Effect of Mucosal Immunomodulation With Fed Cholera Toxin on Healing of Experimental Colonic Anastomosis

Mehmet Kaplan, M.D.,* B. Bülent Menteş, M.D.,* Ertan Tatlıcıoğlu, M.D.,* Başak Kayhan, M.D.,† Cemalettin Aybay, M.D., Ph.D.†

*From the Departments of *Surgery and †Immunology, Gazi University Medical School, Ankara, Turkey*

PURPOSE: The aim of this study was to investigate in rats whether preoperative orogastric administration of low doses of cholera toxin would influence the mechanical strength of experimental colonic anastomosis on the basis of the gut mucosal immunomodulation effect of this antigen. METHODS: The cholera toxin group (n = 14) was fed 10 μg of cholera toxin in phosphate-buffered saline three times before surgery at 10-day intervals, whereas the controls (n = 14) received phosphate-buffered saline only. Twenty-four hours after the last dose of cholera toxin (or placebo in control group), the animals underwent left colonic transection and anastomosis. Seven days after colonic transection-anastomosis, the bursting pressure of the anastomotic segment was recorded *in situ*. Perianastomotic and extra-anastomotic tissue samples were obtained for measurements of tissue transforming growth factor-beta, interleukin-6, and interferon-gamma levels with enzyme-linked immunosorbent assay. RESULTS: Cholera toxin administration resulted in a significantly higher bursting pressure than in the control group (165.78 ± 12.37 *vs.* 138.4 ± 7.87 mmHg; $P < 0.001$). Compared with the control group, the heightened mechanical strength of colonic anastomosis provided by cholera toxin was associated with significant increases in the perianastomotic tissue levels of transforming growth factor-beta (199.34 ± 24.85 *vs.* 70.66 ± 10.63 pg/ml; $P < 0.001$) and interleukin-6 (439.31 ± 95.14 *vs.* 289.57 ± 96.59 pg/ml; $P = 0.001$), whereas interferon-gamma was significantly lower (174.04 ± 44.82 *vs.* 219.00 ± 31.35 pg/ml; $P < 0.05$). This cytokine pattern induced by cholera toxin in the wound milieu was also found to be similar in the extra-anastomotic colon. CONCLUSION: The mechanical strength of uncomplicated experimental colonic anastomosis increased significantly with gut mucosal immunomodulation with repeated low preoperative doses of cholera toxin. This enhanced healing had significant positive correlation with the colonic tissue level of transforming growth factor-beta and inverse correlation with interferon-gamma. If the relevant dose regimen is identified and its safety is assured in humans, gut mucosal immunomodulation might provide an efficient, safe, and inexpensive tool to improve surgical outcome in colorectal surgery, particularly in high-risk situations. [Key words: Mucosal immunity; Immunomodulators; Anastomosis; Repair; Colorectal surgery]

Kaplan M, Menteş BB, Tatlıcıoğlu E, Kayhan B, Aybay C. Effect of mucosal immunomodulation with fed cholera toxin on healing of experimental colonic anastomosis. Dis Colon Rectum 2002;45:819–825.

Presented at the meeting of the Mediterranean Society of Coloproctology, Nazareth, Israel, September 24 to 27, 2000.
No reprints are available.

Anastomotic complications continue to appear to be unavoidable in large-bowel surgery.[1,2] Well-known systemic factors (*e.g.*, age, protein malnutrition, vitamin deficiency, corticosteroids, and hypoxia) and local factors (*e.g.*, blood supply, tension on anastomosis, peritoneal sepsis, fecal loading, radiotherapy, and drains) influence colonic anastomotic healing.[3,4] Even under optimal conditions, clinical complications related to colonic anastomotic leakage have been found in 2 to 8 percent of patients, whereas radiologically detectable but asymptomatic leaks have been demonstrated in up to 50 percent,[5] which indicates that studies on anastomotic healing of the colon are still needed to find ways to improve surgical outcome, particularly in high-risk situations.[2,3] Some experimental data have addressed the possible contributory roles of collagenase inhibitors,[6] free radical scavengers,[7] prostaglandin synthesis inhibitors,[8] or externally applied electromagnetic fields[9] in healing of colonic anastomosis. Conclusions primarily are based on the bursting pressure (BP) of the anastomotic segment, which is a reliable measure of its resistance to increasing intraluminal pressure (for a review, see Hendriks and Mastboom[10]). Nevertheless, no widely applied agent exists that would significantly promote colonic anastomotic healing, and the best the physician can do appears to be to maintain optimal physiologic conditions for the inborn healing mechanisms of the patient to proceed.

