rHuKGF ameliorates symptoms in DSS and CD4\(^+\)-CD45RB\(^{Hi}\) T cell transfer mouse models of inflammatory bowel disease

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Byrne, Fergus R., Catherine L. Farrell, Richard Aranda, Karen L. Rex, Sheila Scully, Heather L. Brown, Silvia A. Flores, Li H. Gu, Dimitry M. Danilenko, David L. Lacey, Thomas R. Ziegler, and Giorgio Senaldi. rHuKGF ameliorates symptoms in DSS and CD4\(^+\)-CD45RB\(^{Hi}\) T cell transfer mouse models of inflammatory bowel disease. Am J Physiol Gastrointest Liver Physiol 282: G690–G701, 2002.—There is an acute need for effective therapy for inflammatory bowel disease (IBD), particularly at the level of repair of the damaged epithelium. We evaluated the efficacy of recombinant human keratinocyte growth factor (rHuKGF) in both the dextran sodium sulfate (DSS) and the CD4\(^+\)-CD45RB\(^{Hi}\) T cell transfer models of IBD. Disease was induced either by the ad libitum administration to normal mice of 4% DSS in the drinking water or by the injection of 4 \times 10^5 CD4\(^+\)-CD45RB\(^{Hi}\) T cells into immunodeficient scid/scid mice. rHuKGF was administered by subcutaneous injection at doses of 1.0 or 3.0 mg/kg in both preventative and therapeutic regimens during both studies. rHuKGF significantly improved survival and body weight loss in the DSS model in both preventative and therapeutic dosing regimens. It also improved diarrhea, hematocrit, and hematological parameters, as well as large intestine histopathology. In the T cell transfer model, rHuKGF improved body weight loss, diarrhea, and levels of serum amyloid A, as well as large intestine histopathology. In both models of IBD, the colonic levels of intestinal trefoil factor (ITF) were elevated by the disease state and further elevated by treatment with rHuKGF. These data suggest that rHuKGF may prove useful in the clinical management of IBD and its effects are likely mediated by its ability to locally increase the levels of ITF.

colitis; growth factor; epithelial repair; intestinal trefoil factor

INFLAMMATORY BOWEL DISEASES (IBD; Crohn’s disease and ulcerative colitis) are widespread, chronic, debilitating diseases of unknown etiology (37, 42). Although the development of novel therapeutic agents for these conditions is an active field, current therapy remains largely symptomatic, and most agents under investigation target their intervention at the level of immune system cells and molecules (42). Much of the damage and ensuing clinical symptoms, however, occur at the level of the single-cell epithelium that lines the gastrointestinal tract, separating the mucosal immune system from the dynamic and poorly understood luminal antigens (9, 14). As yet, no effective therapy operates at this level. Keratinocyte growth factor (KGF), also known as fibroblast growth factor (FGF)-7, is produced by mesenchymal cells adjacent to the epithelium of many organs (40, 41). Unlike many members of the FGF family, KGF exhibits strict specificity for epithelial cells by virtue of its receptor, FGFR-2IIIb (30, 33), which in vitro studies suggest binds in a heparin-dependent fashion (22, 23). Recombinant human keratinocyte growth factor (rHuKGF) has shown evidence of efficacy against the oral mucositis associated with radiation therapy and chemotherapy in cancer treatment (15, 48). Preclinical studies (17) have shown that the effect of rHuKGF is likely mediated by its trophic and cytoprotective effects on the epithelial tissues of the digestive tract. Previous studies (18, 19) have also demonstrated that KGF is overexpressed in IBD and have postulated a role for it in epithelial repair subsequent to tissue damage. Several authors (8, 21) have postulated a potential role for KGF produced by γδ intraepithelial T lymphocytes being involved in repair of the damaged epithelium and protection from intestinal inflammation. Both rHuKGF and another member of the FGF family, KGF-2 (also known as FGF-10), have shown some therapeutic efficacy in the dextran sodium sulfate (DSS) mouse model of IBD (16, 32).

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both the prophylactic and therapeutic potential of increasing doses of rHuKGF in the CD4<sup>+</sup>CD45RB<sup>hi</sup> T cell transfer model as well as the DSS model of IBD.

**MATERIALS AND METHODS**

**Animals.** Female SJL/J, mice aged 8–10 wk were used for the DSS study. For the T cell transfer study 12- to 14-wk-old CB6F1 mice of both sexes and immunodeficient female C.B.17 scid/scid mice aged 8–10 wk were used. All mice were purchased from Jackson Laboratories (Bar Harbour, ME). All animals were cared for according to the standard protocols of the Amgen Institutional Animal Care and Use Committee.

