GM-CSF as a therapeutic target in inflammatory diseases

Annemarie van Nieuwenhuijze\textsuperscript{a,b,c}, Marije Koenders\textsuperscript{b}, Debbie Roeleveld\textsuperscript{b}, Matthew A. Sleeman\textsuperscript{d}, Wim van den Berg\textsuperscript{b}, Ian P. Wicks\textsuperscript{a,c,e,*}

\textsuperscript{a} Inflammation Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia
\textsuperscript{b} Department of Rheumatology and Advanced Therapeutics, University Medical Centre Nijmegen, Nijmegen, The Netherlands
\textsuperscript{c} Department of Medical Biology, University of Melbourne, Parkville, Victoria, Australia
\textsuperscript{d} MedImmune Ltd., Cambridge, United Kingdom
\textsuperscript{e} Melbourne Health, PO Royal Melbourne Hospital, Parkville, Victoria, Australia

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\section*{A B S T R A C T}

GM-CSF is a well-known haemopoietic growth factor that is used in the clinic to correct neutropaenia, usually as a result of chemotherapy. GM-CSF also has many pro-inflammatory functions and recent data implicates GM-CSF as a key factor in Th17 driven autoimmune inflammatory conditions. In this review we summarize the findings that have led to the development of GM-CSF antagonists for the treatment of autoimmune diseases like rheumatoid arthritis (RA) and discuss some results of recent clinical trials of these agents.

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1. Biology of GM-CSF

1.1. Production of GM-CSF

Granulocyte macrophage colony stimulating factor (GM-CSF, or CSF2) was first purified from LPS treated mouse lung-conditioned medium. It was subsequently characterised as a haemopoietic growth factor that stimulates the proliferation of myeloid cells from bone-marrow progenitors (Burgess et al., 1977). Human and mouse GM-CSF share a high level of amino acid homology (Gough et al., 1984; Lee et al., 1985), but are species-specific in terms of receptor binding (Shanafelt et al., 1991). GM-CSF can be produced by a wide variety of cell types, including activated T cells, B-cells, macrophages, endothelial cells, fibroblasts and tumour cells (Fleetwood et al., 2005). The production of GM-CSF can be stimulated by a range of factors, including T cell receptor and co-receptor stimulation (Fraser and Weiss, 1992; Shannon et al., 1995), toll like receptor (TLR) agonists, TNF, IL-1 and prostaglandin E2 (Quill et al., 1989; Hamilton, 2008) (Fig. 1).

1.2. Action of GM-CSF on mature cells

Besides the production and differentiation of haemopoietic cells from precursors, GM-CSF is now recognised to have a range of other functions on mature haemopoietic cells, including enhanced antigen presentation, induction of complement- and antibody-mediated phagocytosis, and promotion of leukocyte chemotaxis and adhesion (Cook et al., 2004; Fleetwood et al., 2005; Gomez-Cambronero et al., 2003; Metcalf, 2008). GM-CSF, in combination with other inflammatory stimuli, can polarise macrophages into “M1-like” inflammatory macrophages (Fleetwood et al., 2007; Mantovani et al., 2002). M1-macrophages produce a range of inflammatory cytokines, such as TNF, IL-6, IL-12p70 and IL-23. In contrast, “M2-like” macrophages are maintained in a non-activated state by M-CSF, and produce anti-inflammatory factors IL-10 and CCL2 (Fleetwood et al., 2007; Mantovani et al., 2002). In the central nervous system, GM-CSF can activate resident macrophage-like microglia and promote neuroinflammation through the upregulation of TLR4 and CD14 (Parajuli et al., 2012). In mature neutrophils, the integrin CD11b is upregulated by GM-CSF, which increases...
adhesion to vascular endothelium and tissue entry (Hansen et al., 2008). Priming of neutrophils by GM-CSF increases antimicrobial functions, such as phagocytosis and oxidative burst (Condiffe et al., 1998; Fleischmann et al., 1986). GM-CSF can have multiple effects on mature DC, such as increased cross-presentation (Zhan et al., 2011) and increased uptake capacity (Daró et al., 2000). GM-CSF also induced proliferation of CD103+ intestinal dendritic cells in vivo in mice (Schulz et al., 2009). How GM-CSF regulates the development and function of dendritic cell subsets is summarised in a recent review (Zhan et al., 2012). GM-CSF can also activate non-haemopoietic cells, such as endothelial cells and sensory neurons (Bussolino and Mantovani, 1991; Schweizerhof et al., 2009). Elevated tissue levels of GM-CSF have been detected in multiple inflammatory conditions, including RA, multiple sclerosis (MS), obesity, lung disease and cancer (Hamilton, 2008).

