Biological Therapies For Inflammatory Bowel Disease: Research Drives Clinics

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Abstract: The better understanding of the mechanisms of inflammatory bowel disease has driven our progress into the development of new biological therapies targeting specific molecules.

Anti-TNF-α biologic compounds have shown great efficacy particularly in Crohn's disease. Infliximab (an IgG1 mouse/human chimeric monoclonal anti-TNF-α antibody fragment) is the most efficacious compound in induction and maintenance therapy of active and fistulizing Crohn's disease, being at present the only biological compound approved for therapy, but with the limit of the immunogenicity; CDP-571 (a humanized anti-TNF-α antibody) and CDP-870 (a PEGylated anti-TNF-α antibody) are less immunogenic, showed some efficacy in induction therapy in Crohn's disease but a rapid loss of response in maintenance therapy. Etanercept and onercept (soluble human recombinant TNF-α receptors fusion proteins) seem not to be efficacious in Crohn's disease demonstrating no class-effect for anti-TNF-α compounds. In preliminary study, adalimumab (an IgG1 humanized monoclonal anti-TNF-α antibody) offers good perspective of efficacy and safety also in infliximab-resistant or allergic patients. Inhibition of lymphocyte trafficking to the gut, through anti-adhesion molecules specific therapies (natalizumab, MLN-02, alicaforsen), has shown promising results: unfortunately, natalizumab, the most effective drug of this class, has recently been suspected to favour serious neurological complications. Other biologic therapies are under evaluation but at present seem to be less promising than infliximab; they consist of antiinflammatory cytokines, inhibitors of proinflammatory cytokines, hormones and growth factors: anti-IL12-antibody, interferon-α, interferon-β, G-CSF, GM-CSF, EGF, growth hormone, anti-interferon-γ, anti-IL-18, anti-IL-2-receptor and anti-CD3 antibodies. The evaluation of other biological drugs has been suspended for severe side effects as happened for anti-CD40L antibody causing thromboembolism and anti-CD4 antibody causing ly.mphopenia. Other compounds as IL-10 and IL-11 have been proven to be ineffective even if an oral formulation of IL-11 is under evaluation. Among the MAP kinases inhibitors BIRB-796 and RDP58 showed to be ineffective while CNI-1493 is under evaluation.

The effort in identifying specific patients features predicting therapy response and the possible combination of different biological therapies represent undoubtedly a very promising perspective. Aim of this article is to review the biological compounds and their efficacy in IBD.

1. INTRODUCTION

Emerging knowledge of the pathogenic steps of Crohn's disease (CD) and ulcerative colitis (UC), the two major forms of inflammatory bowel disease (IBD), has driven our progress into the development of new biological therapies targeting specific molecules.

The pathogenesis of IBD is a very intriguing puzzle, whose tassels are coming together. It is now well established that several mediators are critically involved in inducing and maintaining mucosal inflammation and that targeting each one of these molecules could represent a rational and specific strategy for therapeutic intervention. In the last decade translational medicine has indeed proven that bench work has been solid in providing the major pathway to interrupt or shut down intestinal inflammation.

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In this paper we aim to review recent advance in IBD therapy and future therapeutic approaches now on the horizon.

2. PATHOGENIC BASES

Inflammatory bowel diseases (IBD) are pathological conditions characterized by a dysregulated immune response leading to a persistent inflammatory condition in the bowel.

Normal inflammatory response against pathogens is usually self-limiting and terminates with the eradication of the pathogen itself. Because of the activity of anti-inflammatory cytokines as interleukin-10 (IL-10), transforming growth factor-beta (TGF-β), interleukin-11 (IL-11) or cytoprotective factors as epidermal growth factor (EGF), keratinocyte growth factor (KGF), IL-11 and growth hormone (GH) it is possible to down-regulate inflammation or contribute to tissue restitution, respectively [1]. In IBD patients the balance is altered and the contact with the intestinal mucosal immune system of otherwise innocuous luminal agents results in a persistent chronic inflammation.

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Genetic, microbial, environmental factors certainly contribute to this imbalance [2].

