Role of the β Common (β c) Family of Cytokines in Health and Disease

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The β common (β c)/CD131 family of cytokines comprises granulocyte macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-3, and IL-5, all of which use β c as their key signaling receptor subunit. This is a prototypic signaling subunit-sharing cytokine family that has unveiled many biological paradigms and structural principles applicable to the IL-2, IL-4, and IL-6 receptor families, all of which also share one or more signaling subunits. Originally identified for their functions in the hematopoietic system, the β c cytokines are now known to be truly pleiotropic, impacting on multiple cell types, organs, and biological systems, and thereby controlling the balance between health and disease. This review will focus on the emerging biological roles for the β c cytokines, our progress toward understanding the mechanisms of receptor assembly and signaling, and the application of this knowledge to develop exciting new therapeutic approaches against human disease.

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The β common (β c) cytokines are produced by many cell types in the body and act in the immediate microenvironment or at a distance. They are recognized by specific cell-surface receptors present on hemopoietic and nonhemopoietic cell types (Fig. 1). On binding to their

specific receptors, granulocyte macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-3, and IL-5 signal through heterodimeric receptor complexes comprising β c as the major signaling subunit and cytokine-specific α subunits (Broughton et al. 2012). Cytokine binding

Figure 1. Diverse functions in health and disease for the β common (β c) cytokine family. The β c cytokines are predominantly expressed by activated T cells, but many other cell types have been reported to express selected β c cytokines. Among the β c cytokines, interleukin (IL)-3 and granulocyte macrophage colony-stimulating factor (GM-CSF) have actions on the widest range of cell types, whereas IL-5 is largely restricted to actions on eosinophils. Cytokines are coded IL-3 (blue), IL-5 (purple), GM-CSF (green), and the line thickness indicates the relative biological significance. All β c cytokines have been reported to contribute to diseases in humans, either directly or indirectly, and this includes asthma, AML (acute myeloid leukemia), AMML (acute myelomonocytic leukemia), ALL (acute lymphoblastic leukemia), BPDCN (blastic plasmacytoid dendritic cell neoplasm), CML (chronic myeloid leukemia), CMML (chronic myelomonocytic leukemia), CRSwNP (chronic rhinosinusitis with nasal polyps), DTHS (delayed-type hypersensitivity), HCL (hairy cell leukemia), KD (Kawasaki disease), PAP (pulmonary alveolar proteinosis), RA (rheumatoid arthritis), SLE (systemic lupus erythematosus), and SM (systemic mastocytosis). Agents that block the function of β c cytokines or target their receptors have been generated and are undergoing clinical development in a range of disease settings (red boxes). See Table 1 for details of the listed agents. moDCs, Monocyte-derived dendritic cells.

Table 1. Therapeutic agents targeting the β c family of cytokines or their receptors

Target	Disease	Type	Therapy name	Mode of action	Publications	Clinical trial reference
GM-CSF	RA	MAb	GSK3196165/ MOR103	Blocks GM-CSF	Behrens et al. 2015	NCT01023256 (c)
GM-CSF	MS	MAb	Namilumab	Blocks GM-CSF	Constantinescu et al. 2015	NCT01517282 (c)
	RA				-	NCT01317797 (c)
GM-CSF		MAb	Lenzilumab/ KB003	Blocks GM-CSF		NCT02379091 (o)
	PP				-	NCT02393378 (p)
	RA				-	NCT02129777 (c)
					-	NCT00954449 (t)
GM-CSF GMR α	Asthma	MAb	MORAb-022 Mavrilimumab/ CAM-3001	Blocks GM-CSF Blocks GM-CSF	Molfini et al. 2016	NCT01603277 (c)
	CMML				-	NCT02546284 (p)
	RA				-	NCT01357759 (c)
	RA				Burmester et al. 2011 Burmester et al. 2013	NCT00771420 (c) NCT01050998 (c) NCT01715896 (c) NCT01706926 (c) NCT02152956 (p)
IL3R α + CD3	AML	DART	MGD006/ S680880	Targets cell, ADCC	Al-Hussaini et al. 2016	
IL3R α	AML, MDS	MAb	KHK2823	ADCC	Akiyama et al. 2015	NCT02181699 (p)
IL3R α	AML	MAb	CSL362/INJ- 56022473	Blocks IL-3 and ADCC	Busfield et al. 2014	NCT01632852 (c) NCT02472145 (p)
IL3R α	SLE	Immunotoxin	SL-401/ DT388IL3	Cytokine targets cell that is killed by toxin	Oon et al. 2016a	-
	AML				Feuring-Buske et al. 2002; Frankel et al. 2008;	NCT00397579 (c)
	BPDCN				Tettamanti et al. 2013	
	AML, BPDCN					NCT02113982 (p)
	AML				Frankel et al. 2014; Angelot- Delette et al. 2015	NCT02270463 (p) NCT02268253 (p)
	SM, CMML, PED, MS					

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Table 1. *Continued*

Target	Disease	Type	Therapy name	Mode of action	Publications	Clinical trial reference
IL3R α	AML	CAR		T cell kills AML	Mardiros et al. 2013	NCT02159495 (p)
IL-5	Asthma	MAb	Mepolizumab/ Nucala/SB-240563	Blocks IL-5	Keating 2015; Patterson et al. 2015; Pavord et al. 2012	NCT01000506 (c) NCT02654145 (p) NCT02594332 (p) NCT00086658 (c) NCT02836496 (n) NCT01362244 (c) NCT02105961 (p) NCT02105948 (p) NCT00274703 (c) NCT02452190 (p) NCT02559791 (p)
	HES				Rothenberg et al. 2008	
	NP				-	
	COPD				-	
	EO				Straumann et al. 2010	
IL-5	Asthma	MAb	Reslizumab/ Cinqair/ SCH-55700	Blocks IL-5	Patterson et al. 2015; Pelaia et al. 2016	
IL5R α	Asthma	MAb	Benralizumab	Blocks IL-5 and ADCC	Patterson et al. 2015; Tan et al. 2016 FitzGerald et al. 2016 Bleecker et al. 2016	NCT02821416 (n) NCT02075255 (o) NCT01914757 (c) NCT01928771 (c) NCT02417961 (c) NCT02258542 (o) NCT01227278 (c) NCT02155660 (p) NCT01759849*
β c	COPD				- Castro et al. 2014	
	Asthma	MAb	CSL311	Blocks GM-CSF, IL-3, and IL-5	Panousis et al. 2016	
β c + CCR3	Asthma	Oligo	TPI ASM8	Blocks β c expression	Gauvreau et al. 2011 Imaoka et al. 2011	NCT01158898 (c) NCT00550797 (c)

AML, Acute myeloid leukemia; BPD/CN, blastic plasmacytoid dendritic cell neoplasm; CMML, chronic myelomonocytic leukemia; COPD, chronic obstructive pulmonary disease; EO, eosinophilic oesophagitis; HES, hypereosinophilic syndrome; MDS, myelodysplastic syndrome; ME, myelofibrosis; MS, multiple sclerosis; NP, nasal polyposis; PED, advanced symptomatic hypereosinophilic disorder; PP, plaque psoriasis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SM, systemic mastocytosis. CAR, chimeric antigen receptor expressed on transduced T cells; DART, dual-affinity retargeting proteins; Immunotoxin, cytokine conjugated to diphtheria toxin; MAb, monoclonal antibody; Oligo, antisense oligonucleotide. ADCC, antibody-dependent cell cytotoxicity.

