Scientific paper

Effect of Infliximab on IFN-γ and IL-4 Expression in Peripheral Blood and Inflamed Intestinal Mucosa of Patients with Crohn's Disease

Ivan Ferkolj[1]*, Saša Markovič[1], Željka Večerič[1], Alojz Ihan[2], Maja Pohar[3]

[1] *University Medical Centre Ljubljana, Division of Internal Medicine, Department of Gastroenterology, Ljubljana, Slovenia*

[2] *Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia*

[3] *Institute of Biomedical Informatics, Faculty of Medicine, University of Ljubljana, Slovenia*

** Corresponding author: University Medical Centre Ljubljana, Division of Internal Medicine, Department of Gastroenterology, Japljeva 2, 1000 Ljubljana, Slovenia, Tel: +386 41 519879, Fax: +386 1 4334190, E-mail: ivan.ferkolj@kclj.si*

Received: 24-10-2006

Abstract

Background: Infliximab, a monoclonal anti-tumor necrosis factor alpha (TNF-α) antibody, is an effective treatment for Crohn's disease without prior response to conventional therapy. It binds with high affinity to soluble and membrane-bound TNF-α, neutralizing its biological activity and reducing TNF-α production by mucosal cells. Its influence on other cytokines has been investigated to a much smaller extent. Aim: The aim of the study was to establish the influence of infliximab on cells containing the proinflammatory cytokine interferon-gamma (IFN-γ) and the anti-inflammatory cytokine interleukin-4 (IL-4) in peripheral blood and inflamed intestinal mucosa. Methods: Twenty-five patients with Crohn's disease (11 with the luminal and 14 with the fistular form) underwent treatment with infliximab. IFN-γ and IL-4 containing cells in peripheral blood and inflamed intestinal mucosa were analysed by flow cytometry before and 14 days after the treatment. Results: Twenty-two patients (88%) showed a clinical response to the drug and three (12%) had no response. In the group of responders, the proportion of cells containing IFN-γ and IL-4 in peripheral blood and inflamed intestinal mucosa remained unchanged after the treatment. Conclusions: Infliximab has no direct influence on cells containing IFN-γ and IL-4 in peripheral blood and inflamed intestinal mucosa. The insignificant changes in the proportions of these cells observed after the treatment may be attributed to its direct effect on TNF-α, which resulted in silencing of the inflammation.

Keywords: Crohn's disease, infliximab, interferon-gamma, interleukin-4

1. Introduction

Crohn's disease (CD) is a chronic inflammatory bowel disease of unknown aetiology. The most likely cause of the intestinal inflammation is an inappropriate response of the intestinal immune system to one or more antigens. The inflammation is the result of a complex series of events, starting with the activation of the intestinal immune system by various triggers (bacterial products, viruses, food antigens etc.), followed by the secretion of cellular immunomodulatory peptides-cytokines.[1–4] Cytokines are produced and secreted by lymphocytes (mainly helper T cells of the Th1 and Th2 phenotypes), monocytes, intestinal macrophages, granulocytes, epithelial cells, endothelial cells and fibroblasts.[2,5–7] They are involved in both amplification and termination of the inflammatory response. They modulate the inflammatory response as well as the resulting tissue damage and subsequent repair. They induce the production of adhesion molecules and other inflammatory mediators such as leukotrienes, prostaglandins, platelet-activating factor, reactive oxygen metabolites and nitric oxide. Cytokines attract to the site of inflammation immune cells, which in turn release even more inflammatory mediators and effectors, so that a vicious circle is created.[6] This process leads to destruction of the intestinal epithelium and its underlying structures.