The relationship between the immune system and wound healing has been appreciated for a long time[11,12] on the basis of the common observations that infection, sepsis, trauma, and hemorrhage, as well as antineoplastic agents that suppress immune functions, also impair healing.[4,13–15] Recent data have suggested that immune components such as macrophages and lymphocytes, as well as platelets and fibroblasts, secrete polypeptide growth factors that regulate certain phases of wound healing by binding to their specific cell surface receptors.[16–19] Of these

factors, transforming growth factor (TGF)-beta, which is secreted mainly by the recently identified T helper 3 (TH3) lymphocyte subtype, accelerates wound healing by stimulating fibroblast proliferation and collagen synthesis.[19–24] However, interferon (IFN)-gamma, which is a major product of the TH1 subtype, impairs wound healing by decreasing collagen deposition, neovascularization, and probably collagen cross-linking in a dose-dependent manner.[19,24,25] Systemic administration of IFN-gamma has been reported to impair incisional wound healing,[25] whereas accelerated healing was induced by TGF-beta.[20] In the gastrointestinal tract, selective activation of immune cells by orally administered antigens, with the resultant enhanced secretion of specific cytokines, has been attempted in the treatment of some immune disorders, such as inflammatory bowel disease, arthritis, and allergy.[26] This mucosal immunomodulation is inexpensive, free of significant side effects, and relatively easy to apply.[27] Therefore, selective activation of certain subgroups of cells and enhanced secretion of a certain panel of cytokines from these subgroups might provide a new tool to modulate wound repair.

Cholera toxin (ChT) is a potent mucosal adjuvant that stimulates mucosal immune response, alters oral tolerance, and selectively stimulates TH2 and TH3 and suppresses TH1 lymphocytes.[28–31] Given the theoretical beneficial effects of this lymphocyte menu on wound healing, we investigated whether orally administered ChT would influence the mechanical strength of experimental colonic anastomosis. The BP of the anastomotic segment and tissue levels of some of the related cytokines (IFN-gamma, interleukin (IL)-6, and TGF-beta) were studied.

METHODS

Male Wistar albino rats, 8 to 10 weeks old and weighing 180 to 200 g, were used. They were kept in a temperature-controlled environment and had free access to standard rat food and water.

Study Design and Administration of ChT

Twenty-eight rats were randomly assigned to 2 groups of 14 each. They received a 4 percent $NaCO_3$ solution as drinking water for 24 hours before orogastric intubation was performed under light ether anesthesia, as described previously.[32] Group 1 (ChT group; n = 14) was fed 10 μg of ChT (Sigma Chemical Co., Steinheim, Germany) dissolved in 0.5 ml of phosphate-buffered saline (PBS; 137 mmol/l NaCl, 2.7 mmol/l KCl, 8 mmol/l $Na_2HPO_4 \cdot 7\ H_2O$, 1.5 mmol/l KH_2PO_4). Group 2 (control; n = 14) was fed 0.5 ml of PBS only. This procedure was repeated three times at ten-day intervals. No method of colonic cleansing was attempted.

Surgical Procedures

Twenty-four hours after the last dose of ChT (or placebo in control group), the animals were anesthetized by an intramuscular injection of ketamine hydrochloride (50 mg per kilogram of body weight). Under semisterile conditions and through a 3-cm-long midline abdominal incision, the entire circumference of the left colon was transected 3 cm proximal to the peritoneal reflection. Meticulous attention was paid not to damage the blood supply. An end-to-end anastomosis was then performed by the same surgeon, who was blinded to the study groups, with eight interrupted inverting sutures through all layers, made with 7-0 polypropylene suture material (Prolene®, Ethicon, Birmingham, UK).

