Vol. 20, No. 7 July 1998

CE

Refereed Peer Review

FOCAL POINT

- The use of recombinant human granulocyte colony-stimulating factor (rhG-CSF) in the treatment of infectious diseases in veterinary patients focuses primarily on viral infections.

KEY FACTS

- In a study of parvoviral-enteritis–infected puppies, rhG-CSF did not increase neutrophil counts.
- In animals with septicemia, rhG-CSF has been demonstrated to have beneficial effects.
- Human patients with HIV can benefit from treatment with granulocyte colony-stimulating factor; beneficial effects thus are possible in cats infected with feline immunodeficiency virus.
- In patients with infectious diseases, the use of rhG-CSF has not been extensively studied; further research is particularly warranted with regard to preventing secondary infections.

Veterinary Uses of Recombinant Human Granulocyte Colony-Stimulating Factor. Part II. Infectious Diseases*

Veterinary Specialties of Omaha, PC
Omaha, Nebraska
Jennifer M. Rewerts, DVM

University of Missouri
Carolyn J. Henry, DVM, MS

Hematopoietic growth factors are cytokines that regulate the growth, development, and function of hematopoietic cell lineages. Cytokines that stimulate the leukocyte lineages are important in the pathogenesis of inflammation and response to infection. The first part of this two-part article provided an overview of granulocyte colony-stimulating factor (G-CSF) and recommendations for its use in veterinary patients receiving chemotherapy. Part II discusses the use of G-CSF in treating patients with infectious diseases, primarily viral infections.

OVERVIEW

In veterinary practice, recombinant canine G-CSF (rcG-CSF) reportedly increases neutrophil counts in healthy dogs and is beneficial in treating chemotherapy-induced neutropenia.[1,2] Positive results have been associated with the use of recombinant human G-CSF (rhG-CSF) to treat cyclic neutropenia in gray collies.[3] Recently, rhG-CSF has been advocated in treating neutropenia caused by infectious disease (i.e., parvoviral enteritis) in young puppies.[4] The high cost of rhG-CSF may limit its usefulness in these puppies, many of which have owners who are not affluent.

*Part I of this two-part presentation appeared in the June 1998 (Vol. 20, No. 6) issue of *Compendium*.

NEUTROPENIA

Before discussing when rhG-CSF might be used in veterinary patients, it is important to understand the potential risks. One complication is the production of antibodies to the rhG-CSF. Several studies indicate that neutropenia develops after prolonged use of rhG-CSF in animals.[3,5,6] Patients develop antibodies to the exogenous G-CSF, which also cross-reacts with endogenous G-CSF. Neutropenia is a consequence of the antibody production. If rhG-CSF is discontinued, neutrophil production typically returns to normal over time. The use of recombinant G-CSF (rG-CSF) specific to a particular species (e.g., dogs or cats) alleviates this problem. However, recombinant animal G-CSF is not commercially available at this time.

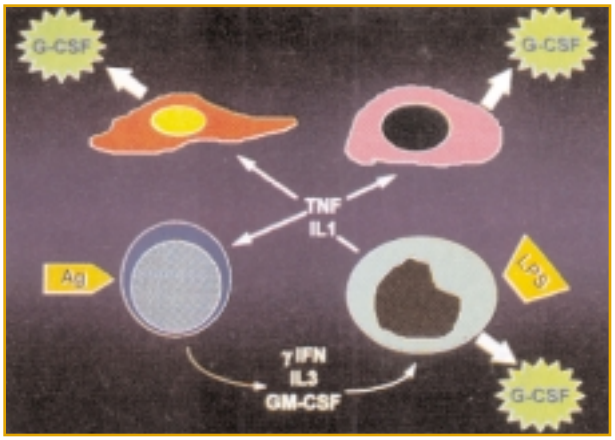

Figure 1—Production of colony-stimulating factors and inflammatory mediators during the inflammatory process (*Ag* = antigen; *G-CSF* = granulocyte colony-stimulating factor; *GM-CSF* = granulocyte-macrophage colony-stimulating factor; γ*IFN* = interferon-γ; *IL* = interleukin; *LPS* = lipopolysaccharide; *TNF* = tumor necrosis factor).