The evidence coming from animal models indicating that colitis development is prevented by the absence of bowel bacteria [3], the observation of tight junctions dysfunction in the intestinal mucosa of IBD patients [4] and the relation between CD and some genes involved in the first line defence against bacteria (the so called pattern recognition receptors, in particular NOD2/CARD15 protein and toll-like receptors 4 linking the lipid A portion of lypopolisaccaride of gram negative bacteria) [5,6] suggest that the first-line defence barrier impairment could be the initial pathogenetic step towards IBD development. This impairment of the innate immune system can be the cause of a subsequent persistent activation of the specific immune system. Specially in CD patients the production by APCs (Antigen Presenting Cells) and macrophages of interleukin-12 (IL-12) and interleukin-18 (IL-18) causes a T helper (Th)1 polarization resulting in a typically Th1-cytokines profile characterized by interferon-gamma (IFNγ), interleukin-2 (IL-2) and tumor necrosis factor (TNF) secretion. These cytokines on their turn stimulate APCs, macrophages and endothelial cells to produce other proinflammatory cytokines as TNF-α, interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), IL-12, IL-18 leading to the creation of a self-sustained cycle [4,7].

Conversely, in UC patients a Th2-like phenotype is observed with an increased production of interleukin-5 (IL-5) and reduction of IFN-γ [4].

Furthermore, another essential step is the specific immune cells recruitment in the side of inflammation that is made possible by adhesion molecules: selectins, integrins and endothelial cells adhesion molecules such as mucosal addressin cell adhesion molecule (MAdCAM)-1, vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1 and -2 [8].

3. GENERALITY ON BIOLOGIC THERAPY

Classical therapy in IBD treatment does not allow completely satisfactory effects. The controversial efficacy of aminosalicylates (sulfasalazine, mesalazine, olsalazine) in CD patients, the poor efficacy of corticosteroids as maintenance agents, the incidence of side effects related to corticosteroids and "classic" immunosuppressant agents sach as thiopurines analogues (azathioprine and 6-mercaptopurine), methotrexate, cyclosporine created the need for new, more efficacious and eventually safer drugs [9]. Medical research, focusing on the pathogenetic mechanisms involved in IBD, permitted the elaboration of compounds targeted to a specific pathogenetic step.

These drugs are indicated as "biological" agents and they can be classified under a structural point of view as recombinant peptides or proteins, antibodies and nucleic acids. Conversely, they can be classified, under a functional point of view, as inhibitors of proinflammatory cytokines, anti-inflammatory cytokines, inhibitors of cell adhesion molecules, inhibitors of Th1 polarization, inhibitors of T cells activation and proliferation, growth factors, immunostimulator, MAPK (mitogen-activated protein kinases) inhibi-tors, immunomodulators, anti-leukocyte molecules (Table **1**).

Some of these drugs have been proven effective, other are ineffective and other ones (the majority) are under evaluation and in most cases are showing to be very promising.

In the following pages we will briefly treat the mechanism of action and the scientific evidence of efficacy and safety of these compounds in CD and UC patients.

Table 1. Classes of Biologic Agents

Class of the drug	Specific biologic agents
Inhibitors of proinflammatory cytokines	Infliximab, CDP571, CDP870, etanercept, onercept, adalimumab, RDP58, antisense NF-kB, IL-6R antibody
Anti-inflammatory cytokines	IL-10, IL-11
Adhesion molecule inhibitors	Natalizumab, MLN-02, ISIS 2302
Inhibitors of Th1 polarization	Anti-IL-12, anti-IL-18, anti-IFNγ
Inhibitors of T cells proliferation	Anti-IL-2 receptor (Daclizumab, Basiliximab)
Inhibitors of T cells activation	Anti-CD40L
Anti-CD4 therapy	cM.T412, Max.16H5, BF-5
Anti-CD3 therapy	Visilizumab
Growth factors	EGF, KGF, GH
Immunostimulators	G-CSF, GM-CSF
Immunomodulators	IFN-β, IFN-α
MAP-kinase inhibitors	BIRB-796, CNI-1493, RDP58

4. ANTI-TNF-α THERAPIES

Tumor necrosis factor-alpha (TNF-α) is a pivotal cytokine in IBD, playing a central role in initiation and amplification of the inflammatory reaction: it has been shown increased in the intestinal mucosa of both UC [10] and CD patients [11,12,13] and correlates with the inflammatory activity [12]. "Classical" drugs (sulfasalazine, mesalazine, thalidomide, pentoxyphylline) [14] interfere with its synthetic pathway at different levels and the efficacy of some biological agents, specifically targeting TNF-α, has now been proven.

Infliximab is the first and, at present, the only approved monoclonal antibody against TNF-α in CD. Unfortunately, its chimeric nature confers immunogenicity to this drug. To overcome this limit other more "humanized" anti-TNF-α agents have been created and are under evaluation: some of these have already obtained preliminary positive results (Table **2**).