Determination of BP and Tissue Sampling

Seven days after colonic transection-anastomosis, the abdominal incision was reopened after ketamine anesthesia. The anastomosis was not freed of adhesions, and great care was taken not to disturb the anastomotic segment and not to cause any bleeding. The rectum was dissected and incised approximately 1 cm distal to the peritoneal reflection. The intact transverse colon (approximately 3 cm proximal to the anastomosis) was also incised and connected to a plastic catheter. This was connected to an infusion pump (IVAC® 770, IVAC Corp., San Diego, CA) with 2 ml of saline given per minute and with the simultaneous intraluminal pressure measured digitally. After clear fluid emerged, the distal end was tied off. The pressure of rupture (BP) was then recorded *in situ*.

After BP was noted, the animals were killed by cardiac blood aspiration. The colon including the anastomosis was dissected at the mesenteric border and resected. It was incised along its antimesenteric border and opened. The sutures were removed, and 0.5 g of perianastomotic colon, including the anastomotic line, was harvested. A fresh tissue sample was obtained from the cecum for extra-anastomotic measurement of tissue cytokine levels. Thus, one peri-

anastomotic and one extra-anastomotic tissue sample were obtained from each animal.

Tissue Cytokine Measurements

The specimens were prepared for measurement of TGF-beta, IL-6, and IFN-gamma levels. Fresh bowel tissue was prepared for enzyme-linked immunosorbent assay by homogenization as described previously.[33] In brief, each specimen was homogenized for 60 seconds in 10 ml of PBS that contained a cocktail of protease inhibitors that included 2 mM phenylmethylsulfonyl fluoride and 2 μg/ml aprotinin, leupeptin, and pepstatin A (Sigma) to inhibit proteolysis of cytokines. All protease inhibitors were used as recommended, and stock solutions were prepared daily when necessary. Then, homogenates were obtained with the stirrer equipped with a tissue grinder with Teflon® (E.I. du Pont de Nemours & Co., Inc., Wilmington, DE) pestle (Jencons Scientific Ltd., Bedfordshire, UK) and were ultra-centrifuged with 10,000 rpm at 4°C for 45 minutes, and the supernatants were sampled by micropipettes and stored at −70°C. Cytokines were measured by enzyme-linked immunosorbent assay with rat IFN-γ (BioSource International Inc., Camarillo, CA), rat IL-6 (BioSource), or rat TGF-β kit (MedSystems Diagnostics GmbH, Vienna, Austria).

Statistical Analysis

Means and standard deviations (SDs) of the data were calculated. Comparisons were made with the Mann-Whitney *U* test, and *P* values of less than 0.05 were accepted as statistically significant. Associations of cytokine levels and BPs were evaluated with the Pearson correlation analysis, and positive and negative *r* values at or below 0.05 *P* levels were accepted as significant positive and negative correlations, respectively.

RESULTS

No deaths occurred after any of the procedures. A few episodes of loose stool were observed in the majority of the rats in the ChT group beginning a few days after each immunization. During the harvesting procedures on the seventh postoperative day, a single rat in the control group was found to have developed anastomotic leakage and abscess. This animal was excluded from the study.

In the control group of rats, BP was 138.4 ± 7.87 mmHg on the seventh postoperative day. ChT administration resulted in a significantly higher BP (165.78 ± 12.37) than in the control group ($P < 0.001$). The heightened mechanical strength of colonic anastomosis provided by ChT was associated with significant increases (compared with the control group) in the perianastomotic tissue levels of TGF-beta (199.34 ± 24.85 *vs.* 70.66 ± 10.63 pg/ml; $P < 0.001$) and IL-6 (439.31 ± 95.14 *vs.* 289.57 ± 96.59 pg/ml; $P = 0.001$), whereas IFN-gamma was significantly lower (174.04 ± 44.82 *vs.* 219.00 ± 31.35 pg/ml; $P < 0.05$; Table 1). A significant positive correlation between BP and the perianastomotic level of TGF-beta was noted ($r = 0.59$, $P = 0.025$; Fig. 1). An insignificant positive correlation existed between BP and the perianastomotic IL-6 level ($r = 0.43$, $P = 0.118$), whereas a significant negative correlation was found with the IFN-gamma level ($r = -0.64$, $P = 0.013$; Fig. 2).

