Local delivery of adenoviral vectors encoding murine interleukin 10 induces colonic interleukin 10 production and is therapeutic for murine colitis

J O Lindsay, C J Ciesielski, T Scheinin, F M Brennan, H J Hodgson

Introduction: Interleukin 10 knockout (IL-10−/−) mice spontaneously develop a Th1 T cell mediated colitis with many similarities to Crohn’s disease. Daily injections of IL-10 are unable to induce remission in mice with established disease. In contrast, we have shown previously that intravenous administration of adenoviral vectors encoding IL-10 (Advmu-10) induces hepatic IL-10 release and leads to long term disease suppression with profound systemic immunoregulatory changes.

Aims: To determine whether rectal delivery of Advmu-10 induces localised colonic IL-10 expression without systemic immune suppression, and assess its therapeutic efficacy in IL-10−/− mice with established colitis.

Results: A single rectal infusion of 5×10⁶ PFU Advmu-10 to 10 week IL-10−/− mice resulted in a median level of 27.3 pg/mg IL-10 in colonic homogenates harvested one week later. IL-10−/− mice with established colitis treated with an enema of 5×10⁶ PFU Advmu-10 entered clinical and histological remission whereas empty cassette adenovirus (Adv0) or phosphate buffered saline (PBS) treated mice developed progressive disease. After four weeks, the histological score of Advmu-10 treated mice [4.4 (1.5)] was significantly lower than that of Adv0 [11.1 (1.1); p<0.001] and PBS [10.9 (1.0); p<0.01] treated controls. In addition, the stool concentration of IL-18 over the four week experiment was significantly higher in mice treated with saline or Adv0 than in those treated with Advmu-10 (p<0.01).

Conclusion: Local Advmu-10 therapy reverses colitis in IL-10−/− mice without the systemic effects seen after intravenous administration. Gene therapy strategies using adenoviral vectors encoding immunoregulatory cytokines may prove to be a potent approach to the treatment of chronic inflammatory diseases such as Crohn’s disease.

Abbreviations: IL-10, interleukin 10; Advmu-10, adenoviral vector encoding murine IL-10; Advβgal, adenoviral vector encoding β-galactosidase; AdvO, empty cassette adenoviral vector; TNF-α, tumour necrosis factor α; IFN-γ, interferon γ; LPS, lipopolysaccharide; FCS, fetal calf serum; PBS, phosphate buffered saline; DMEM, Dulbecco’s modified Eagle’s medium; CAR, Coxsackie virus and adenovirus receptor; IPMN, lamina propria mononuclear cell; MOI, multiplicity of infection.

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of colitis. It is possible that targeted local IL-10 would be sufficient to treat intestinal inflammation while avoiding the side effects associated with systemic therapy. Thus oral administration of non-pathogenic bacteria (Lactobacillus lactis) that have been genetically modified to secrete murine IL-10 led to histological improvement in dextran sodium sulphate and IL-10−/− models of colitis. Concerns raised by the release of genetically modified organisms into the environment may be avoided by the use of replication deficient adenoviral vectors such as AdvmuIL-10. Adenoviruses have strong tropism for epithelial tissues, and adenoviral vectors delivered per rectum have been shown to induce expression of the delivered transgene within colonocytes. In this paper, we demonstrate the ability of rectal AdvmuIL-10 to induce colonic IL-10 expression and ameliorate established colitis in IL-10−/− mice without the generalised side effects associated with systemic therapy. Furthermore, we show that local AdvmuIL-10 results in a diminished host antiadenoviral response compared with control adenoviral vectors.

MATERIALS AND METHODS

General reagents were of research grade and purchased from Sigma (St Louis, Missouri, USA). All reagents used for cell culture were determined to be LPS free using a limulus amoebocyte lysate assay (Biowhittaker, Berkshire, UK), as directed by the manufacturer (sensitivity <10 pg/ml).

Adenoviral vectors

The recombinant E1 deleted type 5 adenoviral vectors, encoding murine IL-10 under the transcriptional control of the rous sarcoma virus promoter (AdvmuIL-10), β-galactosidase under the cytomegalovirus promoter (AdvβGal), or having no insert (Adv0), were generously donated by Professor Dallman (Imperial College, London, UK). Viruses were propagated in the 293 human embryonic kidney cell line (Quantum Biotechnology Inc., Canada) and purified by ultracentrifugation through two caesium chloride gradients (Boehringer Mannheim, Lewes, Inc., Canada) and then stored at −80°C until use.

In vitro epithelial cell infection

The transformed human colonic epithelial cell lines HT29 and SW620 (ATCC, Maryland, USA) were cultured at a density of 1×10⁵/ml in RPMI 1640 medium (PAA Laboratories Ltd, Yeovil, UK) supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, and 100 µg/ml streptomycin (Biowhittaker). Initial experiments demonstrated that at least 95% infection with AdvβGal was obtained with a multiplicity of infection of (MOI) 50:1 and an incubation time of 36 hours; thus these conditions were used in subsequent experiments. Cells were cultured in triplicate, infected with Adv0, AdvmuIL-10, or saline vehicle and cultured for 28 days with weekly passaging. Supernatants were sampled daily and frozen until assay. IL-10 bioactivity was determined by the ability of serial dilutions of the supernatant to inhibit TNF-α release from a murine monocye cell line (RAW cells; ATCC) plated at 1×10⁶/ml in a 96 well plate stimulated with 10 ng/ml LPS. Serial dilutions of recombinant murine IL-10 were used as a standard while the specificity of the effect was determined by preincubation of the supernatants and standards with 10 µg/ml of a rat monoclonal IL-10 antibody (JES52A5; donated by DNAX Research Institute, California, USA) or a rat IgG1 isotype control (OX20; ATCC).

Animals

IL-10−/− mice on a C3HBlb background (purchased from Harlan UK Ltd, Oxon, UK) were backcrossed for one generation onto DBA/1 mice to increase breeding vigour and disease expression. Progeny from the interbreeding of the heterozygous offspring were used in all experiments. Newly bred mice were screened for the homozygote IL-10−/− or wild-type genotype (C57BL/6×DBA1), as described previously. Mice were maintained in specific pathogen free conditions in a laminar flow hood at all times with free access to food and water. All experiments involving animals were approved by the local ethics review process committee and performed under license from the Home Office.