The most important pathogenetic factor in CD is believed to be an imbalance of proinflammatory and anti-inflammatory cytokines and growth factors.[2,8–10] According to their cytokine production profile, helper T cells are classified into three broad types: Th1, Th2 and Th3 cells. Although all three cell populations develop from the same precursor, the naive CD4+ T lymphocyte, they differ in their ability to express immunoregulatory cytokines. Th1 cells produce the proinflammatory cytokines interleukin-2 (IL-2), interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α); Th2 cells secrete the anti-inflammatory cytokines IL-4, IL-5, IL-9, IL-10 and IL-13, and Th3 cells regulate Th1 and Th2 cytokine production by producing transforming growth factor-β.[2,4,5,8] In CD patients, an exaggerated Th1 response has been described. TNF-α is a typical proinflammatory cytokine. Infliximab (Remicade; Centocor, Malvern, PA, USA), a genetically constructed IgG1 murine-human (chimeric) anti-TNF-α monoclonal antibody, is an effective treatment for moderate to severe luminal and fistulizing CD without prior response to conventional treatment with corticosteroids (CS) and immunosuppresive drugs.[11–17]

The effect of infliximab on TNF-α in peripheral blood and inflamed tissue has been demonstrated in several studies.[9,12,13,18–21] The aim of this study was to evaluate its influence on cells containing IFN-γ and IL-4 in peripheral blood and inflamed intestinal mucosa of patients with CD.

2. Materials and Methods

2.1. Patients

Twenty-five patients with active luminal or fistulizing CD of at least 6 months duration were included in an open label, single centre prospective study at the Department of Gastroenterology in Ljubljana (characteristics of the patients are presented in Table 1). The patients' mean age was 40 years (range 19–60) and the mean duration of their illness was 10 years (range 1–22).

Inclusion criteria for active luminal disease (11 patients) comprised: moderately to severely active ileal and/or colonic form of CD not adequately responding to prior conventional treatment with 5-aminosalicylates (5-ASA) 4.5 g/day given for at least 2 months, antibiotics (metronidazole 1.2 g/day and/or ciprofloxacin 1g/day) given for at least 1 month, 6-methyl-prednisolone up to 42 mg/day given for at least 2 months, and azathioprine (AZA) 2.5 mg/kg/day given for at least 6 months. Disease activity was evaluated by Crohn's disease activity index (CDAI).[22] CDAI scores were calculated from patient diaries within a week before infliximab administra-

Table 1: Characteristics of the 25 patients included in the study.

	Gender (M,F)	Age (in years)	Duration (in years)	Type of disease	Localisation of disease	CDAI before therapy	Medication before therapy	EIM before therapy
1.	F	60	22	L	C	320	AZA	+
2.	F	33	12	L	I	310	0	-
3.	F	60	6	L	C	290	AZA	+
4.	F	33	1	F	I		AZA+CST	+
5.	M	30	11	F	IC		AZA	-
6.	M	49	12	F	C		AZA	-
7.	M	26	8	F	I		AZA	-
8.	F	49	21	L	IC	270	AZA+CST	+
9.	F	23	4	F	IC		AZA	-
10.	F	31	12	L	C	180	CST	+
11.	F	49	1	L	C	260	AZA+CST	+
12.	F	60	15	L	C	230	CST	+
13.	F	43	12	L	I	310	0	+
14.	M	43	15	F	IC		AZA+CST	-
15.	M	39	15	F	I		AZA+CST	-
16.	F	50	16	F	IC		AZA+CST	+
17.	F	30	1	F	C		0	-
18.	M	43	20	L	I	310	AZA+CST	-
19.	F	19	5	F	I		AZA	-
20.	M	26	2	F	IC		CST	-
21.	M	41	12	L	C	310	CST	+
22.	F	37	9	L	IC	290	0	+
23.	F	39	9	F	C		AZA+CST	-
24.	M	36	12	F	I		AZA	-
25.	M	41	7	F	IC		AZA+CST	+

Type of disease: L = luminal, F = fistular; Localisation of disease: I = ileum, C = colon, IC = ileum + colon; CDAI = Crohn's disease activity index; Medication: AZA = azathioprin, CST = corticosteroids; EIM = extraintestinal manifestations of the disease, + = present, - = absent

tion. The mean CDAI score of the group was 280 (range 180–320).

The following inclusion criteria were used for fistulizing disease (14 patients): single or multiple draining fistulas occurring as a complication of CD, with no prior response to standard treatment with metronidazole 0.8 g/day and/or ciprofloxacin 1g/day given for at least 2 months, and AZA 2.5 mg/kg/day given for at least 6 months.