Clinical Trials identifier and status: c, completed; n, not started; o, ongoing, not recruiting; p, in progress; t, terminated; *, preclinical.

triggers assembly of the cytokine–receptor complex in a distinct stoichiometry that initiates multiple signaling pathways. These include emerging roles for signaling cross talk with other receptor systems and novel kinases as well as the more familiar Janus kinase (JAK) 2/signal transducer and activator of transcription (STAT) 5, mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase (PI3K) pathways (Martinez-Moczygemba and Huston 2003; Hercus et al. 2013). Signaling by the β c cytokines gives rise to diverse biological responses, including cell survival, proliferation, differentiation, migration, and mature leukocyte effector functions. A putative fourth member of this family, KK34, has recently been described in some species (Yamaguchi et al. 2016), but its ortholog in humans and mice is a pseudogene.

BIOLOGY OF THE β c CYTOKINES

Among the plethora of cell types that secrete β c cytokines, there is a clear distinction between GM-CSF, which is produced by multiple cell types, including macrophages, fibroblasts, and epithelial cells, and IL-3 and IL-5, which are more T-cell restricted (Metcalf 2008; Broughton et al. 2012; Hercus et al. 2013). The spectrum of GM-CSF-producing cells immediately suggests a role for this cytokine in various innate and adaptive immune responses, and in inflammatory diseases (Fig. 1) (Broughton et al. 2012; Bhattacharya et al. 2015; Wicks and Roberts 2016). This concept has been supported by studies in rheumatoid arthritis (RA) (Campbell et al. 1998; Cook et al. 2001; Hamilton 2008), multiple sclerosis (MS) (McQualter et al. 2001; Codarri et al. 2011; El-Behi et al. 2011; Croxford et al. 2015a), asthma (Yamashita et al. 2002; Asquith et al. 2008; Saha et al. 2009), and chronic obstructive pulmonary disease (COPD) (Saha et al. 2009).

In addition to their role in inflammation, the β c cytokines are increasingly being recognized as contributing to several forms of leukemia. In human myeloid leukemia, GM-CSF acts as a growth and survival factor (Hercus et al. 2013) and promotes the survival of chronic myeloid leukemia (CML) even in the presence of

BCR-ABL kinase inhibitors (Hiwase et al. 2010). Blast cells from patients with acute myeloid leukemia (AML) show enhanced GM-CSF receptor expression and proliferation (Riccioni et al. 2009; Mathew et al. 2013), whereas juvenile myelomonocytic leukemia (JMML) (Bernard et al. 2002; Bunda et al. 2013) and chronic myelomonocytic leukemia (CMML) (Ramshaw et al. 2002; Padron et al. 2013) display a characteristic GM-CSF hypersensitivity. This suggests a pathogenic role for GM-CSF and highlights a focus for targeted therapies in leukemias (Fig. 1).

Whereas GM-CSF produced during inflammation promotes hemopoietic cell production and immunity, its role in steady-state hematopoiesis does not appear to be critical, with the notable exception of normal alveolar macrophage function. GM-CSF signaling blockade by autoantibodies (Kitamura et al. 1999; Inoue et al. 2008; Wang et al. 2013) or disruption of GM-CSF signaling by mutation of the genes encoding GM-CSF or its receptor (Suzuki et al. 2008, 2010, 2011; Griese et al. 2011; Tanaka et al. 2011) is associated with pulmonary alveolar proteinosis (PAP), revealing a nonredundant role for GM-CSF in lung function. Thus, emerging therapies that antagonize GM-CSF must factor in the need to maintain lung homeostasis.

An important role for GM-CSF in at least some forms of antiviral immunity is indicated by the existence of a viral protein that antagonizes GM-CSF and IL-2 function. The orf virus infects many species, including humans and sheep, in which it causes a contagious ecthyma, also known as orf (Felix et al. 2016). Intriguingly, the orf virus encodes a protein, GM-CSF/IL-2-inhibition factor (GIF), that is able to inhibit ovine GM-CSF and ovine IL-2 function but does not appear to bind the human or murine orthologs or the other β c cytokines (Deane et al. 2000). GIF has recently been shown to bind ovine GM-CSF and ovine IL-2 through mutually exclusive binding sites and is likely to act as a competitive decoy receptor (Felix et al. 2016). The species-restricted function of GIF has been suggested to be an adaptation by the orf virus to its principal host, the sheep (Deane et al. 2000), but further studies are de-

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sirable to determine the role of GIF in human orf virus infections.

The role of IL-3 as a growth and survival factor for multiple lineages of normal and malignant hemopoietic cells is well recognized (Hercus et al. 2013). Unlike GM-CSF and IL-5, IL-3 also regulates hemopoietic stem cells and megakaryocytes, desirable properties for bone marrow reconstitution, although its stimulation of mast cell and basophil production and activity has restricted its clinical application (Fig. 1) (Eder et al. 1997). Of particular significance is the overexpression of the IL-3 receptor α subunit (IL3R α /CD123) in several hematological malignancies. Originally described in leukemic stem-like cells of patients with AML (Jordan et al. 2000; Testa et al. 2002; Jin et al. 2009; Vergez et al. 2011), this overexpression is also observed in progenitor cells of CML (Nievergall et al. 2014), blastic plasmacytoid dendritic cell neoplasm (BPDCN) (Frankel et al. 2014; Angelot-Delettre et al. 2015), neoplastic mast cells (Pardani et al. 2015), and systemic mastocytosis (Fig. 1) (Pardani et al. 2016). IL3R α overexpression is particularly important in AML where IL3R α expression levels strongly correlate with reduced patient survival (Jordan et al. 2000; Testa et al. 2002; Vergez et al. 2011), and this is being exploited therapeutically through targeting of IL3R α by antibodies (Jin et al. 2009; Busfield et al. 2014; Al-Hussaini et al. 2016), chimeric antigen receptor (CAR) T cells (Tettamanti et al. 2013), and IL-3-toxin conjugates (Frankel et al. 2008), which specifically recognize IL3R α . Of these approaches, the IL3R α -specific antibody CSL362, which blocks IL-3-mediated signaling (Sun et al. 1996; Jin et al. 2009) through a dual mechanism (Broughton et al. 2014) and enhances natural killer (NK) cell-mediated antibody-dependent cell-mediated cytotoxicity (ADCC) (Busfield et al. 2014), is the most advanced and is currently in phase II clinical trials for the treatment of AML.

IL-3 has also been reported to contribute to the development of lupus nephritis in a mouse model of the human autoimmune disease systemic lupus erythematosus (SLE), a process that could be blocked by anti-IL-3 antibodies (Renner et al. 2015). Interestingly, along with leuke-

mic stem-like cells and basophils, human plasmacytoid dendritic cells (pDCs) also show high expression of IL3R α . These cells play a central role in autoimmunity through their capacity to produce type I interferons (Liu 2005; Llanos et al. 2013) and their high expression of IL3R α may present an opportunity to control their function. This notion is supported by recent experiments in which blocking IL3R α or depleting pDCs showed a marked reduction in expression of both type I and III interferons and IL-6 (Oon et al. 2016a) and suggests a new approach to manage autoimmune diseases such as SLE (Oon et al. 2016b).