Chemical reagents. DSS (molecular mass 36–44 kDa) was purchased from ICN Chemicals, (Costa Mesa, CA) made up fresh weekly as a 4% (w/vol) solution in distilled, deionized water and sterilized through a 0.45-μm filter. Water for the negative control groups was similarly filtered. rHuKGF (Amgen) was expressed in *Escherichia coli*, refolded, purified to homogeneity, and demonstrated endotoxin free before lyophilization. HuKGF was reconstituted in phosphate-buffered saline (vehicle) and diluted to the appropriate dose in a volume of 200 μl. Mice were injected subcutaneously.

**DSS colitis.** All experiments were performed on groups of 6–10 mice, housed in the same cage. For disease induction, mice were given a 4% DSS ad libitum as drinking water for 6–8 days. Disease was initiated by the intraperitoneal injection of 4 × 10<sup>6</sup> fluorescein-activated cell-sorted CD4<sup>+</sup>CD45RB<sup>hi</sup> T cells from CB6F1 mice into immunodeficient C.B.17 scid/scid mice (2). CD4<sup>+</sup>CD45RB<sup>hi</sup> T cells were isolated using magnetic beads (Miltenyi Biotec, Auburn, CA) stained with fluorescein isothiocyanate and then incubated with an antibody against L-selectin. All experiments were performed on groups of 10 mice housed in the same cage. Data for any particular study group are representative of at least two separate experiments.

**Survival curves.** All animals were monitored daily, and mice were weighed three times per week. Body weight data were calculated only for groups in which all mice remained alive. The stool score was based on a modification of the system described by Cooper et al. (12). A mouse could score from zero to a maximum of 5. Stool consistency was graded as 0 = solid, 1 = soft, 2 = diarrhea, 3 = diarrhea and perianal stool. Hematochezia was assessed using occult blood kits purchased from Dipro Diagnostic Products (Louisville, KY) per the manufacturer's instructions, and scoring was as follows: 0 = normal, 1 = slight color change, 2 = full and frank color change.

**Postmortem evaluation.** Mice were euthanized by CO<sub>2</sub> asphyxiation. Organs were removed, weighed or measured, and fixed in zine Formalin for at least 24 h, after which they were processed into paraffin blocks and sections were cut in 5-μm sections and stained with hematoxylin and eosin. Blood was taken for complete blood count using a Techcon H1E (Bayer, Newark, NJ), and serum was frozen at −80°C for subsequent analysis. Levels of plasma insulin-like growth factor-1 (IGF-1) were determined using a mouse-specific radioimmunoassay kit incorporating acid ethanol extraction as described by the manufacturer (Diagnostic Systems Laboratories, Webster, TX). Levels of serum amyloid A (SAA) were determined by ELISA using a kit from BioSource International (Camarillo, CA). Samples of the large intestine were designated proximal (1.5 cm in from rectum), middle (middle of proximal and distal), and distal (1.5 cm in from the anus).

Histopathological damage of the proximal, middle, and distal large intestine was scored as a global assessment of inflammation based on inflammatory infiltrate, mucin depletion, and epithelial cell atypia as follows: 0 = absent, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, and 5 = severe. All samples were scored in a blinded fashion.

**Immunohistochemistry.** Intestinal trefoil factor (ITF) protein expression was determined using a polyclonal anti-ITF antibody (kindly provided by Dr. Daniel K. Podolsky, Massachusetts General Hospital, Boston, MA) raised in rabbits against a synthetic peptide reproducing the 18 COOH-terminal amino acids of rat ITF. Immunohistochemistry was performed using an avidin biotin-complex technique and pressure cooker antigen retrieval (DAKO, Carpinteria, CA). Negative controls had the primary anti-ITF antibody replaced by buffer. Semiquantitative grading of ITF expression in the proximal, middle, and distal small and large intestine was performed by scoring individual colonic sections on a scale of 1 to 4 indicating the relative intensity and extent of ITF staining in the entire section. All samples were scored in a blinded fashion. An overall score for both small and large intestines was generated by averaging the proximal, middle, and distal score.

**In situ hybridization.** An antisense RNA probe for ITF (nucleotides 19–450 from GenBank accession no. D38410) was synthesized from a linearized plasmid template with <sup>[33P]</sup>UTP (1,000–3,000 Ci/mol; Amersham, Piscataway, NJ) and T7 RNA polymerase (Ambion, Austin, TX). Formalin-fixed, paraffin-embedded, sections were deparaffinized, rehydrated, and pretreated with proteinase K. After dehydrating and drying, the sections were conditioned in hybridization mix (49) and hybridized with 2 × 10<sup>6</sup> cpm of probe. Sections were then digested with RNase A, dehydrated, and dipped in NTB-2 emulsion (Kodak, Rochester, NY); exposed for 2–3 wk, developed with D-19 developer (Kodak), and counterstained with hematoxylin and eosin. Semiquantitative grading of ITF mRNA expression in the proximal, middle, and distal small and large intestine was performed by scoring individual colonic sections on a scale of 1 to 4, indicating the relative intensity and extent of ITF staining in the entire section. All samples were scored in a blinded fashion. An overall score for both small and large intestine was generated by averaging the proximal, middle, and distal score.