Exclusion criteria for the study were: other complications of CD (e.g. symptomatic strictures or stenosis, abscesses, previous total colectomy), a history of allergy to murine proteins, a serious infection in the previous 3 months, and prior treatment with any anti-TNF-α medication.

The protocol allowed concomitant treatment with 5-ASA, antibiotics, CS or AZA, provided that the dosage had been stable for at least 2 months before enrolment. All 19 patients who tolerated AZA continued to receive the drug at a stable dose of 2.5 mg/kg/day. Six patients continued to receive CS at a stable dose. Smoking was not allowed for 2 months before and 14 days after enrolment.

The study was approved by the Medical Ethics Committee of the Republic of Slovenia (approval No. 57/03/20). Informed consent was obtained from all patients.

2.2. Normal Control Subjects

Control data were obtained from specimens of peripheral blood (19) and normal colonic mucosa (6) collected from 25 healthy subjects who ranged in age from 26 to 62 years (mean age 37.4 years) and were matched for age and sex with the patients.

2.3. Treatment Protocol

All patients were admitted to our day care center. Infliximab was administered by a single infusion at a dose of 5 mg/kg. The drug was filtered, diluted with 500 ml of normal saline, and infused through a 0.22 micron low-protein binding filter over a period of two hours.

2.4 Peripheral Blood

Blood samples for routine laboratory tests and flow cytometry were collected directly before and two weeks after infliximab administration.

2.5. Biopsy of Inflamed Intestinal Mucosa

Colonoileoscopy was performed within 2 days before and 14 days after infliximab administration. During the procedure, 3 specimens of macroscopically inflamed mucosa were collected from the vicinity of ulcerations or fistulas. In each patient, both endoscopic procedures were performed with the same instrument and by the same endoscopist, and the specimens were collected from the same sites. The location of lesions was recorded by their distance from the anus and clockwise orientation. The colon was involved in 9 patients, the ileum in 8, and both the colon and the ileum in 8.

2.6. Evaluation of the Treatment Response

The response to the drug was evaluated 14 days after the infusion. For luminal CD, disease activity was assessed using the CDAI score. A positive response was defined as a CDAI score of 150 points or less, or a decrease by 70 points or more from the baseline value. For fistulizing disease, a positive response was defined as closure of fistulas or a decrease in secretions by at least 50% from baseline.

2.6.1. Evaluation of Safety

The patients were monitored for possible adverse effects of the drug. Their blood pressure, pulse and temperature were measured at 30-minute intervals during the infusion and over the next 2 hours. At the follow-up visit 14 days after the treatment, they were questioned about any adverse experiences and their outcome.

2.6.2. Flow Cytometry

Flow cytometry of peripheral blood and specimens of inflamed intestinal mucosa was performed using a fluorescence cell counter (FACS Calibur Becton Dickinson, Mountain View, CA). Fresh whole blood was collected into EDTA-Vacutainer tubes while biopsy material was rinsed with RPMI and mechanically dispersed by Medimachine (Becton Dickinson, Mountain View, CA). The suspension was then transferred to a tube containing RPMI, supplemented with collagenase (5 mg/ml, Sigma, Missouri, USA). The capped tube was incubated at 37 °C for 20 min, and the suspension was triturated gently every 5 min through a wide-bore pipette to assist disintegration. The cells were washed twice with PBS and prepared for flow cytometry.

Stimulation, fixation and permeabilisation of lymphocytes for the detection of intracellular IL-4 and IFN-γ was accomplished by means of a Cytodetect kit (Immuno-Quality Products, Groningen, Netherlands). Intracellular concentration of cytokines was achieved with the use of the exocytosis inhibitor brefeldin A (5M, Sigma). Histograms of fluorescence distribution were generated by plotting cell numbers (*y* axis) versus fluorescence intensities (log scale; *x* axis). The median fluorescence intensity (MFI) which reflects the overall level of expression of the epitope specifically recognized by Mab was considered. Isotype controls (Becton Dickinson Mountain View, CA) and cell viability controls (Live/Dead Kit, Molecular Probes, Oregon USA) were included in the analysis.

