2 Animal Models of Hematopoietic Growth Factor Perturbations in Physiology and Pathology

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1. INTRODUCTION

The clinical use of hematopoietic growth factors (HGFs) is built on nearly 20 years of in vitro studies followed by preclinical animal studies. These laboratory and animal studies, undertaken before first use in humans, provided the basis for expectations of what the biologic effects in humans would be.

Reflecting the available technologies, the initial animal studies primarily evaluated the in vivo effects of factor excess after administration of factors to various animal species and included transgenic models, particularly when the supply of factor itself was limiting or issues of chronic factor exposure were to be addressed. With the development of genetic technologies to disrupt genes in mice selectively, animal models of

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factor deficiency were developed in the 1990s. These models were particularly useful for defining the indispensable and physiologic roles of factors and their multicomponent receptors. Increasing sophistication of the technologies for transgenesis and targeted gene modification enabled generation of animal models with inducible and tissue-specific genetic modifications that included not only gene disruptions but also truncations, point mutations, and gene replacement. Animal models incorporating these latter changes were usually generated to test hypotheses regarding the role of specific lesions in gene function or disease pathogenesis. This range of approaches collectively contributes to the preclinical evaluation of new biologic agents or to the modeling of particular disease processes so that pathogenic mechanisms can be better understood and therapeutic strategies can be assessed.

This chapter presents a descriptive overview of animal models of perturbed amounts of HGF, with a particular emphasis on genetic models, and focuses on those factors in clinical use: erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin (IL)-11. Since diseases are often acquired and not infrequently present somatic rather than germline genetic lesions, animal models with acquired rather than congenital perturbations of HGF concentrations and signaling are also described.

2. ANIMAL MODELS OF HEMATOPOIETIC GROWTH FACTOR DEFICIENCY

Early models of induced factor deficiency relied on immunologic mechanisms to neutralize factor activity. The ability to disrupt individual genes selectively by gene targeting provided a powerful method of generating mice with deficiencies of either selected ligands, receptors, or downstream-signaling molecules. Such engineered deficiencies have usually been designed to be absolute and are life-long, providing insight into the cumulative effects of nonredundant roles of the absent gene product. This genetic approach has been pre-eminent in defining the essential physiologic role of various factors. However, animal models with less-than-total factor deficiency have been generated using other methodologies, both before the gene targeting era and more recently. These models offer several advantages: although they may not result in absolute factor deficiency, they offer flexibilities including inducibility, reversibility, and nonlethality. A new approach, not yet applied to studying HGF, is the use of RNA interference *(1)*. Various experimental approaches to factor ablation are listed in Table 1 with some comparative relative advantages and disadvantages.

2.1. Spontaneously Arising Mutants With Hematopoietic Factor Deficiency

The first durable models of HGF deficiency resulted fortuitously from spontaneously arising or induced mutations in the genes encoding growth factors or their receptors. The two examples of this are mutants deficient in stem cell factor (SCF) and colony-stimulating factor-1 (CSF-1; also known as macrophage colony-stimulating factor [M-CSF]). These models presented prototypes for the models of other factor deficiencies generated by gene targeting.

The *steel (Sl)* mutation arose in 1956 *(2)*. In its most severe form, animals homozygous for the original *Sl* allele die before birth with macrocytic anemia, absent germ cell

Table 1
Comparison of Various Approaches to Impair Hematopoietic Growth Factor Action or Reduce Factor Production

Method of factor deficiency or impairment	Durability *Degree of impairment*	*Applicable for use*		*Technical difficulty and issues*	*Comments*
		In vitro	*In vivo*		
Neutralization by antibody	Transient *Incomplete*	Yes	Yes	If antibody available, straightforward	Standard approach to demonstrate specificity of factor effects in vitro; systemic administration may not achieve neutralization in all body compartments and local sites
Antisense RNA from transfected construct	Transient *Incomplete*	Yes	No	Oligonucleotide stability and potential toxicity	Specificity must be demonstrated
Antisense RNA from stable transgene	Permanent *Incomplete*	Yes	Yes	Similar to other transgenic projects	Expression of transgene may be variable in different tissues leading to variable degrees of factor impairment
Administration of antagonist	Transient *Incomplete*	Yes	Yes	Specific antagonist must be developed and validated	Antagonism at level of receptor most appropriate to study factor physiology
Induced innate autoimmunity to factor	Transient in long term *Incomplete*	No	Yes	Requires immunogenic form of factor	No control over induced immune response, which may be nonspecific or nonneutralizing
Natural or randomly-induced mutation	Permanent *Complete or incomplete*	No	Yes	Capricious and unreliable	Structure of disrupted allele must be characterized; may involve several adjacent or separate loci.
Targeted gene disruption	Permanent *Complete*	Yes	Yes	Difficult multistage process requiring several mouse generations	Total factor deficiency must still be formally proven at the protein level
Targeted gene modification or inducible disruption	Under experimental control *Incomplete*	Yes	Yes	Difficult multistage process requiring several mouse generations	More flexible than germline gene disruption—can be controlled in both time and anatomical location
RNA interference	Depends on methodology *Incomplete*	Yes	Yes	Techniques still under development in mammalian systems	Not yet applied to growth factor models

development, and defective skin pigment cell development *(2,3).* Heterozygous *Sl*/+ animals have diluted hair pigment and mild macrocytic anemia and are fertile. Other alleles were noted that resulted in less severe phenotypes in homozygous animals, e.g., Sl^d (steel-Dickie), for which homozygotes are viable but have severe anemia, sterility, and a black-eyed/white-coated phenotype. A full list of characterized *Sl* alleles is found in Peters et al. *(4),* and an overview of the major phenotypic subtleties is described in Russell *(5).* When in 1990 the ligand for the cellular proto-oncogene c-*kit* was cloned by several groups *(6–8),* it was shown to be the product of the *steel* locus on mouse chromosome 10 *(7,8).* The *steel* gene product was a previously unknown growth factor that, among other functions, acts as a hematopoietic CSF in vitro *(8,9)* and was designated variously as kit-ligand, steel factor, mast cell growth factor, or stem cell factor.

Table 2
Animal Models of Reduced Eythropoietin Levels or Signaling

Animal	*Method of reduced erythropoietin signaling*	*Major phenotypic consequences*	*Reference*
Rabbit	Passive immunization with serum containing presumed anti-EPO antibodies	Anemia	*26, 27*
Mice	Passive immunization with serum containing presumed anti-EPO antibodies	Anemia	*25*
Monkey	Immunization during GM-CSF EPO hybrid protein administration resulting in anti-EPO antibodies crossreacting with simian EPO	Anemia	*28*
Mice	Targeted disruption of EPOR gene	Death *in utero* at E13.5 Ventricular hypoplasia Vascular abnormalities	*29, 30*
Mice	Targeted disruption of EPOR receptor gene	Death *in utero* at E13.5 Ventricular hypoplasia Vascular abnormalities Haploinsufficiency	*29, 30*

ABBREVIATIONS: E, embryonic day; EPO, erythropoietin; EPOR, EPO receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor.

Mice with spontaneously arising mutations at the dominant spotting *W* locus have long been known *(10,11);* this locus was only relatively recently molecularly characterized as being the SCF receptor c-*kit (12).*

The *osteopetrosis (op)* mutant arose in 1970 and was characterized in 1976 *(13).* The mutation was characterized as a base insertion generating a premature stop codon in the *Csfm* (M-CSF) gene on mouse chromosome 3 *(14). op/op* mice have severe osteopetrosis with disordered bone remodeling and osteoclast deficiency *(13,15),* marked but not absolute monocyte and tissue macrophage deficiency *(16–21),* impaired female fertility *(22),* a lactation defect *(23),* and reduced survival *(13).* Mice lacking the CSF-1 receptor were generated by gene targeting that largely replicate the ligand-deficiency phenotype *(24).*

A challenge in interpreting the phenotype of naturally occurring mutations is to know whether the factor deficiency is absolute or partial. This question can be addressed by combining knowledge of necessary functional domains, gene expression analysis, and determination of amounts of bioactive and immunoreactive protein. Comparison of mice lacking ligand with those lacking the corresponding receptor can be helpful. Some spontaneous mutations involve deletions, which may potentially encompass several genes, thus potentially confounding the phenotype.

