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G-CSF：嗜中性球產生的關鍵調節器，但這還不是全部！

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G-CSF: A key regulator of neutrophil production, but that's not all!

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Abstract
G-CSF is a major extracellular regulator of haemopoiesis and the innate immune system. Named for its relatively specific stimulation of the growth of neutrophil progenitor cells *in vitro* in semi-solid cultures (Burgess and Metcalf 1980, Nicola et al. 1983), G-CSF influences the survival, proliferation and differentiation of all cells in the neutrophil lineage, from haemopoietic stem cell through to mature neutrophil. Further, G-CSF influences the function of mature neutrophils. These actions underpin its rapid uptake into clinical medicine as a drug that increases the production of neutrophils in patients with chemotherapy-induced neutropenia.

Ongoing research has uncovered initially unsuspected polyfunctionality for G-CSF. G-CSF is well recognised as a potent mobiliser of haemopoietic stem cells from the bone marrow into the blood, and now is being increasingly accepted as a regulator of immune responses. These two "new" actions of G-CSF first came to light through observations made during clinical trials of G-CSF. Subsequent investigations into the cellular and molecular basis for this polyfunctionality have generated exciting new knowledge about the biology of G-CSF. This review emphasises recent advances in knowledge about G-CSF signalling, mechanisms of G-CSF-induced stem cell mobilisation, and how G-CSF influences T-cell function and dendritic cell activation. An attempt is made to link the current issues about the biology of G-CSF with its clinical uses, both present and future.

Keywords: *G-CSF, haemopoietic stem cell, neutrophil, dendritic cell*

G-CSF: Basic biology

G-CSF is a 25 kD secreted glycoprotein encoded by the *CSF3* gene. Gene deletion studies in mice have revealed the central physiological roles G-CSF plays in the regulation of neutrophil production in health and particularly in emergency responses to infections and bone marrow aplasia. G-CSF-deficient mice and G-CSF receptor-deficient mice are characterised by a resting neutropenia, a markedly reduced capacity to mount a neutrophilic response to invoked bacterial and fungal infections, an increased mortality from these infections, a susceptibility to bacterial pneumonia and a propensity to develop reactive amyloidosis with age (Lieschke et al. 1994, Liu et al. 1996, Seymour et al. 1997, Basu et al. 2000).

In keeping with these functional data from mouse models, serum concentrations of G-CSF are normally undetectable or detectable at very low levels in humans (Cebon et al. 1994). However, in response to infection, serum levels are markedly elevated and fall in parallel with recovery from infection (Kawakami et al. 1990). All tissues within the body are capable of producing G-CSF, but generally do so only after stimulation. Early experiments with tissue-conditioned media revealed that inflammatory stimuli including IL-1, LPS, TNF-α induced G-CSF production by macrophages, endothelial cells, fibroblasts and related mesenchymal cells (reviewed in Roberts and Nicola 1996). More recently, IL-17 has been implicated as a major upstream extracellular regulator of G-CSF production by tissues, particularly the bone marrow stroma (Fossiez et al. 1996). *In vivo*, blockade of IL-17 reduces G-CSF serum levels and G-CSF-driven neutrophilia (Forlow et al. 2001).

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Linking IL-17 production by T cells to physiological initiating signals for G-CSF production, such as bacterial infection, are the Toll-like receptors (TLRs) and IL-23. Emerging data suggest that tissue macrophages and dendritic cells sense Gram-negative bacteria through TLRs (e.g. LPS binding TLR4), inducing IL-23 production which in turn is a potent stimulus for IL-17 production by T cells (Happel et al. 2003).

The mechanisms by which G-CSF regulates neutrophil production and influences neutrophil function are now well elucidated (reviewed in Roberts and Nicola 1996). At the cellular level, G-CSF induces proliferation in all mitogenic cells in the granulocytic lineage from uncommitted haemopoietic stem cells through to myelocytes. While co-stimulation with other cytokines are required to sustain stem cell proliferation, G-CSF alone is sufficient stimulus for committed myeloid progenitor cells to generate many thousands of progeny. Concomitantly, G-CSF enhances differentiation along the neutrophil lineage, and accelerates maturation of metamyelocytes into mature neutrophils. G-CSF also prolongs the survival of neutrophils and their precursors, including stem cells. For mature neutrophils, G-CSF enhances, but does not primarily instigate, key functions such as superoxide production, phagocytosis, and bacteriocidal killing.