2.6.3. Statistical Analysis

Because of the relatively small sample size and nature of the covariates, no normality assumptions could be made. Consequently the Wilcoxon signed rank test was used for comparing values measured before and after the treatment. For easier understanding, the data were presented also in the form of box plots. The analysis was done using Statistical Software R for Windows, version 2.2.1.

3. Results

3.1. Efficacy and Safety of Treatmenty

Fourteen days after the treatment, 22 patients (88%) had a clinical response and 3 (12% – 1 in the luminal and 2 in the fistular group) had no response. Extraintestinal manifestations (EIM) of CD, originally present in 12 patients, disappeared in 10 of them soon after the therapy. The remaining 2 patients did not respond to the therapy and also had no improvement of EIM.

In both groups, infliximab was well tolerated and no serious side reactions were recorded. Six patients, 2 in the luminal and 4 in the fistulizing group, had minor adverse events (hypertension, flu-like symptoms, fever, angina-like syndrome, weakness, psychiatric disorders and chest pain). No changes in routine biochemistry and urinary analysis were recorded.

3.2. Flow Cytometric Analysis of Peripheral Blood Cells

The results of flow cytometric analysis of peripheral blood cells obtained from patients who responded to the therapy are presented in Figure 1 (fluorescence intensities of IFN-γ containing cells) and Figure 2 (fluorescence intensities of IL-4 containing cells). The results varied widely, and statistical analysis revealed no significant differences either between the values for the control subjects and the patients before the therapy, or between the patients' pre- and post-treatment values.

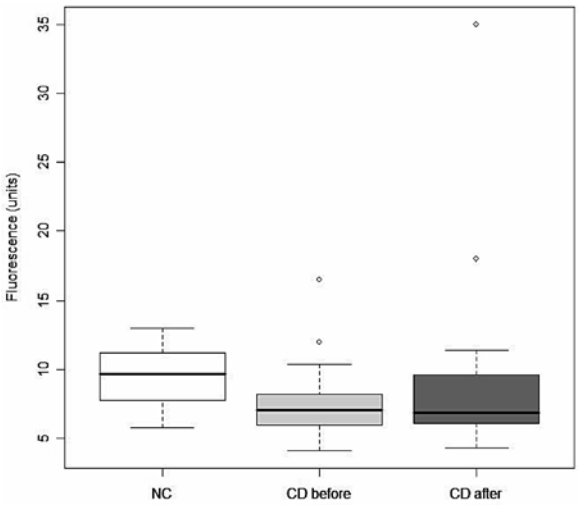

Figure 1: Fluorescence intensities of IFN-g containing peripheral blood cells from normal control subjects (NC) and CD patients who responded to the therapy. (CD before: CD patients before treatment; CD after: CD patients 14 days after treatment)

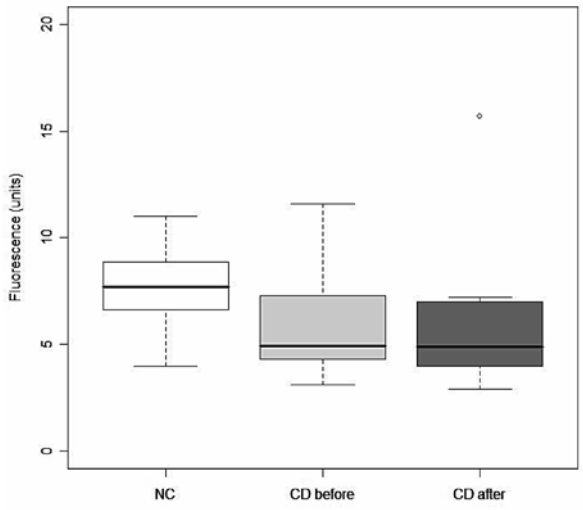

Figure 2: Fluorescence intensities of IL-4 containing peripheral blood cells from control subjects (NC) and CD patients who responded to the therapy. (CD before: CD patients before treatment; CD after: CD patients 14 days after treatment)