This cytokine pattern induced by ChT in the wound milieu was found to be similar in the extra-anastomotic colon as well. Compared with control levels, ChT resulted in significantly higher cecal levels of TGF-beta (211.20 ± 34.99 *vs.* 50.66 ± 10.63 pg/ml; $P < 0.001$) and IL-6 (841.58 ± 148.84 *vs.* 521.62 ± 59.86 pg/ml; $P < 0.001$) but a significantly lower level of IFN-gamma (148 ± 23.86 *vs.* 191.66 ± 43.70 pg/ml; $P < 0.05$; Table 2).

Table 1.
Bursting Pressure and Perianastomotic Tissue Cytokine Levels of the Study Groups

	BP (mmHg)	TGF-Beta (pg/ml)	IL-6 (pg/ml)	IFN-Gamma (pg/ml)
ChT group	165.78 ± 12.37	199.34 ± 24.85	439.31 ± 95.14	174.04 ± 44.82
Control group	138.4 ± 7.87	70.66 ± 10.63	289.57 ± 96.59	219.00 ± 31.35
P value	<0.001	<0.001	0.001	<0.05

BP = bursting pressure; TGF = transforming growth factor; IL = interleukin; IFN = interferon; and ChT = cholera toxin.

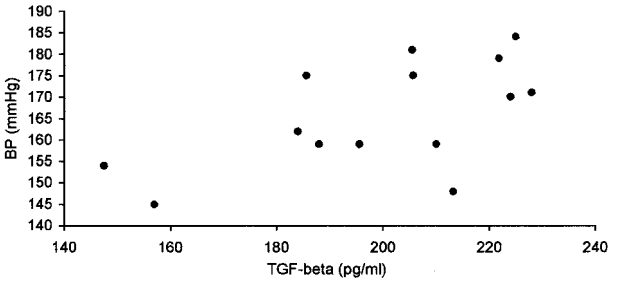

Figure 1. Bursting pressure (BP) and perianastomotic transforming growth factor (TGF)-beta levels are positively correlated ($r = 0.59$; $P = 0.025$).

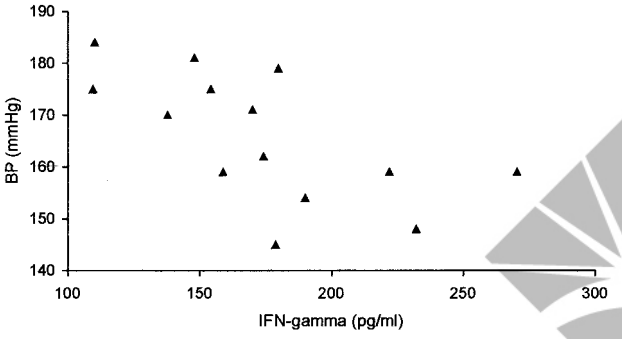

Figure 2. Bursting pressure (BP) and perianastomotic interferon (IFN)-gamma levels are negatively correlated ($r = -0.64$; $P = 0.013$).

DISCUSSION

The results of this study demonstrate for the first time that an oral antigen (ChT) increases the mechanical strength of uncomplicated experimental colonic anastomosis. Significantly higher BP values were obtained seven days after the creation of colonic anastomoses when repeated low doses of ChT were administered to rats preoperatively via orogastric tubes ($P < 0.001$). The heightened mechanical strength of colonic anastomosis provided by ChT was correlated with the perianastomotic tissue level of TGF-beta, whereas an inverse correlation was noted with IFN-gamma. No side effects were noted in rats within the time limits of the study. Similarly, no toxic effects of equivalent immunogenic doses of ChT have been described in humans.[34–36] There are substantial differences in the gut immunity between rats and humans. Although only whole ChT is strongly immunogenic in rats, nontoxic but immunogenic subunits of ChT are used safely in humans. Therefore, these original findings may suggest a new, practical, and safe adjunctive modality in colorectal surgery with regard to the enhancement of surgical wound healing.