1.3. GM-CSF: A T cell cytokine

Several recent findings have sparked interest in the relationship between GM-CSF and T cells. The discovery that GM-CSF promotes the induction and survival of Th17 cells via IL-6 and IL-23 and that GM-CSF is required for the pathogenicity of Th17 cells in experimental allergic encephalomyelitis (EAE), shows that GM-CSF is relevant outside of the myeloid cell lineage (Codarri et al., 2011; El-Behi et al., 2011). In these studies, GM-CSF was shown to be induced via ROR-γt, a lymphocyte-specific transcription factor that was initially described as the master regulator for Th17 development (Ivanov et al., 2007). Production of GM-CSF by T cells is also regulated by the transcription factor NF-κB (Campbell et al., 2011a; Holloway et al., 2003; Osborne et al., 1995). It was shown that T cells specifically require the NF-κB1 subunit (p50, most likely as part of the active p50/p65 NF-κB heterodimer) for production of GM-CSF.
and that T cell-derived GM-CSF was crucial for the local differentiation of monocyte derived inflammatory dendritic cells in synovial tissue during a model of inflammatory arthritis (Campbell et al., 2011a).

2. The GM-CSF receptor

The GM-CSF receptor (CSF2R) is a heterodimer, composed of a specific ligand-binding α-chain (CSF2RA), which binds GM-CSF with low affinity and a signal-transducing β-chain (CSF2RB) (Gearing et al., 1989; Hayashida et al., 1990). High affinity binding follows dimerization of both chains. For signal transduction, the cytoplasmic domains of both the α and β chain are required, but it is mainly the β chain that binds JAK2 (Hansen et al., 2008). Signalling from the CSF2R complex activates the Ras/MAPK and JAK/STAT/SOCS pathways (Hansen et al., 2008; Jenkins et al., 1998; Sato et al., 1993). Alternative signalling pathways, such as the c-fos/fes pathway, have also been suggested (Hanazono et al., 1993). The CSF2R is expressed on myeloid cells and on some non-haemopoietic cells, such as endothelial cells (Colotta et al., 1993; Rosas et al., 2007), but interestingly, not on T cells (Hercus et al., 2009). In both mice and humans, the β-subunit is shared with the GM-CSF, IL-3, and IL-5 receptors, and is known as the common β chain (βc) (Hayashida et al., 1990; Kitamura et al., 1991; Tavernier et al., 1991). In the mouse, an additional IL-3-specific β-chain exists, known as βL-3 (Itoh et al., 1990), which is used in preference to βc for signalling by IL-3 (Nicola et al., 1996).

In addition to the membrane-bound form of the CSF2Rα, multiple splice variants of soluble CSF2R have been described, both in vitro and in vivo (Brown et al., 1995; Pelley et al., 2007; Prevost et al., 2002). It is not clear whether these soluble receptors are of functional importance, but they may inhibit the ligand-membrane bound CSF2R interaction (Heaney and Golde, 1998).

3. Too much or too little?

Abnormalities in GM-CSF production or CSF2R function have been implicated in multiple human pathologies such as RA (Bell et al., 1995), juvenile myelomonocytic leukaemia (Birnbaum et al., 2000), chronic myelomonocytic leukaemia (Ramshaw et al., 2002) and pulmonary alveolar proteinosis (PAP) (Dirksen et al., 1998; Seymour et al., 1998). Many of the features of these human conditions have been studied in mice deficient for GM-CSF or the CSF2R and mice that overexpress GM-CSF. In addition, therapeutic blockade of GM-CSF in animal models using neutralising antibodies has yielded crucial information.