3.3. Flow Cytometric Analysis of Cells from Specimens of Intestinal Mucosa

The results of flow cytometric analysis of mucosal cells obtained from the patients who responded to the therapy are presented in Figure 3 (fluorescence intensities

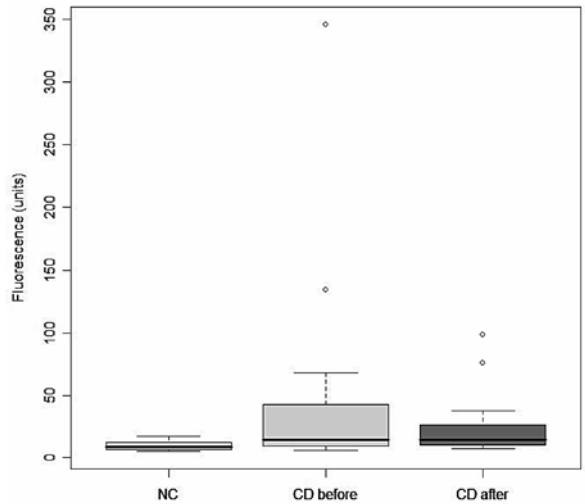

Figure 3: Fluorescence intensities of IFN-g containing mucosal cells from normal control subjects (NC) and CD patients who responded to the therapy. (CD before: CD patients before treatment; CD after: CD patients 14 days after treatment)

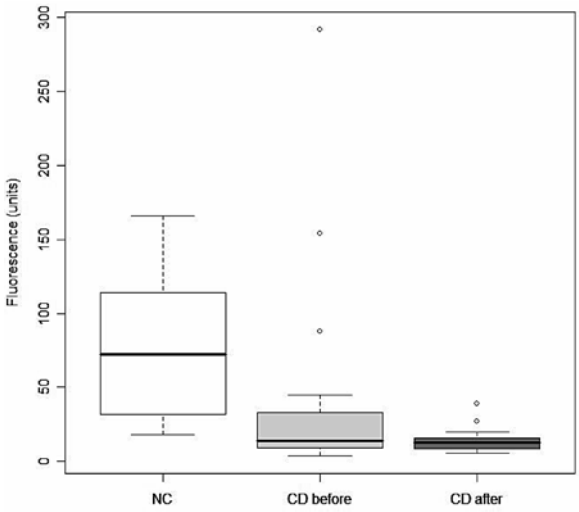

Figure 4: Fluorescence intensities of IL-4 containing mucosal cells from normal control subjects (NC) and CD patients who responded to the therapy. (CD before: CD patients before treatment; CD after: CD patients 14 days after treatment)

of IFN-γ containing cells) and Figure 4 (fluorescence intensities of IL-4 containing cells). Again, the results varied widely, and there were no significant differences either between the values for the control group and the patients' prior to treatment, or between the patients' pre- and post-treatment values.

4. Discussion

Infliximab is an effective treatment for CD in patients with poor prior response to conventional therapy. In our study, 22 patients (88%) had a clinical response and only three (12%) had no response 14 days after a single infusion of infliximab. The evaluation of the clinical response was so early due to standard protocol of the treatment at the time of study (only 1 infusion for luminal CD and 3 infusions for fistulizing CD at week 0, 2, 6) and was done just before the patients with fistulizing form od CD receive the second infusion of infliximab. The treatment was well tolerated and no serious side reactions were recorded. The aim of this study was to assess its potential effect on the presence of cells containing IFN-γ and IL-4 by flow cytometric examination of peripheral blood and inflamed intestinal mucosa. In the group of patients who responded to the treatment, the results for both cytokines were very dispersed, and we found no significant differences between the pre- and post-treatment values.