G-CSF receptor and G-CSF-induced intracellular signalling

All cellular responses to G-CSF are the consequence of signals arising from the cytoplasmic domain of the G-CSF receptor (G-CSFR), after ligation of the extracellular domain of the receptor by G-CSF (reviewed in Avalos 1996). Direct responses to G-CSF therefore require cells to express the G-CSFR, and most of our understanding of the biology of G-CSF actions is based on studies of common cell types (such as neutrophils) that uniformly express G-CSFRs. As will be discussed later, G-CSF also imparts significant indirect effects on tissues and whole animals.

G-CSFR distribution

The G-CSFR is expressed by all neutrophils and their precursors including metamyelocytes, myelocytes, promyelocytes, myeloblasts, myeloid progenitor cells, and primitive haemopoietic stem cells (Nicola and Metcalf 1985, McKinstry et al. 1997). In the latter, transcription of the G-CSFR gene (*CSF3R*) is thought to occur intermittently and stochastically. While expressing the G-CSFR, undifferentiated HSC are susceptible to influence by G-CSF.

Other cell types, not usually considered to be targets of G-CSF action, have been demonstrated to express G-CSFRs. Endothelial cells (Bussolino et al. 1989), placental cells (Nicola et al. 1985) and activated T lymphocytes (Franzke et al. 2003) express functional G-CSFRs, and many non-haemopoietic tumour cell lines have also been reported to express G-CSFR (reviewed in Roberts and Nicola 1996). For each of these cell types, it remains an ongoing challenge to define the physiological relevance of this expression.

G-CSFR structure and proximal signalling pathways

G-CSFR is a transmembrane protein with a structure typical of the Type I cytokine receptor family. The extracellular component contains an N-terminal immunoglobulin domain, a cytokine recognition domain within a paired fibronectin module, and three other fibronectin III repeats (Avalos 1996). Precisely how G-CSF and its receptor associate remains to be completely elucidated, but analyses of the structure of G-CSF bound to a truncated receptor reveal a 2:2 complex (Aritomi et al. 1999). Within this complex, each G-CSF molecule binds to both receptors. It is likely that the activated form of the G-CSFR is at least a dimer.

Ligation of the extracellular domain of the G-CSFR results in activation of multiple intracellular signalling cascades, some of which rely on phosphorylation of one or more of four tyrosine residues in the C-terminal region of the receptor. Mutational analyses of the receptor and interacting proteins have enabled detailed definition of these signalling cascades. Figure 1 outlines in cartoon form key components of the signalling complex created by activation of the G-CSFR, and the broad downstream pathways activated. To date, most knowledge is restricted to describing linear pathways. Our understanding of how these pathways interact, and how they are influenced by cellular context and competing or complementing extracellular signals, remains embryonic. Ultimately, signalling from the G-CSFR influences cell survival, proliferation and differentiation, and these behaviours represent the net effects of discrete pathways acting in concert.

Lacking any intrinsic kinase activity, the G-CSFR signals via several classes of non-receptor kinases. Members of the Jak tyrosine kinase family, Jak1, Jak2 and Tyk2 are phosphorylated after receptor ligation (Nicholson et al. 1994, Tian et al. 1994). These proteins are constitutively associated with the receptor and bind to the membrane-proximal region of the receptor within the Box 1 and 2 domains (which are conserved among other members of the Type I cytokine receptor family). Similarly, members of the Src kinase family, Lyn and Hck, are recruited to this region (Corey et al. 1994, Ward et al. 1998a). Phosphorylation of Jak family kinases in turn activates STAT1, STAT3 and STAT5 which homodimerise. While STAT3 is the most studied of these in G-CSF