**Statistical analysis.** Survival curves were compared by the χ<sup>2</sup> probability associated with the log-rank life test procedure. Weight loss curves were compared by the probability associated with Dunnett's correction for the t statistic in ANOVA. Continuous variables such as length of the large intestine and discrete variables such as histopathological scoring were compared by the probability associated with the unpaired, heteroscedastic Student's t-test. Figures illustrate mean values with SE bars, and data in the text are given as means ± SE.

**RESULTS**

rHuKGF prevents lethality and body weight loss in the DSS model of IBD. We conducted three different studies on the effect of rHuKGF in the DSS model of colitis: 1) a long-term study to day 21 that, given the relatively quick lethality of this disease model, proved useful as an initial screening tool for appropriate dosing regimens of the drug; 2) an acute necropsy study using doses judged to be optimal where animals were subjected to full necropsy on day 8; and 3) a recovery study where mice were given DSS from day 0 until day...
7 and then DSS was withdrawn and replaced with ordinary water. In the recovery study, dosing with rHuKGF was begun on day 4 and continued until all animals were subjected to full necropsy on day 19. This recovery study was intended to mimic a clinical situation in IBD where a patient would be placed on immunosuppressive medication and rHuKGF might hasten recovery and/or repair of the damaged gastrointestinal tract.

In the long-term study, several different doses of rHuKGF were evaluated for therapeutic efficacy. Data shown illustrate the effects of 1.0 and 3.0 mg/kg, both given every other day starting on day 0. In an attempt to mimic a clinical dosing regimen, a separate arm of the study delayed dosing until day 4 after administration of the 4% DSS drinking water, at which point, clinical symptoms are grossly manifest. Figure 1 shows that control mice receiving vehicle only started to die on day 6 and were all dead by day 9 of DSS administration. In contrast, mice receiving 1.0 mg/kg rHuKGF maintained 80% survival to day 20 (P < 0.0001) and 3.0 mg/kg of rHuKGF maintained 70% survival up to day 20 (P < 0.0001). If rHuKGF administration was delayed until day 4 of the study, then the 1.0- and 3.0-mg/kg doses achieved survival rates of 80% (P < 0.0001) and 90% (P < 0.0001), respectively. To control for any possible pathological effect of rHuKGF, a group of normal SJL/J mice drinking ordinary water were dosed with 3.0 mg/kg of rHuKGF every other day for the 21-day duration of the study and they maintained 100% survival with no clinical symptoms.

Figure 2A illustrates the results from the acute necropsy study. Control animals receiving vehicle only lost body weight steadily over the 8-day course of the experiment until all appeared moribund at the time of necropsy with an average body weight loss of ~25%. In contrast, the animals receiving either 1.0 or 3.0 mg/kg of rHuKGF every other day from day 0 exhibited minimal body weight loss relative to the vehicle control group on day 8 (P < 0.0001 in both cases). These two disease groups of mice treated with rHuKGF maintained their original body weight in a manner similar to normal SJL/J mice given 3.0 mg/kg rHuKGF or normal age- and sex-matched mice (data not shown). Figure 2B shows data from the recovery study. Therapeutic dosing with rHuKGF started on day 4 and DSS was withdrawn and replaced with ordinary water on day 7. By day 9, all animals had started to regain weight but both the 1.0- and 3.0-mg/kg rHuKGF treatment groups were not significantly different from the vehicle control group with respect to body weight loss (P = 0.2289 and P = 0.8850, respectively). Weight regain continued until the end of the experiment on day 19 when all animals had almost returned to their starting body weight and the two treatment groups were once again not significantly different from the vehicle control group (P = 0.2093 for 1.0 mg/kg and P = 0.9993 for 3.0 mg/kg rHuKGF). At all time points, normal SJL/J mice receiving 3.0 mg/kg of rHuKGF gained weight in a manner similar to normal, untreated mice (data not shown).

rHuKGF improves diarrhea and hematological disease markers in the DSS model of IBD. Analysis of various disease-associated parameters further substantiated the observation that ongoing administration of rHuKGF ameliorates disease in this model of IBD. Mice in the acute necropsy study were assessed for the presence of diarrhea as described in MATERIALS AND METHODS. In the acute necropsy study, the stool score for the vehicle control mice rose steadily from 0 on day 0 to 3.4 ± 0.4 on day 8. This was significantly improved in both rHuKGF treatment groups at this terminal time point with a score of 1.7 ± 0.2 for the 1.0 mg/kg (P < 0.0001) and 1.6 ± 0.3 for the 3.0 mg/kg...
Normal mice, either untreated or given 3.0 mg/kg rHuKGF, scored 0.0 throughout the entire course of the experiment. Vehicle control mice had an average white blood cell (WBC) count of $17.38 \pm 0.80 \times 10^3/\mu l$ compared with $11.37 \pm 0.41 \times 10^3/\mu l$ ($P = 0.0220$) for the 1.0 mg/kg group and $10.25 \pm 0.58 \times 10^3/\mu l$ ($P = 0.0121$) for the 3.0 mg/kg rHuKGF treatment group. Normal mice receiving rHuKGF had an average WBC count of $4.23 \pm 1.18 \times 10^3/\mu l$, which is within the limits of normal values (Amgen unpublished data). The differential analysis revealed that this ele-