Under normal circumstances, regulatory mucosal T cells maintain the intestinal mucosa in a state of »controlled inflammation«.[2,4,5,8,18] An imbalance between proinflammatory and anti-inflammatory cytokines is important in the pathogenesis of CD. TNF-α, a typical proinflammatory cytokine, is considered to play a pivotal role in CD; its production in the intestinal mucosa of patients with CD is increased and often correlates with the severity of the inflammation. Infliximab binds with high affinity to soluble and membrane-bound TNF-α, thus neutralizing its biological activity, and reduces TNF-α production by mucosal cells. In the majority of patients with CD, it suppresses the inflammation in the gut mucosa and induces clinical improvement.[12,13,19–21,23–25] Its mechanism of action is not limited to the simple elimination or blockage of TNF-α. In addition, it appears to cause sustained downregulation of activated T cells capable of producing other proinflammatory and anti-inflammatory cytokines.[2,18–20] To test this hypothesis, we investigated the effect of infliximab on cells containing IFN-γ and IL-4 in the peripheral blood and in inflamed intestinal mucosa of patients with CD.

IFN-γ, also a cytokine of the Th1 profile, is an activator of phagocytes and neutrophils and promotes the differentation of T cells. Its role in CD is less well characterized and both normal as well as decreased or increased levels of IFN-γ have been reported in patients with CD.[8,10,18,23,26–30] It is a potent proinflammatory cytokine implicated in the inflammation and treating of active CD with fontolizumab, a humanised anti IFN-γ antibody was well tolerated and resulted in increased rates of clinical response and remission compared with placebo.[31] Our investigations did not reveal any significant differences in IFN-γ values between normal controls and CD patients either before or after the treatment. However, all our patients had been treated for a long time with immunosuppressive agents and CS. Agnholt and co. found an insignificant decrease in IFN-γ production in cell cultures at 8 weeks after infliximab administration.[26] Plevy and co., Cornillie and co. and Agnholt and Kaltoft found a significant decrease in the number of IFN-γ producing cells in the lamina propria of CD patients after infliximab therapy. However, all above authors used different techniques than were used in our study.[23,18,21,26] *In vitro* studies have shown that infliximab reduces IFN-γ production by T-cells in tissue, suggesting that IFN-γ synthesis may be dependent on TNF-α levels.[18,23,26] When TNF-α is neutralized by infliximab binding to activated T-cells, the stimulus for lymphocytes to produce IFN-γ may be removed.[18] This cytokine apparently reflects some of the essential mechanisms involved in active inflammation. Its production seems to be in intimate balance with TNF-α levels and may be an end-product of the changes observed after infliximab treatment.

The principal cellular sources of IL-4 are CD4+ T lymphocytes of the Th2 subset as well as activated mast cells and basophils. IL-4 is an important stimulus for the production of IgE antibodies and for the development of Th2 cells from naive CD4+ helper T cells. It functions as an autocrine growth factor for differentiated Th2 cells, acting both as an inducer and an effector of this subset.[6] By antagonizing the macrophage-activating effects of IFN-γ, it inhibits cell-mediated immune reactions.[6,10]

Cytokines of the Th2 profile, such as IL-4, have widespread inhibitory effects on the synthesis of proinflammatory cytokines and may inhibit tissue damage elicited by Th1 cells. By suppressing the production of cell-mediated immunity and inducing proinflammatory cytokines, Th2 cells may function as "suppresor cells" controlling immune responses, particulary inflammatory Th1 responses. Therefore, Th2 cytokines may be inappropriately downregulated in CD, and CD patients are generally reported to have decreased IL-4 levels in their tissue.[8,32,33] In our study, the patients' IL-4 levels before the treatment did not differ significantly from those measured in the control group, probably as a result of concomitant treatment with imunosuppressive agents and CS. Fourteen days after the infusion of infliximab, the fluorescence intensity of IL-4 containing cells from the patients' peripheral blood and intestinal mucosa did not differ significantly from the pretreatment values. Baert and co., using the immunohistochemical technique, found decreased IL-4 values in their patients' mucosa 8 weeks after infliximab therapy and concluded that decrease of IL-4 in tissue may be explained by a general restoration of the mucosa and an answer on a downregulation of inflammation (less inflammation, less IL-4).[20]

In our patients, the values of both cytokines were very dispersed both before and after the treatment. A similar dispersion of the cytokine levels was observed also in the control group. Because of the small number of patients and the broad range of the values, all our findings could not be translated into statistically significant results. Moreover, CD is focally distributed inflammation and a sampling effect cannot be avoided despite our efforts to take several biopsy specimens from the macroscopically most diseased segments.