2.2. Erythropoietin

Early studies used serum from rabbits immunized with concentrated EPO-containing urine to achieve neutralization of endogenous EPO in recipient rabbits *(25–27).* Passively immunized rabbits and mice developed anemia. In a more recent study involving active rather than passive immunization, monkeys treated with a human (Hu)GM-CSF-EPO fusion moiety developed anti-EPO (but not anti-GM-CSF) antibodies (Ab), with resultant anemia *(28)* (Table 2).

Mice with targeted disruption of the *EPO* gene or EPO receptor (EPOR) gene develop similar phenotypes. $EPO^{-/-}$ and $EPOR^{-/-}$ embryos die *in utero* at d 13.5 with failure of fetal liver erythropoiesis *(29)* and with cardiac defects including ventricular hypoplasia and epicardial and vascular abnormalities *(30).* Although the $EPO^{-/-}$ and $EPOR^{-/-}$ mice had erythropoietic failure, fetal liver erythroid blast-forming units (BFU-E) and erythroid-colony-forming units (CFU-E) progenitor cells were isolated and capable of terminal differentiation in vitro, implicating EPO in the terminal proliferation and survival of erythroid lineage cells *(29).* Comprehensive analysis of $EPOR^{+/-}$ mice showed evidence of haploinsufficiency, with lower hematocrits and reduced CFU-E frequencies in both bone marrow and spleen *(31).*

A human EPO mutant in which Arg103 is replaced by Ala [Epo(R103A)] acts as a competitive inhibitor of EPO in vitro in human EPO signaling systems; its effects in vivo and in murine systems have not been reported, although an intent to study the molecule in animal models was foreshadowed *(32).*

2.3. Granulocyte Colony-Stimulating Factor

Neutralizing polyclonal *(33)* and monoclonal *(34)* antibodies (MAbs) to HuG-CSF have been available; they formed the basis for determination of immunoreactive HuG-CSF levels and for showing specificity in HuG-CSF bioassays *(35).* A polyclonal neutralizing antiserum to murine (Mu)G-CSF has been used for G-CSF neutralization in vitro *(36).* Despite the availability of these reagents, no attempts to neutralize endogenous MuG-CSF in vivo were reported. One experiment in rats involved passive immunization with a rabbit anti-G-CSF Ab 2 h before pulmonary challenge with *Pseudomonas aeruginosa (37).* Anti-G-CSF Ab pretreatment reduced pulmonary neutrophil recruitment and intrapulmonary bactericidal activity at 4 h after infection without affecting the number of circulating neutrophils, suggesting that a local pulmonary G-CSF response to the infection had been impaired.

The hematologic consequences of neutralization of endogenous G-CSF were first observed in dogs, resulting from Ab induced to HuG-CSF crossreacting against canine G-CSF *(38)* (Table 3). Dogs administered HuG-CSF developed an initial neutrophilia, but with ongoing HuG-CSF administration, neutropenia supervened. On cessation of HuG-CSF administration, neutrophil counts slowly returned to normal, but after a nontreatment interval, neutropenia rapidly recurred upon retreatment with HuG-CSF. Anti-HuG-CSF Abs in serum were seen, and passive immunization of dogs by plasma infusion was achieved.

Induction of autoimmunity to murine MuG-CSF required the use of immunostimulatory MuG-CSF conjugates *(39).* Immunized mice developed neutropenia coincident with an IgG autoantibody response, without effect on other peripheral blood parameters or on the number of marrow progenitor cells. The neutropenia was sustained for >9 mo. Hematologically, these mice phenocopied mice with absolute G-CSF deficiency owing to disruption of either the G-CSF ligand *(40)* or receptor *(41)* genes.

Mice with absolute G-CSF deficiency induced by targeted disruption of either the G-CSF or G-CSF receptor (G-CSFR) gene have similar hematologic phenotypes *(40,41).* $G\text{-}CSF^{-/-}$ mice display chronic neutropenia, reduced marrow granulopoiesis, and impaired G-CSF-provoked neutrophil mobilization *(40).* Kinetic analysis of granulopoiesis revealed a reduced transit time through the mitotic compartment of $G\text{-}CSF^{-/-}$ mice, a normal transit time through the postmitotic compart-

Table 3
Animal Models of Reduced G-CSF Levels or Signaling

Animal	*Method of reduced G-CSF signaling*	*Major phenotypic consequences*	*Reference*
Dog	Immunization during HuG-CSF administration resulting in anti-HuG-CSF antibodies crossreacting with canine G-CSF	Transient neutropenia Rapid neutropenia on rechallenge	*38*
Rat	Passive immunization with anti-MuCSF antibodies	↓ Local response to pulmonary bacterial infection	*37*
Mouse	Active immunization with MuG-CSF-conjugates resulting in anti-MuG-CSF autoantibodies	Prolonged neutropenia	*39*
Mouse	Targeted disruption of G-CSF gene	Chronic neutropenia ↓ marrow granulopoiesis Pathogen susceptibility ↑ neutrophil apoptosis Haploinsufficiency	*40*
Mouse	Targeted disruption of G-CSF receptor gene	Chronic neutropenia ↓ marrow granulopoiesis ↓ progenitor cell and neutrophil mobilization ↓ neutrophil chemotaxis Haploinsufficiency	*41*

ABBREVIATIONS: G-CSF, granulocyte colony-stimulating factor; Hu, human; ↑, increased; ↓, decreased.

ment, and an increase in the proportion of Gr-1+ cells that have initiated apoptosis as detected by mercocyanine 540 staining *(42)*. G-CSF deficiency results in increased susceptibility to pathogens including *Listeria monocytogenes* and *Candida albicans (43)*. Surprisingly, despite the unexpected impairment of monocyte/macrophage responses in G-CSF$^{-/-}$ mice during *Listeria* infections *(40,44,45), Mycobacterium avium* infections were not exacerbated in G-CSF$^{-/-}$ mice, and high levels of interferon (IFN)-γ production accompanied infection with this pathogen *(46)*. *Candida* infection of G-CSF$^{-/-}$ mice was accompanied by a vigorous neutrophilia, exceeding the magnitude of that in wild-type mice, and early control of the pathogen load. However, after 1 wk of infection, deep tissue infection with high *Candida* pathogen loads persisted in G-CSF$^{-/-}$ mice at a time the infection was resolving in wild-type mice *(43)*.

The hematologic profile of G-CSFR$^{-/-}$ mice largely resembled that of the ligand-deficient mice, with chronic neutropenia, reduced marrow granulopoiesis, and a propensity of Gr-1+ marrow cells to undergo apoptotic death in vitro *(41)*. The G-CSFR$^{-/-}$ mice have enabled distinctions to be drawn between G-CSF-dependent and G-CSF-independent neutrophil functions. Neutrophil primary granule myeloperoxidase activity was normal, and neutrophil migration induced by chemical peritonitis was preserved. However, progenitor cell and neutrophil mobilization into the peripheral blood by cyclophosphamide and IL-8 was impaired *(47)*. Neutrophils from G-CSFR$^{-/-}$ mice had defective chemotactic responses to IL-8 and other chemoattractants in vitro, despite

intact metabolic responses to several agents *(48).* The intrinsic defect in G-CSFR$^{-/-}$ cells has enabled experiments to be designed to distinguish between cell-autonomous and -nonautonomous functions. For example, radiation chimeras were established with either wild-type or G-CSFR$^{-/-}$ hematopoietic cell populations in wild-type or G-CSFR$^{-/-}$ stromal backgrounds to study the phenomenon of G-CSF-stimulated progenitor cell mobilization. Expression of the G-CSFR on the hematopoietic cells (and then only a subpopulation of them) and not the stromal cells was necessary for G-CSF-stimulated mobilization to occur *(49),* although interpretation of this experiment assumes that little reconstitution of the marrow stroma by the transplanted marrow cells occurred.