Fig. 2. Administration of rHuKGF attenuates disease associated body weight loss in the DSS model of IBD (A) and favors body weight regain when recovery from DSS exposure is allowed (B). A: mice received a sterile filtered 4% aqueous solution of DSS ad libitum from day 0 and were injected with rHuKGF or vehicle alone every other day starting from day 0, as indicated. Normal mice were fed ordinary, sterile filtered water and injected with 3.0 mg/kg of rHuKGF every other day from day 0. B: mice received a sterile filtered 4% aqueous solution of DSS ad libitum from day 0 until day 4 when normal water was substituted and were injected with rHuKGF or vehicle alone every other day starting from day 4 until necropsy on day 19. Normal mice were fed ordinary, sterile filtered water and injected with 3.0 mg/kg of rHuKGF every other day from day 0 until necropsy on day 19. In all cases, results represent the average of 10 animals. Statistical significance vs. the DSS vehicle control was calculated by ANOVA. *$P < 0.05$; **$P < 0.001$.

($P < 0.0001$). Normal mice, either untreated or given 3.0 mg/kg rHuKGF, scored 0.0 throughout the entire course of the experiment. Hematological analysis was performed on mice in the acute necropsy study at the time of euthanasia and a complete blood count with differential was obtained. Vehicle control mice had an average white blood cell (WBC) count of $17.38 \pm 0.80 \times 10^3/\mu l$ compared with $11.37 \pm 0.41 \times 10^3/\mu l$ ($P = 0.0220$) for the 1.0 mg/kg group and $10.25 \pm 0.58 \times 10^3/\mu l$ ($P = 0.0121$) for the 3.0 mg/kg rHuKGF treatment group. Normal mice receiving rHuKGF had an average WBC count of $4.23 \pm 1.18 \times 10^3/\mu l$, which is within the limits of normal values (Amgen unpublished data). The differential analysis revealed that this ele-

Fig. 3. Representative colons from normal mice with (A) and without rHuKGF (B) and mice with DSS-induced colitis treated with vehicle (C and E) or 3.0 mg/kg of rHuKGF (D and F). In the acute necropsy study, mice received a sterile filtered 4% aqueous solution of DSS ad libitum from day 0 and were injected with vehicle alone (C) or 3.0 mg/kg of rHuKGF (D) every other day starting from day 0 until necropsy on day 8. In the recovery study, mice received a sterile filtered 4% aqueous solution of DSS ad libitum from day 0 until day 4 when normal water was substituted and were injected with vehicle alone (E) or 3.0 mg/kg of rHuKGF every other day starting from day 4 (F), until necropsy on day 19.
vation in the WBC count in the disease group was composed mainly of neutrophils, which is consistent with the increased inflammatory infiltrate seen in the colon in this model. Hemoglobin levels in the vehicle control group averaged 10.76 ± 0.24 g/dl. rHuKGF exhibited a tendency to improve these levels in the two treatment groups: 11.45 ± 0.13 g/dl (P = 0.2266) for the 1.0 mg/kg group and 12.65 ± 0.34 g/dl (P = 0.0394) for the 3.0 mg/kg group. Normal mice receiving 3.0 mg/kg of rHuKGF had an average hemoglobin of 14.32 ± 0.10 g/dl, which is within the limits of normal values (Amgen unpublished data).

**rHuKGF improves gross anatomy and histopathology in the DSS model of IBD.** Although parameters such as body weight, stool score, and WBC count are important and relevant to clinical IBD, the histological findings of inflammatory cell infiltrate and destruction of the tissue of the gastrointestinal tract are disease hallmarks connected to severe intestinal upset. Shortening of the large intestine is also known to be a reliable disease marker in this model (16). In the acute necropsy study, colon lengths from the diseased mice treated with either the 1.0-mg/kg (9.66 ± 0.20 cm) or the 3.0-mg/kg dose (10.17 ± 0.19 cm) of rHuKGF were significantly closer to the average length for normal, untreated mice (10.01 ± 0.32 cm) than those from the vehicle control groups (6.75 ± 0.30 cm, P < 0.0001 in both cases). Similarly, in the recovery study, the 1.0 mg/kg (10.31 ± 0.10 cm) and 3.0 mg/kg (10.20 ± 0.19 cm) treatment groups were significantly closer to normal than the vehicle control group (7.27 ± 0.16 cm) (P < 0.0001 in both cases).