3.1. GM-CSF and CSF2R knock out mice

Mice have been generated that are deficient in GM-CSF (Stanley et al., 1994). Surprisingly, these mice do not have a defect in myeloid cell development, but display infiltration of the lungs with lymphocytes and defective maturation of alveolar macrophages (Stanley et al., 1994). As a consequence, there is accumulation of lung surfactant, which results in the development of a lung phenotype equivalent to idiopathic PAP (Dranoff et al., 1994; Stanley et al., 1994; Trapnell et al., 2003; Uchida et al., 2007). In humans, PAP is a rare lung disease in which patients present with an accumulation of surfactant proteins in the lungs that interferes with gas exchange. In some patients this can render them more susceptible to infections (Trapnell et al., 2003). Notably, GM-CSF deficient mice have been reported to have a somewhat increased mortality from microbial infections (Dranoff et al., 1994; Stanley et al., 1994). This suggests that GM-CSF plays an important role in the host response to infectious agents, particularly in the lungs, possibly through regulating macrophage function (Fleetwood et al., 2005). More recently it was shown that GM-CSF deficient mice also have a defect in the maturation of invariant natural killer cells (Bezbradica et al., 2006), which could be important in innate immunity.

Mice lacking GM-CSF are protected from a wide range of autoimmune disease models, such as methylated BSA(mBSA)/IL-1 induced arthritis (Lawlor et al., 2005), collagen induced arthritis (CIA) (Campbell et al., 1998), EAE (McQuilter et al., 2001) and experimental autoimmune myocarditis (EAM) (Sonderegger et al., 2008). Intriguingly, these models all require T cells to develop pathology.

Mice deficient for the β-chain (CSF2RB) of the GM-CSF receptor (Nishinakamura et al., 1995; Robb et al., 1995) have also been generated. Although the phenotype of these mice cannot be attributed to perturbed signalling of GM-CSF alone, due to the activation of this receptor subunit by IL-3 and IL-5 (Hayashida et al., 1990; Kitamura et al., 1991; Tavernier et al., 1991), the phenotype was in many aspects similar to that of the GM-CSF deficient mice. CSF2RB gene knockout mice displayed a lung phenotype resembling PAP, and had normal development of haemopoietic cells (Nishinakamura et al., 1996; Robb et al., 1995). Taken together, the data from GM-CSF gene knockout animals clearly illustrates that GM-CSF and the GM-CSF receptor are not essential for steady state haemopoiesis, but play more complex biological roles.

3.2. Overexpression of GM-CSF

Systemic administration of GM-CSF is used in the clinic for treatment of neutropenia after chemotherapy (Metcalf, 2008), and as an adjuvant in anti-tumour immunity (Disis et al., 1996; Slimgull, 2003). However, when GM-CSF is administered to patients with Felty’s syndrome, a flare of RA can occur (Hazenbg and et al., 1989). Likewise, administered GM-CSF also exacerbated arthritis in mice (Campbell et al., 1997). In addition, GM-CSF given as an adjuvant with a whole-cell melanoma vaccine, caused systemic recruitment of eosinophils and basophils and there was a trend towards worse survival (Faries et al., 2009). These findings illustrate that not only the relative absence, but also the abundance, of GM-CSF can have significant effects on the immune system.

Besides exogenous administration of GM-CSF, there have been numerous studies in which GM-CSF was overexpressed endogenously, either transiently using adenoviral vectors (Steinwede et al., 2011; Xing et al., 1996) or constitutively using transgenic mice (Biondo et al., 2001; Breuhahn et al., 2000; Lang et al., 1987; Paine et al., 2003; Van Nieuwenhuijze et al., unpublished observations). A common feature of these transgenic animal models was local macrophage and DC recruitment/differentiation, with phenotypes including blindness due to accumulation of macrophages in the eye, myelo-proliferation and inflammatory tissue destruction (Lang et al., 1987), the induction of autoimmune gastritis (Biondo et al., 2001), and the development of disseminated histiocytosis (Van Nieuwenhuijze et al., unpublished observations). These models emphasize the importance of regulating the level of bioactive GM-CSF to prevent exaggerated myelopoiesis or a break of immune tolerance.