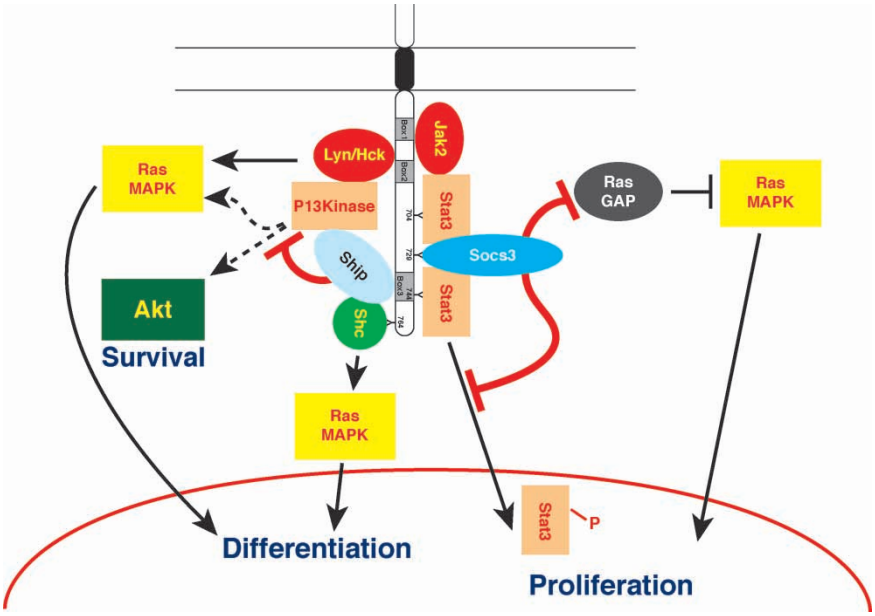

Figure 1. Signalling pathways emanating from the cytoplasmic tail of the G-CSF receptor after activation by ligand binding are shown in stylised form. G-CSF binds the extracellular domains (not shown), triggering receptor dimerisation which is required for activation of signalling. For simplicity however, the activated receptor is shown as a monomer in this cartoon. As described in the text, Box 1 and Box 2 regions define the key membrane-proximal region required for delivery of a proliferative signal. The four tyrosine residues present in the carboxy-terminus of the receptor are required for full receptor function, including differentiative signals and negative regulation. Binding sites for signalling molecules are shown, as are the general pathways activated from various regions of the G-CSF receptor. Black arrows indicate positive effects on downstream pathways, while red lines indicate negative effects. Two key negative regulator elements are shown, SOCS3 and SHIP. SOCS3 binds the activated G-CSF receptor directly at phosphorylated Y729, while SHIP does not bind the G-CSF receptor directly, but associates via Shc after it has bound phosphorylated Y764. For references to specific activation or interaction events, see the main text.

signalling (Tian et al. 1994), heterodimers of STAT1/3 and STAT3/5 are also activated (de Koning et al. 1996a, Ward et al. 1999a). Similarly, Lyn phosphorylation recruits c-Cbl which when phosphorylated binds and activates PI3-kinase (Wang et al. 2002) and subsequently Akt (Dong and Larner 2000). Other downstream kinases activated by the G-CSFR in a Lyn-dependent fashion include Syk and Bmx (Ekman et al. 2000).

Whilst the membrane-proximal 57 amino acids of the G-CSFR (containing Box 1 and 2 domains) are sufficient to deliver a proliferative signal to the cell, the distal 150 amino acids are crucial for the normal functioning of the receptor, and particularly for the negative regulation of G-CSF signalling. The C terminus includes internalisation motifs (Aarts et al. 2004) and four tyrosines, which when phosphorylated upon receptor dimerisation and Jak activation, provide docking sites for additional signalling proteins with both positive and negative functions. A series of elegant functional studies in primary haemopoietic cells (Akbarzadeh et al. 2002, Hermans et al. 2003) and biochemical investigations in cell lines and primary cells have characterised the pivotal roles that each of these phospho-tyrosines plays in cellular responses to G-CSF, but almost certainly knowledge remains incomplete as to the full spectrum of proteins which compete for binding at each site. STAT3 binds both Y704 and Y744 (Ward et al. 1999b), with Y704 being required for maximal STAT3 phosphorylation (Tian et al. 1996). In contrast, STAT1 and STAT5 activation are unaffected in truncation mutants lacking all C-terminal tyrosines. While studies in the cell line 32D indicate Y704 and Y744 are required for normal proliferation (Ward et al. 1999b), the data from experiments with primary cells suggest a more subtle contribution to cellular responses to G-CSF (Akbarzadeh et al. 2002, Hermans et al. 2003). Isolated mutations of Y704 or Y744 have little effect on the proliferation and differentiation of bone marrow cells stimulated with G-CSF (Akbarzadeh et al. 2002), however, the capacity of progenitor cells stimulated with G-CSF to be recloned is reduced if either of these tyrosines is present (Hermans et al. 2003).