In contrast to IL-3 and GM-CSF, the actions of IL-5 are restricted to eosinophils and basophils, with IL-5 being directly linked to the hypereosinophilic syndrome (Fig. 1) (Broughton et al. 2012; Tan et al. 2016). Interestingly, although IL-5 is eosinophil-specific, all three β c cytokines stimulate eosinophil production and function, both under steady-state (Nishinakamura et al. 1996) and inflammatory conditions such as asthma (Panousis et al. 2016). Thus, anti-IL-5 therapies that target eosinophil-mediated inflammation may be successful in cases in which IL-5 is the predominant cytokine involved (Broughton et al. 2012; Tan et al. 2016), but ultimately blocking all three cytokines by targeting β c may yield the best results.

While mutations of the β c cytokine receptors are only infrequently observed, a recent publication reported a germline mutation in the β c gene (*CSF2RB*) from a patient with T-cell acute lymphoblastic leukemia (T-ALL) that is able to stimulate factor-independent cell proliferation in vitro (Watanabe-Smith et al. 2016). This is consistent with previous in vitro studies that identified the potential for mutations in β c to give rise to factor-independent cell growth (D'Andrea et al. 1994; Jenkins et al. 1998) and represents the first demonstration in vivo that such mutations are associated with leukemia.

A ROLE FOR β c CYTOKINES IN SEPSIS AND INFLAMMATORY DISEASE

Sepsis is a serious and often fatal complication of infection that has been attributed to uncon-

trolled inflammatory responses. Clinical trials to control sepsis have been largely unsuccessful, probably owing to the heterogeneous nature of the septic syndrome. As the mechanisms underpinning sepsis are beginning to be dissected, it appears that β c cytokines play distinct roles in this setting. On the one hand, GM-CSF can be viewed as a beneficial factor owing to its antimicrobial immune function, as sepsis-associated immunosuppression is becoming recognized as a critical feature in disease progression (Hotchkiss and Sherwood 2015). In fact, clinical trials with GM-CSF have shown some reversal of sepsis-associated immunosuppression (Meisel et al. 2009; Leentjens et al. 2012). This protective role is consistent with the identification of GM-CSF-expressing innate response activator (IRA) B cells that protect mice from death in a cecal ligation and puncture (CLP) model of sepsis (Rauch et al. 2012) and that produce immunoglobulin M (IgM) through an autocrine GM-CSF loop to protect against pulmonary bacterial infections (Weber et al. 2014). On the other hand, IL-3 produced by B cells appears to play a pathogenic role in sepsis. A recent study reported that IL-3 amplifies acute inflammation in a mouse model of sepsis and could be blocked by anti-IL-3 agents (Weber et al. 2015). These findings may suggest differences in inflammatory responses mediated by GM-CSF and IL-3, fundamental differences between chronic human disease and the acute animal models (Hotchkiss and Sherwood 2015), or may simply be a reflection of sepsis representing a complex set of related diseases rather than a single condition.

Experimental autoimmune encephalomyelitis (EAE) is a rodent model of MS, an inflammatory disease of the central nervous system (CNS). EAE is driven by autoreactive CD4⁺ T helper (Th) cells and it has become clear that Th cell production of GM-CSF in response to IL-23 and IL-1 β or IL-7 (McQualter et al. 2001; Codarri et al. 2011; El-Behi et al. 2011; Sheng et al. 2014) is a critical factor in the recruitment and activation of myeloid cells and ultimately CNS demyelination (Croxford et al. 2015b). Recent studies have reported elevated numbers of Th cells expressing GM-CSF in patients with MS

(Rasouli et al. 2015) and, in some patients, this has been linked to MS-associated polymorphic variants of the IL-2 receptor α gene (*IL2RA*) (Hartmann et al. 2014). Expression of GM-CSF by a subset of human memory B cells has also been linked to MS (Li et al. 2015). GM-CSF production by human Th cells is regulated differently from their murine counterparts, as IL-2 and IL-23 promote and repress its expression, respectively (Noster et al. 2014). The essential role of GM-CSF in EAE pathogenesis is because of its activation of CCR2⁺Ly6C^{hi} monocytes and subsequent programming of inflammatory function in their progeny (Croxford et al. 2015a). The presence of these activated or inflammatory monocyte-derived dendritic cells (moDCs) in the CNS has been directly linked to demyelination in EAE (Yamasaki et al. 2014). GM-CSF stimulation of moDCs induces expression of multiple genes linked to EAE, including IL-1 β , which expands the GM-CSF-expressing Th cell pool and thereby amplifies the inflammatory response (Croxford et al. 2015b; Paré et al. 2016). These studies highlight a potential role for pathogenic GM-CSF activity in MS, and prompted a recently completed phase Ib clinical trial in MS patients using a human anti-GM-CSF antibody (Constantinescu et al. 2015).

Recent reports have indicated an unexpected role of GM-CSF in cardiomyopathies. GM-CSF has recently been identified as a primary initiator of cardiac inflammation in a model of Kawasaki disease (KD), which is the leading cause of pediatric heart disease in developed countries (Stock et al. 2016). Using a CAWS (*Candida albicans* water-soluble fraction) model of KD, Stock et al. reported that expression of GM-CSF by cardiac fibroblasts was required for the expression of multiple proinflammatory chemokines by cardiac macrophages that then promote neutrophil and monocyte recruitment into the heart where they mediate cardiac pathology. Although using different cellular sources, the indirect role of GM-CSF in the KD model is reminiscent of the indirect role of GM-CSF in EAE (Croxford et al. 2015a) and suggests the therapeutic potential of GM-CSF antagonists in patients with KD.

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THE β c CYTOKINES IN CANCER

In recent years, there has been increasing interest in the role of the β c cytokines outside the hematopoietic compartment. GM-CSF activity is reported to be relevant in a number of solid tumor settings and through multiple mechanisms. Although the balance between antitumor and protumor effects is complex, the activity of GM-CSF appears to be predominantly protumorigenic based on its expression by certain tumors and their recruitment of tumor suppressor cells. Expression of GM-CSF is associated with protumorigenic outcomes in squamous cell carcinomas (Obermueller et al. 2004; Gutschalk et al. 2006), breast cancer (Su et al. 2014; Vilalta et al. 2014), colon cancer (Rigo et al. 2010), and glioma (Revoltella et al. 2012). GM-CSF may act directly on tumors in an autocrine manner (Obermueller et al. 2004; Gutschalk et al. 2006; Revoltella et al. 2012; Vilalta et al. 2014) or indirectly through GM-CSF-mediated responses from tumor-associated macrophages (TAMs) (Rigo et al. 2010; Su et al. 2014). Several studies have also found that tumor-derived GM-CSF recruits, expands, and activates myeloid-derived suppressor cells (MDSCs), a heterogeneous group of immature myeloid cells that suppress other immune cell types, such as T cells, to contribute to the metastasis of glioma, pancreatic, and liver cancers (Bayne et al. 2012; Pylayeva-Gupta et al. 2012; Kohanbash et al. 2013; Thorn et al. 2016). Furthermore, the recent recognition that neutrophils can be manipulated by the tumor microenvironment to facilitate tumor growth (Coffelt et al. 2016) and the associated expression of GM-CSF in some tumors (Garcia-Mendoza et al. 2016) suggests an unanticipated role for GM-CSF on tumor-associated neutrophils. In contrast, GM-CSF has been reported to contribute to antitumor responses in colon cancer (Urduingio et al. 2013), and, through expression in oncolytic viral therapies (Andtbacka et al. 2016; Kuryk et al. 2016), is being used to stimulate host antitumor responses. It would be important to clarify the contribution of GM-CSF activity in different solid tumor

settings to harness its protective effects or to inhibit its function.