Recently, a critical role in the wound-healing process has been attributed to polypeptide growth factors, which are naturally found in the wound milieu. These growth factors, such as interleukins, function as cytokines, and they appear to mediate the critical relationship between the immune system and wound healing. For example, impaired left colonic anastomotic healing in rats induced by 5-fluorouracil was proposed to be associated with a significant decrease in the perianastomotic TGF-beta level.[15] Accelerated healing of incisional wounds with the administration of TGF-beta was reported.[20,21,23] Recently, this contributory effect of TGF-beta has been attributed, at least in part, to the decreased production of nitric oxide (NO), a well-known factor that disturbs wound healing.[37] It has been suggested that the primary regulator of inducible NO synthase (iNOS) and collagen metabolism might be TGF-beta.[38] TGF-beta induces arginase activity in fibroblasts, inhibits iNOS activity, produces ornithine from L-arginine, and stimulates fibroblast proliferation and collagen synthesis.[39] In the present study, the significantly higher BP values obtained in the ChT group were correlated with the significantly higher TGF-beta levels in the wound milieu, which supports the possible contributory role of this cytokine in wound repair.

On the other hand, it has been reported that IFN-gamma antagonizes the secretion of TGF-beta, as well as its beneficial effects on wound healing.[24] IFN-gamma impairs healing of skin wounds by decreasing collagen deposition and neovascularization and probably affecting collagen cross-linking in a dose-dependent manner.[25,40] The activity of iNOS also increases with high levels of IFN-gamma.[39] These negative effects of IFN-gamma on wound healing are intensified by high levels of tumor necrosis factor-alpha.[41] In this respect, IFN-gamma has been used successfully in the treatment of hypertrophic scars.[42] The inverse correlation with the BP values and perianastomotic tissue levels of IFN-gamma observed in the present study supports the hypothesis that IFN-gamma impairs early phases of wound repair, and any interference to reduce tissue IFN-gamma levels might aid in enhanced healing.[43]

Another cytokine investigated in the present study was IL-6, a proinflammatory cytokine with suggested roles in the systemic response to trauma, inflammation, and tissue repair.[44] Locally, IL-6 is present in the wound environment, and it is thought to regulate several biologic events, such as differentiation of macrophages, growth of keratinocytes, and inhibition of

Table 2.
Extra-Anastomotic Tissue Cytokine Levels of the Study Groups

	TGF-Beta	IL-6	IFN-Gamma
Cholera toxin group	211.20 ± 34.99	841.58 ± 148.84	148.00 ± 23.86
Control group	50.66 ± 10.63	521.62 ± 59.86	191.66 ± 43.70
P value	<0.001	<0.001	<0.05

TGF = transforming growth factor; IL = interleukin; IFN = interferon.
Figures are picograms per milliliter except where otherwise specified.

fibroblast proliferation in the late phases of wound healing.[45] The relationship between experimental anastomotic healing and IL-6 levels in endotoxin-induced sepsis was investigated by Ishimura and coworkers.[46] They found that the perianastomotic tissue IL-6 level increased in the septic group in the first 24 hours of healing but decreased thereafter. In the same study, no correlation was shown between anastomotic healing and IL-6. A role for IL-6 in the restitution of damaged bowel epithelium was denied in another study.[47] We also failed to demonstrate a significant correlation between IL-6 and the mechanical strength of uncomplicated colonic anastomosis, even though significantly higher perianastomotic and extra-anastomotic IL-6 levels were induced by ChT. In brief, significantly higher perianastomotic TGF-beta and lower IFN-gamma levels were associated with the higher BP values induced by ChT. We cannot rule out that other growth factors/cytokines might work in concert with the cytokines investigated in the present study to mediate anastomotic healing.