Preliminary experiments demonstrated that under standard laboratory conditions, IL-10−/− animals developed a progressive colitis from four weeks of age. Clinical manifestations of disease included the passage of mucous, diarrhoea, rectal prolapse, and weight loss of greater than 5% of total body weight. Mice were examined weekly, and given a clinical score that consisted of one point for each of the above signs. Previous work has demonstrated a close correlation between the clinical score and histological severity of colitis (correlation coefficient 0.865; Dr T Scheinin, personal communication).

Experimental protocols

Ten week old IL-10−/− mice with established colitis received 5×10⁶ PFU of AdvmuIL-10, Adv0, or phosphate buffered saline (PBS) vehicle as a 100 µl rectal infusion under sedation. Mice were held vertically for one minute and then suspended on tilted racks for a further 10 minutes to prevent seepage. Wild-type C37BL/6×DBA1 mice were used as a negative control. The clinical score of each animal was assessed and stool samples were collected weekly throughout the experiment. Animals were sacrificed by cervical dislocation four weeks after adenoviral therapy. Serum was collected via cardiac puncture, spleens were harvested, stool samples collected, and serial segments of colon, caecum, and ileum were fixed in 10% neutral buffered formalin for histological analysis. In a separate experiment, IL-10−/− mice that had received saline or AdvmuIL-10 by rectal instillation were sacrificed after seven days. The liver, spleen, and colon of these animals were weighed and homogenised in 5 µl PBS per mg tissue. Supernatants were harvested after centrifugation and stored at −20°C until assay.

Histological analysis

Samples were routinely processed, embedded in paraffin, sectioned at 5 µm, and stained with haematoxylin and cosin for light microscopic examination. Histological assessment was performed by an investigator (TS) blinded to the treatment group. The terminal ileum and five segments of colon were examined per mouse and each given a histological score from 0 to 4 as described previously; thus a total score for each mouse from 0 (no change in any segment) to 24 (grade 4 changes in all segments) was obtained. Scores of less than 5 were deemed to be within normal limits.

Stool samples

Stool samples were collected weekly from all animals and weighed. Samples were emulsified in 500 µl per 100 µg stool weight of a solution of 1 mg/ml soy trypsin inhibitor and 1 mg/ml phenylmethylsulphonyl fluoride in PBS. Supernatants were collected after centrifugation at 10 000 g for 15 minutes and stored at −20°C.

Spleen cell cultures

After sacrifice, each spleen was placed in RPMI 1640 supplemented with 10% FCS, 100 U/ml penicillin, and 100 µg/ml streptomycin. Cell suspensions were obtained by passing tissue through a 200 µm nylon mesh. After erythrocyte lysis, cells were washed in Hanks balanced salt solution three times before resuspension in medium. Cells were plated at 2×10⁶/well in 12 well plates (Falcon, Becton Dickinson Labware, USA) in a final volume of 1 ml of medium.

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with or without LPS 10 µg/ml recombinant mull-10 10 ng/ml (Schering Plough, New Jersey, USA), 10 µg/ml neutralising rat anti murine IL-10 antibody (JESS2A5; donated by DNAX Research Institute), or a rat IgG1 isotype control (OX20; ATCC). Cultures were maintained for 24 hours before supernatants were harvested and stored at −20°C.

Measurement of serum antiadenoviral antibody response
The neutralising antiadenovirus antibody response was analysed in serum from untreated or treated IL-10−/− mice four weeks after gene transfer with 5×10⁶ PFU of Advmull-10, Adv0, or PBS vehicle. Serum samples (100 µl) were heat inactivated at 56°C for 30 minutes and diluted twofold in serum free Dulbecco’s modified Eagle’s medium (DMEM). Each dilution was incubated for 90 minutes at 37°C with 2×10⁶ PFU of Adβgal, and applied in duplicate to 80% confluent 293 cells on a 96 well plate. After one hour at 37°C, 50 µl of DMEM containing 10% FCS was added to each well, and cells were cultured for a further 36 hours. Cell supernatants were then removed and replaced with 30 µl of 0.23 mM Tris HCl, pH 7.8.

RESULTS
In vitro infection of epithelial cell lines with Advmull-10 induces bioactive murine IL-10 release
In order to ascertain whether Advmull-10 induces IL-10 secretion from epithelial cells, cultures of the human colon epithelial cell lines HT29 and SW620 were infected with Advmull-10 at an MOI of 50:1 for 36 hours; separate wells were infected with Adv0 or saline vehicle as controls. Supernatants were sampled at 36 hours after gene transfer with 5×10⁶ PFU of Advmull-10 or saline vehicle either by tail vein injection (A) or rectal infusion (B) under light sedation (n=3/group). After seven days, mice were sacrificed by cervical dislocation. The liver, spleen, and colon were homogenised in 5 µl phosphate buffered saline (PBS) per mg tissue. After centrifugation, supernatants were assayed for murine IL-10 by ELISA (sensitivity 8 pg/mg).

Plates were vortexed, frozen, and then thawed for three cycles to detach and lyse cells, and centrifuged at 1000 rpm for 20 minutes. The supernatant (10 µl) from each well was mixed with 90 µl of a β-galactosidase substrate solution containing 1 mg/ml D- nitrophenyl-β-D-galactopyranoside, 1 mM MgCl₂, 45 mM 2-mercaptoethanol, in 0.1 M sodium phosphate buffer, pH 7.5. The enzyme reaction was stopped after five minutes with addition of 150 µl of 0.1 M Na₂CO₃, and plates were read at 405 nm. Mean optical density of serum from five animals for each group was compared.

Cytokine analysis
Cytokine concentrations were measured by sandwich ELISA using paired antibodies according to the manufacturer’s recommendations (IL-10 and TNF-α were purchased from Pharmingen, Sorentino, California, USA; interferon γ (IFN-γ) was purchased from Genzyme diagnostics, USA; IL-1β was purchased from R&D Systems (Abingdon, Oxon, UK)).