In certain solid tumors, IL-3 may also play a pathogenic role by acting on both the vasculature and on the tumor cells directly. IL-3 receptors are expressed on endothelial cells (Korpelainen et al. 1993), endothelial progenitor cells, mediating their rapid expansion (Moldenhauer et al. 2015), and on tumor-derived endothelial cells thus contributing to angiogenesis and tumor growth (Dentelli et al. 2011). Furthermore, the concomitant expression of IL-3 receptors in certain breast cancers (C Bonder and A Lopez, unpubl.) and the activating effects of IL-3 on these cells suggest that IL-3 and its receptor may constitute previously unappreciated targets to simultaneously inhibit the growth and activity of some breast cancer cells directly and also the angiogenic microenvironment.

β c CYTOKINES IN THE NERVOUS SYSTEM

A fascinating aspect of β c cytokine biology has emerged from reports that describe a role for the β c cytokines in the nervous system. Whereas the molecular basis for many of these actions remains to be firmly established, the data suggest an important role for β c cytokines in neural development, diseases such as MS and schizophrenia, as well as a direct role in nociception. Expression of GM-CSF, IL-3, and IL-5 receptors in brain tissue (Lins and Borojevic 2001; Schabitz et al. 2008; Luo et al. 2012) support a role for these cytokines in the CNS. GM-CSF promotes the in vitro regeneration of retinal ganglion cells (Legacy et al. 2013; Hanea et al. 2016), has been shown to have neuroprotective effects in a transient focal cerebral ischemia model in rats (Kong et al. 2009), restores motor function in a mouse model of stroke (Shanmugalingam et al. 2016; Theoret et al. 2016), and provides a neuroprotective response in mouse models of Alzheimer's disease (Boyd et al. 2010) and Parkinson's disease (Kim et al. 2009; Kosloski et al. 2013). GM-CSF may also provide a neuroprotective effect by triggering the entry of microglia, tissue-resident macrophages of the CNS, into the brain across the

blood–brain barrier (Boyd et al. 2010; Shang et al. 2016), suggesting a beneficial role in patients with Alzheimer’s disease or Parkinson’s disease (Boyd et al. 2010; Heinzelman and Priebe 2015).

IL-3 and the IL-3 receptor subunits, IL3R α and β c, are also expressed in mouse embryonic brain (Luo et al. 2012), and IL-3 has been shown to stimulate the proliferation of microglial cells (Frei et al. 1986) as well as neuronal progenitor cells (Luo et al. 2012). Intriguingly, a number of recent studies have now linked the expression and function of IL-3 with CNS development and schizophrenia. Genome-wide association studies (GWAS) have identified single-nucleotide polymorphisms (SNPs) upstream of or within the *IL3* gene sequence that are associated with variations in human brain volume (Luo et al. 2012; Li et al. 2016b) or are linked to schizophrenia (Chen et al. 2007; Edwards et al. 2008). Other studies have identified variants in the genes for β c (*CSF2RB*) and IL3R α (*IL3RA*) that are associated with schizophrenia (Lencz et al. 2007; Chen et al. 2008) and depression (Chen et al. 2011). Consistent with these observations, elevated levels of serum IL-3 are associated with more severe symptoms among patients with chronic schizophrenia (Xiu et al. 2015; Fu et al. 2016). It remains to be seen whether these observations are causally linked.

In addition to their actions on the CNS, the β c cytokines also play a role in the peripheral nervous system, in particular in regulating pain. Models of inflammatory pain and arthritis in GM-CSF-deficient *Csf2*^{-/-} mice have shown that pain is dependent on GM-CSF activity and that GM-CSF-mediated pain but not arthritis is cyclooxygenase-dependent (Cook et al. 2013). Pain associated with bone cancer was mediated by GM-CSF in a mouse sarcoma model and specific knockdown of GM-CSF receptor α subunit (GMR α /CD116) expression in the sensory nerves was able to significantly reduce tumor-induced pain, suggesting that GM-CSF may act directly on peripheral nerves (Schweizerhof et al. 2009) and potentially open up new indications for anti-GM-CSF therapies.

INTERACTIONS OF β c CYTOKINE RECEPTORS WITH OTHER MEMBRANE RECEPTORS

Although the β c cytokines are the classical activators of the β c receptor family, a surprising number of membrane proteins have been reported to interact and signal with β c (Broughton et al. 2012). These include the receptor for stem-cell factor (c-Kit), β 1 integrin, the FcR γ subunit of the high-affinity immunoglobulin E (IgE) receptor, vascular endothelial growth factor (VEGF) receptor 2 (VEGFR2), and the receptor for erythropoietin (EPOR). As a result of these interactions there is emerging evidence that signaling through the β c subunit can be regulated by noncanonical cytokines. Transforming growth factor β 1 has been shown to regulate cellular functions controlled by IL-3 (Broide et al. 1989) and the cell-surface density of GM-CSF receptor (Jacobsen et al. 1993). Furthermore, VEGFR-2 has been shown to interact with β c, leading to enhanced p38 activation (Saulle et al. 2009) and tumor angiogenesis (Dentelli et al. 2005). However, the mechanisms underlying the cross talk between these receptors remain to be elucidated. Gene expression data obtained from publicly available datasets suggest that, in some epithelial and hematological cancers, the ratio of β c and α subunit gene expression differs significantly from that observed in corresponding normal tissues (Fig. 2). Whereas it is well known that IL3R α is overexpressed relative to β c in AML (Jordan et al. 2000; Testa et al. 2002; Jin et al. 2009; Vergez et al. 2011), the genes for GMR α and the IL-5 receptor α subunit (IL5R α /CD125) are also differentially expressed relative to the β c subunit in certain malignant tissues (Fig. 2). These observations led us to hypothesize that noncanonical interactions between β c and other receptors may be a feature of malignancy, with specific aberrant interactions driving the phenotype in different cancers.