Their avidity and affinity for TNF-α, their capacity to bind either soluble and/or membrane TNF-α, to fix complement, to mediate antibody-dependent cytotoxicity, to cause T cells apoptosis are probably at the basis of the different efficacy of anti-TNF-α inhibitors and justify the absence of a class effect for these drugs (Table **3**) [15].

4.1. Infliximab

Infliximab (REMICADE®) is a mouse (25%)-human (75%) chimeric IgG1 monoclonal antibody that is intravenously administered. It is able to block both membrane and soluble TNF-α. Infliximab has been proven efficacious in inducing remission in active [16] and perianal fistulizing Crohn's disease [17]. However, response to infliximab is poorer in non-perianal and non-enterocutaneous fistulas [18].

Large placebo-controlled clinical trials evaluated the efficacy and safety of infliximab also in maintenance therapy and obtained statistical significant results: in the ACCENT 1 (A Crohn's Disease Clinical Trial Evaluating Infliximab in a New Long Term Treatment Regimen) maintenance study patients were treated with infliximab every 8 weeks after an induction therapy (infusion at 0, 2 and 6 week). In the group of patients receiving maintenance therapy at 5 mg/kg of infliximab the remission rate was significant over the placebo group [19].

The efficacy of infliximab in maintaining remission of perianal and enterocutaneous fistulas in refractory fistulizing CD has been evaluated through week 54 in the ACCENT 2 maintenance study. In the group treated with infliximab at the dose of 5 mg/Kg every 8 weeks the remission rate was 36% vs 19% of placebo group (p=0,0009) and the time to

Table 2. Anti-TNF Agents Efficacy

Drug	Structure	Indication	Phase of investigation	Results
Infliximab	Chimeric anti-TNF-α IgG1	CD/RCU	IV/III	effective/promising
CDP571	Humanized anti-TNF-α IgG4	CD/RCU	III/IIa	Effective in CRP > 10 / modest
CDP870	Pegylated Fab fragment of a humanized anti-TNF-α antibody	CD	II,III underway	Effective in CRP > 10
Etanercept	Human soluble p75 fused with the Fc domain of human IgG1	CD	II	Ineffective
Onercept	Human soluble p55 fused with the Fc domain of human IgG1	CD	II	Ineffective
Adalimumab	Full-humanized anti-TNF-α IgG1	CD	III	Effective

Table 3. Mechanisms of Action of Anti-TNF-α DRUGS (MODIFIED from Sandborn 2005, [15])

Drug	Binding to both soluble and membrane TNF-α	Complement fixation	Antibody dependent cytotoxicity	Induction of apoptosis
Infliximab	Yes	Yes	Yes	Yes
Etanercept	Yes	No	No	No
Adalimumab	Yes	Yes	Yes	Yes
CDP870	Yes	No	No	?
CDP571	Yes	No	No	?
Onercept	Yes	No	No	No

loss of response was more than 40 weeks vs 14 weeks of the placebo group (p<0,001) [20].

On the basis of these favourable results, infliximab has been approved for induction and maintenance of clinical remission in moderate to severe CD patients with a previous inadequate response to conventional therapy and for induction and long-term maintenance (when conventional maintenance therapy failed) in fistulizing CD with draining enterocutaneous or perianal fistulas [21].

Infliximab results in the treatment of UC seem not to be as satisfactory as in CD. Preliminary uncontrolled results and data coming from two controlled trials on severe steroid-dependent UC are quite conflicting [22-23].

Results of two large phase III trials (ACT1 and ACT2), both on 364 patients with moderate or severe UC refractory to at least one standard therapy have been published. For both trials, patients were randomized to receive placebo or infliximab 5mg/Kg or 10 mg/Kg at week 0, 2 and 6 and than every 8 weeks through week 46 for ACT 1 and through week 22 for ACT 2 (ACT1, ACT2) [23,24].