Neutropenia is caused by decreased production of neutrophils by the bone marrow, consumption in tissue, or immune-mediated destruction. Parvovirus involves two of these mechanisms. The virus has a predilection for rapidly dividing cells, making the granulocyte pool in the bone marrow a prime target for destruction. The virus also attacks the intestinal crypt epithelial cells, stimulating infiltration of neutrophils and macrophages by releasing chemotactants, initiating the process of inflammation (Figure 1), and consuming neutrophils already in the circulation. Bone marrow studies in puppies with parvoviral enteritis indicate that immune-mediated destruction contributes more to neutropenia than does primary destruction in the bone marrow.[7]

PARVOVIRAL INFECTION

Because neutropenia is a common sequela to parvoviral enteritis, it might be presumed that supplementation with G-CSF would alleviate neutropenia and reduce mortality in infected animals. To date, few clinical studies have evaluated the use of G-CSF in this setting.

A recently published study did demonstrate that supplementation of rhG-CSF to parvovirally infected puppies increased neutrophil counts compared with those in parvovirus-infected control dogs.[4] The puppies were given rhG-CSF subcutaneously at 5 µg/kg two to three times a day. Cats infected with panleukopenia were treated in a similar manner. Treated cats did not have higher neutrophil counts than did controls.

One possible explanation for this lack of response is that cats are inherently resistant to the effects of exogenous G-CSF. Several studies in normal cats suggest that this is not the case.[5,8] Normal cats given rhG-CSF had significant initial increases in neutrophil counts.[5] Another possible explanation is a significant decrease in the number of stem cell precursors as a result of viral destruction. In dogs and cats infected with parvovirus, it is relatively common to observe rebound neutrophilia during recovery from illness; such recovery would indicate that precursor cells remain. As mentioned, significant consumption in the gastrointestinal tract contributes as much or more to the neutrophilia associated with parvoviral infections.[7,9]

Contrary to the study by Kraft and Kuffer,[4] a study performed at the Veterinary Medical Teaching Hospital of the University of Missouri demonstrated no increase in neutrophil counts in puppies naturally infected with parvovirus that were given rhG-CSF (compared with those in control dogs).[10] Neutropenia was defined as less than 1000 cells/µl. At first observation of neutropenia, the puppies were randomly divided into a control group and an rhG-CSF treated group. Puppies treated with G-CSF were given a subcutaneous rhG-CSF dose of 5 µg/kg/day until neutrophil counts increased to 1500 cells/µl. Neutrophil response was similar in both groups. There were no significant differences in survival rates, duration of hospitalization, or the time from neutropenia to neutrophil recovery. A similar increase in neutrophil numbers was observed in both groups after clinical recovery from the virus.

The conflicting findings of these two studies may result from several factors. First, the average neutrophil counts of the affected puppies in Kraft's study were higher than those found at the University of Missouri when G-CSF was first instituted. A second difference in the studies was related to the dosage of rhG-CSF: Kraft administered 5 µg/kg two to three times a day, whereas the study performed at the University of Missouri used 5 µg/kg once a day.