Statistical analysis
Data that exhibited a normal distribution were analysed using a two tailed t test; for comparison of more than two means a two way analysis of variance (ANOVA) was performed.
ng/ml muIL-10, respectively (mean (SEM)). IL-10 release continued for the 28 days of the experiment, as shown in fig 1. In order to confirm that this IL-10 was bioactive, the ability of the supernatants from AdvmuIL-10 infected HT29 cells to inhibit LPS induced TNF-α release from cultures of RAW cells was compared with recombinant murine IL-10. muIL-10 10 ng/ml reduced TNF-α release to 17.5 (0.7)% of LPS stimulated levels whereas a 1/10 dilution of the supernatant of AdvmuIL-10 infected HT29 cells reduced TNF-α release to 15.8 (0.29)%. Addition of a monoclonal antimurine IL-10 antibody (but not isotype control antibody) abrogated this inhibitory effect completely (fig 1).

Rectal instillation of AdvmuIL-10 results in localised colonic IL-10 expression

Systemic delivery of adenoviral vectors predominantly targets hepatocytes although some splenic and colonic expression of the delivered transgene has been reported. In order to assess whether rectal AdvmuIL-10 delivery results in localised colonic IL-10 release, 5x10^8 PFU of AdvmuIL-10 or PBS vehicle were delivered to 10 week old IL-10−/− mice either by tail vein injection or by rectal instillation (n=3/group). Mice were sacrificed one week after adenoviral administration. IL-10 concentration in colonic homogenates from mice that had received rectal AdvmuIL-10 was mean 26.5 (SEM 8.7) pg/mg.
Local delivery of adenoviral vectors

compared with 13 (6.5) pg/mg in mice that had received an intravenous injection. In contrast, while liver and spleen IL-10 concentrations after rectal AdvmuIL-10 administration did not differ significantly from the background seen in PBS treated controls (the sensitivity of the ELISA was approximately 8 pg/mg), IL-10 concentrations in the liver and spleen of mice given an intravenous injection were significantly elevated (22.5 (22.3) pg/ml and 5.7 (4.3) pg/ml, respectively) (fig 2).

**Local AdvmuIL-10 treatment is therapeutic for established colitis in IL-10−/− mice**

IL-10−/− mice develop a spontaneous enterocolitis associated with weight loss, passage of mucous, rectal prolapse, and diarrhea. It has been reported that daily injections of recombinant murine IL-10 prevent disease but are not sufficient to reverse established colitis. In order to determine the therapeutic efficacy of local AdvmuIL-10 delivery, 10 week old IL-10−/− mice with established colitis received a single rectal instillation of 5x109 PFU of AdvmuIL-10, Adv0, or PBS vehicle. Wild-type mice received the same treatment to act as a negative control. IL-10−/− mice that received AdvmuIL-10 demonstrated a significant improvement in their colitis scores over the four week experiment whereas those that had received Adv0 or vehicle continued to develop progressive disease (fig 3). Thus the mean (SEM) clinical score in the AdvvmuIL-10 group fell from 1.8 (0.13) to 2.5 (0.27) and from 1.8 (0.22) to 2.6 (0.13) in the PBS and Adv0 treated groups, respectively (p<0.001 for each animal were assayed in triplicate; results are expressed as mean (SEM) for each group. *p<0.05; **p<0.01; ***p<0.001 versus pooled wild-type (WT) mice.

**Figure 5** Rectal adenoviral vector encoding murine interleukin 10 (AdvmuIL-10) did not diminish the lipopolysaccharide (LPS) splenocyte response in IL-10−/− mice. Splenocytes were isolated at sacrifice from both IL-10−/− (n=10/group) and wild-type (n=5/group) mice treated with 5x109 PFU empty cassette adenoviral vector (Adv0), saline, or AdvmuIL-10 by rectal instillation four weeks previously. Cells (2x106 per well) were cultured for 24 hours in the presence of LPS 10 ng/ml. Supernatants were harvested and assayed for tumour necrosis factor α (TNF-α) by ELISA. Cells from each animal were assayed in triplicate, results are expressed as mean (SEM) for each group. *p<0.05; **p<0.01; ***p<0.001 versus pooled wild-type (WT) mice.

Neutralising antiadenovirus antibody response is diminished in AdvmuIL-10 treated IL-10−/− mice

Previous studies have shown elevated titres of antiadenovirus antibodies in mice treated with adenoviral vectors. We hypothesised that the neutralising antibody response to AdvmuIL-10 vectors would be diminished, as the protein encoded for by this virus will act to suppress both T cell activation and antigen presentation. A bioassay was used to detect the presence of antibodies directed to adenoviral proteins in the serum of mice that had received a rectal infusion of Adv0, AdvmuIL-10, or saline vehicle four weeks previously. Serum from both IL-10−/− and wild-type mice that had received rectal Adv0 contained neutralising antiadenovirus antibodies were compared with serum from mice that had received saline (p<0.001 two way ANOVA; fig 6). However, there was no difference in the antiadenoviral antibody titre in serum from IL-10−/− mice that had received rectal AdvmuIL-10 compared with those that had received saline. Interestingly, the diminished antiadenoviral response to AdvmuIL-10 was less pronounced in wild-type mice than in IL-10−/− mice.
DISCUSSION

We have explored the potential of local AdvmuIL-10 administration as a therapy for intestinal inflammation. This strategy has the advantage that IL-10 delivery is targeted to the sites of inflammation which may prevent the side effects associated with high dose systemic IL-10 administration. The use of the IL-10−/- model of colitis allows accurate determination of the site of IL-10 expression after local AdvmuIL-10 delivery. In addition, as daily systemic injections of murine IL-10 are unable to reverse established colitis in this model,4 it allows a comparison of the therapeutic efficacy of local AdvmuIL-10 and recombinant IL-10 injections.