Clinical response at 8 weeks ranged from 62% (10 mg/Kg group of ACT 1) to 69% (5 mg/Kg group of ACT 1 and 10 mg/Kg group of ACT 2) vs 37% (ACT 1) and 29% (ACT 2) of placebo (p<0.001). Remission rate at 8 weeks ranged from 28% (10 mg/Kg group in the ACT2) to 39% (5 mg/Kg group of ACT 1) vs 15% (ACT1) and 6% (ACT 2) of placebo (p<0.001 and p<0.002). Clinical response at week 30 ranged from 47% (5 mg/Kg group of ACT 2) to 60% (10 mg/Kg group of ACT 2) vs 30% (ACT 1) and 26% (ACT 2) of placebo (p<0.001 and p< 0.002). Remission rate at week 30 ranged from 26% (5 mg/Kg group in the ACT 2) to 37% (10 mg/Kg group in the ACT 2) vs 16% (ACT 1) and 11% (ACT 2) of placebo (p<0.001). These data were confirmed by mucosal healing results that achieved 57% (10 mg/Kg group of ACT 2) at week 30 vs 30% of placebo (p<0.001). Anyway, the rate of discontinuation of corti-costeroids at week 30, even if significantly higher than placebo (3%) ranged from only 18% (5 mg/Kg group of ACT 2) to 27% (10 mg/Kg group of ACT 2) [23].

The safety profile of infliximab in the trials and in clinical practice [21] is quite good but it is well-known the capacity of this drug to favour infections (and among these tuberculosis reactivation) [24,25] and to cause acute infusion reactions (shortness of breath, chest pain, palpitations, flushing, fever, headache, urticaria and sometimes hypotension) that are reported in about 20% of patients and delayed hypersensitivity reactions (similar to a serum sickness-like reaction) occurring 5-9 days after the infusion with a frequency of 0.2%. All these adverse reactions occur more frequently in patients with a long drug-holiday interval (more than 3 months) [15]. They are probably related to infliximab immunogenicity, as shown by the strict relation existing with the HACAs (human anti-chimeric antibodies) serum concentration. At the same time, HACAs level seems to be a predictor of loss of response to infliximab [26].

The strategies aimed to decrease HACAs formation (co-administration of immunosuppressive therapy, use of a 3-dose induction regiment, pre-treatment with hydrocortisone and a regular maintenance therapy with infliximab) certainly reduced HACA formation (to less than 10%) and immuno-derived side effects, without abolishing them [27]. Research is looking for less immunogenic anti-TNF-α compounds (Table **4**) and alternative, fully or more humanized anti-TNF-α drugs have been tested. However, immunogenicity partially persists also with these humanized drugs, as, for example, is already known for fully-human recombinant insulin causing specific anti-insulin antibodies until 44% of patients [28]. Furthermore, new and less immunogenic anti-TNF-α compounds have not shown to be as efficacious as infliximab.

4.2. CDP571

CDP571 (HUMICADE™) is a humanized IgG4 mono-clonal anti-TNF-α antibody administered intravenously that is in 95% human and in 5% murine, thus potentially less immunogenic than infliximab.

A first phase II dose finding trial showed short-term efficacy [29], while other three controlled trials testing this compound did not completely confirm this positive result. In the first one, 169 patients with moderate to severe CD were treated with an initial dose of CDP571 followed by re-treatment at 8 or 12 weeks [30]. The short term response (at week 2) was significantly higher in the 10 mg/Kg group (54% of clinical response vs 27% of placebo), but unlikely statistical significance was lost at week 24 of follow up. Another trial on 71 corticosteroid-dependent CD patients evaluated efficacy of CDP571 as corticosteroid-sparing [31].

Table 4. Anti-TNF-α Immunogenicity

Drug	Infusion reaction frequency	Anti-drug antibody	Delayed hypersensitivity reaction
Infliximab	20% [15]	30%-75% [15]	2% [15]
Adalimumab	20% (site of infusion) [15]	5,5% [15]	/
CDP571	21% [32]	11% [32]	0% [32]
CDP870	0% [36]	/	/
Etanercept	49% (site of infusion) [39]	2% [39]	0% [39]
Onercept	30% [41]	0% [41]	

Also in this case, results have been not completely satisfactory: after 2 infusions (at week 0 and 8) steroid-sparing rate was significant at week 16 (46,2% vs 21,9%) but the primary endpoint (steroid-sparing at week 10) was not achieved.

In a large pivotal phase III trial, 396 patients with moderate or severe CD were treated with CDP571 (10 mg/Kg) or placebo every 8 weeks to week 24 [32]. The primary endpoint (28-week clinical response) was not achieved (response rate of 30.4% vs 23.5%; p=non significant) and only the secondary end-point (2-week clinical response) was achieved (34.2% vs 21.2% of placebo; p=0,011).