The best characterized of the noncanonical β c interactions is the innate repair receptor (IRR), a complex of EPOR and β c (Brines et al. 2004) that is expressed in many tissues under stress and triggers a tissue protection

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and repair response (Collino et al. 2015). Tissue-protective activation of the IRR in vivo is thought to preferentially use hyposialated EPO (hsEPO). EPO variants that activate the IRR, but not the classical EPOR homodimer (Leist et al. 2004) have also been developed. A short peptide fragment of EPO (pHBSP/ARA-290) (Brines et al. 2008) selectively activates IRR and is undergoing clinical development to reduce symptoms of small fiber neuropathy in patients with sarcoidosis (Heij et al. 2012; Dahan et al. 2013) and as a therapy in type 2 diabetes (Brines et al. 2014). Although the interaction of βc with other cell-surface receptors may not be required for classical or established signaling outcomes from these receptors (Scott et al. 2000), it is important to keep in mind that noncanonical heteromeric receptors, such as the IRR complex, may exist. Novel proteomic-based approaches coupled to appropriate biological characterizations will be needed to fully appreciate the importance of βc signaling beyond hematopoiesis.

Although all known activities of the βc cytokines use $\alpha/\beta c$ heteromeric receptor complexes, a recent report describes the direct sensitization of persister cells of *Pseudomonas aeruginosa* strains to a range of antibiotics by incubation with low doses of human GM-CSF (Choudhary et al. 2015). Although the mechanism that drives this surprising response is unknown, this novel action for GM-CSF could represent a new activity for the βc cytokine family and perhaps other cytokine families.

βc CYTOKINE RECEPTOR ASSEMBLY AND INITIATION OF SIGNALING

A major advance in understanding how the βc family of receptors assemble and signal has been the determination of the three-dimensional (3D) structure of the receptor's extracellular components as apo, binary, and ternary complexes. Crystal structures of the GM-CSF and IL-5 cytokines (PDB IDs: 1CSG, 2GME, 1HUL [Milburn et al. 1993; Rozwarski et al. 1996]), the βc subunit (PDB IDs: 1GH7, 2GYS [Carr et al. 2001, 2006]), the IL-5:IL5R α binary complex (PDB IDs: 3QT2, 3VA2 [Patino et al. 2011; Ku-

sano et al. 2012]), and the GM-CSF binary and ternary receptor complexes (PDB IDs: 4RS1, 4NKQ, respectively [Fig. 3A] [Hansen et al. 2008; Broughton et al. 2016]) have been solved. Apart from unbound βc , the only published components of the IL-3 receptor signaling complex are a nuclear magnetic resonance (NMR) structure of IL-3 (PDB ID: 1JLI [Feng et al. 1996]) and IL3R α bound to a therapeutic antibody (Broughton et al. 2014). IL-3 and GM-CSF adopt a typical cytokine four-helical bundle motif (helices A–D), whereas IL-5 forms an intertwined dimer of two four-helix bundles, with the fourth helix of each monomer being swapped (Milburn et al. 1993). The three α subunits share a similar architecture consisting of three fibronectin III (FNIII) domains (NTD, D2, and D3) that adopt a “wrench-like” conformation relative to the bound cytokine (Fig. 3A). The shared βc subunit has four linked FNIII domains (D1–D4), which, in solution and the solid state, form an arched intertwined homodimer (Fig. 3A).

Receptor activation is initiated when the cytokine binds to its specific α subunit (site 1, Fig. 3A). Residues from all three FNIII domains of the α subunit participate in the interaction with the cytokine to form a binary complex. This is a low-affinity interaction that differs markedly between the three cytokines with K_D values of ~ 100 nM for IL-3, 1–10 nM for GM-CSF, and 1 nM for IL-5 (Broughton et al. 2012). In both the GM-CSF and IL-5 binary complexes, the α subunit amino-terminal domain (NTD) interacts with its specific cytokine via extensive inter- β -strand main-chain hydrogen bonds (Patino et al. 2011; Kusano et al. 2012; Broughton et al. 2016). Residues within GMR α D2 and D3 make significant polar interactions with GM-CSF (Fig. 3B), whereas D2 and D3 of IL5R α make very few polar interactions with IL-5 and instead interact with the cytokine via numerous van der Waals interactions. Mutagenesis studies have shown that a small patch of residues in the IL5R α NTD appears to drive the formation of the binary complex, whereas residues in D2 and D3 are critical in the GM-CSF binary complex formation. In contrast to GMR α and IL5R α , residues in the IL3R α

Figure 2. Relative gene expression for the β common (β c) and α subunits in normal and malignant tissue. Mean rank gene expression data extracted from Oncomine of genes for β c (*CSF2RB*), granulocyte macrophage colony-stimulating factor (GM-CSF) receptor α (GMR α) (*CSF2RA*), IL3R α (*IL3RA*), and IL5R α (*IL5RA*) in normal versus malignant tissue. Key indicates percentage mean rank enrichment. Datasets used: Roth normal, Roth normal 2, Su multicancer, Wouters leukemia, Xu melanoma, and Ge normal. N, Normal; Ca, cancer; NSC, non-small-cell lung cancer; SqC, squamous cell lung cancer; BM, bone marrow; AML, acute myeloid leukemia; Mel, melanoma; haem, hematopoietic system.

NTD and D3 have been shown to be important in the formation of the IL-3 binary complex (Broughton et al. 2014). The binary complexes then interact with the β c subunit to form a ternary complex, as observed in the GM-CSF ternary structure (sites 2 and 3, Fig. 3A) (Hansen et al. 2008; Broughton et al. 2016); this is a high-affinity interaction with K_D values of 100 pM for GM-CSF, 250 pM for IL-5, and 140 pM for IL-3 (Broughton et al. 2012). The symmetrical nature of the β c homodimer allows two binary complexes to bind to one β c subunit and to form either a hexameric complex (IL-3 and GM-CSF) or an octameric complex (IL-5). Crystal packing in the GM-CSF receptor structure revealed a second hexamer stacked against the first to create a dodecamer (sites 4 and 5, Fig. 3A). The physiological relevance of this dodecamer in JAK/STAT signaling has been shown by (1) functional studies of site 4 mutants, and (2) site-4-specific antibodies, in which both abolished JAK/STAT signaling (Hansen et al. 2008; Broxmeyer et al. 2012). Although the IL-3 and IL-5 ternary complexes are yet to be solved, it is highly likely that a similar higher-order receptor assembly occurs during IL-3 (dodecamer) and IL-5 (hexadecamer) signaling.

During the assembly of the ternary complex, the structure shows that GM-CSF appears

to actively transition toward the β c subunit, becoming locked into place by numerous interactions in the site 2 interface (Fig. 3C) while maintaining critical site 1 interactions with GMR α (Fig. 3B) (Broughton et al. 2016). The recently solved structures of the GM-CSF: GMR α binary and the improved GM-CSF: GMR α : β c ternary complexes (Broughton et al. 2016) have revealed key residues involved in the transition from the binary to ternary complex. These include GMR α R283, which acts as a pivot point for GM-CSF (Fig. 3B), and β c Y421, which forms critical interactions with GM-CSF E21 (Fig. 3C). E21 is structurally conserved across the β c family of cytokines and mutation of the equivalent residues in IL-3 (E22) and IL-5 (E13) has been shown to abrogate binding and signaling (Barry et al. 1994; Tavernier et al. 1995). The ternary complex structure showed a shift in the amino-terminal region of GM-CSF helix A and reorientation of the β c D4 BC loop to prevent steric clashes between residues in β c and GM-CSF (Broughton et al. 2016) during the binary to ternary transition. These movements accommodate β c D4 on complexation, and avoid steric hindrance with residues in the membrane proximal domain of β c and facilitate β c contact with E21 in GM-CSF (site 2, Fig. 3A,C). In addition, significant remodeling

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Figure 3. β Common (β c) cytokine receptor complex assembly and initiation of signaling. (A) The α and β c receptor subunits for this cytokine family each consist of an extracellular domain connected, via a short juxtamembrane region and helical transmembrane domain, to an intracellular tail. The granulocyte macrophage colony-stimulating factor (GM-CSF) dodecamer is shown bound to the cell membrane as a cartoon. GM-CSF is colored green, GM-CSF receptor α (GMR α) pink, and the β c dimer blue and teal. (Legend continues on following page.)

within the membrane proximal region of GMR α D3 was observed during the binary to ternary transition (Fig. 3D). These different structures illustrate the dynamic nature of receptor assembly and how conformational changes in the receptor complexes initiate signaling (Broughton et al. 2016).