In the present study, the method of mucosal immunomodulation with ChT was used to change the colonic tissue cytokine pattern. Although it is possible and relatively easy to administer these agents systemically or locally, such an approach is impractical because of the expense and short life of cytokines and the possible side effects. On the other hand, mediation of the cytokine pattern by selective antigenic stimulation of certain subsets of immune components utilizes the inborn mechanisms of the organism, and therefore, it is prone to feedback control, in addition to being inexpensive, free of side effects, and of longer duration.[26,27,34,48–50] ChT is a potent mucosal adjuvant, and it stimulates mucosal immune response, alters oral tolerance, and switches antibody secretion from immunoglobulin G to immunoglobulin A isotype when given orally.[31] ChT selectively stimulates the differentiation of TH2 and TH3 lymphocytes, whereas it suppresses the TH1 subtype.[30,31] TH2 lymphocytes mainly secrete IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13, whereas the more recently identified TH3 subtype is the main producer of TGF-beta.[51] TGF-beta further promotes TH3 differentiation and its own synthesis, and it suppresses TH1 lymphocytes that secrete IFN-gamma.[26,51] This sequence of events, with the resultant enhanced TGF-beta and reduced IFN-gamma levels, was demonstrated to occur in gut-associated lymphoid tissue when ChT was administered orally in low doses.[51] We demonstrated that repeated low doses (10 μg) of ChT introduced via orogastric tubes into the stomach of rats resulted in significantly higher tissue TGF-beta and IL-6 levels and lower IFN-gamma. The use of repeated doses is a common procedure in oral immunization to ascertain adequate intestinal uptake, and we cannot state that a single dose would not be equally effective.[35,52] This immunomodulation was prevalent throughout the whole colon, at the very least, as confirmed by analysis of the extra-anastomotic tissue samples. Although not investigated in the present study, it is probable that selective antigenic stimulation by ChT of the TH2 and TH3 lymphocyte subsets (as reflected by high IL-6 and TGF-beta levels, respectively) and inhibition of TH1 (as reflected by low IFN-gamma) were responsible for this cytokine menu. We again cannot dispute that other immune components might be influenced by ChT and involved in the mediation of this enhanced healing phenomenon.

The present study demonstrated that the mechanical strength of uncomplicated experimental colonic anastomosis increased significantly with gut mucosal immunomodulation with repeated low preoperative doses of ChT. This enhanced healing had significant positive correlation with the colonic tissue TGF-beta level and inverse correlation with IFN-gamma, possibly resulting from selective activation of TH2 and TH3 lymphocyte subgroups and inhibition of TH1 by ChT. The next step should be to confirm that a similar intestinal cytokine menu can be produced in humans with oral ChT or other antigens. The most important concern with ChT may be the development of diarrhea, which may occur in a dose-dependent manner. Nevertheless, it is now possible to produce immuno-

genic but completely nontoxic subunits of ChT with recombinant DNA technology.[52] If the relevant dose regimen is identified and its safety is assured, gut mucosal immunomodulation might provide an efficient, safe, and inexpensive tool to improve surgical outcome in colorectal surgery, especially in high-risk situations.