Data from multiple studies indicate that Y764 is essential for transmission of a maximal proliferative signal (Akbarzadeh et al. 2002, Hermans et al. 2003), and this activity is readily explained by the recruitment of Shc to phosphorylated Y764 and the consequent activation of Ras via the adapter proteins Grb2 and SOS (de Koning et al. 1996b). G-CSF also activates Ras-MAPK via Lyn/Hck, but biochemical and functional data suggest that the Y764-dependent

signal is dominant (Rausch and Marshall 1997). In addition to activating Ras-MAPK, Shc phosphorylation also activates JNK and p38MAPK (Rausch and Marshall 1997), and recruits SHIP, a negative regulator of PI3K signals, to the receptor complex (Hunter and Avalos 1998). Y764 has also been implicated as the binding site for another phosphatase, SHP-2 (Ward et al. 1998b).

Negative regulation of G-CSF signalling

The theme of recruiting negative as well as positive regulators to the activated G-CSFR is further exemplified by Y729, defined consistently in functional studies as an essential motif for reducing proliferation and clonogenicity, and for enhancing differentiation in 32D cells and primary progenitors (Ward et al. 1999b, Akbarzadeh et al. 2002, Hermans et al. 2003). Recently, it was discovered that suppressor of cytokine signalling-3 (SOCS3) binds selectively to the human G-CSFR at Y729 when this residue is phosphorylated (pY729) (Hortner et al. 2002). In cell-based overexpression systems, binding of SOCS3 to phosphorylated Y729 leads to the inhibition of STAT-dependent gene expression after stimulation of the cell with G-CSF (Hortner et al. 2002). These data, together with the functional data from mutation analyses, suggested that SOCS3 is a negative regulator of G-CSF-induced cellular responses. This hypothesis was proved in conditional knockout mice in which all haemopoietic cells were deficient in SOCS3. When stimulated with G-CSF *in vitro*, SOCS3-deficient cells of the neutrophilic granulocyte lineage exhibited prolonged STAT3 activation and enhanced cellular responses to G-CSF, including an increase in cloning frequency, survival and proliferative capacity (Croker et al. 2004). Consistent with the *in vitro* findings, mutant mice injected with G-CSF displayed enhanced neutrophilia, progenitor cell mobilisation, splenomegaly, and neutrophil infiltration into multiple tissues, with unexpectedly marked tissue destruction and inflammation. These data confirmed that SOCS3 is a key negative regulator of G-CSF signaling in myeloid cells, and that this is of particular physiological significance during G-CSF-driven emergency granulopoiesis. SOCS3 may also increase MAPK activation indirectly by inhibition of Ras-GAP, as observed when T cells were stimulated with IL-2 (Cacalano et al. 2001). To date this has not been investigated for the G-CSFR, however, no major perturbations of MAPK phosphorylation were observed in SOCS3-deficient cells stimulated with G-CSF (Croker et al. 2004). SOCS3 appears to function in a closed negative feedback loop to limit the intensity, duration and scope of G-CSF signals. The expression of SOCS3 is induced within 30 min by G-CSF (Hortner et al. 2002) (and many other cytokines) in a STAT3-dependent manner (Lee et al. 2002), and limits signalling by inhibiting STAT3 directly and by targeting the activated receptor for ubiquination and proteasomal degradation.

