²⁰⁷. Thus, virus-like particle-based vaccines selectively targeting IL-17A, soluble TNF α or IL-1 β have shown promising results in the protection against autoimmune arthritis and myocarditis in mouse models^{37,208–210}.

Outlook

Given their central role in the regulation of immune responses, cytokines are clearly appealing targets for therapeutic intervention. Emphasis has been placed on cytokines that are produced early in the inflammatory cascade such as TNF α and IL-6, and therapeutics neutralizing these cytokines or their receptors are already on the market or in late-phase development. More focus is now being placed on the extensive range of downstream cytokines that have been identified in recent years. Perhaps these cytokines will allow diseases to be modulated with greater specificity; certainly no broad-spectrum magic bullet has been identified. Rather, it is becoming clearer how heterogeneous diseases are between individuals and the numerous levels of redundancy that have evolved in cytokine networks. New technologies to block individual cytokines are progressing rapidly; however, alone, they are unlikely to provide the key to modulating inflammatory diseases. These advances in technology combined with our improved understanding of cytokine networks lend themselves to the development of highly target-specific therapeutics aimed at disease pathways in individuals or in certain patient cohorts. Rather than blocking early pleiotropic cytokines, future success may lie in the combined neutralization of effector cytokines with narrower ranges of defined activity.

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Competing interests statement

The authors declare [competing financial interests](#): see web version for details.