Adenoviral vectors bind to cell surface integrins (αβ3 and ανβ3) and gain entry by receptor mediated endocytosis using a receptor such as the Coxsvirus and adenovirus receptor (CAR).24 CAR mRNA can be detected in human intestinal tissue by northern blotting.25 Thus infection of human colonic epithelial cells with AdvmuIL-10 in vitro resulted in the release of high levels of functional IL-10 as determined by a specific bioassay.23 The effect of the secreted murine IL-10 on epithelial cell function was not assessed, as despite 73% amino acid homology between murine and human IL-10 proteins, murine IL-10 has no activity on human cells.26

The presence of CAR on murine colonic epithelial cells has not been determined.27 However, recent reports have suggested that the MHC class I receptor may also function as a high affinity receptor for adenoviral vectors.28 Irrespective of this, previous studies in mice have demonstrated that colonic administration of adenoviral vectors leads to colonic reporter gene expression, which peaks at 48–72 hours.29–31 These findings were confirmed by the significant levels of IL-10 that were detected in colonic homogenates of IL-10−/- mice one week after local AdvmuIL-10 administration. The duration of IL-10 expression in this experiment is surprising considering that colonic epithelial cell turnover occurs every 2–3 days.32 However, Foreman et al also reported low level β-galactosidase expression for up to 180 hours after colonic Advβ-gal administration.26 Although rectal administration of adenoviral vectors predominantly targets the colonic epithelial cell, some expression has been reported in lamina propria mononuclear (LPMN) cells.33 Thus the late phase of gene expression after local adenoviral vector administration may represent LPMN cell infection. Furthermore, Wirtz et al have reported that the ratio of LPMN cells to epithelial cells infected by rectal adenoviral administration is increased in mice that have colitis.34 This may explain the prolonged duration of IL-10 expression that we report after AdvmuIL-10 delivery to IL-10−/- mice with established colitis. In addition, AdvmuIL-10 induces a diminished host antiviral response in IL-10−/- mice compared with control vectors that may also permit prolonged IL-10 expression.

Whereas systemic AdvmuIL-10 administration leads to IL-10 expression in the liver, spleen, and colon, IL-10 protein was not detected in the liver and spleen of IL-10−/- mice that received AdvmuIL-10 as an enema. The absence of systemic IL-10 activity after local AdvmuIL-10 therapy was confirmed by experiments examining the response of harvested splenocytes to stimulation with LPS. Local delivery of replication deficient adenoviral vectors was well tolerated and did not exacerbate intestinal inflammation. Studies of adenoviral vectors encoding non-immunoregulatory proteins have demonstrated host antivirus immune responses that limit the duration of gene expression and prevent retreatment.35 However, in contrast with the marked antibody response to control vectors, local AdvmuIL-10 delivery significantly diminished the host antiviral response in IL-10−/- mice. It is interesting that the reduction in antiviral response seen with AdvmuIL-10 administration is not as marked in wild-type mice as in IL-10−/- mice. The explanation for this dichotomy is not clear but is likely to represent alterations in B cell maturation in IL-10−/- mice.4

IL-10−/- mice develop a spontaneous Th1 cell mediated enterocolitis with many similarities to Crohn's disease.36 Steidler et al investigated the therapeutic efficacy of local IL-10 delivery using bacteria that had been genetically modified to secrete high levels of IL-10.37 A four week oral course of these bacteria led to a 75% reduction in the histological severity of colitis in IL-10−/- mice. Furthermore, clinical remission was maintained for at least four weeks after therapy. The dramatic improvement in clinical score was associated with a fall in the levels of the proinflammatory cytokine IL-1β that was detected in high concentrations in the stool of IL-10−/- mice with colitis. Most strikingly, rectal AdvmuIL-10 therapy led to a 60% reduction in the histological severity

Figure 6 Rectal adenoviral vector encoding murine interleukin 10 (AdvmuIL-10) induced a diminished adenoviral response in IL-10−/- mice. The neutralising antiviral antibodies titre was analysed in serum from (A) IL-10−/- and (B) wild-type mice four weeks after rectal instillation of 5×1010 PFU of AdvmuIL-10, empty cassette adenoviral vector (Adv0), or saline vehicle. Sera were incubated for 60 minutes at 37°C with 2×106 PFU of adenoviral vector encoding β-galactosidase (Advβ-gal) and applied in duplicate to 80% confluent 293 cells on a 96 well plate. After one hour at 37°C, 50 μl of Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum was added to each well, and cells were cultured for a further 36 hours. Cell supernatants were then removed and assayed using a β-galactosidase substrate solution. In this assay, a low optical density reflects a high titre of serum antiviral antibodies. Results are expressed as mean (SEM) optical density for each group. ***p<0.001 compared with saline treated mice, two way ANOVA).
colitis compared with control treated mice. Thus a single dose of IL-10 gene therapy delivered directly to the colon was sufficient to suppress established disease in IL-10−/− mice for at least four weeks.

The mechanisms by which IL-10 induced by AdvmuIL-10 therapy could have diminished disease severity in the intestine were not addressed directly in our experiments. However, they include downregulation of antigen presenting cell activity and inhibition of proinflammatory cytokine production. The fact that the therapeutic effects of AdvmuIL-10 therapy persisted for at least four weeks and extended throughout the colon suggests that its mechanism of action may extend beyond the local suppression of proinflammatory cytokine release. An analogous situation occurs in mice with collagen induced arthritis in which an intratractive injection of AdvmuIL-10 is therapeutic for both the treated and contralateral paw.37 As in our experiments, the duration of this therapeutic effect persisted far longer than the period in which local IL-10 can be detected. Thus it is possible that local IL-10 release induces differentiation or activation of regulatory T cell clones.38 This hypothesis is supported by studies of transgenic mice that overexpress IL-10 in the intestinal epithelium.39 The mucosal lymphocyte population of these transgenic mice contains a higher proportion of immunoregulatory CD4+CD25+ T cells than wild-type controls. Furthermore, stimulated intestinal lymphocytes from IL-10 transgenic mice secrete lower levels of Th1 cytokines and higher levels of transforming growth factor-β than controls.40 Thus delivery of AdvmuIL-10 directly to the intestinal lumen provides local IL-10 production minimising the side effects associated with systemic therapy. Most strikingly, local AdvmuIL-10 therapy induces clinical and histological remission in IL-10−/− mice with established colitis. The concept of local gene delivery as a therapy for intestinal inflammation is supported by the demonstration that intrarectal administration of an E1 deleted adenoviral vector expressing IL-18 antiseNSE mRNA inhibits mucosal IFN-γ release and suppresses inflammation in the transfer model of colitis.41 However, patients with Crohn’s disease are not IL-10 deficient and may mount an immune response to AdvmuIL-10, as seen in wild-type mice. This would limit the duration of a therapeutic response and prevent retreatment. Alternative viral vectors may be more appropriate for clinical trials of IL-10 gene therapy than the replication deficient adenoviral vectors used in the current study. Adeno associated viruses are less immunogenic, and by integrating into the host genome have been shown to provide long term gene expression in the intestinal tract.42 Furthermore, therapy could be targeted to sites of active disease using an immunization inducible C3-tat/HIV promoter that induces IL-10 release in response to inflammatory stimuli such as TNF-α.43

In conclusion, local gene therapy strategies using viral vectors encoding immunoregulatory cytokines may prove to be a potent approach to the treatment of chronic inflammatory diseases such as Crohn’s disease.