Gross observation also proved revealing. Normal, untreated mice and normal mice treated with 3.0 mg/kg of rHuKGF every other day and euthanized on day 19 both had large intestines that were unremarkable in length and appearance (Fig. 3A and 3B) with well-defined cecum and normal, thin bore, and several visible solid stool pellets along the length of the organ. The colons from the vehicle controls (Fig. 3C and 3E) were generally shorter and thicker than normal with a less well-defined cecum and held no solid stool pellets (indicative of diarrhea). Colons of the mice treated with rHuKGF were considerably more normal in appearance (Fig. 3D and 3F), although the rHuKGF-treated colons in the recovery study showed some degree of shortening and thickening (Fig. 3F). This gross observation is supported by the histological analysis of cross sections of the large intestine stained with hematoxylin and eosin. Sections were prepared and scored as described in MATERIALS AND METHODS. Figure 4 illustrates that in the acute necropsy study, only the 3.0-mg/kg dose significantly improved the average histopathological score for the entire colon (1.0 mg/kg vs. vehicle, P = 0.8609; and 3.0 mg/kg vs. vehicle, P = 0.0252). In the recovery study, both doses significantly improved the histopathology score relative to the control, but the effect was more pronounced with the 3.0-mg/kg dose (1.0 mg/kg vs. vehicle, P = 0.0285; and 3.0 mg/kg vs. vehicle, P = 0.0004). Normal mice given

![Image](http://ajpgi.physiology.org/)

**Fig. 4.** Administration of rHuKGF improves the histopathological score of the large intestine in the DSS model of IBD. In the acute necropsy study, mice received a sterile filtered 4% aqueous solution of DSS ad libitum from day 0 and were injected with rHuKGF or vehicle alone every other day starting from day 0 until necropsy on day 8. In the recovery study, mice received a sterile filtered 4% aqueous solution of DSS ad libitum from day 0 until day 4 when normal water was substituted and were injected with rHuKGF or vehicle alone every other day starting from day 4 until necropsy on day 19. Normal mice were fed ordinary, sterile filtered water and injected with 3.0 mg/kg of rHuKGF every other day from day 0 until necropsy on day 19. Hematoxylin- and eosin-stained biopsies of the proximal, middle, and distal large intestine were assessed for extent of inflammation on a scale of 0–5 (0 = absent, 5 = severe) by a pathologist in a blinded fashion. In all cases data represent the average value for the whole colon of 5–9 animals ± SE. Statistical significance vs. the DSS vehicle control was calculated by the probability associated with the unpaired heteroschedastic Student’s t-test. *P < 0.05; **P < 0.001.
3.0 mg/kg of rHuKGF every other day and euthanized on day 19 exhibited scores similar to normal, untreated mice.

Figure 5 illustrates the improved appearance of the large intestine tissue when treated with rHuKGF relative to the vehicle controls. Figure 5A and 5B are colon tissues from normal, untreated mice and normal mice dosed with 3.0 mg/kg of rHuKGF every other day and euthanized on day 19, respectively. Both are unremarkable in appearance. Colons from vehicle-control mice exhibited mucosal erosion and crypt abcessation with an accompanying inflammatory infiltrate composed primarily of lymphocytes, with fewer macrophages and neutrophils (Figs. 5D and 5F). Colonic damage in the acute necropsy study was primarily characterized by mucosal inflammatory infiltrate and regularly dispersed crypt hyperplasia and some crypt abcessation along with early destruction of the surface epithelium (Fig. 5D). Colonic damage in the recovery study was even more pronounced with inflammatory infiltrate of the mucosa as well as submucosal edema and lymphocytic and histiocytic infiltrate (Fig. 5F). There was almost complete destruction of both superficial and crypt epithelial cells. Treatment with 3.0 mg/kg of rHuKGF effected a substantial improvement in both studies (Figs. 5C and 5E). rHuKGF maintained crypt length and general crypt architecture as well and preserved the superficial mucosal surface. The degree of inflammation in the rHuKGF-treated colons was also substantially decreased. Histological analysis was also performed on samples from the small intestine, lungs, liver, and spleen, but no significant lesions were found.