3.3. Experimental blockade of GM-CSF

Genetic ablation of GM-CSF or CSF2R has provided many insights into the role of GM-CSF and GM-CSF signalling in steady...
state and emergency haemopoiesis and autoimmunity. However, for the development of neutralising GM-CSF therapeutics for inflammatory disorders, it is paramount that inhibition is evaluated during pathological processes. For the treatment of RA for instance, it is anticipated that GM-CSF blockade might be an option for patients who do not respond adequately to conventional treatment, so these patients will have pre-existing inflammation. The therapeutic effect of GM-CSF blockade after disease onset was shown in CIA (Cook et al., 2000), streptococcal cell wall-induced arthritis (SCW) (Plater-Zyberk et al., 2007), mBSA/IL-1 experimental monarthritis (Yang and Hamilton, 2001), Alzheimer’s disease (Manczak et al., 2009) and LPS-induced lung inflammation (Bozinovski et al., 2004). In all of these studies, there was rapid improvement in established disease features.

An important issue for the development of GM-CSF inhibitors is the possibility of adverse effects, in particular PAP and increased susceptibility to microbial lung infections (Seymour et al., 1998). To date, these effects have not been observed in animals treated with anti-GM-CSF. It is not clear if this is due to the duration and extent of GM-CSF deficiency, locally controlled regulation of GM-CSF during PAP, or because experimental animals are housed under controlled conditions and are exposed to a reduced infectious load. As many cytokine inhibitors have been trialled and approved in a broad range of inflammatory conditions, physicians and regulatory authorities have a strong understanding of managing the risk of opportunistic infections, however additional lung monitoring is likely required in human clinical trials of GM-CSF inhibitors in which patients may present with a different side effect profile.

4. GM-CSF and Th17 cells

Perhaps surprisingly, GM-CSF is actually produced in high levels by T cells during inflammation (Wada et al., 1997; Ponomarev et al., 2006; Shi et al., 2006) and it has been found recently that GM-CSF is linked to the IL-23/IL-17 pathway (Campbell et al., 2011a; Codarri et al., 2011; El-Behi et al., 2011; Poppensieker et al., 2012; Van Nieuwenhuijze et al., unpublished observations). GM-CSF promoted the induction and survival of autoimmune Th17 effector cells in a CD4 T cell-mediated model of myocarditis (Sonderegger et al., 2008). During models of acute inflammatory arthritis or periodontitis, GM-CSF production by T cells was dependent on NF-κB1 and was required for the local differentiation of inflammatory monocyte derived DCs (Campbell et al., 2011a). It was also shown that GM-CSF is essential for the pathogenicity of Th17 cells and for the CCR4-dependent production of IL-23 by DC in EAE (Codarri et al., 2011; El-Behi et al., 2011; Poppensieker et al., 2012). The production of GM-CSF was regulated not only by the transcription factor NF-κB1, but also by ROR-αt (Codarri et al., 2011). As noted in chapter 2, T cells lack GM-CSF receptors and so the effect of T cell derived GM-CSF on Th17 mediated inflammation occurs via enhancement of IL-6 and IL-23 production from antigen presenting cells (APC) (El-Behi et al., 2011). Through binding to their respective receptors on T cells, IL-6 and IL-23 enhance GM-CSF production by T cells, creating a positive feedback loop (El-Behi et al., 2011). The close relationship between GM-CSF and IL-17 in inflammatory conditions suggests that therapeutic inhibition of one of these molecules might have an effect on the other. It will be interesting to see what happens to the level of GM-CSF in patients treated with IL-17 inhibitors that are currently in clinical trials (Patel et al., 2012). Since IL-17 inhibitors reduce the number of Th17 cells in the circulation, one would assume there will also be a reduction in the level of GM-CSF production (Patel et al., 2012). However, it is also possible that the effects on Th17-derived GM-CSF are mostly local, at the site of inflammation, and difficult to measure due to the generally low levels of GM-CSF in the circulation. Likewise, the levels of IL-17 and the number of Th17 cells are likely to be affected in patients treated with GM-CSF inhibitors. The potential of IL-17 and the IL-17 receptor as therapeutic targets in inflammatory conditions has recently been reviewed (Roeleveld et al., 2013).