REFERENCES

1. Khoury GA, Waxman BP. Large bowel anastomoses, I: the healing process and sutured anastomoses: a review. Br J Surg 1983;70:61–3.
2. Fielding LP, Stewart-Brown S, Blesovsky L, Kearney G. Anastomotic integrity after operations for large-bowel cancer: a multicentre study. BMJ 1980;281:411–4.
3. Brasken P. Healing of experimental colon anastomosis. Eur J Surg 1991;566(Suppl):1–51.
4. Thornton FJ, Barbul A. Healing in the gastrointestinal tract. Surg Clin North Am 1997;77:549–73.
5. Cohen IK, Diegelmann RF, Crossland MC. Wound care and wound healing. In: Schwartz SI, ed. Principles of surgery. Sixth ed. McGraw-Hill, New York: 1994: 279–303.
6. Högström H, Haglund U, Zederfelt B. Beneficial effects on intestinal anastomosis of S-2441, a synthetic kallikrein-kinin antagonist: experimental studies in the rat. Am J Surg 1985;150:312–3.
7. Högström H, Haglund U. Neutropenia prevents decrease in strength of rat intestinal anastomosis: partial effect of oxygen free radical scavengers and allopurinol. Surgery 1986;99:716–20.
8. Brennan SS, Foster ME, Morgan A, Leaper DJ. Prostaglandins in colonic anastomotic healing. Dis Colon Rectum 1984;27:723–5.
9. Menteş BB, Tascılar O, Tatlıcıoğlu E, Bor MV, İşman F, Türközkan N, Çelebi M. Influence of pulsed electromagnetic fields on healing of experimental colonic anastomosis. Dis Colon Rectum 1996;39:1031–8.
10. Hendriks T, Mastboom WJ. Healing of experimental intestinal anastomoses: parameters for repair. Dis Colon Rectum 1990;33:891–901.
11. Peterson JM, Barbul A, Breslin RJ, Wasserkrug HL, Efron G. Significance of T-lymphocytes in wound healing. Surgery 1987;102:300–5.
12. Barbul A. Immune aspects of wound repair. Clin Plast Surg 1990;17:433–42.
13. Schaffer M, Barbul A. Lymphocyte function in wound healing and following injury. Br J Surg 1998;85:444–60.
14. Angele MK, Knoferl MW, Ayala A, *et al.* Trauma-hemorrhage delays wound healing potentially by increasing pro-inflammatory cytokines at the wound site. Surgery 1999;126:279–85.
15. Fukuchi SG, Seeburger JL, Parquet G, Rolandelli RH. Influence of 5-fluorouracil on colonic healing and expression of transforming growth factor-beta 1. J Surg Res 1999;84:121–6.
16. McGrath MH. Peptide growth factors and wound healing. Clin Plast Surg 1990;17:421–32.
17. Bennet NT. Growth factors and wound healing: biochemical properties of growth factors and their receptors. Am J Surg 1993;165:728–37.
18. Steenfos HH. Growth factors and wound healing. Scand J Plast Reconstr Hand Surg 1994;28:95–105.
19. Steed DL. The role of growth factors in wound healing. Surg Clin North Am 1997;77:575–87.
20. Mustoe TA, Pierce GF, Thomason A, Gramates P, Sporn MB, Deuel TF. Accelerated healing of incisional wounds in rats induced by transforming growth factor-beta. Science 1987;237:1333–6.
21. Mustoe TA, Landes A, Cromack DT, *et al.* Differential acceleration of healing of surgical incisions in the rabbit gastrointestinal tract by platelet-derived growth factor and transforming growth factor, type beta. Surgery 1990;108:324–30.
22. Bernstein EF, Harisiadis L, Salomon G, *et al.* Transforming growth factor-β improves healing of radiation-impaired wounds. J Invest Dermatol 1991;97:430–4.
23. O'Kane S, Ferguson MW. Transforming growth factor βs and wound healing. Int J Biochem Cell Biol 1997;29: 63–78.
24. Tredget EE, Wang R, Shen Q, Scott PG, Ghahary A. Transforming growth factor-beta mRNA and protein in hypertrophic scar tissues and fibroblasts: antagonism by IFN-alpha and IFN-gamma in vitro and in vivo. J Interferon Cytokine Res 2000;20:143–51.
25. Miles RH, Paxton TP, Zacheis D, Dries DJ, Gamelli RL. Systemic administration of interferon-gamma impairs wound healing. J Surg Res 1994;56:288–294.
26. Faria AM, Weiner HL. Oral tolerance: mechanisms and therapeutic applications. Adv Immunol 1999;73: 153–264.
27. Garcia G, Weiner HL. Manipulation of Th responses by oral tolerance. Curr Top Microbiol Immunol 1999;238: 123–45.
28. Xu-Amano J, Jackson RJ, Fujihashi K, Kiyono H, Staats HF, McGhee JR. Helper Th1 and Th2 cell responses following mucosal or systemic immunization with cholera toxin. Vaccine 1994;12:903–11.
29. Marinaro M, Staats HF, Hiroi T, *et al.* The mucosal adjuvant effect of cholera toxin in mice results from induction of T helper 2 (Th2) cells and IL-4. J Immunol 1995;155:4621–9.
30. Kim PH, Eckmann L, Lee WJ, Han W, Kagnoff MF. Cholera toxin B subunit induce IgA switching through the action of TGF-beta 1. J Immunol 1998;160: 1198–203.
31. Freytag LC, Clements JD. Bacterial toxins as mucosal adjuvants. Curr Top Microbiol Immunol 1999;236: 215–36.