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REFERENCES

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Interstitial pneumonia associated with autoimmune pancreatitis

We read with interest the article by Kamisawa et al reporting IgG4 positive plasma cells in peripancreatic tissue, extrahepatic bile duct, gall bladder, and salivary gland (Gut 2003;52:683–7). The association of retroperitoneal fibrosis and sclerosing pancreatitis with IgG4 bearing plasma cells in the tissues of both lesions has been also reported.1

We would like to report the first case of interstitial pneumonia associated with autoimmune pancreatitis and IgG4 positive plasma cells in the interstitium.

Hyperamylasemia was detected in a routine blood examination in a 63 year old man who had been treated for duodenal ulcer at a clinic. He was admitted to our hospital for further examination. He did not complain of epigastralgia or back pain. Serum amylase was 323 (39–130 IU/l), IgG was elevated to 2150 (800–1600 mg/dl), and IgG4 was 1690 (<80 mg/dl). Antinuclear antibody, anti-SS-A antibody, anti-SS-B antibody, rheumatoid factor, and antismooth muscle antibody were all negative. Abdominal ultrasonography and computed tomography (CT) showed swelling of the head and tail of the pancreas. Endoscopic retrograde pancreatography showed irregular narrowing of the main pancreatic duct in the head and tail. Magnetic resonance cholangiography showed stricture stenos in the lower common bile duct. The patient was diagnosed with autoimmune pancreatitis but he refused steroid therapy and was followed as an outpatient.

Three months later, hombocyming of the bilateral lower lung field was detected in a follow up abdominal CT. Chest CT revealed ground glass attenuation in the middle and lower lobe, and honeycombing predominantly at the back of the lower lobe, bilaterally (fig 1A). (Figure 1 (A–D) is available for viewing online at http://gut.bmjournals.com/cgi/eletters/52/5/683 #127.) Retrospectively, a slight reticular shadow in the lower lung field was detected in the chest roentgenogram taken at the first admission but the lesion had progressed over three months. He was readmitted for further examination. He had a history of smoking 30–40 cigarettes a day for approximately 40 years. IgG was 3934 mg/dl, IgG4 was 2690 mg/dl, KL-6 was 1440 (<500 u/ml), serum amylose was 142, and lipase was 121 (0–49 IU/l). Schirmer’s test indicated a decrease in lacrimal secretion. Swelling of the head and tail of the pancreas were not changed on abdominal ultrasonography and CT.

With galium scintigraphy, uptake was observed bilaterally at the back of the lower lobe, suggesting active pneumonia. Histology obtained by transbronchial lung biopsy from segment 8a of the right lobe showed marked thickening of the alveolar septum with marked infiltration of plasma cells and lymphocytes (fig 1B). Immunostaining with IgG4 was performed using the immunoperoxidase method (mouse anti-human IgG4; ICN Biomedicals, Inc, Ohio, Canada). Infiltration of IgG4 positive plasma cells was detected in the alveolar septum (fig 1C).

Macrophages in the alveoli are considered to be due to smoking which often coexists with interstitial pneumonia in smokers.2 Because interstitial pneumonia associated with autoimmune pancreatitis was strongly suggested, prednisolone (40 mg/day) was administered for two weeks and then the dose was tapered. Chest CT taken two weeks after treatment showed that the ground glass attenuation in the middle and lower lobe had disappeared whereas the honeycombing remained (fig 1D).

Abdominal ultrasonography performed two weeks after treatment showed a marked decrease in the swelling the pancreas.

In the present case, infiltration of IgG4 positive plasma cells in the interstitium strongly suggests that the interstitial lung disease was associated with autoimmune pancreatitis. Interstitial pneumonia associated with Sjogren’s syndrome is unlikely in this case although there was decreased lacrimal secretion. Sicca syndrome observed in Sjogren’s syndrome is distinguishable from classical Sjogren’s syndrome in that it is negative for anti-SS-A or anti-SS-B antibodies, serum IgG4 is elevated, and infiltration of IgG4 positive plasma cells in the salivary glands is observed.3

Autoimmune pancreatitis, in some cases, may be part of a systemic disease associated with IgG4.

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References

Author’s reply
We thank Dr Taniguchi et al for the interesting presentation of interstitial pulmonary fibrosis associated with autoimmune pancreatitis (AIP).

We have experienced 24 cases of AIP but no cases showed interstitial pulmonary fibrosis. Recently, we histologically examined the organs of eight patients with AIP using anti-IgG4 antibody. IgG4 positive plasma cell infiltration was detected in the portal area of the liver, gastric mucosa, colonic mucosa, and bone marrow as well in the pancreas, peripancreatic tissue, extrahepatic bile duct, gall bladder, salivary gland, liver, and bile duct.
and lymph nodes of patients with AIP. However, few IgG4 positive plasma cells were observed in identical control specimens. From these findings, we proposed a new clinicopathological entity of IgG4 related autoimmune disease, and stressed that AIP is not simply pancreatitis but a pancreatic lesion involved in this systemic autoimmune disease.2 As IgG4 positive plasmacytic infiltration was observed in the transbronchially biopsied pulmonary specimens of the patient with AIP (unpublished data), it is likely that interstitial pneumonia occurs in association with AIP.