To define signals mediated specifically by the G-CSFR, gene-targeted mice have been generated in which the G-CSFR was replaced by a chimeric receptor comprising the extracellular and transmembrane portions of the G-CSFR (capable of binding G-CSF) connected to the intracellular portion of the EPOR *(50).* Hematologically, these mice resemble G-CSFR$^{-/-}$ mice with peripheral blood neutropenia and a modest marrow granulopoietic defect. Although this chimeric receptor supported granulocytic lineage commitment and differentiation, some specific defects were demonstrable: there was impaired G-CSF-stimulated progenitor cell mobilization and reduced IL-8-induced chemotaxis *(50,51).*

2.4. Granulocyte-Macrophage Colony-Stimulating Factor

Neutralizing polyclonal antibodies to MuGM-CSF have been characterized *(52),* and well-characterized monoclonal anti-MuGM-CSF Abs *(53,54)* are now commercially available. Such Abs form the basis of enzyme-linked immunosorbent assays (ELISAs) for determination of immunoreactive MuGM-CSF levels and have been used to show specificity in MuGM-CSF bioassays. Although no studies attempting to neutralize basal levels of endogenously produced MuGM-CSF by passive immunization in vivo have been reported, Abs have been used to neutralize GM-CSF activity in disease models. The effect of GM-CSF pretreatment to aggravate lipopolysaccharide (LPS)-induced mortality and hepatic toxicity could be ameliorated by the administration of GM-CSF Abs *(55).* Administration of an anti-GM-CSF Ab attenuated the severity of arthritis in two murine arthritis models, one in which erosive arthritis is induced by bovine serum albumin (BSA) and IL-1 administration *(56),* and in one model of collagen-induced arthritis *(57).*

A competitive antagonist of HuGM-CSF has been developed named E21R, which is a ligand analog in which amino acid 21 is changed from glutamic acid to arginine *(58).* Owing to the high species specificity of GM-CSF, preclinical in vivo studies with the moiety were performed in baboons, administering E21R for up to 21 d *(59)* (Table 4). E21R resulted in a transient eosinophilia and neutrophilia and granulocyte infiltrates in lymph nodes and duodenal submucosa. The transient eosinophilia was unexpected but was also seen in patients receiving E21R on a phase 1 study *(59),* and so is an effect of this agent accurately predicted by the animal model.

Two mouse lines with absolute GM-CSF deficiency owing to targeted gene disruption have been independently generated *(60,61);* both lines show identical phenotypes. Baseline hematopoiesis is unperturbed despite GM-CSF deficiency *(61),* although reduced frequencies of marrow CFU-E sensitive to low EPO concentrations in vitro have recently been documented *(31).* During *M. avium* infection, GM-CSF$^{-/-}$ mice fail

Table 4
Animal Models of Reduced GM-CSF Levels or Signaling

Animal	*Method of reduced GM-CSF signaling*	*Major phenotypic consequences*	*Reference*
Mouse	Passive immunization with anti-MuGM-CSF antibodies	↓ LPS-induced mortality ↓ LPS hepatic toxicity	*55*
Mouse	Targeted disruption of GM-CSF gene	Normal basal hematopoiesis Pulmonary alveolar proteinosis ↓ hematopoiesis during chronic *M. avium* infection ↓ zymocel-induced hepatic granulomatous inflammation	*60–62, 68*
Mouse	Targeted disruption of IL-3/GM-CSF/IL-5 receptor β_c subunit	Normal basal hematopoiesis except ↓ eosinophil production Pulmonary alveolar proteinosis Failure to develop eosinophila to parasitic infections	*68, 69*
Baboon	Competitive peptide antagonist (E21R)	Transient eosinophilia and neutrophilia	*59*

ABBREVIATIONS: GM-CSF, granulocyte-macrophage colony-stimulating factor; LPS, lipopolysaccharide; IL, interleukin; Mu, murine; ↑, increased; ↓, decreased.

to sustain hematopoietic cell production *(62),* suggesting a role for GM-CSF under emergency if not basal conditions of hematopoiesis. GM-CSF$^{-/-}$ mice have been exploited to examine the role of this factor in several models of inflammation; different effects have been seen in different models. Acute peritoneal inflammation after casein injection was normal in GM-CSF$^{-/-}$ mice *(63).* GM-CSF deficiency delayed zymocel-induced hepatic granuloma formation and impaired monocyte infiltration and proliferation, although macrophages within granulomata expressed markers suggesting normal activation *(64).* Normal activation of peritoneal macrophages was observed during *L. monocytogenes* infection *(45).* GM-CSF deficiency attenuated inflammation in a murine model of arthritis induced by BSA and IL-1 injection *(56)* and also in murine models of immune-mediated glomerulonephritis *(65).* GM-CSF$^{-/-}$ mice have moderately impaired reproductive capacity and reduced long-term survival *(66).*

GM-CSF$^{-/-}$ mice develop a striking pulmonary pathology with extensive peribronchial B-cell infiltrates and alveolar accumulation of surfactant phospholipid, protein, and intra-alveolar macrophages, a disorder resembling pulmonary alveolar proteinosis *(60,61).* The pathophysiology relates to impaired surfactant clearance and catabolism *(216)* and can be reversed by local GM-CSF expression *(67),* evidence collectively indicating a local defect in alveolar macrophages.

GM-CSF signaling is initiated by ligand binding to a heterodimeric receptor comprising a specific α-subunit (GM-CSFRα) and a β-subunit (IL-3/GM-CSF/IL-5Rβ_c) shared in common with the analogously heterodimeric IL-3 and IL-5 receptors. (In mice, but not in humans, there are two rather than one IL-3 receptor β-subunits.) GM-CSF deficiency has been mimicked by targeted disruption of the IL-3/GM-CSF/IL-5Rβ_c gene *(68,69),* and these mice develop a similar, but less severe, pulmonary pathology *(70).*

Additionally, they showed additional manifestations of defective IL-5 signaling such as low baseline eosinophil numbers *(68)* and impaired eosinophil response to *Nippostrongylus brasiliensis (68,69,71).* In this cell-autonomous model of the pulmonary disease, bone marrow transplantation with wild-type hematopoietic cells reversed the pulmonary pathology *(72),* albeit not completely *(73).* IL-3/GM-CSF/IL-5Rβ_c-deficient mice displayed an attenuated cutaneous reaction to *Leishmania major (74).*

2.5. Interleukin-11

Despite its potent action on hematopoietic progenitor cell development in vitro, mice with a targeted disruption of the IL-11 receptor-α (IL-11Rα) had normal baseline hematopoiesis, immune function, and erythroid reserves *(75)* but displayed a defect in postimplantation decidualization that impaired the fertility of female mice *(76).* Mice deficient in gp130, the partner of IL-11-Rα in the heterodimeric IL-11 receptor, display additional defects that reflect defective signaling from other ligands that share gp130 as a component of their heterodimeric receptors: IL-6, leukemia inhibitory factor (LIF), ciliary neurotropic growth factor (CNTF), oncostatin M, and cardiotropin (CT). Absolute gp130 deficiency results in embryonic lethality from multiple defects including impaired fetal liver hematopoiesis *(71).* When embryonic lethality was circumvented by a genetically based inducible *Cre*-lox gene targeting approach, adult gp130-deficient mice developed multisystem defects including thrombocytopenia, leukocytosis, and impaired hematopoietic recovery after 5-fluorouracil (5-FU) stem-cell ablation or after antiplatelet antiserum *(77).*

IL-11 has been neutralized in mice by passive immunization using a sheep anti-MuIL-11 Ab in a study investigating the role of IL-11 in bone changes after oophorectomy *(78).*

2.6. Other Hematopoietic Growth Factors

Over the last decade, murine models of HGF deficiency have been generated for most factors, and in many cases, for their receptors (Tables 5 and 6).

2.7. Combined Hematopoietic Growth Factor Signaling Deficiencies

By combining genetically based factor-deficiencies, the interacting roles of growth factors can be studied in vivo. Sometimes interactions have been achieved by combining ligand-deficiency for one factor and receptor deficiency for another, often for reasons of utility and availability. Occasionally, genetic constraints due to the proximity of loci influence the approach. Some combinations merely result in the simple addition of the phenotypic traits of the two individual factor deficiencies, suggesting independent roles for the two factors. Others result in the emergence of new phenotypic features, or the accentuation of component phenotype traits, suggesting that one factor can assume a compensatory role in the deficiency phenotype of another factor, although compensation requires that activation of a process over the usual normal amount be shown as well. The emergence of new phenotypic traits in combination with deficiency genotypes allows for the possibility that independent, separately regulated mechanisms may contribute to a particular process, and the integrity of the process requires one or the other mechanism to be intact, but only when both mechanisms are impaired does the process fail.