To understand how movements of the β c-family heterodimeric receptor extracellular domains might be transmitted through to the intracellular domains, we can examine the simpler homodimeric human growth hormone receptor (hGHR), the archetypal cytokine receptor system. Waters and coworkers recently showed that adjacent juxtamembrane and transmembrane domains interact to keep hGHR as a dimer (Brooks et al. 2014). In this preformed homodimer, the two hGHR intracellular domains are held in a parallel inactive state, with the pseudokinase domain of one JAK2 molecule inhibiting the kinase domain of the JAK2 molecule bound to the adjacent hGHR intracellular domain. Binding of human growth hormone (hGH) to the hGHR extracellular domains results in the rotation of the juxtamembrane regions and transmembrane helices from the inactive parallel state to a left-

handed crossover configuration, splaying apart of the carboxy-terminal end of the transmembrane helices and separation of the intracellular domains. The movement of the hGHR intracellular domains uncouples the JAK2 pseudokinase domain from the adjacent JAK2 kinase domain, thereby allowing the two adjacent kinase domains to interact and trigger cross-activation of JAK2.

The receptor activation mechanism for the β c cytokine family is more complicated than for the hGH by virtue of their heterodimeric nature; however, the basic signaling principles appear similar. For the GM-CSF receptor the major signaling configuration appears to be a dodecamer or higher-order complex (Hansen et al. 2008; Broughton et al. 2016). The distance between the β c juxtamembrane domains in the center of the GM-CSF dodecameric complex (i.e., immediately below site 4, Fig. 3A) is similar to the distance between the juxtamembrane domains of the hGHR homodimer (~ 10 Å). The movement of the membrane proximal domains of GMR α (D3) and the β c subunit (D4) observed on cytokine binding and affinity conversion (Fig. 3A,E,F) suggests that the transmembrane helices of the GM-CSF receptor

Figure 3. (*Continued*) The juxtamembrane domains are shown as black rods, whereas the transmembrane and intracellular domains are indicated as rectangles and colored as above. Janus kinase 2 (JAK2) (FERM, purple circle; SH2, yellow circle; JH2, light green rectangle; JH1, dark blue rectangle) is shown bound via the FERM domain to the intracellular domain of β c. The direction of signaling through the transmembrane and intracellular regions of β c is indicated by white arrows, and the productive interaction of the JH1–JH2 domains of the adjacent JAK2 molecules resulting in signaling is indicated by a white “P” and jagged red arrow. The sites of interaction within the extracellular dodecamer complex are indicated. (B) Interaction of GMR α D2 and D3 with GM-CSF (part of site 1) in the ternary complex. R283 in the FG loop of GMR α D3 acts as a pivot point for GM-CSF movement during ternary complex formation. This region of site 1 is almost identical in both the binary and ternary complexes. Some residues key to the formation of the binary complex are shown in the panel. Polar interactions are depicted by black dashed lines, colors as in A. (C) View of site 2 illustrating the reorientation of GM-CSF helices A and C on ternary complex formation. The position of GM-CSF in the binary complex is shown in orange. Ternary complex colored as in A. (D) Remodeling of the GMR α D3 domain D strand to a two-turn coiled loop on ternary complex formation. GMR α D3 in the binary complex is colored yellow and pink in the ternary complex. (E) Movement of the GMR α D3 domain on recruitment of β c (site 3) to form the ternary complex. The binary and ternary complexes have been aligned via GM-CSF. In the binary complex, GMR α is colored yellow and GM-CSF is orange. Components of the ternary complex are colored as in A. Direction of domain movement is indicated by the arrow. (F) Movement of the β c D4 domain on formation of the ternary complex (site 3). The uncomplexed β c dimer was aligned with the β c dimer in the GM-CSF ternary complex. Ternary complex colored as in A. The uncomplexed β c dimer is colored gray. Direction of domain movement is indicated by the arrow. A full description of the structural changes that occur during the binary to ternary transition can be found in Broughton et al. (2016).

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might also cross over into an active state as the cytokine “rolls” from its binary to ternary complex orientation. At the center of the GM-CSF receptor dodecamer, the rotation of the β_c D4 domains is likely to translate to a splaying out of the central transmembrane domains, effectively positioning the JAK2 molecules associated with the β_c intracellular domains to initiate JAK/STAT signaling (Fig. 3A).

RECEPTOR PROXIMAL ACTIVATION OF SIGNALING

The β_c subunit is the main signaling subunit of the GM-CSF, IL-3, and IL-5 receptors and mutational analysis of β_c has provided insight into the mechanisms of β_c activation and the location of distinct functional domains (Hercus et al. 2013). However, it is becoming increasingly clear that while the α subunits provide ligand specificity they also regulate some signaling outcomes and this may explain some of the signaling diversity arising from the β_c receptor family.

Formation of the multimeric receptor complex, consisting of ligand, a ligand-specific α subunit, and β_c , triggers the activation of kinases associated with these receptors and leads initially to activation of the JAK2/STAT5 and PI3 kinase pathways (Fig. 4). JAK2 is thought to be the tyrosine kinase responsible for β_c subunit phosphorylation and data from JAK2-deficient mouse models support this view because JAK2 is essential for IL-3-dependent biological responses (Silvennoinen et al. 1993). However, it is now clear that other tyrosine kinases associate with the receptor complex and contribute to β_c signal transduction. For example, whereas fetal liver myeloid progenitor cells from JAK2-deficient mice fail to respond to IL-3 in a colony-formation assay (Parganas et al. 1998), IL-3 induced colony formation from the cells isolated from JAK1-deficient mice, albeit with reduced number and size compared with those from wild-type mice. This suggests that JAK1 is contributing to the full effect of IL-3 receptor signaling, but is not required for signaling. This may be specific for the IL-3 receptor because deletion of JAK1 had no observable effect on

GM-CSF- and IL-5-dependent colony formation (Rodig et al. 1998).