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References

The diagnostic dilemmas in discrimination between pancreatic carcinoma and chronic pancreatitis

In response to the letter of Harlozinska-Szmyrka and Strutynska-Karpinska (Gut 2003; 52: 1500–4) commenting on our study, we agree with the remarks made in relation to the difficulties in discriminating between chronic pancreaticitis and adenocarcinoma using currently employed diagnostic imaging and tumour marker analysis. Our study was aimed at determining the risk of cancer development in patients with proven chronic pancreatitis, examining age and sex standardised incidence ratios calculated from the number of observed cases of pancreatic cancer in our cohort of 373 patients with predominantly alcohol related chronic pancreatitis to the number of cases expected in the National Cancer Registry. Our study design did not take into consideration diagnostic dilemmas and focused purely on cancer risk in our cohort of patients using defined stringent criteria. Indeed, we pre-

In search of the correct strategy for preventing the spread of HCV infection

Hepatitis C virus (HCV) infection is an emerging global healthcare issue. Apart from affecting approximately 3% of the world population, HCV is also a silent disease—the majority of incidences go unrecognised and serve unknowingly as sources of infection to others.1 Add to that the scant information defining the transmission routes and rates of HCV, the programme presented by Skipper et al in their paper evaluating the diagnosis and prevention of HCV in a prison outreach clinic (Gut 2003; 52: 1500–4) seems a model that could significantly constrain a situation that appears to be reaching epidemic proportions, especially here in Southern Italy.

Studies on the epidemiology of HCV infection in Italy have shown that this infection represents a major health problem throughout Southern Italy, with a prevalence of up to 12% in the general population.4-6 Also, HCV has been linked to the high incidence and mortality rates for liver cancer found in our region (standardised incidence rates per 100 000 inhabitants: 18.9 male, 13.2 female; mortality rates 15.2 male, 9.0 female).3 Between January 2000 and December 2001, we performed a cross sectional study of 5844 individuals (4260 men; 1584 women) from the general population and from five select groups in the city of Naples, with the purpose of assessing the prevalence of HCV infection in select groups with different exposure patterns in Southern Italy. Below are the preliminary data from this study:

• general population (n = 1972; prevalence 8.5%);
• imprisonment (n = 524; prevalence 37.4%);
• intravenous drug use (n = 1436; prevalence 31.8%);
• haemodialysis (n = 678; prevalence 2.9%);
• routine medical examination of patients (n = 453; prevalence 12.6%); and
• health care workers at the National Cancer Institute of Naples (n = 781; prevalence 6.4%).

Our findings confirm the high prevalence among drug users (31.8%) but the highest prevalence was found among male prisoners (37.4%), particularly those in the 30–49 year age range (47.3%). In fact, we concur with Skipper et al that “those involved with IDU frequently pass through the penal system and spend time in prisons” thus indicating a probable interrelationship between these two groups.

Our other findings from this study shed light on the unusually high prevalence of this disease in the general population of Southern Italy. Previous reports have shown that the prevalence of HCV infection is generally low in the general population of most industrialised countries (<5%). In the South of Italy, the high prevalence of HCV infection may be the result of past iatrogenic transmission, aggravated by:

1. extensive use of glass syringes or non-sterile syringes; and
2. social conditions which may indirectly favour the spread of infection:

• general poverty,
• poor education etc.,7,8, and
• especially among females, haemodialysis procedures.

Prisons do play a significant role in the hepatitis C epidemic, especially as the same social conditions mentioned above which may facilitate spread of infection also predict imprisonment.9 In fact, a disproportionate share of the burden of HCV infection is found among those who pass through correctional facilities.10 As stated previously, HCV sero-prevalence is high among this group. A history of incarceration is one of the strongest associations with HCV seropositivity.10 Yet despite these high levels, reports of HCV transmission in the prison setting are uncommon.11 In fact, studies have revealed that an overwhelming number of these infections are being brought into prisons via inmates who are already previously infected; drug users are most likely to become infected with HCV at the beginning of their addiction—long before being imprisoned for the first time.12 Yet it must be taken into account the fact that the dynamic movement of people in and out of prisons makes it very difficult to detect transmission.13 While the available data do not prove that infections are acquired in prison, they do indicate prisons as high risk institutions for the spread of HCV.14

What is of great concern to us are the implications of the previous and following data:

• HCV is easily transmitted parenterally.
Our prisons are overcrowded (the Secondigliano prison located in Naples, for example, has 1350 prisoners and only 790 beds).

In Southern Italy, the health system in general is less efficient and less meticulous than that in the north of Italy (and in the rest of the EU and in the USA).\(^7\)

We have no harm reduction programmes in place.

The limited availability of prevention methods has been linked to the transmission of HCV infection.\(^3\) This association, and its relation to inadequate management of a marginalised problem, surely opens up our National Health System to costly retaliations. If it can be proven that an inmate contracted HCV while incarcerated, due to a lack of sufficient care and prevention on the part of the system, he then has the right to seek judicial indemnification—a costly process for all concerned.

How much more economical to initiate admission screening programmes in our overcrowded prisons where, as detailed above, there is an identifiable elevated risk. By so doing, we move one step closer to correcting a problem that is grossly out of control. As HCV is associated with different kinds of diseases (liver, possibly non-Hodgkins lymphoma) and with autoimmune diseases (criglobulinaemia, thyroiditis, Hashimoto thyroiditis), which develop after the virus has caused immune system alterations,\(^4\) routine health screening on admission to prison presents a unique opportunity to identify health needs and plan health services at an early stage.\(^5\) In fact, studies have found that screening provides a preventive function to those who had previously been presented for a hepatitis C test, regardless of the result, were less likely to have recently engaged in high risk behaviour (that is, sharing injecting equipment).\(^6\)

How much more economical to initiate a good educational harm reduction programme such as that implemented by Skipper et al.\(^8\) Correctional interventions of this kind stand to benefit not only the inmates themselves and their families and partners, but also the public health of the communities to which the vast majority of inmates return.\(^9\) By implementing such a programme, the healthcare system would be doing its job, demonstrating efficient management of a social problem and sustaining the welfare of its people.

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References

Cross reactivity due to positive canrenone interference
Canrenone is a selective competitive inhibitor of the aldosterone receptor and a diuretic drug commonly used in the treatment of cirrhotic patients both with and without ascites.\(^1\) The aim of our observation was to determine if canrenone cross reacts with aldosterone in an immunoradiosorbent assay kit used for the routine function test, according to the Child-Pugh classification for liver cirrhosis.\(^2\) Canrenone, for in vitro experiments, was a gift from GElene Pharma (Group Therabel) Diagnostics. Canrenone, potassium canrenenate, and spironolactone are often used with digoxin in clinical practice and can cause false low results in common assays for digoxin (that is, ASyM MEIA-Abott) due to negative cross-reactivity, and falsely elevated serum digoxin concentrations with the fluorescence polarization immunomassay for digoxin.