Table 5
Genetic Models of Deficiency of CSFs and Other Factors Affecting Hematopoiesis in Mice

Factor	*Genetic basis (allele)*	*Major phenotypic features*[a]	*Reference*
G-CSF	Targeted gene disruption	–/– Chronic neutropenia ↓ Progenitor cells Infection vulnerability	*40*
GM-CSF	Targeted gene disruption	–/– Unperturbed hematopoiesis Alveolar proteinosis Lung infections	*60, 61*
M-CSF	Natural point mutation *(op)*	–/– Osteopetrosis ↓ Monocyte/macrophages ↓ Osteoclasts	*13, 20*
SCF	Natural mutation *(Sl)*[b]	–/– Lethal *in utero* Impaired hematopoiesis +/– Pale coat Mild macrocytic anemia Small gonads	*3, 5* *3*
LIF	Targeted gene disruption	–/– Maternal infertility ↓ Splenic CFC and CFU-S Normal peripheral blood	*172* *173*
IL-1β	Targeted gene disruption	–/– Fever-resistant ↓ Acute-phase response Hematopoiesis not analyzed	*174*
IL-2	Targeted gene disruption	–/– Perturbed B-cell function Ulcerative colitis	*175* *176*
IL-3	Transgenesis (antisense RNA, partial IL-3 deficiency only)	+/– Lymphoproliferative disorder Neurologic dysfunction	*177*
IL-3	Targeted gene disruption	–/– ↓ Delayed-type hypersensitivity ↓ Tissue mast cells in nematode infection	*81, 82*

IL-4	Targeted gene disruption	–/– ↓ Th2 responses	*178*
		↓ Reactive eosinophilia	*178*
		↓ IgG1 switching	*179*
		↓ Mucosal immunity	*180*
IL-5	Targeted gene disruption	–/– Normal Th-dependent B-cell responses	*181*
		Normal immunoglobulins	
		Normal eosinophils ↑ in parasitic infection; normal parasite killing	
IL-6	Targeted gene disruption	–/–↓ Acute-phase and anti-infective response	*182, 183*
		↓ Mucosal immunity	*184*
		↓ Pre-CFU-S, CFU-S and lineage-committed CFCs	*185*
		↑ Bone turnover	*186*
IL-7	Targeted gene disruption	–/– ↓ B lymphopoiesis	*187*
		↓ Thymic cellularity	
		↓ Splenic lymphocytes	
EPO	Targeted gene disruption	–/– Lethal at E 13	*29*
		Hepatic erythropoiesis fails	
TPO	Targeted gene disruption	–/– ↓ Platelets (>80%)	*188, 189*
		↓ Marrow megakaryocytes and megakaryocyte-CFCs	
		↓ Megakaryocyte ploidy	
		+/– ↓ Platelets (67%)	*188, 189*

ABBREVIATIONS: CFC, colony-forming cell; CFU, colony-forming unit; EPO, erythropoietin; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; LIF, leukemia inhibitory factor; M-CSF, macrophage colony-stimulating factor; TPO, thrombopoietin; ↑, increased; ↓, decreased.

[a] Factor-deficient genotype indicated as heterozygous (+/–) or homozygous (–/–).

[b] Numerous different alleles exist. *Sl* refers to that originally described *(2,3)*. Other alleles are indicated by superscripts, e.g., Sl^d (Steel-Dickie): Sl^d/Sl^d and Sl/Sl^d mice are viable but severely anemic and sterile with black eyes and white coats *(190)*.

Table 6
Genetic Models of Chronic Deficiency of Components of Receptors for Factors Affecting Hematopoiesis in Mice

Ligands	*Receptor component*	*Genetic basis (allele)*	*Major phenotypic features*[a]	*Comments and comparison With ligand absence or impairment*	*Reference*
SCF	c-*kit*	Spontaneous and mutagen-induced mutation *(W)*[b]	–/– Perinatal lethality Severity macrocytic anemia Sterility Absent coat pigmentation +/– Normal hematopoiesis Fertile White spotting	Similar	*5,10,12*
IL-3	β_{IL3} (AIC2A)	Targeted gene disruption	–/– Normal	IL-3 signaling via αIL_3 βc receptor complex still possible; Basal hematopoiesis normal in IL-3$^{-/-}$ mice *(82)*	*68*
IL-3, IL-5 GM-CSF	βc (AIC2B)	Targeted gene disruption	–/– Lung alveolar proteinosis Normal CFC levels (assayed with SCF/IL-6/EPO) ↓ eosinophils ↓ reactive eosinophila	Resembles GM-CSF$^{-/-}$ mice *(60,61)* IL-5$^{-/-}$ mice have normal basal eosinophil numbers but ↓ reactive eosinophila *(181)*	*68,69*
IL-3	α_{IL3}	Spontaneous mutation	–/– IL-3 hyporesponsivness	Still have low numbers of high-affinity receptors on marrow cells; *probably not a null allele*	*191–194*
TPO	c-*mpl*	Targeted gene disruption	–/– Thrombocytopenia	Resembles TPO$^{-/-}$ mice *(195)*	*196, 197*
IL-2, IL-4, IL-7, IL-9, IL-15	γ, (γ_c)	Targeted gene disruption (X-linked gene)	–/– Males lacking γ_c: Perturbed T lymphopoiesis Perturbed B lymphopoiesis	Reflects IL-2$^{-/-}$ mice *(176)* Reflects IL-7-receptor$^{-/-}$ mice *(198)*	*203*

			Absent dendritic epidermal T cells Typhlitis and colitis	Not ulcerative as in IL-2$^{-/-}$ mice *(176)* or T-cell receptor α–/–, β–/– and β–/–, δ–/– mice *(199–202)*	
IL-2	β	Targeted gene disruption	–/– ↓ survival, activated CD4+ cells B-cell activation with IgG1 & IgE Perturbed T- and B-cell responses Hemolytic anemia Myeloproliferative disorder ↑ Splenic granulopoiesis	No colitis as in IL-2$^{-/-}$ mice *(176)*	*204*
IL-8	mIL-8Rh	Targeted gene disruption	–/– Splenomegaly B-cell hyperplasia ↑ Neutrophils and granulopoeisis ↓ Neutrophil migration	IL-8$^{-/-}$ mice not yet described	*205–207*
EPO	EPO-R	Targeted gene disruption	–/– Lethal at embryonic d 13 Failure of liver erythropoiesis CFU-E, BFU-E develop, but fail to survive	Resembles EPO$^{-/-}$ mice *(29)*	*29*
CSF-1 (M-CSF)	c-*fms*	Targeted gene disruption	–/– Osteopetrosis ↓ monocytes and tissue macrophages reproductive defects ↑ serum CSF-1 20-fold	Resembles *op/op* mice *(14)*	*24*

ABBREVIATIONS: SCF, stem cell factor; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; TPO, thrombopoietin; CFU-E, colony-forming unit-erythroid; BFU-E, erythroid burst-forming unit; EPO, erythropoietin; CSF, colony-stimulating factor; ↑, increased; ↓, decreased.

[a] Factor-deficient genotype indicated as heterozygous (+/–) or homozygous (–/–).

[b] At least 27 alleles exist. *W* refers to that originally described *(10)* and molecularly characterized by Nocka et al. *(12)*. Other alleles are indicated by superscripts, e.g., W^v, a qualitatively different allele that occurred in C57BL/J6 results in homozygous mice that are viable but severely anemic, sterile, black-eyed, and white coated.

2.7.1. Combined Deficiencies Involving Erythropoietin

EPO-R$^{-/-}$ mice were interbred with GM-CSF$^{-/-}$ and IL-3$^{-/-}$ mice *(31)*. A reduced frequency of marrow CFU-E was observed in EPO-R$^{+/-}$ haploinsufficient GM-CSF$^{-/-}$ or IL-3$^{-/-}$ mice, although CFU-E frequencies were reduced in mice with isolated GM-CSF or IL-3 deficiency. This finding was of functional significance in the mice with combined factor signaling deficiencies, since GM-CSF$^{-/-}$EPO-R$^{+/-}$ and IL-3$^{-/-}$EPO-R$^{+/-}$ mice were more anemic after exposure to phenylhydrazine than mice of the single-component genotypes.