**rHuKGF increases colonic ITF production in the DSS model of IBD.** In an attempt to uncover a mechanistic explanation for the beneficial effects of rHuKGF in this model, we performed immunohistochemistry for the presence of ITF in large intestine sections, as rHuKGF is known to upregulate expression of this factor in the gastrointestinal tract (28). It has also been shown that mice lacking ITF are more susceptible to DSS colitis (31), leading us to believe that rHuKGF may well be mediating its beneficial effects at least partially through regulation of ITF production. Immunohistochemistry was performed as described in MATERIALS AND METHODS to generate an overall score for the

**Fig. 5.** Administration of rHuKGF protects against the colonic mucosal damage associated with disease in the DSS model of IBD. A: the large intestine from a normal SJL/J mouse; B: large intestine from a normal mouse treated with 3.0 mg/kg of rHuKGF every other day from day 0 until death on day 19. In the acute necropsy study, mice received a sterile filtered 4% aqueous solution of DSS ad libitum from day 0 and were injected with 3.0 mg/kg of rHuKGF (C) or vehicle alone (D) every other day starting from day 0 until necropsy on day 8. In the recovery study, mice received a sterile filtered 4% aqueous solution of DSS ad libitum from day 0 until day 4 when normal water was substituted and were injected with 3.0 mg/kg of rHuKGF (E) or vehicle alone (F) every other day starting from day 4, until necropsy on day 19. Shown here are hematoxylin and eosin stained biopsies of the middle section of the large intestine from the various different groups. All samples are from the middle cross section of the mouse large intestine.
presence of ITF in the large intestine. In the acute necropsy study, mice treated with rHuKGF (1.0 mg/kg) had scores of colonic ITF (3.0 ± 0.20) higher than vehicle control mice (2.0 ± 0.09, P = 0.0058). In turn, vehicle control mice had ITF scores higher than normal mice given rHuKGF (1.3 ± 0.27, P = 0.0594). However, normal mice given rHuKGF had ITF scores not significantly different from normal, untreated mice (1.1 ± 0.07, P = 0.2697). Similarly, in the recovery study, mice treated with rHuKGF (1.0 mg/kg) had scores of colonic ITF (2.4 ± 0.19) higher than vehicle control mice (1.3 ± 0.22, P = 0.0650). We also assessed the levels of ITF mRNA by in situ hybridization, as described in the MATERIALS and METHODS. At variance with the immunohistochemistry scores, the scores for ITF mRNA were not significantly different between normal and diseased mice, treated or not treated with rHuKGF (data not shown).

rHuKGF ameliorates body weight loss, diarrhea, and levels of SAA in the CD4+CD45RBhi T cell transfer model of IBD. We also sought to evaluate the efficacy of rHuKGF in another model of IBD more relevant to immune-mediated chronic intestinal inflammation. The CD4+CD45RBhi T cell transfer model of IBD is well characterized, has a requirement for intestinal microbiota, and involves the full spectrum of the immunoinflammatory cascade (2). Disease was induced as described in MATERIALS and METHODS and rHuKGF was dosed at 3.0 mg/kg three times per week, either starting on day 0 for the prevention cohort or after an individual mouse had lost 10% of its original weight for the therapeutic cohort. Negative control in this model is represented by scid/scid mice injected with CD4+CD45RBlo T cells. These were also dosed with 3.0 mg/kg of rHuKGF three times per week from day 0. Figure 6 illustrates the effect of rHuKGF on the body weight loss associated with disease development in this model. Mice given disease-causing CD4+CD45RBhi T cells and vehicle control lost body weight uniformly and steadily until all the animals were moribund with an average body weight loss of ~25% by day 30. Administration of rHuKGF from day 0 completely prevented body weight loss (P < 0.0001). At the end of the experiment, rHuKGF-treated mice had a body weight similar to the mice receiving nonpathogenic CD4+CD45RBlo cells and rHuKGF. If administration of rHuKGF was delayed until individual animals had lost 10% of their original body weight, then the body weight loss was significantly reduced relative to administration of vehicle control (P < 0.0001). The scid/scid mice injected with CD4+CD45RBlo T cells and dosed with rHuKGF from day 0 gained body weight in a manner identical to untreated CD4+CD45RBlo-injected scid/scid mice and no abnormal effects were observed (data not shown). In certain studies, we housed all the animals individually to assess food and water consumption, and it was repeatedly observed that, on average, diseased and nondiseased animals consumed the same amount of food and water on a weekly basis (data not shown). Hence, the body weight loss that occurs seems to be related to malabsorption of nutrients and not decreased food intake. Administration of these doses of rHuKGF in either the disease or nondisease state did not alter food and water consumption (data not shown).
The administration of rHuKGF was found to be undetectable or minimal at the beginning of the experiment and in all cases were found to be undetectable or minimal (<2.0 μg/ml). As for the DSS model, hematological analysis was performed on all mice at the time of euthanasia and a complete blood count with differential was obtained. There were trends toward improvements in disease-associated hematological markers in the rHuKGF treatment groups, particularly with respect to the levels of WBCs and hemoglobin (data not shown).