Human hepatic stellate cells, isolated from wedge sections of normal human liver unsuitable for transplantation, were separated, after digestion with collagenase/protease, from other liver non-parenchymal cells by ultracentrifugation over gradients of strophanthidin. Cells were cultured on plastic culture dishes in Iscove's modified Dulbecco's medium, supplemented as described elsewhere.\(^3\) Cells were isolated (1 × 10^5 cells in well dishes) and incubated with increasing concentrations of canrenone or with no drug (SFIF or control conditions), after a 24 hour incubation period in serum free insulin free (SFIF) medium. After 24 hours, medium was replaced, and cells were lyophilised in a dry vacuum. Pellets were then resuspended in a total volume of 1 ml and aliquots of 200 μl were processed for aldosterone assay (Radim kit, Italy; KS171CT, RIA method). The results were expressed as percentage linearity (0.007 ± 0.003; 0.018 ± 0.004; 0.027 ± 0.003) nmol/l (mean ± SD); n = 3. As values obtained in SFIF samples were below the lower limit of the assay (0.009 ± 0.001), it is conceivable that these cells do not produce aldosterone under basal conditions.\(^4\) To further validate this observation, we then spiked sera with increasing concentrations of canrenone (10, 50, 100 μM) and, subsequently, aldosterone concentrations were determined. Sera were collected from patients with different degrees of liver disease (from acute hepatitis (n = 1), to non-alcoholic steatohepatitis (n = 1), or chronic active hepatitis with (n = 3) or without (n = 6) cirrhosis). Aldosterone concentrations were as follows: 0.32 (0.18), 0.63 (0.22), 0.85 (0.26), and 1.07 (0.35) nmol/l (n = 11). Comparing these concentrations with the increasing concentrations of canrenone spiked into sera, a significant correlation was found (r = 0.874, r² = 0.988; p < 0.001).

To date, only positive interferences leading to falsely high digoxin readings, including those due to spironolactone and canrenone, have been reported.\(^5\) Negative interference is much more dangerous. Toxic concentrations may remain undetected. Less severe negative interferences or interferences from clinically less significant cross reactants have been reported. Assay manufacturers should assess potential cross reactivity in the presence of the primary ligand. This difficulty may apply to immunoassays and cross reactants other than digoxin and canrenone, Pathologists and clinicians should be aware of negative interference so that intoxication due to drug dosing guided by monitoring of its concentration in serum can be avoided. For positive interference of a low magnitude substance, such as in our observation, false positive test results for hormonal and electrolyte disturbances during liver cirrhosis can be avoided.

These in vitro results can be considered effective in measuring aldosterone concentrations in plasma under in vivo conditions using a more cautious approach, as cross
Reactivity could hardly affect biological determinations.

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References

Responses to endothelin-1 in patients with advanced cirrhosis before and after liver transplantation

In response to Helmy’s comments in his recent letter (Gut 2004;53:470–1), we wish to emphasise the following points, many of which were clearly stated in our original paper.

In agreement with the comments on “generalised vasodilatation” in cirrhosis, we made it clear that basal forearm blood flow was normal in our patient cohort despite the presence of a vasodilated circulation, as evidenced by a reduced systemic vascular resistance index. As pointed out in our paper, this observation is consistent with findings of previous studies and suggests that beds other than the forearm circulation, such as those of the splanchnic and pulmonary circulation, were diluted in our patients.

With regard to the issues raised about the use of one arm plethysmography, our original results and those of others have shown that under well controlled circumstances the effects of external stimuli on results obtained using this approach are minimal.1 Indeed, in our study, the results of forearm plethysmography were very consistent across both the control and cirrhotic patient groups. However, we acknowledge that single forearm plethysmography could be affected by changes in systemic haemodynamics (due for example to the effects of drug infusions). However, as stated in the text, neither heart rate nor blood pressure altered significantly throughout the course of the experiment.

In forearm resistance arteries (and elsewhere), ET receptors on vascular smooth muscle and endothelial cells mediate opposing effects on vascular tone.2 Thus ETB blockade could result in either vasodilatation or vasoconstriction, depending on which receptor subpopulation is most affected. In our hands, preliminary experiments with the ETB blocker BQ788 yielded ambiguous responses, even in control subjects, causing vasocostriction in some and vasodilatation in others. Until a selective ETB receptor antagonist (for VSMC or endothelium) is available, interpretation of the results remains difficult.

As for the concern that similar vasodilatation was observed with endothelin-1 (ET-1) and BQ123 (an ETB agonist), we wish to re-emphasise these were two very different experiments in two separate groups of patients, asking two different questions. We observed:

1 that ET-1 infusion in these advanced cirrhotics produced generalised vasodilatation.
2 that we observed no difference between cirrhotics and controls in the effects of BQ123 on vascular tone.

We put these two results together to propose that it is likely that the abnormal response to ET-1 infusion reflects alterations in ETB mediated responses in cirrhotics (either via receptor changes or downstream pathways such as changes in nitric oxide synthesis, production, or proteolysis). This is not totally unexpected as it has previously been demonstrated that ET-3 (an ETB receptor agonist) causes early vasodilatation in control subjects; similarly, a trend towards an early vasodilatory effect of ET-1 has been observed in healthy subjects.

Regarding the use of concomitant drug therapy, all medications were ceased more than 24 hours prior to the experiments. While some residual effect of these agents is possible, more prolonged cessation of drug therapy in these decompensated patients was not considered safe or ethical. With regard to measurement of ET-1, as detailed in our paper, a commercially available assay with cross-reactivity between big ET-1 and ET-1 was used. The study was not powered (nor was it designed to) to pick up small differences in brachial artery ET-1 levels.

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References
1 Vaughan RB, Angus PW, Chin-Dusting JF. Evidence for altered vascular responses to exogenous endothelin-1 in patients with advanced cirrhosis with restoration of the normal vasoconstrictor response following successful liver transplantation (Gut 2003;50:1505–10).