2.7.2. Combined Deficiencies Involving Granulocyte Colony-Stimulating Factor

G-CSF-deficient mice were interbred with GM-CSF-deficient mice to create mice deficient in both factors *(66)*. G-CSF$^{-/-}$GM-CSF$^{-/-}$ mice were more neutropenic than G-CSF$^{-/-}$ mice in the early neonatal period, had higher neonatal mortality, and showed a propensity to the development of the amyloidosis evident in G-CSF$^{-/-}$ mice. Mice deficient in G-CSF and IL-6 signaling have been generated, both by creating mice deficient in both ligands *(43)* and by creating G-CSFR$^{-/-}$IL-6$^{-/-}$ mice *(79)*. G-CSFR$^{-/-}$IL-6$^{-/-}$ mice had an exacerbated neutropenia compared with G-CSFR$^{-/-}$ mice *(79)*. Although infection of G-CSF$^{-/-}$ mice with *C. albicans* resulted in a neutrophilia with increased amounts of serum IL-6, indicating that factors other than G-CSF can drive the emergency granulopoietic response, G-CSF$^{-/-}$IL-6$^{-/-}$ mice also showed this phenomenon, indicating that IL-6 was not the sole driver of this infection-related granulopoietic response *(43)*. Thrombopoietin (TPO)-deficient mice and G-CSFR$^{-/-}$ mice have been interbred, testing the role of either factor in modulating the other-factor deficiency-phenotype. G-CSF deficiency did not further exacerbate the thrombocytopenia of TPO$^{-/-}$ mice, but TPO deficiency augmented the granulopoietic defect of G-CSFR$^{-/-}$ mice, with a consequent increased early infective mortality *(80)*.

2.7.3. Combined Deficiencies Involving Granulocyte-Macrophage Colony-Stimulating Factor

Since mice have a second IL-3 receptor (βIL-3), IL-3/GM-CSF/IL-5Rβ_c-deficient mice are not absolutely deficient in IL-3 signaling. IL-3-deficient mice have been generated by gene targeting *(81,82),* but the close chromosomal location of GM-CSF and IL-3 precluded bringing these mutations together efficiently by interbreeding. GM-CSF deficiency has been combined with IL-3 deficiency by a sequential gene targeting approach *(83);* these mice have a basal eosinophila but had impaired contact hypersensitivity reactions. They have also been used to evaluate the role of these cytokines in vivo in murine models of leukemia and myeloproliferative disease based on BCR-ABL and several leukemogenic TEL-tyrosine kinase fusion oncoproteins *(84,85);* in all models, combined deficiency of these two factors did not impact on the in vivo phenotype of the model leukemia. Mice completely lacking GM-CSF, IL-3, and IL-5 signaling were generated by creating IL-3$^{-/-}$IL-3/GM-CSF/IL-5R$\beta_c^{-/-}$ mice; these mice have surprisingly normal basal hematopoiesis and showed normal hematopoietic responses to *L. monocytogenes* infection and after 5-FU administration *(86)*. GM-CSF$^{-/-}$IL-3$^{-/-}$IL-3/GM-CSF/IL-5R$\beta_c^{-/-}$ mice have been created, which sum to the same growth factor signaling defect *(83)*.

GM-CSF deficiency has been combined with CSF-1 (M-CSF) deficiency by interbreeding CSF-1-deficient *op* mutant mice with GM-CSF$^{-/-}$ mice *(87,88)*. Concomitant

CSF-1 (M-CSF) deficiency accentuated the pulmonary disease of GM-CSF-deficient mice, but mice deficient in both factors still had residual macrophages, indicating that other factors are still able to affect macrophage development and differentiation in vivo *(87)*. Conversely, GM-CSF deficiency was shown not to be the mediator of age-related corrections in macrophage development observed in *op/op* mice *(88)*.

GM-CSF deficiency has also been combined with TPO signaling deficiency by generating GM-CSF$^{-/-}$c-*mpl*$^{-/-}$ mice. On an inbred background, no further effect of GM-CSF deficiency on the thrombocytopenia of c-*mpl*$^{-/-}$ was observed. This study demonstrated one of the pitfalls of this approach: on a noninbred background, a partial amelioration of the c-*mpl*$^{-/-}$ thrombocytopenia was seen, suggesting existence of other modifier genes of this phenotype.

2.7.4. Combined Deficiencies Involving Interleukin-11

To combine IL-11 and TPO deficiency, IL-11R$\alpha^{-/-}$ mice and mice deficient in the TPO receptor c-*mpl* were interbred *(89)*. Despite the ability of pharmacologic doses of IL-11 to stimulate megakaryocytopoiesis and thrombopoiesis, combined IL-11R$\alpha^{-/-}$c-*mpl*$^{-/-}$ mice did not have accentuation of the platelet and megakaryocyte production defects that characterize c-*mpl* deficiency.

3. ANIMAL MODELS OF HEMATOPOIETIC GROWTH FACTOR EXCESS

Administration of an HGF to a normal animal superimposes an acute excess of circulating factor on otherwise normal hematopoiesis, potentially mimicking factor-driven emergency hematopoiesis. Numerous preclinical evaluations of this type have been done, and only some are summarized in this chapter. A particular advantage of this approach is its flexibility for comprehensive testing of the in vivo effects of combinations of multiple different factors, including enabling a range of scheduling issues to be evaluated.

Genetic models of HGF overproduction have the advantage of durability and provide additional information about the effects of chronic long-term exposure to the factor (Table 7). When the model is based on germline transgenesis, the model is able to be propagated, and populations of uniformly affected animals can be generated for study. Genetic approaches are particularly useful for evaluating the effects of excess factor production in vivo when there are limited amounts of factor available for direct administration and for defining the toxicity of long-term factor exposure.

3.1. Erythropoietin

Numerous studies have reported the effects of EPO administration to a wide range of species. Recombinant EPO administration induces polycythemia in a dose-related manner; summaries of these early preclinical studies are found in several comprehensive reviews *(90,91)*. Mice have also been used for comparative evaluations of the in vivo activity of the EPO-related moiety darbepoietin alfa, an EPO derivative with a modified polypeptide and glycosylation structure (reviewed in ref. *92*), and to demonstrate the activity of small-molecule EPO mimetics *(93)*. rHuEPO has a wide cross-species activity that apparently extends from mammals to fish *(94)*. Collectively, these studies indicate that in many species, EPO is a potent and highly specific stimulant of erythropoiesis.

A transgene including 0.4 kb of endogenous 5′ untranslated sequences flanking the 5 exons of the human genomic *EPO* gene sequences resulted in high serum EPO

Table 7
Genetic Models of Chronic Elevation of Hematopoietic Growth Factor Amounts in Mice

Factor	*Genetic basis of model*	*Reference*
EPO	Transgenesis	*95*
		96
		97
EPO	Reconstitution with hematopoietic cells infected with EPO-expressing recombinant retrovirus	*98*
G-CSF	Reconstitution with hematopoietic cells infected with G-CSF-expressing recombinant retrovirus	*104*
GM-CSF	Transgenesis	*107*
GM-CSF	Reconstitution with hematopoietic cells infected with GM-CSF-expressing recombinant retrovirus	*111*
IL-11	Transgenesis	*117*
		118
IL-11	Reconstitution with hematopoietic cells infected with IL-11-expressing recombinant retrovirus	*115*
		114

ABBREVIATIONS: EPO, erythropoietin; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin.

concentrations and sustained polycythemia in mice *(95)*. Subsequent transgenic studies exploited the transcriptional activity of this short EPO promoter fragment to identify more distant but contiguous regulatory elements that together regulate EPO expression in liver and kidney and in response to hypoxia *(96,97)*. Murine reconstitution experiments using marrow cells over-expressing monkey EPO resulted in a severe, progressive, and ultimately fatal polycythemia with marked expansion of erythropoiesis *(98)*.