**rHuKGF improves histopathology and increases colonic ITF production in the CD4+CD45RBHi T cell transfer model of IBD.** As in the DSS model, histological analysis of the small intestine, lungs, liver, spleen, and large intestine was performed. However, the primary histological damage in this model occurs in the colon (3). The diseased vehicle control mice had an average histopathological score of 3.0 ± 0.1 for this organ at the time of necropsy (Fig. 9A). Mice receiving rHuKGF from day 0 had a substantially improved average score of 2.5 ± 0.2 (P = 0.0791 relative to vehicle control mice) and mice receiving rHuKGF after 10% body weight loss had a significantly improved average score of 1.9 ± 0.1 (P < 0.0001 relative to vehicle control mice). Although the rHuKGF therapeutic group scored lower than the preventative group, they were not significantly different from each other (P = 0.2912). The nondiseased CD4+CD45RBLo-injected scid/scid mice, either untreated or given rHuKGF, had no detectable colonic damage. Colonic mucosal protection afforded by rHuKGF is illustrated in Fig. 10. Figure 10A shows the middle section of the colon from a diseased vehicle control mouse. There is mucosal hyperplasia with superficial erosions, crypt abscission and marked mucin depletion. There is also a prominent, accompanying mixed-inflammatory cellular infiltrate.

As in the DSS model, we performed immunohistochemical analysis for the presence of ITF in these sections. Results show specific expression of ITF localized to goblet cells along the entire colonic crypt to the luminal surface with extrusion of ITF into the crypt and colonic lumens. Figure 9B demonstrates that administration of rHuKGF elevated the levels of ITF

**Fig. 7. Administration of rHuKGF attenuates diarrhea in the CD4+CD45RBHi T cell transfer model of IBD.** Disease was induced by the injection of 4 × 10⁵ sorted CD4+CD45RBHi T cells into scid/scid mice. RBLo mice received 4 × 10⁵ nonpathogenic CD4+CD45RBLo cells. Preventative treatment consisted of subcutaneous injection of 3.0 mg/kg of rHuKGF or vehicle 3 times per week starting on day 0. Therapeutic treatment with rHuKGF was started when individual animals had attained 10% body weight loss. Treatment was continued in all cases until necropsy on day 30. Score was assigned from 0–3 on the following criteria: 0 = absent, 1 = slight perianal stool, 2 = marked loose stool, 3 = frank and complete perianal stool. Data shown represent the average from an n = 10 for the disease groups and n = 4 for the RBLo control mice. Statistical significance vs. the CD45RBHi vehicle control group was calculated by ANOVA. *P < 0.05; **P < 0.001.

**Fig. 8. Administration of rHuKGF reduces the levels of serum amyloid A in the CD4+CD45RB T cell transfer model of IBD.** Disease was induced by the injection of 4 × 10⁵ sorted CD4+CD45RBHi T cells into scid/scid mice. RBLo mice received 4 × 10⁵ nonpathogenic CD4+CD45RBLo cells. Preventative treatment consisted of subcutaneous injection of 3.0 mg/kg of rHuKGF or vehicle 3 times per week starting on day 0. Therapeutic treatment with rHuKGF was started when individual animals had attained 10% body weight loss. Treatment was continued in all cases until necropsy on day 30. Data shown represent the average with an n = 10 for the disease groups and an n = 4 for the RBLo control group ± SE. Statistical significance vs. the CD4+CD45RBHi vehicle control group was calculated by the probability associated with the unpaired heterogeneous Student’s t-test. ***P < 0.001.
DISCUSSION

This study demonstrates that administration of rHuKGF can be of considerable benefit in animal models of IBD, improving the mucosal integrity of the large intestine as well as several distinct systemic parameters usually associated with the most debilitating symptoms in humans. Although there is currently intensive study of human IBD, the majority of therapy is still symptomatic and even recently approved specific biological therapies are far from appropriate for all patients (36, 47). There are few agents that putatively target the more downstream events in the immunoinflammatory cascade at the level of tissue destruction. rHuKGF has been shown to be efficacious in treating the mucositis associated with chemotherapy and radiation therapy in animal studies (15, 17, 48) and clinical trials (11, 44). Clarke and colleagues (11) demonstrated a significant benefit in chemotherapy-induced oral mucositis in colorectal cancer, which is supportive of the conceptual appeal of using rHuKGF as a therapeutic for other gastrointestinal disorders, including intestinal inflammatory conditions.