32. Strobel S, Ferguson A. Immune responses to fed protein antigens in mice, 3: systemic tolerance or priming is related to age at which antigen is first encountered. Pediatr Res 1984;18:588–93.
33. Wu Y, Kudsk KA, DeWitt RC, Tolley EA, Li J. Route and type of nutrition influence Ig-A-mediating intestinal cytokines. Ann Surg 1999;229:662–8.
34. Holmgren J, Svennerholm AM, Jertborn M, *et al.* An oral B subunit: whole cell vaccine against cholera. Vaccine 1992;10:911–4.
35. Jertborn M, Svennerholm AM, Holmgren J. Safety and immunogenicity of an oral recombinant cholera B subunit-whole cell vaccine in Swedish volunteers. Vaccine 1992;10:130–2.
36. Taylor DN, Cardenas V, Perez J, Puga R, Svennerholm AM. Safety, immunogenicity, and lot stability of the whole cell/recombinant B subunit (WC/rCTB) cholera vaccine in Peruvian adults and children. Am J Trop Med Hyg 1999;61:869–73.
37. Bauer JA, Harr ME, Smith DJ. Diminished nitric oxide production following administration of transforming growth factor-beta1 using a noninflammatory wound repair model. Nitric Oxide 1999;3:402–8.
38. Vodovotz Y. Control of nitric oxide production by transforming growth factor-beta1: mechanistic insights and potential relevance to human disease. Nitric Oxide 1997;1:3–17.
39. Shearer JD, Richards JR, Mills CD, Caldwell MD. Differential regulation of macrophage arginine metabolism: a proposed role in wound healing. Am J Physiol 1997; 272:181–90.
40. Ganstein RD, Deak MR, Jacques SL, *et al.* The systemic administration of gamma interferon inhibits collagen synthesis and acute inflammation in a murine skin wounding model. J Invest Dermatol 1989;93:18–27.
41. Nanes MS, McKoy WM, Marx SJ. Inhibitory effects of tumor necrosis factor-alpha and interferon-gamma on deoxyribonucleic acid and collagen synthesis by rat osteosarcoma cells (ROS 17/2.8). Endocrinology 1989; 124:339–45.
42. Harrop AR, Ghahary A, Scott PG, Forsyt N, Uji-Friedland A, Tredget EE. Regulation of collagen synthesis and mRNA expression in normal and hypertrophic scar fibroblasts in vitro by interferon-gamma. J Surg Res 1995; 58:471–7.
43. Mateo RB, Reichner JS, Albina JE. Interleukin-6 activity in wounds. Am J Physiol 1994;266:R1840–4.
44. Riche FC, Cholley BP, Panis YH, *et al.* Inflammatory cytokine response in patients with septic shock secondary to generalized peritonitis. Crit Care Med 2000;28:433–7.
45. Hirano T. The biology of interleukin 6. Chem Immunol 1992;51:153–80.
46. Ishimura K, Tsubouchi T, Okano, Maeba T, Maeta H. Wound healing of intestinal anastomosis after digestive surgery under septic conditions: participation of local interleukin-6 expression. World J Surg 1998;22: 1069–75.
47. Dignass AU, Podolsky DK. Cytokine modulation of intestinal epithelial cell restitution: central role of transforming growth factor β. Gastroenterology 1993;105: 1323–32.
48. Ferguson SS, Ferguson A. Modulation of intestinal and systemic immune responses to a fed protein antigen, in mice. Gut 1986;27:829–37.
49. Pfeiffer C, Murray J, Madri J, Bottomly K. Selective activation of Th1-and Th2-like cells in vivo: response to human collagen IV. Immunol Rev 1991;123:65–84.
50. Barnett ML, Kremer JM, St. Claair EW, *et al.* Treatment of rheumatoid arthritis with oral type II collagen: results of a multicenter double-blind placebo controlled trial. Arthritis Rheum 1998;41:290–7.
51. Seder RA, Marth T, Sieve MC, *et al.* Factors involved in the differentiation of TGF-β producing cells from naïve $CD4^+$ T-cells: IL-4 and IFN-γ have opposing effects, while TGF-β positively regulates its own production. J Immunol 1998;160:5719–28.
52. Holmgren J, Lycke N, Czerkinsky C. Cholera toxin and cholera B subunit as oral-mucosal adjuvant and antigen vector systems. Vaccine 1993;11:1179–84.