3.2. Granulocyte Colony-Stimulating Factor

Several studies reporting the effect of HuG-CSF administration to mice for short periods up to 3 wk are listed (Table 8). In a study of neutrophil kinetics after HuG-CSF administration to mice (10 μg/kg/d for 4 d), the peripheral blood neutrophil count increased 14.5-fold, but neutrophil half-life remained normal, and the neutrophilia resulted from a calculated 3.8 extra maturation divisions in neutrophil formation *(99)*. Even after only 4 d of HuG-CSF administration, bone marrow showed increased granulopoiesis morphologically. Later, weakly labeled neutrophils were released that presumably reflected maturation and release of neutrophils that were the progeny of immature neutrophil progenitor cells labeled at the time of tritiated thymidine pulsing. Interestingly, the number of peripheral blood monocytes increased during HuG-CSF administration (primarily owing to amplified release of labeled cells 6–9 h after HuG-CSF administration), and HuG-CSF-treated mice had markedly increased numbers of several types of circulating nonerythroid progenitor cells *(100)*. Therefore, although the major acute effect of excess G-CSF was on the distribution of neutrophils and their immediate precursors, the effect of G-CSF was not completely lineage-specific, as G-CSF administration also affected the distribution of monocytes and progenitor cells of other lineages. Nonhematopoietic effects were reported in these studies of short courses of G-CSF

Table 8
Studies of Excess G-CSF Amounts in Mice

	Major phenotypic consequences	
Study type (reference)	*Hematologic*	*Tissues/survival*
Recombinant factor administration (16 µg/mouse/d, 14 d) *(208)*	↑ Blood neutrophils (×10) ↑ Splenic CFCs	Not assessed
Recombinant factor administration (3 µg/kg/d, 14 d) *(209)*	↑ Blood neutrophils (×9) ↑ Blood monocytes (×3) ↑ Spleen cellularity (×3–4) ↑ Splenic GM-CFCs	Not assessed
Recombinant factor administration (10–2,500 µg/kg/d, 4 d) *(210,211)*	↑ Blood neutrophils ↑ Marrow granulopoiesis ↓ Marrow cellularity ↓ Marrow CFCs and CFU-S	Not assessed (effects accentuated by splenectomy)
Recombinant factor administration (5 µg/kg/d, 8 d) *(100)*	↑ Peripheral blood CFC (multiple types)	Not assessed
Recombinant factor administration (10 µg/kg/d, 4 d) *(99)*	↑ Blood neutrophils (×14) early monocyte release (×17)	Not assessed
Recombinant factor administration (2.5 µg/d, 21 d) *(101)*	↑ Blood neutrophils (×20) ↑ Marrow granulopoiesis Splenomegaly	↑ Endosteal osteoclasts ↑ Medullary cavity ↑ Periosteal bone
Recombinant factor administration (10 µg/d, 10 d) *(212)*	↑ Splenic dendritic cells (×2.3) Normal dendritic cell IFN production	Not assessed
Reconstitution with hematopoietic cells infected with G-CSF-expressing recombinant retrovirus *(104)*	↑ $[G\text{-}CSF]_{serum}$ ↑ Blood neutrophils ↑ Blood CFCs	↑ Neutrophils in lung and liver No tissue damage Normal 30-wk survival
Recombinant PEGylated factor administration (30–1000 µg/kg, 1 dose) *(102,103)*	↑ Blood neutrophils proportional to dose for up to 6 d ↑ Blood CFCs	Not assessed

ABBREVIATIONS: CFC, colony-forming cell; G-CSF, granulocyte colony-stimulating factor; GM-CFC, granulocyte-macrophage colony-forming cell, CFU-S, spleen colony-forming unit; IFN, interferon; ↑, increased; ↓, decreased.

administration to mice, but after 21 d, femoral bone morphology was altered, with increased numbers of endosteal osteoclasts, periosteal bone deposition, and increased size of the medullary cavity *(101)*. A recently developed polyethylene glycol-conjugated form of filgrastim (pegfilgrastim) has also been evaluated after administration to mice and shown to share many of the granulopoietic effects of filgrastim, but for a sustained duration and with less dosing-related fluctuation *(102,103)*.

Chimeric G-CSF transgenesis in adult mice was achieved by reconstituting mice with marrow infected with a retrovirus leading to G-CSF overproduction *(104)*. These mice developed very high serum G-CSF concentrations (equivalent to 20–260,000 ng/mL recombinant HuG-CSF) but had normal survival of up to 30 wk. No tissue damage was seen despite considerable tissue infiltration with neutrophils, suggesting that high circulating G-CSF amounts are well tolerated for long periods

Table 9
Studies of Excess GM-CSF Amounts in Mice

	Major phenotypic consequences	
Study type (reference)	*Hematologic*	*Tissues/survival*
Recombinant factor administration (18–600 ng/d, 6 d) *(150)*	↑ blood neutrophils (×2) ↑ Peritoneal macrophages ↑ Splenic hematopoiesis	Lung and liver macrophages
Recombinant factor administration (10 μg/kg/d, 4 d) *(99)*	↑ Blood neutrophils (×1.5) Early monocyte release (×2)	Not assessed
Recombinant factor administration (450 ng/d, 21 d) *(101)*	↑ Peritoneal macrophages Peripheral blood normal Bone marrow normal	↑ Endosteal osteoclasts ↑ Medullary cavity
Recombinant factor administration (1–10 μg/kg/d ≤ 11 wk) *(106)*	↑ Splenic hematopoiesis ↑ Peritoneal macrophages Peripheral blood normal	No toxicity
Transgenesis *(107–110, 213, 214)*	↑ $[GM\text{-}CSF]_{serum}$ ↑ $[IL\text{-}1]_{serum}$ ↑ Peritoneal macrophages Peripheral blood normal	Eye damage Muscle lesions Wasting Premature death
Reconstitution with hematopoietic cells infected with GM-CSF-expressing recombinant retrovirus *(111)*	↑ $[GM\text{-}CSF]_{serum}$ ↑ Blood granulocytes ↑ Blood macrophages	Lesions in liver, lung Lesions in muscle, eye Early death
Recombinant pegylated factor administration (2–5 μg/d, 5 d) *(212, 215)*	↑ Splenic dendritic cells (×12) Impaired dendritic cell IL-12 production	Not assessed

ABBREVIATIONS: IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; ↑, increased; ↓, decreased.

and that the resultant neutrophils are not innately destructive. The changes in distribution of hematopoiesis and hematopoietic cell types were similar to those observed after short courses of G-CSF administration, indicating that these changes can be sustained for long periods. Dysregulated G-CSF expression in hematopoietic cells did not result in malignant transformation.

3.3. Granulocyte-Macrophage Colony-Stimulating Factor

Several studies report the effects of MuGM-CSF administration to mice for short periods (≤3 wk) *(99,105),* and one study assessed the effects of MuGM-CSF administration for 11 wk *(106)* (Table 9). A short course of MuGM-CSF administered either intravenously *(99)* or intraperitoneally *(105)* increased peripheral blood neutrophils only 1.5–2-fold, and the effects on myeloid kinetics were modest *(99).* GM-CSF had similar effects on bone morphology to those observed in G-CSF-treated mice, despite its less dramatic effects on marrow myelopoiesis *(101).* During 11-wk MuGM-CSF courses (1–10 μg/kg/d, sc administration), the short-term effects of MuGM-CSF to increase the relative frequency of marrow and splenic progenitor cells subsided (this was not owing to the development of circulating GM-CSF inhibitors), although the

early increase in number and enhanced function of macrophages was sustained. Compared with G-CSF, excess amounts of GM-CSF had only modest effects on myelopoiesis, and with long-term administration, these effects were transient.

Two genetically based models of mice ectopically overexpressing GM-CSF are of interest. Transgenic mice carrying an MuGM-CSF transgene were characterized by high serum GM-CSF concentrations, ocular opacity and retinal damage, striated muscle lesions, and reduced survival with death at 2–4 mo, but the mice had unperturbed hematopoiesis *(107).* The tissue lesions appeared to be mediated by autostimulated macrophages *(107–109)* and macrophage-derived cytokines such as IL-1α, tumor necrosis factor-α (TNF-α), and basic fibroblast growth factor *(109,110).* In the second model, mice transplanted with marrow cells infected with a retrovirus leading to MuGM-CSF production had 100-fold higher amounts of serum GM-CSF as well as extensive neutrophil and macrophage infiltration in many tissues, and they died within 1 mo of transplantation *(111).* The mice also had perturbed hematopoiesis: peripheral blood neutrophils, monocytes, and eosinophils were increased by 15-, 7-, and 9-fold, respectively, with reduced numbers of marrow progenitor cells and variable changes in number of splenic progenitor cells. The differences between these two genetic models of GM-CSF overproduction may be owing to the different types of cells overexpressing GM-CSF, the effect of the transplantation itself, and the 100-fold difference in GM-CSF production. Both models suggest that although high concentrations of GM-CSF are capable of driving myelopoiesis, the body tolerates these extremely high supraphysiologic circulating GM-CSF amounts poorly.