G-CSF in the clinic

G-CSF and the amelioration of neutropenia

Many years before signalling pathways downstream of the G-CSFR were elucidated, G-CSF was rapidly integrated into clinical medicine as a therapeutic agent (Lieschke and Burgess 1992a,b). Neutropenia is a significant side effect of many cytotoxic chemotherapy regimens used to treat cancers, predisposing patients to serious infections and compromising the delivery of treatments on schedule and at full dosage. G-CSF was initially tested in clinical trials for its ability to prevent or reduce severe neutropenia and its complications. Randomised trials demonstrated that G-CSF accelerates the recovery of neutrophil numbers and reduces the duration of severe neutropenia after chemotherapy (reviewed in Ozer et al. 2000). As a direct consequence of this accelerated neutrophil recovery, G-CSF administration also reduced the incidence of inflammation of the oral mucosa, the duration of fever, the need for antibacterial and antifungal antibiotics and the duration of hospitalisation (also see Clark et al. 2003 for meta-analysis of trials in febrile neutropenia). The benefits are more significant when more intensive chemotherapy is used, and greatest after myeloablative chemo-radiotherapy and autologous bone marrow transplantation. Evidence-based guidelines for the cost-effective use of G-CSF have been published by the American Society of Clinical Oncology (Ozer et al. 2000) and EORTC (Repetto et al. 2003). G-CSF is used routinely to support chemotherapy of moderate intensity in patients with curable malignancies including lymphoma, leukaemia and breast cancer.

G-CSF mobilises stem cells into the blood

While the acceleration of recovery of granulopoiesis was a predicted effect of G-CSF, the mobilisation of large numbers of progenitor and stem cells from the bone marrow into the peripheral blood was entirely unexpected (Duhrsen et al. 1988). It was discovered in the first Phase I studies of G-CSF that the numbers of multiple lineages of progenitor cells (myeloid, erythroid, megakaryocytic) in the blood were elevated dramatically (about 100-fold) 4–7 days after the beginning of G-CSF treatment. These blood stem cells proved easier to collect in large numbers than bone marrow stem cells, and to reconstitute the haemopoietic system significantly faster than bone

marrow when infused following myeloablative chemo-radiotherapy (Sheridan et al. 1992). In particular, platelet recovery was accelerated by 5–10 days. Stem cell mobilisation is augmented further if G-CSF is administered after a myelosuppressive dose of chemotherapy, and for autologous stem cell transplantation this is now the most common method of obtaining a stem cell inoculum.

Patients undergoing allogeneic hemopoietic stem cell transplantation receive cells from normal donors. G-CSF administration to volunteer donors for patients undergoing allogeneic transplantation has been shown to be safe and effective, and G-CSF-mobilised blood stem cells now have replaced bone marrow as the standard source of hemopoietic stem cells for allogeneic bone marrow transplantation. Normal donors injected with G-CSF develop a marked neutrophilia within 4 h of injection, mobilise stem cells after 3–4 days (peak days 4–5), and subclinical spleen enlargement over 4–6 days (Grigg et al. 1995, Stroncek et al. 2003). Between normal individuals, a 3–10-fold variation in magnitude of these responses is observed (Grigg et al. 1995), and splenic rupture is a rare life-threatening side effect of G-CSF mobilisation (Falzetti et al. 1999). Each of these responses occurs in mice injected with G-CSF (Roberts et al. 1997a), and research over the last decade using genetically manipulated mice has elucidated the mechanism of stem cell mobilisation by G-CSF.

In keeping with the early observation that progenitor cells of all lineages (including those not expressing G-CSFRs such as erythroid, megakaryocytic, lymphoid progenitors) are mobilised (Duhrsen et al. 1988, Roberts et al. 1997b), G-CSF-induced mobilisation is a *trans* effect (Liu et al. 2000), dependent on the presence of G-CSFR-bearing neutrophils (Liu et al. 1997, 2000). In response to G-CSF, increased secretion of proteases by the expanding neutrophil mass within the bone marrow cleaves critical adhesion molecules and chemoattractant receptors which normally function to retain progenitor and stem cells within bone marrow microenvironmental niches (Levesque et al. 2001, 2002, 2003, 2004). These factors also serve to mobilise neutrophils (Semerad et al. 2002), and the 2–3 day delay between neutrophil and stem cell mobilisation most likely reflects the requirement for concomitant G-CSF-driven stem and progenitor cell proliferation and expansion. Within the bone marrow, stem and progenitor cells are rapidly cycling after G-CSF stimulation. In contrast, circulating cells are almost always found to be in G0 or early G1 phase of the cell cycle (Roberts and Metcalf 1995), suggesting either that mobilisation only occurs immediately after the completion of mitosis, or clearance mechanisms prevent cycling cells from remaining in the circulation after marrow release.