EFFECTS OF INFLAMMATION ON NEUTROPHIL COUNTS

The lack of response might be explained by an understanding of the mechanism of neutrophil production in the bone marrow in light of inflammation (Figure 2). Administration of rG-CSF is associated with a rapid (less than 24-hour) increase in neutrophil counts despite a bone marrow neutrophil maturation time of 4 to 6 days.[11] Although this time can be accelerated to approximately 3 days in the presence of exogenous G-CSF or inflammation, the nearly immediate increase in neutrophil counts associated with exogenous G-CSF is related to the release of marrow storage-pool neutrophils into the peripheral circulation.[12]

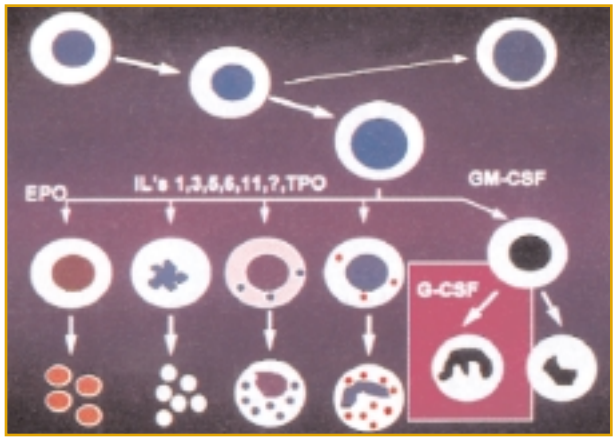

Figure 2—Differentiation and maturation of hematopoietic cell lineages in response to hematopoietic growth factors in the bone marrow (*EPO* = erythropoietin; *G-CSF* = granulocyte colony-stimulating factor; *GM-CSF* = granulocyte-macrophage colony-stimulating factor; *IL* = interleukin; *TPO* = thrombopoietin).

Because many puppies infected with parvovirus may be neutropenic and have severe intestinal inflammation at presentation, the storage pool probably has already been depleted.[7,13,14] Thus, rhG-CSF–induced increases in neutrophil counts would depend on accelerated precursor maturation, which requires at least 2 to 3 days. In our experience, the neutrophil counts of most neutropenic parvovirus-infected puppies rebound within several days with supportive care and clinical recovery from the virus.

SEPTICEMIA

Another consumptive cause for neutropenia is septicemic infection caused by gram-negative bacteria. Neutropenia develops as a result of sepsis and endotoxemia in young animals.[15] The ongoing concern for gram-negative infections in neonates has sparked interest in the use of rhG-CSF in this setting. In a study performed by Eichaker and coworkers, supplementation of rhG-CSF reduced levels of serum endotoxin, tumor necrosis factor, and blood bacterial counts in beagles with induced septicemia.[16]

Septicemia is a leading cause of death in foals.[17] In normal foals, rcG-CSF and recombinant bovine G-CSF produce a significant increase in neutrophil counts. For this reason, supplementation of colony-stimulating factors has been speculated to be prophylactically useful in neonatal foals at risk for septicemia.[18] As shown by Eichaker and coworkers, increased neutrophil numbers should reduce the chance of bacterial infections if G-CSF is supplemented before the onset of septicemia.[16]

FELINE IMMUNODEFICIENCY VIRUS AND FELINE LEUKEMIA VIRUS

Another area that warrants further investigation of the use of G-CSF is neutropenia secondary to feline immunodeficiency virus (FIV) and feline leukemia virus. Positive results have been observed in patients with AIDS supplemented with G-CSF, which can improve neutrophil function and erythropoiesis, enhance fungicidal activity of polymorphonuclear leukocytes, and permit the use of antiviral therapy (which results in neutropenia), as is discussed in Part I of this article.

Because of the strong similarity between HIV and FIV, comparable results would be expected in cats infected with FIV. However, rhG-CSF would have to be administered long-term in patients with FIV, and antibody production would be expected.[5] Recombinant feline G-CSF would be a better choice in such cats, but this product is not commercially available at this time. To our knowledge, no controlled studies have been reported to date concerning the use of G-CSF in FIV-infected cats.

CONCLUSION

In our experience, supplementation of rhG-CSF has had beneficial effects in veterinary patients with myelosuppression from chemotherapy and in gray collies with cyclic neutropenia. It is debatable whether similar benefits occur in puppies infected with parvovirus. Further studies are indicated to determine the efficacy of this cytokine in preventing secondary infections in cats infected with FIV.