3.4. Interleukin-11

The effects of IL-11 administration in preclinical models have been comprehensively reviewed *(112,113).* Genetic overexpression of human IL-11 was achieved in mice transplanted with marrow cells transduced with a retrovirus leading to IL-11 production *(114,115);* mice had high concentrations of serum IL-11, moderately increased platelet counts, increased splenic myeloid progenitor cell numbers, and evidence of system chronic IL-11 toxicity (loss of fat tissue, thymic atrophy, eyelid inflammation, and occasional hyperactivity). Several models of stable germline IL-11-expressing transgenes exist. An IL-11 transgene driven by the *Mx* promoter resulted in mice with constitutive expression on IL-11 in bone and bone marrow cells (this promoter was selected because its transcriptional activity can be upregulated by IFN); the major phenotype of these mice was increased bone formation *(116).* Transgenic mice with IL-11 expression restricted to the airways have been generated *(117),* including an inducible model using the reverse tetracycline transactivator system *(118);* these models have elucidated the role of IL-11 in airway inflammation, lung fibrosis, and the response to acute lung injury *(119).*

4. ANIMAL MODELS OF HEMATOPOIETIC GROWTH FACTOR ADMINISTRATION AFTER CHEMOTHERAPY OR RADIOTHERAPY

HGFs have found their most prominent role clinically in supporting hematopoietic recovery after anticancer chemotherapy and myeloablative regimens. The development of these approaches and the therapeutic principles underpinning them are based on appropriate animal models. Some examples selected from the large number of such studies follow.

4.1. Granulocyte Colony-Stimulating Factor

Many studies focus primarily on hematopoietic parameters after chemotherapy. For example, HuG-CSF accelerated granulopoietic recovery after cyclophosphamide in mice *(120)* and rats *(121),* after etoposide in mice *(122),* and after mitoxantrone and cyclophosphamide combination therapy in dogs *(123).* Animal models can allow evaluation of novel approaches to scheduling and drug delivery. The effectiveness of rectal administration of G-CSF by suppositories has been shown in cyclophosphamide-treated rabbits *(124).*

Scheduling issues can be more readily evaluated in animal models than in patients, particularly when theoretical risks exist. The risks and benefits of different schedules of exogenous G-CSF administration before and after a cyclophosphamide dose have been studied in mice *(125).* Exogenous G-CSF administration immediately before chemotherapy and continued after chemotherapy accelerated neutrophil recovery, although neutrophil nadirs were lower than with other schedules. Exogenous G-CSF administration stopping several days before therapy and restarting after chemotherapy resulted in the greatest granulopoietic effect. The effect of exogenous G-CSF to minimize the interval between cyclophosphamide administrations has been studied *(126).* Another scheduling evaluation showed that with exogenous G-CSF administration through 7 d of etoposide therapy, protection from neutropenia could still be achieved *(122).* A comparison of the granulopoietic effects of pegfilgrastim and filgrastim after 5-FU effectively addressed a scheduling issue *(103).*

More sophisticated studies have modeled the infective complications of chemotherapy. To model culture-positive febrile neutropenic complications of chemotherapy, cyclophosphamide-treated mice were treated with intraperitoneal exogenous G-CSF for 4 d and challenged with bacterial and fungal pathogens *(P. aeruginosa, Serratia marcescens, Staphylococcus aureus, C. albicans) (127).* This short G-CSF treatment protected mice from otherwise lethal inoculums of these pathogens, and synergism with antibiotics was demonstrated for *P. aeruginosa* infections. Another study assessed the effects of exogenous G-CSF and antibiotics in vancomycin-resistant *Enterococcus faecalis*-infected mice *(128).* Cyclophosphamide was administered to induce neutropenia, *E. faecalis* was inoculated, and then exogenous G-CSF was administered either alone or with antibiotics in various doses. The combination of exogenous G-CSF and antibiotics was more effective at enhancing survival than either antibiotic or exogenous G-CSF alone. Beneficial effects of G-CSF on the course of Gram-positive infections have been documented after cyclophosphamide administration in mice *(129);* interestingly, this study did not find comparable effects after irradiation.

Animal models are useful for evaluating novel agents in combination with or compared with HGF. SCH 14988 is a small molecule that enhances endogenous G-CSF production; it accelerated neutrophil recovery after cyclophosphamide administration in association with increased G-CSF concentrations *(130).* In combination with exogenous G-CSF, dipyridamole and adenosine monophosphate enhanced post-5-FU granulopoietic recovery *(131).*

4.2. Granulocyte-Macrophage Colony-Stimulating Factor

Murine studies of GM-CSF are complicated by the lack of cross-species reactivity of HuGM-CSF, necessitating the use of MuGM-CSF.

A brief report demonstrated efficacy of exogenous GM-CSF in a murine model of melphalan-induced neutropenia *(132).* The duration of post-melphalan neutropenia

was shortened, and the mortality was reduced by approx 50%. Exogenous GM-CSF was shown to accelerate neutrophil and platelet recovery in monkeys after total body irradiation and autologous marrow transplantation *(133,134).*

In non-neutropenic mice, administration of exogenous GM-CSF increased the number of peripheral blood neutrophils and monocytes and number of peritoneal macrophages but did not alter the course of *Listeria monocytogenes* infection. In mice rendered neutropenic by either cyclophosphamide administration or irradiation, exogenous GM-CSF had little effect; even these quantitative changes were not observed *(135).*

Dose-for-dose comparison of exogenous G-CSF and exogenous GM-CSF has been undertaken in mice *(126).* GM-CSF had 5% of the potency of G-CSF on neutrophil counts. Interestingly, during exogenous G-CSF administration, neutrophils egressed to an inflammatory site, but this did not occur during exogenous GM-CSF administration, consistent with other studies describing reduced neutrophil mobility with exogenous GM-CSF exposure.

4.3. Erythropoietin

Anemia, although a common accompaniment of cancer, is not usually viewed as an acute complication of myelotoxic chemotherapy, although it is well recognized that chemotherapeutic agents and regimens are associated with anemia, particularly with multiple dosings or repeated courses. Recombinant EPO preparations were effectively the first HGF to be evaluated for their ability to stimulate hematopoietic recovery in murine models, e.g., after irradiation *(136)* or 5-FU *(137).* Relatively few animal studies have directly examined the effects of exogenous EPO administration along with chemotherapy, although the role of exogenous EPO in alleviating the anemia associated with cancer and its treatment is well established *(138).*

One particularly interesting study in mice combined 7 d of etoposide (VP-16) therapy with simultaneous exogenous EPO administration. At lower VP-16 doses with exogenous EPO, higher reticulocytes and hematocrits were observed, but overall a negative interaction between VP-16 and exogenous EPO was evident: VP-16 had a larger antierythropoietic effect in EPO-treated compared with non-EPO-treated animals *(122).* Although combining this relatively non-cell cycle-specific agent with a lineage-specific growth factor resulted in net advantageous outcome over part of the chemotherapeutic agent's dose range, in fact over all doses, growth factor stimulation of erythropoiesis was found to occur but was largely cancelled out by the abrogative effect of concomitant cytotoxic drug administration.

A number of recent animal studies have focused on the effect of EPO-stimulated anemia alleviation to improve the anticancer efficacy of chemotherapeutic agents including cyclophosphamide *(139)* and cisplatin *(140),* of radiotherapy *(141,142),* and of phototherapy *(143).* These beneficial effects are thought to result from improved oxygen delivery to tumors resulting in sensitization to the cytotoxic modality. In one instance, a murine myeloma model, exogenous EPO itself was observed to stimulate immunologically mediated tumor regression *(143,144).*

4.4. Interleukin-11

The preclinical studies of IL-11 effects in the context of myelotoxic or myeloablative therapies have been comprehensively reviewed *(112,113).* Studies were conducted in syngeneic mouse transplant models and in mice given radiotherapy, or chemotherapy, or a combination of these. Some studies included assessment of

impact on modeled infectious and bleeding complications *(145).* Exogenous IL-11 promoted hematopoietic recovery, including accelerated platelet recovery, but another beneficial effect consistently observed has been an IL-11-related reduction in gastrointestinal mucosal toxicity. High-dose exogenous IL-11 protected rats rendered neutropenic with cyclophosphamide from *P. aeruginosa* infection *(146).*

There has been an ongoing interest in IL-11 combinations with other growth factors, for example, to drive a more rapid multilineage hematopoietic recovery, or to combine the mucosal protective effects of IL-11 with the granulopoietic potency of other agents. In one study, 5-FU-related mortality was abrogated by exogenous IL-11 plus exogenous SCF *(147).* In the rat neutropenia and *P. aeruginosa* infection model, G-CSF did not prove protective alone, but IL-11 combined with G-CSF was more protective than IL-11 alone *(148);* additionally, there was significantly superior mucosal integrity in the combination group at histologic analysis at a fixed post-treatment timepoint.