G-CSF, acute myeloid leukaemia and severe chronic neutropenia

An early concern of investigators was that acute myeloid leukaemia (AML) cells expressing G-CSFRs may survive and proliferate in response to G-CSF treatment after intensive chemotherapy. Randomised trials have shown no such deleterious consequences, and the benefits of G-CSF treatment after anti-leukaemia therapy are similar to those seen for patients with other diseases receiving similarly myelosuppressive chemotherapy (reviewed in Bradstock 2002).

G-CSF may have a direct beneficial effect on some leukaemias with occasional patients achieving remission with G-CSF therapy alone (see for example Nimubona et al. 2002) and for some patients with good prognosis myelodysplasias, although the effects in these circumstances are non-uniform. Very recently, G-CSF-priming of leukaemia patients immediately prior to chemotherapy has been shown to improve outcome (Lowenberg et al. 2003). Although unproved, the hypothesised mechanism of action in this circumstance is postulated to be the increased susceptibility to chemotherapy of AML blast cells recruited into cell cycle by the G-CSF.

The care of patients with severe congenital neutropenia (SCN) has been revolutionised by G-CSF treatment. Regular use of G-CSF increases the neutrophil counts towards normal, allowing previously refractory infections to resolve, and preventing mouth ulceration, fevers and recurrent infections (Dale et al. 2003). Adverse events with chronic G-CSF administration include mild splenomegaly, thrombocytopenia, osteoporosis and transformation into myelodysplastic states and AML in 10–15% of patients. The first three complications are directly attributable to G-CSF effects, whereas it remains a contentious issue as to whether transformation to malignancy is a direct consequence of therapy, particularly as severe chronic neutropenia is well recognised as a pre-leukaemic state. Intriguingly, truncation mutants of the G-CSFR are now recognised as an acquired event that can be associated with subsequent progression to AML. These mutations delete or functionally disrupt the distal three tyrosine residues in the cytoplasmic tail of the receptor, interfering with binding of negative (as well as positive) regulatory elements, and apparently act in a dominant negative fashion (Dong et al. 1997). While the important perturbations of signalling events consequent to this mutation remain incompletely defined, functionally cells bearing these receptors have increased survival, and, in knock-in mouse models, have increased responsiveness to G-CSF (Hermans et al. 1998, McLemore et al. 1998). Together these features may well explain the apparent selective advantage seen for G-CSFR mutant clones in patients

with SCN treated with G-CSF. However, acquisition of these mutations does not invariably lead to AML, and some SCN patients evolving to AML do not harbour these mutations (Ancliff et al. 2003).

G-CSF as treatment for serious infection

Given the observation that serum levels of G-CSF are markedly increased during infection, G-CSF has been tested in non-cancer patients with serious bacterial or fungal infections, in anticipation of accelerated and improved recovery rates. Meta-analyses of trials of G-CSF in adult patients with life-threatening sepsis and/or pneumonia (Cheng et al. 2004b), or neonates in intensive care units (Carr et al. 2003) indicate no benefit in these patient populations, and G-CSF is not indicated in these settings. Tantalising data do exist that in specific circumstances or specific infections, G-CSF may favourably change the course of the disease. The highly fatal tropical infection, meliodosis, is one such example (Cheng et al. 2004a).