About the Authors

Dr. Rewerts is affiliated with Veterinary Specialties of Omaha, PC, Omaha, Nebraska. Dr. Henry is with the Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, Missouri. Both authors are Diplomates of the American College of Veterinary Internal Medicine.

REFERENCES

1. Obradovich JE, Ogilvie GK, Powers BE, et al: Evaluation of recombinant canine granulocyte colony-stimulating factor as an inducer of granulopoiesis. A pilot study. *J Vet Intern Med* 5:75–79, 1991.
2. Ogilvie GK, Obradovich JE, Cooper MF, et al: Use of recombinant canine granulocyte colony-stimulating factor to decrease myelosuppression associated with the administration of mitoxantrone in the dog. *J Vet Intern Med* 6:44–47, 1992.
3. Lothrop CDJ, Warren DJ, Souza LM, et al: Correction of canine cyclic hematopoiesis with recombinant human granulocyte colony-stimulating factor. *Blood* 72:1324–1328, 1988.
4. Kraft W, Kuffer M: Treatment of severe neutropenias in the dog and cat with Filgrastim. *Tierarztl Prax* 23:609–613, 1995.
5. Tams TR: Management of the canine viral enteritis patient. *Proc TNAVC*:5–10, 1995.
6. Macartney L, McCandlish IAP, Thompson H, et al: Canine parvovirus enteritis 1: Clinical, haematological and pathological features of experimental infection. *Vet Rec* 115: 201–210, 1984.
7. Fulton R, Gasper PW, Ogilvie GK, et al: Effect of recombinant human granulocyte colony-stimulating factor on hematopoiesis in normal cats. *Exp Hematol* 19:759–767, 1991.
8. Obradovich JE, Ogilvie GK, Stadler-Morris S, et al: Effect of recombinant canine granulocyte colony-stimulating factor on peripheral blood neutrophil counts in normal cats. *J Vet Intern Med* 7:65–67, 1993.
9. Boosinger TR, Rebar AH, DeNicola DB, et al: Bone marrow alterations associated with canine parvoviral enteritis. *Vet Pathol* 19:558–561, 1982.
10. Brunner CJ, Swango LJ: Canine parvovirus infection: Effects on the immune system and factors that predispose to severe disease. *Compend Contin Educ Pract Vet* 7(12):979–989, 1985.
11. Maloney MA, Patt HM: Granulocyte transit from bone marrow to blood. *Blood* 31:195–201, 1968.
12. Welte K, Gabrilove J, Bronchud MH, et al: Filgrastim (r-metHuG-CSF): The first 10 years. *Blood* 88:1907–1929, 1996.
13. Meunier PC, Cooper BJ, Appel MJG, et al: Pathogenesis of canine parvovirus enteritis: The importance of viremia. *Vet Pathol* 22:60–71, 1985.
14. Cudd T, Koterba AM: The ISVP survey of equine neonatal disease: 1987. *Newslett Int Soc Vet Perinatol* 1:7–14, 1988.
15. Koterba AM, Brewer BJ, Tarplee F: Clinical and clinicopathologic features of septicaemia in the newborn foal: A review of 38 cases. *Equine Vet J* 16:312–318, 1984.
16. Eichaker PQ, Waisman Y, Natanson C, et al: Cardiopulmonary effects of granulocyte colony-stimulating factor in a canine model of bacterial sepsis. *J Appl Physiol* 77:2366–2373, 1995.
17. Zinkl JG, Madigan JE, Fridmann DM, et al: Haematological, bone marrow and clinical chemical changes in neonatal foals given canine recombinant granulocyte-colony stimulating factor. *Equine Vet J* 26:313–318, 1994.
18. Madigan JE, Zinkl JG, Fridmann DM, et al: Preliminary studies of recombinant bovine granulocyte-colony stimulating factor on haematological values in normal neonatal foals. *Equine Vet J* 26:159–161, 1994.