5. ANIMAL MODELS EVALUATING HEMATOPOIETIC GROWTH FACTOR SIGNALING IN PATHOLOGIC PROCESSES

The phenotypic characterization and experimental validation of the animal models included descriptions of several pathologic states such as acute inflammation or experimental infection in which the role of a particular HGF has been assessed. To understand particular nuances regarding the role of HGF signaling in disease pathogenesis, particularly at the level of the receptor, several models have been generated by targeted gene modification rather than disruption. Particularly interesting questions about the contribution of HGF to disease pathogenesis can be addressed by interbreeding two murine models together, or by exploiting the range of genetic backgrounds available and the transplantability of the hematopoietic system to assess the role of paracrine and host factor production in disease pathogenesis.

5.1. G-CSF, G-CSFR, and Neutrophil Elastase in Severe Chronic Neutropenia

Although G-CSF-deficient mice have life-long neutropenia, congenital or acquired neutropenia in humans caused by G-CSF deficiency itself has not been described. Approximately 20% of patients with severe chronic neutropenia have associated carboxyl truncations of the G-CSF receptor (G-CSFR) *(149,150),* although these appear to be acquired somatic mutations rather than germline mutations *(150).* Representative examples of this mutation have been modeled in mice by targeted gene modification. In one model, based on a G-CSFR truncated at position 715, mice displayed baseline neutropenia, a milder haploinsufficiency phenotype, and a hyperproliferative response to exogenous G-CSF resulting in neutrophilia *(151,152).* In the other model, both heterozygous and homozygous mice displayed a normal basal granulopoietic phenotype with only a modest reduction in circulating neutrophil numbers, despite the lesion resulting in a hyperproliferative response to exogenous G-CSF in vivo *(153).* The reason for the difference between the two models is not clear, although the gene-targeting strategy used retained the selectable marker in one model *(153)* but not the other *(151).*

Recently, the genetic lesion resulting in cyclical neutropenia was located to the neutrophil elastase gene *(154).* A high prevalence of heterozygous neutrophil elastase mutations in severe congenital neutropenia implicates these lesions epidemiologically

in the pathogenesis of the disease *(155).* Normal granulopoiesis was observed in a murine model of one of these mutations *(156),* suggesting that the pathogenesis of the neutropenia may be more complex than the effects of a single mutation.

5.2. Erythropoietin Receptor and Familial Erythrocytosis

In contrast to the situation with the G-CSF receptor, heterozygous carboxyl truncations of the human EPOR are associated not with anemia but with polycythemia and are often transmitted in the germline *(157,158).* A multistep targeted gene modification approach has been used to replicate one mutated HuEPO receptor (C5964 → G) in a murine model *(159).* These mice show a haploinsufficiency phenotype with polycythemia. Mice homozygous for this mutation (a situation not observed clinically) develop severe polycythemia, but are viable.

5.3. Granulocyte-Macrophage Colony-Stimulating Factor in Pulmonary Disease and Other Experimental Disease Models

The development of alveolar proteinosis in GM-CSFR and GM-CSF-Rβ_c-deficient mice has highlighted the role of GM-CSF signaling in pulmonary pathophysiology. The pathogenic and therapeutic insights contributed by this model have been reviewed *(160).* GM-CSF-deficient mice have been exploited to evaluate the contribution of GM-CSF in other lung pathology. The acute lung injury associated with an experimental model of acute pancreatitis was ameliorated in GM-CSF$^{-/-}$ mice despite comparable degrees of pancreatic inflammation *(161).* Similarly, other immunologically mediated inflammatory diseases have been shown to be ameliorated in GM-CSF$^{-/-}$ mice including collagen-induced arthritis *(162)* and experimental autoimmune encephalitis *(163).*

5.4. Hematopoietic Growth Factors in Myeloid Leukemia

The role of HGF signaling in leukemogenesis and the notion of growth factor dependence as a potential Achilles' heel of leukemic cells that could be targeted therapeutically have been recognized for several decades *(164).* Genetic models of murine myeloid leukemia have been generated based on transgenic expression of several common fusion oncogenes, both for the transformation of marrow cells, which are then used to reconstitute recipient animals, or by germline transgenesis. These leukemia models, when combined with animals with defective HGF signaling, provide a means to assess the role that HGF signals play in the leukemogenic process. Several examples of this approach have been reported.

Mice deficient in the neurofibromatosis 1 (NF1) gene die *in utero (165,166),* but NF1-deficient fetal liver cells induce a myeloproliferative disorder in transplant recipients reminiscent of the juvenile myelomonocytic leukemia (JML) seen in humans with NF1 *(167,168).* Like their human counterparts, murine NF1$^{-/-}$ fetal hematopoietic cells show hypersensitivity to GM-CSF in vitro *(167,169).* NF$^{-/-}$ and GM-CSF$^{-/-}$ mice were interbred and used as a source of NF1$^{-/-}$GM-CSF$^{-/-}$ fetal liver hematopoietic cells for transplant studies to test directly whether the GM-CSF production ability of the fetal liver cells themselves, the host stroma, or both contributed to the murine myeloproliferative phenotype *(170).* GM-CSF production by either the host or engrafting cells was sufficient to induce the myeloproliferative disease, but the myeloproliferative process was suppressed when neither the host nor graft could make GM-CSF. Frankly myeloproliferative marrows transplanted into GM-CSF-deficient recipients

resulted in an attenuated phenotype compared with GM-CSF-replete recipients. Exogenous GM-CSF treatment of secondary recipients (i.e., mice with NF1$^{-/-}$GM-CSF$^{-/-}$ graft cells into GM-CSF$^{-/-}$ recipients) unmasked the myeloproliferative phenotype. Collectively, these data present an elegant use of these animal models to implicate endogenous GM-CSF production and signaling as necessary for the full manifestation of this myeloproliferative disease.

Hematopoietic cells carrying other leukemogenic fusion oncogenes have been tested for their dependence on HGF in similar models. Marrow cells transduced to overexpress *BCR/ABL* resulted in identical diseases in transplant recipients regardless of whether the graft donor, transplant recipient, or both lacked either GM-CSF, or IL-3, or both these factors *(84)*. Similarly, mice deficient in both GM-CSF and IL-3 were used to show that three TEL-protein tyrosine kinase fusion oncogenes induced similar myeloproliferative disorders despite absence of factor production by both the donor cells and recipient animal *(85)*.

We have reported preliminary observations from an experiment in which mice carrying a leukemogenic PLZF-RARα transgene were backcrossed onto G-CSF-deficient or GM-CSF-deficient backgrounds. Surprisingly, mice carrying the invariably lethal PLZF-RARα transgene on a G-CSF-deficient background failed to develop chronic myeloproliferation and lived a normal life span, whereas GM-CSF-deficient mice carrying the transgene died over 6–18 mo, like their transgenic wild-type background counterparts *(171)*. These observations suggest that this murine myeloproliferative disorder requires signals exclusively provided by G-CSF for its full manifestation.

6. CONCLUSIONS

Although much can be learned about the cellular effects of HGFs from their activities in vitro, animal models have been indispensable for understanding the basic physiology of the HGFs. Loss-of-function models and overexpression models have particularly contributed to this understanding of physiologic roles. Animal studies are a mandatory part of the preclinical development of new biologic therapeutics, and many specific models of particular therapeutic scenarios have delineated the potential beneficial activities of HGF in current clinical use, including studies exploring dosing and scheduling parameters that inform the clinical use of these agents in the hematologic support of anticancer treatments, such as those described in later chapters of this book. The animal models have provided sometimes unexpected insights into the pathogenesis of both nonmalignant and malignant disease, which suggest possibilities for exciting new therapeutic approaches.

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