5. GM-CSF autoantibodies

Intriguingly, serum GM-CSF in both mice and humans is generally present in a complex with GM-CSF autoantibodies (Uchida et al., 2009). Free GM-CSF is maintained at very low, often undetectable levels in the serum and tissues (Carraway et al., 2000; Uchida et al., 2009), but is nevertheless essential for the maintenance of some myeloid cell functions in the lungs. In humans, autoimmune PAP is characterized by high levels of auto-antibodies to GM-CSF, or the defective expression of the CSF2R (Browne and Holland, 2010; Dirksen et al., 1998, 1997; Sakagami et al., 2009; Suzuki et al., 2008; Trapnell et al., 2003). As described above, the features of human PAP are similar to those seen in GM-CSF knockout mice (Stanley et al., 1994). Human GM-CSF autoantibodies inhibit GM-CSF bioactivity (Uchida et al., 2004), thereby reducing GM-CSF-dependent cell functions (Dranoff et al., 1994; Stanley et al., 1994; Trapnell et al., 2003; Uchida et al., 2007). GM-CSF autoantibodies are also found at low levels in healthy individuals (Uchida et al., 2009). It is possible that these autoantibodies, together with soluble CSF2R molecules, function as additional regulators of GM-CSF bioactivity in vivo (Heaney and Golde, 1998; Uchida et al., 2009). To prevent autoimmune pathology and the development of PAP, anti-GM-CSF antibodies and the levels of free GM-CSF need to be maintained in a tightly controlled balance. This balance will need to be considered with the introduction of anti-GM-CSF therapeutics for inflammatory conditions (Fleetwood et al., 2005; Uchida et al., 2009).

6. GM-CSF inhibitors in the clinic

Conventional disease-modifying anti-rheumatic drugs (DMARDs), such as methotrexate, are routinely used alone or in combination for the treatment of RA. However, despite the beneficial effect on clinical symptoms and joint damage, DMARDs do not yield the desired outcome for some patients (Smolen et al., 2007). In the past decade, new biological therapies have been introduced in the clinic or are currently in clinical trials for RA and other inflammatory conditions, which has significantly improved clinical outcomes (Furst et al., 2012). These therapies include TNF inhibitors (infliximab, etanercept, adalimumab, certolizumab and golimumab), a B-cell-depleting anti-CD20 antibody (rituximab), an inhibitor of costimulation (cytotoxic T-lymphocyte antigen–4 fusion (CTLA-4) protein abatacept), an IL-1 receptor antagonist (anakinra) and an inhibitor of the IL-6 receptor (tocilizumab) (Campbell et al., 2011b; Smolen et al., 2007). However, about 30% of patients treated with anti-TNF agents show no clinical benefit, and only a minority of patients achieve disease remission (Campbell et al., 2011b). The other four biologicals abatacept, rituximab, anakinra and tocilizumab have now been approved for use in RA patients, and have shown variable clinical efficacy in reducing disease activity in patients for whom TNF inhibitor therapy fails (Nam et al., 2010). There is great interest in trying to understand the basis for clinical response, or lack of response, to these agents in order to better tailor anti-cytokine therapy for individual patients.