The tyrosine kinase Lyn interacts with the GM-CSF receptor complex, having been shown to bind β_c (Adachi et al. 1999; Dahl et al. 2000) and coimmunoprecipitate with GMR α (Perugini et al. 2010). The functional role of Lyn in β_c receptor signaling and whether it is required for specific signaling outcomes is not yet known. Some indications of a functional link between Lyn and the β_c receptor come from in vivo studies of Lyn-deficient mice in which bone marrow-derived macrophages showed increased sensitivity to GM-CSF stimulation and B cells showed increased IL-3-dependent cell survival and STAT5 phosphorylation (Infantino et al. 2014). These data support a model in which Lyn acts as a negative regulator of GM-CSF and IL-3 receptor signaling (Scapini et al. 2009). There are also data from the use of Src-family kinase inhibitors suggesting that Lyn kinase recruitment to the GM-CSF receptor contributes to GM-CSF-dependent survival signaling; a caveat, however, is that the specificity of such inhibitors is not known (Perugini et al. 2010).

IL-3/IL-5/GM-CSF stimulation also activates the PI3K signaling pathway. In other receptor systems, PI3K binds to phosphotyrosine residues of an activated receptor through its SH2 domain. However, in β_c cytokine signaling, most studies suggest that PI3K interacts with a proline-rich motif within the intracellular region of the α subunit, probably through the SH3 domain of PI3K (Dhar-Mascareno et al. 2005; Perugini et al. 2010). Recruitment of PI3K to the receptor complex results in phosphorylation of Ser585 of β_c and binding of the chaperone molecule 14-3-3, giving rise to survival signals that specifically repress apoptosis (Guthridge et al. 2000, 2004). The β_c Ser585 residue is part of a bidentate motif that includes an Shc-binding site at the β_c Tyr577 residue (Guthridge et al. 2006). Phosphorylation of these two residues is mutually exclusive, and they act as a binary switch to activate distinct signaling pathways associated with either survival alone or both proliferation and survival (Guthridge et al. 2006). One experimental

method that modulates this binary switch is to vary the ligand concentration. At low concentrations of GM-CSF (<10 pM), β c Ser585 is selectively phosphorylated and this results in a cell survival signal through recruitment of 14-3-3 and activation of the PI3K pathway. At higher GM-CSF concentrations, β c Tyr577 is phosphorylated, resulting in recruitment of Shc and activation of cell survival and cell-proliferation pathways (Guthridge et al. 2004). The ability to maintain a survival-only signal at low concentrations of GM-CSF may be critical for steady-state maintenance of cell viability, with an emergency response initiated by high GM-CSF levels in response to stress, injury, or infection. Recent reports have shown that physiological concentrations of cytokine in the picomolar range were sufficient to activate PI3K protein kinase activity, leading to β c Ser585 phosphorylation and hemopoietic cell survival but did not activate PI3K lipid kinase signaling or promote proliferation (Thomas et al. 2013). The recently determined GM-CSF receptor complex structure (Broughton et al. 2016) can allow one to speculate whether altering the concentration of ligand can influence the probability of the formation of intermediate and multimeric receptor complexes.

Despite numerous examples of β c phosphorylation regulating receptor activity, there is no convincing evidence to suggest that the α subunits undergo similar posttranslational modifications. However, it is also clear that the short intracellular domain of the α subunits is absolutely required for signaling (Sakamaki et al. 1992; Weiss et al. 1993; Takaki et al. 1994; Cornelis et al. 1995; Barry et al. 1997) and contribute to cytokine-specific ligand stimulation (Evans et al. 2002). These data suggest that the intracellular domains of the α subunits are important for the assembly of an active receptor complex or provide an initiating role in activation through interactions with kinases or signaling molecules required to initiate downstream signaling events (Polotskaya et al. 1993; Ebner et al. 2003; Perugini et al. 2010; Lontos et al. 2011). Identifying the proteins recruited to the α subunits, and how these fit into the active conformation of the receptor complexes, will

provide a more complete understanding of how signaling through β c may have specific outcomes dependent on the α subunit and the ligand engaged.

DEVELOPMENT OF β c CYTOKINE THERAPEUTIC MOLECULES: WHY, WHERE, AND HOW

The β c cytokines offer direct therapeutic utility by their ability to stimulate the production and function of macrophages and neutrophils. Conversely, they can also contribute to pathology in a number of cancer and inflammation settings thereby prompting the development of various targeted therapies. Because GM-CSF enhances host responses to certain cancers by recruitment and activation of antigen-presenting cells (APCs) such as dendritic cells (Mach et al. 2000), talimogene laherparepvec (T-VEC) has been developed as a vaccine engineered from the herpes simplex virus to kill cancer cells and to express GM-CSF to stimulate the host anti-immune response (Andtbacka et al. 2016). T-VEC is the first oncolytic virus to gain approval for use in the treatment of advanced melanoma in the United States and Europe (Liu et al. 2003; Andtbacka et al. 2015; Hoeller et al. 2016; Kaufman et al. 2016). Recent clinical trials have shown that T-VEC, in combination with ipilimumab, had increased efficacy compared with either agent alone (Puzanov et al. 2016). Another oncolytic virus that expresses GM-CSF, ONCOS-102, reduced tumor growth in a murine mesothelioma xenograft model, whereas 40% of patients in phase I clinical trials responded to the ONCOS-102 treatment (Kuryk et al. 2016; Ranki et al. 2016). In contrast, IL-3 is not used therapeutically owing to undesirable side effects (Eder et al. 1997), although a recent report from our group suggests a role for IL-3 in the repair of cardiac tissue in patients following acute myocardial infarction (AMI). We showed that IL-3 has potent growth factor activity on human CD133⁺ cells, which have proangiogenic properties while maintaining low immunogenic potential (Moldenhauer et al. 2015).

Monoclonal antibodies (MAbs) are versatile therapeutic molecules increasingly used clini-

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Figure 4. Intracellular signaling pathways arising from β common (βc) receptor activation. Schematic of key protein interactions following activation of the βc cytokine receptor family. Low concentrations of cytokine promote the activation of phosphoinositide 3-kinase (PI3K) and subsequent phosphorylation of βc on Ser585 (yellow circle) resulting in the recruitment of 14-3-3 and promoting cell survival. High concentrations of cytokine result in Janus kinase (JAK) 1 and JAK2 transphosphorylation and subsequent phosphorylation of βc at eight intracellular tyrosine residues (yellow circles), activating signal transducers and activators of transcription 5 (STAT5) signaling. Cytokine binding activates the tyrosine kinase, Lyn, which interacts with either βc or the α subunit. Phosphorylation of βc on Tyr577 is mutually exclusive with Ser585 phosphorylation and leads to the recruitment of Shc and the loss of 14-3-3 binding, while promoting cell survival and proliferation. SHP2 has been shown to interact with Tyr612 to promote activation of the mitogen-activated protein kinase (MAPK) signaling pathway.

cally for their specificity and well-characterized in vivo properties that have allowed U.S. Food and Drug Administration (FDA) fast tracking. Several MAbs are being developed against βc cytokines and their receptors (Table 1). The GM-CSF blocking antibody GSK3196165/MOR103 has shown preliminary evidence for efficacy in patients with RA (Behrens et al. 2015; Shiomi and Usui 2015) and was well tolerated in a phase Ib clinical trial in patients with MS (Constantinescu et al. 2015). A number of other GM-CSF blocking antibodies have been developed, including lenzilumab/KB003 (Molino et al. 2016), MORAb-022, and namilumab,

and are currently progressing through clinical trials in patients with RA, plaque psoriasis, CMML, or asthma (Table 1). The anti-GMR α antibody, mavrimumab/CAM-3001, has produced clinically significant responses in a phase IIa study in patients with RA with generally mild-to-moderate adverse events (Burmester et al. 2011, 2013). Importantly, there was no evidence of lung toxicity in patients treated with mavrimumab, which might arise from GM-CSF inhibition, possibly because of limited exposure of the lung to systemically administered antibody (Campbell et al. 2016) or the possibility that effective GM-CSF blockade in

patients with PAP requires a polyclonal GM-CSF autoantibody response (Piccoli et al. 2015).