In conclusion, the dramatic clinical, endoscopic and histopathologic responses observed in CD patients treated with a single i.v. infusion of infliximab illustrate that TNF-α has a central role in the pathogenesis of mucosal inflammation in CD. The data presented in our study suggest that this medication has no direct influence on IFN-γ and IL-4 containing cells in peripheral blood and inflamed intestinal mucosa. The insignificant changes in the levels of these two cytokines found after the treatment may be attributed to the direct effect of infliximab on TNF-α, which resulted in silencing of the inflammation.

5. References

1. A. Forbes, in: A. Forbes (Ed.): Clinicians' guide to inflammatory bowel disease, Chapman-Hall Medical, London, **1997**, pp. 1–44.
2. S. R. Targan, *Can. J. Gastroenterol.* **2000**, *14 Suppl C*, 13C–16C.
3. J. Schoelmerich, *Eur. J. Gastroenterol. Hepatol.* **2003**, *15*, 585–586.
4. C. Schmidt, A. Stallmach, *Minerva. Gastroenterol. Dietol.* **2005**, *51*, 127–145.
5. G. Rogler, T. Andus, *World. J. Surg.* **1998**, *22*, 382–389.
6. J. J. Oppenheim, M. Feldman, in: J. J. Oppenheim, M. Feldman (Ed.): Cytokine reference, Academic press, London, **2001**, pp. 3–20.
7. M. G. Neuman, *Rom. J. Gastroenterol.* **2004**, *13*, 309–316.
8. S. J. McClane, J. L. Rombeau, *J. Parent. Enteral. Nutr.* **1999**, *23*, S20–S24.
9. J. F. Mouser, J. F. Hyams, *Clinical Therapeutics* **1999**, *21*, 932–942.
10. S. Schreiber, *Hepato-Gastroenterology* **2000**, *47*, 15–28.
11. H. M. van Dullemen, J. H. van Deventer, D. W. Hommes, H. A. Bijl, J. Jansen, G. N. J. Tytgat, J. Woody, *Gastroenterology* **1995**, *109*, 129–135.
12. S. R. Targan, S. B. Hannauer, S. J. H. van Deventer, L. Mayer, D. H. Present, T. Braakman, K. L. DeWoody, T. F. Schaible, P. J. Rutgeerts, *N. Engl. J. Med.* **1997**, *337*, 1029–1035.
13. S. B. Hanauer, B. B. G. Feagan, G. R. Lichenstein, L. F. Mayer, S. Schreiber, J. F. Colombel, *Lancet* **2002**, *359*, 1541–1549.
14. M. Bewtra, G. R. Lichtenstein, *Expert. Opin. Biol. Ther.* **2005**, *5,* 589–599.
15. R. Panaccione, W. J. Sandborn, *Curr. Opin. Gastroenterol.* **2004**, *20*, 351–359.
16. G. Van Assche, S. Vermeire, P. Rutgeerts, *Curr. Opin. Gastroenterol.* **2005**, *21*, 443–447.
17. W. Kruis, *Schweiz. Rundsch. Med. Prax.* **2005**, *94*, 1595–1598.
18. S. E. Plevy, C. J. Landers, J. Prehn, N. M. Carramanzana, R. L. Deem, D. Shealy and S. R. Targan, *J. Immunol.* **1997**, *159*, 6276–6282.
19. F. J. Baert, P. R. Rutgeerts, *Int. J. Colorectal. Dis.* **1999**, *14*, 47–51.
20. F. J. Baert, G. R. D'Haens, M. Peeters, M. I. Hiele, T. F. Schaible, D. Shealy, *Gastroenterology* **1999**, *116*, 22–28.
21. F. Cornillie, D. Shealy, G. R. D'Haens, K. Geboes, G. van Assche, J. Ceuppens, *Aliment. Pharmacol. Ther.* **2001**, *15*, 463–473.
22. W. R. Best, J. M. Bektel, J. W. Singleton, F. Kern, *Gastroenterology* **1976**, *70*, 439–444.
23. J. Agnholt, J. F. Dahlerup, S. Buntzen, A. Tottrup, S. L. Nielsen, E. Lundorf, *Aliment. Pharmacol. Ther.* **2003**, *17*, 703–710.
24. K. Geboes, P. Rutgeerts, G. Opdenakker, *Curr. Med. Res. Opin.* **2005**, *21*, 1741–1754.
25. G. R. D'Haens, S. J. H. Van Deventer, R. Van Hogezand, D. Chalmers, C. Kothe, F. Baert, *Gastroenterology* **1999**, *116*, 1029–1034.
26. J. Agnholt, K. Kaltoft, *Cytokine* **2001**, *15*, 212–222.
27. B. Y. Lieberman, C. Fiocchi, K. R. Youngman, W, K. Sapatnekar, M. R. Proffitt, *Dig. Dis. Sci.* **1988**, *33*, 1297–1304.
28. A. Stallmach, F. Schafer, S. Hoffmann, S. Weber, I. Muller-Molaian, T. Schneider, G. Kohne, K. W. Ecker, G. Feifel, M. Zeitz, *Gut* **1998**, *43*, 499–505.