A post-hoc laboratory subgroup analysis on 159 patients having baseline C reactive protein (CRP) levels greater than 10 mg/dL demonstrated a significant response also at 28 weeks (28.7% vs 12% of placebo). Considering this sub-group analysis results further prospective investigation on these specific patients could be justified. Moreover, CDP571 showed a good safety profile in these studies: anti-idiotype antibodies were recorded in 11% of patients, infusion reaction in 21% and delayed hypersensitivity reactions in no case [32].

Furthermore, a little open-label trial on 22 CD patients intolerant to infliximab evaluated CDP571 as rescue-therapy. After the administration of a single dose (10 mg/Kg) of CDP571, 23% of patients showed a short-term decrease of disease activity and 50% of patients experienced mild or moderate adverse events (Hanauer S abstract 2003) [33].

Effects of a single infusions of CDP571 on UC have been evaluated in a small open-label pilot study on 15 patients with very modest results [34].

The limited effects in short- and especially long-term therapy and the limited steroid-sparing capability of CDP571, even with promising results in infliximab-intolerant and in higher inflammatory state patients led its manufacturer to discontinue production and so no further clinical development of this compound is expected.

4.3. CDP870

CDP870 is a PEGylated Fab fragment of a humanized anti-TNF-α antibody. The most representative study is a phase II trial on 292 patients with active CD: patients were randomized to receive subcutaneous CDP870 at dose of 100, 200, or 400 mg or placebo at weeks 0, 4, and 8 [35]. In the highest dose group there was a significant response rate from week 2 to week 10 (52.8% vs 30.1% of placebo; p=0.006), but it was lost at week 12 suggesting difficulty to sustain response. A post-hoc analysis showed that in patients with elevated CRP (>10 mg/dL at baseline) CDP870 at any dose (with an optimal dose of 400 mg) had significant higher response and remission rates vs placebo (remission rate of 41.9% vs 10.7% of placebo at week 10 in the 400 mg group) at all time points until 12 weeks.

In another phase II trial on 92 patients with active CD a single intravenous administration of CDP870 failed to achieve the primary end-point (clinical response and remission at week 4) even if this result was achieved at week 2 [36]. The agent's tolerability in all these studies has been reassuring.

In January 2004, 2 phase III studies called PRECISE-1 (Pegylated antibody fRagment Evaluation in Crohn's disease: Safety and Efficacy-1) and PRECISE-2 have been announced and are expected to enrol more than 1.300 patients; they will evaluated CDP870 efficacy as induction and maintenance of remission in patients with moderate to severe CD.

4.4. Etanercept and Onercept

TNF-α interacts on cell surface with 2 different specific receptors: p55 and p75 proteins. Soluble truncated version of these receptors are present in body fluids and have thought to be involved in regulating TNF-α activity [14].

A strategy to block TNF-α is based on two fully-humanized genetically-engineered TNF-α receptors, indicated as etanercept and onercept that prevent TNF-α interaction with its native receptors.

Etanercept (ENBREL®) is a fusion protein consisting of 2 identical chains of recombinant human soluble p75 protein fused with the Fc domain of human IgG1. It binds both TNF-α and TNF-α [37], it is not able to induce T cells apoptosis (Table **3**) [38] and has been shown effective in the treatment of rheumatoid arthritis [39].

However, two controlled trials conducted respectively on 43 [40] and 49 (Amgen, data on file) patients with active CD failed to demonstrate efficacy of etanercept administered subcutaneously twice weekly at a dose of 25 mg. It could be possible that higher doses may have some efficacy, but no dose ranging trial has been performed.

Onercept is a fusion protein consisting of the human soluble p55 protein fused with the Fc domain of human IgG1. Preliminary results from a randomized open-label dose-finding study [41] showed efficacy in active CD at higher dose (67% of clinical remission at week 2 in the 50 mg 3 times per week group) with a good safety profile but these data haven't been confirmed by a following placebo-controlled phase II study (Serono, data on file).

At the moment, on the basis of these negative findings, soluble TNF-α receptors seem to have no perspective of development in the IBD treatment.

4.5. Adalimumab

Adalimumab (D2E7/Humira®), a full humanized anti-TNF-α monoclonal IgG1 antibody administered subcutaneously, is one of the most promising alternative biologic drug to infliximab. It seems to act through the same mechanism of infliximab, since it is able to block both soluble and membrane TNF-α, to fix complement, to mediate ADCC (antibody dependent cytotoxicity) (Lorenz HM 2002) [42] and cause apoptosis in a caspase-dependent way (Table **3**) [38]. Because of these properties, it should have the same efficacy of infliximab with the advantage of less immunogenicity.