Our data in the DSS study suggest that ongoing administration of 1.0 or 3.0 mg/kg of rHuKGF can significantly ameliorate both the systemic and colonic effects of disease in the DSS model of IBD, either in an acute treatment scenario or in a more prolonged recovery scenario, once the primary source of immunoinflammatory damage has been controlled. Survival data demonstrate that delaying treatment until day 4 (relatively late in this acute model) can significantly prolong survival, suggesting that this agent may be useful in a wide range of clinical scenarios. Although both doses of 1.0 or 3.0 mg/kg of rHuKGF significantly improve systemic symptoms, only the dose of 3.0 mg/kg of rHuKGF markedly improved the actual histological appearance of the large intestine. Although the relative beneficial effects of rHuKGF were more evident in the necropsy study, the overall histopathological scores were higher in the recovery study. This observation, coupled with the large intestine length data and the gross observations, suggests that the development of gross and histological damage of the colon may be a lagging disease indicator in this model. Future studies should determine the optimal dosing regimen in both preclinical and clinical settings.
The data presented here with rHuKGF in the DSS model is similar to the data reported for KGF-2 by Miceli et al. (32). However, the schedules were different in the two studies with KGF-2 being dosed daily and rHuKGF dosing every other day. Although KGF and KGF-2 are both members of the FGF superfamily (7), they share only 57% homology (27) and are quite distinct in their respective affinities for the FGF receptors and in the phenotypes of the their respective knockouts. KGF exhibits strict specificity for the FGFR2iiib receptor (24) with a $K_i$ of 0.1 nM, whereas KGF-2 binds the FGFR2iiib receptor with 10-fold less affinity (32) and also binds the FGFR1iiib receptor (27). Several publications document a role for KGF-2 in lung and limb development (10, 50), and KGF-2 knockout mice die perinatally, having failed to develop lungs and limbs (34). KGF knockout mice, in contrast, survive to maturity and exhibit a rough hair coat (20), but are otherwise normal, possibly due to the greater affinity and narrower specificity of KGF for its receptor. It has been reported that KGF knockout mice are more susceptible to DSS-induced colitis (32), and this may be due to the fact that KGF is more intimately involved with the mucosal immune system. In fact, KGF has been reported to be secreted by γδ intraepithelial lymphocytes (8); hence, we judged it likely to be efficacious in a T cell-dependent model of intestinal inflammation.

Although it is a useful model and its general recalcitrance to other effective therapeutic modalities makes our data with rHuKGF compelling, the acute DSS model lacks features of an immune-mediated model of chronic intestinal inflammation, particularly the requirement for intestinal microflora (5). For these reasons, we also evaluated rHuKGF in the CD4+ CD45RBhi T cell transfer model of IBD. Here, rHuKGF also proved to be efficacious, ameliorating mucosal damage of the large intestine as well as improving various systemic parameters, most notably body weight loss and SAA levels. Diarrhea was also significantly resolved by rHuKGF in both disease models.

Previous studies in humans and in rodent models of colitis indicated that plasma levels of the somatic and IGF-1 are decreased in IBD (6, 46). In an attempt to elucidate the relevant mechanism of action of the drug in this disease state, we examined plasma levels of IGF-1 at necropsy in the T cell transfer disease model to gain insight as to whether potential modulation of colitis by KGF involved changes in plasma IGF-1 concentrations. There was no significant difference in plasma concentrations between disease and treatment groups, and the values were within the normal range, indicating that the decreased colonic mucosal inflammation with KGF does not involve modulation of systemic levels of IGF-1 as a potential mechanism.

We also examined levels of ITF in the small and large intestine for both the DSS and the CD4+ CD45RBhi T cell transfer model. This molecule is known to be an important regulator of epithelial integrity in the gastrointestinal tract and rHuKGF has been shown to increase the numbers of goblet cells and the mucosal expression of ITF (28, 31). Furthermore, mice lacking ITF are significantly more susceptible to DSS colitis (31). There were no significant differences among groups in the small intestine. In the large intestine, the presence of disease alone was sufficient to raise the expression levels of ITF protein, but administering rHuKGF raised levels of ITF significantly higher still. This phenomenon was observed in both disease models. Hence, we hypothesize that this event would lead to increased production of mucin and its many compo-
ments. This could effectively improve the barrier between the afferent component of the mucosal immune system (antigen presenting cells and T cells) and the antigen-rich environment of the lumen, arresting the cycle of immune system activation and inflammation. The considerable discrepancy between the levels of ITF protein detected by immunohistochemistry and the mRNA coding for ITF detected in any one individual sample is noteworthy. Our results suggest that production of ITF, whether induced by disease or rHuKGF, may be regulated at the posttranscriptional level. The molecular biology of the pathway by which KGF exerts its effects on ITF is now being elucidated (25, 26) and future studies should prove revealing. However, the levels of ITF protein would be of more functional relevance in these analyses.

A role for KGF has been demonstrated in many different physiological pathways. Previous studies (35) have shown gross pathology in embryonic KGF transgenic mice, but comparisons of long-term high local concentrations of KGF to relatively short-term systemic doses may not be clinically relevant. KGF has been shown to be an important mediator of hair follicle growth and development (13), skin regeneration (38), as well as prostate gland and seminal vesicle development (1, 45). Therefore, it is likely that there are some pharmacologic effects on other organ systems, but this did not appear to interfere with the beneficial effects on the gastrointestinal tract in this disease model. Furthermore, no gross lesions in any major organ system were found in the rHuKGF-treated mice in this study. In conclusion, we believe that this study demonstrates that administration of rHuKGF may be of considerable benefit in the management of IBD.

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REFERENCES


TREATMENT OF MOUSE MODELS OF IBD WITH rhKGF