Collectively, a wealth of pre-clinical studies now highlight GM-CSF as a key mediator of inflammatory and immune disorders,
suggesting it might be an excellent candidate for therapeutic intervention (Cormish et al., 2009; Hamilton, 2008). The action of GM-CSF can be inhibited by at least two different approaches: inhibition of GM-CSF itself by neutralising antibodies, or the specific blockade of GM-CSF binding to its receptor by antibodies against the GM-CSF receptor α chain. These approaches have resulted in humanized or fully human anti-GM-CSF and anti-CSF2Rα therapeutics that are currently being tested in clinical trials. Mavrilimumab, a fully human antibody that binds CSF2Rα, thereby preventing signalling through this receptor, has now successfully been tested in Phase I and Phase II clinical trials in RA patients (Burmester et al., 2011, 2012). In a recent study of patients with active RA in spite of methotrexate, 55.7% of all participants treated with subcutaneous injection of mavrilimumab (every two weeks for 12 weeks) met the primary endpoint of achieving ≥1.2 decrease from baseline in the disease activity score (DAS28-CPR) at week 12. At the highest dose of mavrilimumab (100 mg), 66.7% of subjects met the primary endpoint compared with 34.7% of placebo subjects. Treatment responses were often rapid, with differences observed between placebo as early as two weeks, and were sustained during 12 weeks of follow up. Throughout the duration of this study adverse events were reported as mild or moderate in intensity. Of note is that there were no significant pulmonary complications reported during the study (Burmester et al., 2012). Likewise, MOR103, another fully human high affinity anti-GM-CSF monoclonal antibody, has also recently completed evaluation in a Phase Ib/2a trial in RA patients, with an acceptable safety profile and indications of efficacy ( Morphophys AG). Whilst both clinical studies were different in size, route of administration and duration, it is noteworthy that both report a rapid onset of action and provide evidence of clinical efficacy that support further clinical investigation. In addition to RA, MOR103 is currently being tested in a Phase Ib trial for multiple sclerosis (MS) ( Morphophys AG). A panel of other GM-CSF inhibitors are also under investigation, however results have not yet been reported. Of particular note is KB003, which is a “humanised” anti GM-CSF antibody that is currently undergoing clinical trials in severe asthma ( KaloBios Pharmaceuticals). Whilst there is compelling evidence that this pathway also plays a role in severe respiratory diseases such as asthma and chronic obstructive pulmonary diseases (COPD) (Botelho et al., 2011; Saha et al., 2009), a fine dosing balance may have to be achieved so as not to promote additional pulmonary complications.

7. GM-CSF and pain

Pain is one of the main symptoms of inflammatory diseases like RA, and rated as one of the highest priorities by RA patients (Lee, 2013; Wolfe and Michaud, 2007). The relatively recent and surprising finding that functional GM-CSF receptors are present on sensory nerves has led to the investigation of GM-CSF as a mediator of pain (Schweizerhof et al., 2009). In this study, GM-CSF secreted by tumour cells was linked to signal transduction in nociceptors, and directly mediated bone tumour-induced pain. It was suggested that the locally produced GM-CSF at sites of inflammation or tumour could sensitize nociceptors. The high levels of GM-CSF found in inflammatory synovium could therefore be linked to joint pain experienced by RA patients. This link has recently been examined in animal models of inflammatory arthritis and osteoarthritis (Cook et al., 2013, 2012), where it was shown that GM-CSF was required for arthritic pain. GM-CSF deficient mice were completely protected from pain associated with the induction of inflammatory arthritis. Interestingly, GM-CSF mediated pain could be relieved by the administration of the cyclooxygenase inhibitor indomethacin, but this did not have any effect on joint inflammation (Cook et al., 2013).

These results raise the possibility that GM-CSF inhibitors might be of particular value in relieving pain as well as inflammation.

8. Concluding remarks

There are many facets to GM-CSF biology – haemopoietic growth factor, inducer of myeloid and T cell differentiation, pro-inflammatory mediator and colony-stimulating factor. The preclinical data provides a strong rationale for considering GM-CSF as a potential therapeutic target for inflammatory diseases. Recent trials with specific GM-CSF or CSF2R inhibitors promise improved treatment options for patients with inflammatory conditions. Careful monitoring for potential side effects, particularly PAP, remains paramount, but we anticipate anti-GM-CSF therapeutics will be of value for many autoimmune and inflammatory conditions and perhaps tumour associated pain. Continued study of the in vivo consequences of GM-CSF inhibition in patients with inflammatory diseases will no doubt provide further insights into this fascinating cytokine.

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References