Several clinical trials are underway to develop antibody therapies that target IL3Rα for patients with leukemia or myelodysplastic syndromes (MDSs). CSL362, an anti-IL3Rα MAb that blocks IL-3 binding and signaling (Sun et al. 1996; Jin et al. 2009) through a dual mechanism (Broughton et al. 2014), is optimized for antibody-dependent cell-mediated cytotoxicity (ADCC) (Busfield et al. 2014). CSL362/JNJ-56022473 has recently progressed to phase II clinical trials in patients with AML in combination with decitabine (ClinicalTrials.gov identifier: NCT02472145). CSL362 may also be effective at targeting leukemic stem cells in patients with CML as these cells are thought to be insensitive to tyrosine kinase inhibitors (TKIs) (Corbin et al. 2011). We recently observed that IL3Rα is overexpressed in CD34⁺/CD38[−] cells from patients with CML and that CSL362-mediated killing of these cells by ADCC was enhanced by treatment with the TKI nilotinib (Nievergall et al. 2014). This MAb is being progressed in parallel as a potential therapy for SLE given its ability to deplete pDCs and, therefore, down-regulate type I and III interferon pathways (Oon et al. 2016a). The anti-IL3Rα MAb, KHK2823 (Akiyama et al. 2015), is currently in phase I clinical trials in patients with AML or MDS (ClinicalTrials.gov identifier: NCT02181699) as is the bispecific antibody MGD006 (Al-Hussaini et al. 2016) that targets IL3Rα and CD3 (ClinicalTrials.gov identifier: NCT02152956). The development of an anti-CD123 antibody–drug conjugate that directs a chemotherapy agent, camptothecin, to the target cell (Li et al. 2016a) represents an alternative use for anti-IL3Rα MAbs. In related approaches, engineered T cells expressing CARs have been developed to target IL3Rα expressed on AML blast cells and leukemic stem cells (Testa et al. 2002). These studies indicate that CD123-directed CAR T cells (CART123) are effective at inducing cell killing (Tettamanti et al. 2013) and are able to eradicate primary AML cells engrafted in immunodeficient mice (Mardiros et al. 2013; Gill et al. 2014). Although normal hemopoietic stem cells are not reported

to express IL3Rα, CART123 impact on normal human myelopoiesis in one study (Gill et al. 2014) suggests some caution is needed with this approach.

An alternate to antibody therapy for hematological malignancies that overexpress IL3Rα is the fusion protein SL-401/DT388IL3, which fuses the catalytic and translocation domains of diphtheria toxin (DT) to IL-3 (Feuring-Buske et al. 2002). Initial phase I clinical trials with DT388IL3 in patients with chemorefractory AML or myelodysplasia showed that the treatment was well tolerated and some favorable clinical responses were observed (Frankel et al. 2008). Phase II clinical trials of SL-401 are continuing in patients with BPDCN (Frankel et al. 2014; Angelot-Delettre et al. 2015) and AML while improved fusion proteins are being developed (Liu et al. 2004; Testa et al. 2005; Hogge et al. 2006; Frankel et al. 2008; Frolova et al. 2014).

The prominent role played by IL-5 and eosinophils in asthma has prompted development and extensive clinical testing of a number of antibodies to IL-5 itself and to the IL-5 receptor (Table 1; and in Keating 2015; Patterson et al. 2015; Nixon et al. 2016). Initial clinical studies with these antibodies in patients with mild-to-moderate asthma were disappointing until a responder subset of asthma patients were identified with elevated blood eosinophil counts (≥ 300 cells/ μ l). This patient population showed successful depletion of the key pharmacodynamic biomarkers, eosinophils (in blood, bone marrow, and sputum), and basophils (in blood), which confirmed target engagement and provided an understanding of mechanism of action (Haldar et al. 2009; Nair et al. 2009). In more recent multicenter phase III trials, an anti-IL5Rα MAb, benralizumab, showed efficacy in reducing annual exacerbation rates and safety for uncontrolled severe asthma patients with elevated eosinophils, further indicating that this patient population will likely receive the greatest benefit from benralizumab treatment (Bleecker et al. 2016; FitzGerald et al. 2016). Mepolizumab/Nucala/SB-240563, an anti-IL-5 MAb, was the first candidate therapeutic targeting the IL-5 pathway to enter clinical studies

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and, since the early 2000s, there have been multiple trials in a range of asthmatic populations. Although in earlier studies mepolizumab showed evidence of target engagement, as evidenced by a reduction in circulating eosinophils, there was no improvement in FEV₁ or other clinical asthma measures (Leckie et al. 2000; Menzies-Gow et al. 2007). Similar to benralizumab, the efficacy of mepolizumab is now being explored for treatment of severe asthma with an eosinophilic phenotype and it is also currently in clinical trials for a number of other conditions (Table 1), including hypereosinophilic syndrome (HES) (Rothenberg et al. 2008) and COPD.

The failure of IL-5 targeting therapies to treat endotypes of asthma other than eosinophilic asthma reflects the heterogeneous nature of asthma, the role of multiple cell types including neutrophils, as well as redundancy in β c cytokine signaling. Targeting the β c subunit allows simultaneous targeting of all three β c cytokines, which may be a more effective therapeutic approach. In one approach, TPI ASM8 contains two antisense oligonucleotides targeting the expression of β c and the chemokine receptor CCR3. Preliminary clinical studies in patients with mild asthma indicated that TPI ASM8 was safe and reduced eosinophil accumulation after allergen challenge (Imaoka et al. 2011). Alternatively, MAbs against the common cytokine-binding site in β c, site 2, are being developed. Initially, MAb BION-1 (Sun et al. 1999) showed proof-of-principle for blocking site 2 in β c. This has been followed by MAb CSL311 (Panousis et al. 2016), which also blocks the function of all three β c cytokines. CSL311 is a fully human MAb that binds β c at the cytokine-binding surface (site 2, Fig. 3A) with high affinity and is a potent antagonist of GM-CSF, IL-3, and IL-5 function, inhibiting the survival of cells isolated from inflammatory airway disease tissue. Although the pharmacodynamic and pharmacokinetic properties of CSL311 and TPI ASM8 are likely to be quite different, the indicated safety profile of TPI ASM8 is encouraging and suggests that blocking β c function with CSL311 might yield a safe and efficacious intervention and be of broad utility

in a range of asthma endotypes owing to its ability to simultaneously block activity of all three β c family cytokines.

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