Cluster's last stand

Guthrie and colleagues (Gut 2003;52:1616–22) described the results of cluster analysis in a patient sample with severe irritable bowel syndrome (IBS). Their analysis investigated a broad range of factors in addition to symptom thresholds; these included psychosocial measures (psychiatric intervention, health service encounters, quality of life) and physiological parameters (rectal thresholds). The authors have demonstrated that severe IBS can be classified according to non-medical characteristics and, in particular, according to the level of psychological distress, service encounters, and rectal sensitivity. They describe three groups which they labelled “distressed high utilisers”, “distressed low utilisers”, and “tolerant low utilisers.” The authors defend their analysis on clinical grounds and point to treatment implications for each of these groups.

We feel that there are some fundamental points about the nature of cluster analysis that readers of this paper should not overlook. Cluster analysis was initially developed to confirm the existence of biological entities and to evaluate specific syndromes, as described by the current Rome criteria.1,3 Following traditional clinical approaches, cluster solutions have generally been derived from symptom based parameters, including frequency, severity, and predominant complaint.

The term “cluster analysis” describes a range of procedures which use empirical methods to form groups of highly similar entities. While the notion that cluster analysis is solution seeking, operation of these techniques is essentially solution imposing: that is, clustering methods will always place objects into groups. Furthermore, as there are no formal statistical procedures to evaluate the resulting solution, the reasonableness of any solution is determined only on the basis of personal judgement. This is a problem. Indeed, critics of the approach have argued that cluster analysis encourages “naive empiricism”—that is, inclusion of as many variables as possible in the hope that a meaningful structure will come out. However, proponents of cluster analysis have suggested that careful selection of variables on theoretical grounds can overcome these problems. It is intuitively obvious that any single entity can be classified according to a broad range of dimensions, and Guthrie et al have certainly demonstrated this with respect to IBS. However, we rarely classify any object or entity according to all possible dimensions simultaneously; this would lead to a complex set of descriptors which may be unwieldy and contain many redundancies. Rather, we tend to select out a subset of meaningful dimensions that best suit our purposes in forming a classification.

There are certainly theoretical grounds for considering psychological involvement when evaluating patients with IBS. However, we rarely classify any object or entity according to all possible dimensions simultaneously; this would lead to a complex set of descriptors which may be unwieldy and contain many redundancies. Rather, we tend to select out a subset of meaningful dimensions that best suit our purposes in forming a classification.

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may stigmatise some patients with this complaint; one of the unfortunate consequences of classification is the tendency to attach labels to the subgroups that emerge.

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References

Small bowel carcinoma and coeliac disease

We thank Howdle et al for their comments on our study, detailed recently in their letter (Gut 2003;53: 470). In their British Society of Gastroenterology (BSG) National Survey,1 Howdle et al relied mainly on gastroenterologists and surgeons to report cases of small bowel carcinoma and whether they were associated with either coeliac or Crohn’s disease. This may have resulted in underestimation of associated coeliac disease. In our series, we had two cases in which the original pathologist had failed to recognise the histological features of coeliac disease in mucosa adjacent to the adenocarcinoma.2 The diagnosis of coeliac disease was made after review of the original resections. This problem has been recognised previously3 and results in the underdiagnosis of coeliac disease and further diagnostic delay for the patient with coeliac disease.

While the individual risk for patients with coeliac disease in developing adenocarcinoma of the small intestine is not great, poor survival should prompt rapid evaluation when symptoms occur. In addition, there should be a consideration of whether there is a subset of patients with coeliac disease who would benefit from screening for these cancers. Because patients with coeliac disease do not have a significantly increased risk of duodenal adenomas,4 the role of video capsule endoscopy of the entire small intestine needs to be explored.

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References

RETRACTION

Due to an administrative error, one article has been published on two occasions. The journal would like to retract the paper by Lindsay et al in the July issue (Gut 2003;52: 981–7) as it is a replicated version of a paper by the same authors in the March issue (Gut 2003;52: 363–9). The journal apollogises for this error.

NOTICES

British Society of Gastroenterology
Paul Brown Travel Fellowships

The Paul Brown Travel Fellowships are awarded by the Endoscopy Committee of the BSG. They are intended to assist trainee gastroenterologists and established consultants in visits to units outside the United Kingdom for specialist experience and training in endoscopy.

Specialist registrars who have not achieved their CCT are expected to have the approval of their Postgraduate Dean and their Regional Training Director before applying for a Travel Fellowship. Applicants are expected to provide confirmation that they have been accepted for training in the unit that they wish to visit. Successful applicants will be expected to provide a brief written report to the Endoscopy Committee of the outcome of their visit.

Application forms are available from the British Society of Gastroenterology Office, 3 St Andrew’s Place, London NW1 4LB. Email: bsg@mailbox.ule.ac.uk

14th International Workshop of Digestive Endoscopy, Ultrasonography and Radiology

The 14th International Workshop of Digestive Endoscopy, Ultrasonography and Radiology will be held in Marseille on 27–28 May 2004. For further information, please contact: Nathalie Fontan, Atelier Phenix, 41 rue Docteur Monru, 13006 Marseille (tel: (33) 04-91-37-15-28; e-mail: nfontant@aphenix.com).

European Postgraduate Gastro-surgical School (EPGS) Courses 2004

The EPGS at the Academic Medical Center of the University of Amsterdam will be hosting the following courses during the year: ‘Benign Hepato-Biliary Disorders’ will be held on 22 & 23 April 2004, ‘Endosonography live in Amsterdam’ will be held on 2, 3 & 4 June 2004, and ‘Update in Coloproctology’ will be held on 28 & 29 October 2004. For further information, please contact: J Goedkoop (tel: (31) 566 3926; fax: (33) 267 5594; e-mail: j.goedkoop@amc.uva.nl; website: www.epgs.nl).

8th Southeast European Symposium of Paediatric Surgery

The 8th Southeast European Symposium of Paediatric Surgery will focus upon ‘Infectious Problems in Paediatric Surgery’. The event will be held between 24–25 September 2004, at the University of Graz, Austria. For further information, please contact: Professor M E Höllwarth, Department of Paediatric Surgery, Medical University of Graz, Austria, Auenbruggerplatz 34, 8036 Graz; tel: + 43 316 385 3762; fax: tel: + 43 316 385 3775; e-mail: kinderchirurgie@uni-graz.at