G-CSF as an immune regulator

G-CSF and T cells

Having been named for its effects on myeloid cells, G-CSF was not predicted to regulate T cell behaviour. Resting T cells do not express G-CSFRs, and indeed, G-CSF was not observed to influence T cell numbers in early preclinical and clinical studies. However, investigations into the effects of G-CSF upon T cell populations was prompted by the clinical observation that G-CSF-mobilised blood stem cell allogeneic transplants did not elicit an increased incidence of T cell-driven acute graft-versus-host-disease (GVHD) when compared with standard bone marrow transplants, despite a 10-fold increase in numbers of T cells infused. Investigations revealed that exposure to G-CSF *in vivo* induced changes in T cell function, including polarisation towards a T-2 phenotype (secreting IL-4 and IL-10) rather than interferon-γ secreting T-1 phenotype, and reduced proliferation in response to allo-antigens (Pan et al. 1995, Mielcarek et al. 1997). While some activated T cells are now known to express G-CSFRs (Franzke et al. 2003), and when exposed to G-CSF *in vitro* demonstrate altered expression of GATA3 mRNA and cytokine production, a significant component of this effect is indirect. IL-10 in the serum of G-CSF treated donors reduces T cell responses to antigens, as does the presence of monocytes and other myeloid cells within the mononuclear cell fraction (Mielcarek et al. 1998, Rutella et al. 1998). Emerging evidence suggests that G-CSF induces the production of regulatory dendritic cells (Arpinati et al. 2000, Rutella et al. 2004) and also DC-like myeloid cells (G Hill, Queensland Institute of Medical Research, personal communication) which in turn influence T cell development and function (Morris et al. 2004). The net effect of donor treatment with G-CSF on the stem cell inoculum is to reduce the incidence of acute GVHD by diminishing T cell alloreactivity and inflammatory cytokine production (interferon-γ and TNF-α) in the recipient in the early engraftment period. This effect is relatively short-lived, and chronic GVHD is eventually increased in recipients of mobilised stem cell transplants. Further, the effect cannot be recapitulated by administration of G-CSF to the recipient following transplantation. In this scenario, toxicity from GVHD appears to be increased (Ringden et al. 2004).

G-CSF and inflammation

Not surprisingly, given the accumulating evidence described above that exposure to G-CSF *in vivo* influences dendritic cell and T cell function, G-CSF has been found to modulate inflammation in other settings. In mice, pretreatment with G-CSF reduces Th1 cytokine production (Shaklee et al. 2004), protecting mice from lethal septic shock induced by bacterial superantigens (Aoki et al. 1995), and hapten-induced colitis (Egi et al. 2003). However, G-CSF is also a key driver of disease in some murine models, such as inflammatory arthritis, where anti-G-CSF antibody therapy at the onset of arthritis is highly effective (Lawlor et al. 2004). Similarly, in humans, G-CSF can modulate inflammatory processes to either the benefit or detriment of patients, depending on the context. For example, when given peri-operatively, G-CSF was found to reduce monocyte and lymphocyte activation post-operatively, with possible positive effects on morbidity (Schneider et al. 2004), and in preliminary studies G-CSF may be beneficial in some patients with inflammatory bowel disease (Dejaco et al. 2003). In contrast, G-CSF is recognised to exacerbate acute lung injury related to chemotherapy (White and Cebon 1995, Niitsu et al. 1997), and G-CSF can exacerbate inflammation in patients with rheumatoid arthritis (Snowden et al. 1998).

G-CSF: The future

As can be discerned by the discussion above, G-CSF is now recognised as a bone-fide polyfunctional cytokine. While its predominant physiological influence is on neutrophil production and function, it exerts subtle effects on stem cell, T cell and dendritic cell function, particularly *in vivo*. Its availability as a pharmacological agent has changed the face of curative cancer chemotherapy, and ushered in an era of blood stem cell transplantation. Its use clinically is becoming more selective, and, as experimental data resolve just how G-CSF modulates inflammation *in vivo*, will almost certainly become more sophisticated.

Future challenges also include elucidation of the complete signalling circuitry between G-CSFR, gene expression and cellular responses; definition of the physiological role G-CSF plays in endothelial cell biology (Bussolino et al. 1989); and clinically, how to best tailor G-CSF therapy for patients with severe chronic neutropenia, myelodysplasia and AML.

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