29. F. Cominelli, K. O. Arseneau, T. T. Pizarro, in: S. R. Targan, F. Shanahan, L. C. Karp (Ed.): Inflammatory bowel disease: from bench to bedside, Kluwer academic publishers, Dordrecht, **2003**, pp. 147–176.
30. K. A. Papadakis, *Curr. Allergy. Asthma. Rep.* **2004**, *4*, 83–89.
31. D. W. Hommes, T. L. Mikhajlova, S. Stoinov, D. Stimac, B. Vucelic, J. Lonovics, M. Zakuciova, G. R. D'Haens, G. Van Assche, S. Ba, S. Lee, T. Pearce, *Gut* **2006,** *55*, 1131–1137.
32. R. Karttunnen, E. J. Breese, J. A. Walker-Smith, T. T. MacDonald, *J. Clin. Pathol.* **1994**, *47*, 1015–1018.
33. G. A. West, T. Matsuura, A. D. Levine, J. S. Klein, C. Fiocchi, *Gastroenterology* **1996**, *110*, 1683–1695.

Povzetek

Infliximab, monoklonsko protitelo proti tomor razpadajočemu faktorju alfa (TNF-α) je učinkovito zdravilo za zdravljenje tistih oblik Crohnove bolezni, ki se niso odzvale na običajna zdravila. Z visoko afiniteto se veže na topne in membransko vezane oblike TNF-α, izniči njegovo delovanje, zmanjša število celic, ki ga izločajo in umiri vnetje Vpliv infliximaba na ostale citokine pa do sedaj še ni bil dobro raziskan. Namen raziskave je bil ugotoviti vpliv infliximaba na celice (v krvi in v vnetem črevesnem tkivu), ki izločajo vnetni citokin interferon-gama (IFN-γ) in protivnetni citokin interleukin-4 (IL-4). Zdravili smo 25 bolnikov s hudo obliko Crohnove bolezni (11 z vnetno luminalno obliko in 14 s fistulizirajočo obliko). Pred zdravljenjem in 14 dni kasneje smo z metodo pretočne citometrije analizirali celice, ki izločajo IFN-γ in IL-4. Dvaindvajset bolnikov (88 %) se je odzvalo na zdravljenje, pri treh (12 %) pa ni bilo odziva. Pri bolnikih, ki so se odzvali na zdravljenje, se razmerje celic, ki izločajo IFN-γ in IL-4, po zdravljenju ni spremenilo.
Ugotavljamo, da infliximab nima neposrednega vpliva na krvne in tkivne celice, ki izločajo IFN-γ in IL-4. Neznačilne spremembe, ki smo jih zaznali po zdravljenju, pripisujemo neposrednemu delovanju infliximaba na TNF-α in posledični umiritvi vnetja.