Adalimumab has been approved for the treatment of moderate to severe active rheumatoid arthritis on the basis of the successful results obtained in large population trials [43,

44]; consequently safety profile informations prevalently come from these large studies involving about 2400 rheumatoid arthritis patients. Adalimumab seems less immunogenic than infliximab: anti-adalimumab antibodies were identified in 5.5% of patients, with a substantial difference according to methotrexate co-administration (0.6%) or not (12.4%); moreover, 12.6% of adalimumab-treated patients vs 7,3% of placebo-treated patients developed ANA and only a case of lupus-like syndrome has been signalled [15] (Table **4**). The most common observed adverse events have been injection site reactions (about 20% of patients) and other undesirable effects were qualitatively similar to those observed with infliximab even if less frequent.

In CD patients adalimumab has been firstly evaluated in a pilot short-term open-label study on 8 patients with active luminal CD who had experienced immediate- or delayed-hypersensitivity reaction to infliximab (7 patients) or infliximab-induced lupus (1 patients). In this study patients were treated with an initial dose of 80 mg followed by 40 mg every 2 weeks until 8 weeks. Seven patients experienced a clinical response and one patient (with a history of previous exposure to human immunoglobulins) did not respond and experienced a facial rash [45].

In a larger open-label trial 24 patients with active CD intolerant (20 patients) or with a loss of response to infliximab have been treated with adalimumab 80 mg, followed by 40 mg every 2 weeks until week 12 [46]. Results have been quite encouraging, since it was observed a progressive increase of clinical outcome throughout 12 weeks (response and remission rate respectively of 59% and 29% at week 12). Similarly, the analysis of fistula response has been positive, with a rapid and persistent effect of the drug (33% of fistula complete closure and 56% of fistula improvement).

In another open-label study [47] on 15 patients with active CD who experiencing an attenuated response to infliximab, adalimumab obtained a response rate of 85%.

These preliminary studies suggest good efficacy (also in infliximab allergic/intolerant patients) of adalimumab with a good safety profile, but it should be noticed that in more than 50% of patients dose escalation has been necessary to achieve clinical effects, suggesting that doses commonly used in rheumatoid arthritis could be too low in CD. If this finding should be confirmed, attentive safety evaluation of high dosages will be necessary.

Preliminary results from a recent short-term, large multicenter, randomized, phase III, placebo-controlled trial, called CLASSIC (Clinical Assessment of Adalimumab Safety and Efficacy Studied as Induction Therapy in Crohn's Disease), on active CD patients have been presented at Digestive Disease Week 2004 [48]. 299 patients with no previous exposure to anti-TNF-α drugs with moderate to severely active CD were divided into four groups treated at week 0 and at week 2, respectively with: 120 mg/80 mg of adalimumab, 80 mg/40 mg of adalimumab, 40 mg/20mg of adalimumab or placebo/placebo.

The primary endpoint (significant remission rate with the two higher doses of adalimumab vs the placebo at week 4) was achieved: a remission rate of 24% in the 80mg/40 mg group and of 36% in the 160mg/80mg group was obtained (12% in placebo group). Furthermore all adalimumab treatment groups obtained a significant decrease of CDAI scores of at least 70 points from baseline and about 50% of patients in the 160/80 mg group obtained a decrease of the CDAI score of at least 100 points at week 4.

Adalimumab safety profile in this large study was similar to the previous findings in other adalimumab-treated populations: adverse events were dose-indipendent and did not differ from placebo. The most frequent adverse events were injection site reactions; only 3 severe adverse events (perianal abscess, abdominal pain and pneumonia) were observed in the 160/80mg adalimumab group, in one case leading to treatment withdrawal [48].

In conclusion, adalimumab has shown efficacy in CD patients with active luminal CD as induction therapy and certainly is the best candidate to become a concrete alternative to infliximab. Controlled trials are needed to evaluate its efficacy in maintenance of remission and in fistulizing CD.

5. ANTI-ADHESION MOLECULES THERAPIES

Leukocytes trafficking into the gut is a pivotal step in intestinal inflammation. Several endothelial adhesion molecules (E-selectin, ICAM-1, ICAM-2, VCAM-1, mucosal addressin Mad-CAM-1), over-expressed after cytokines exposure, interact with specific integrins on leukocytes permiting their adherence and transmigration across the blood vessels to inflammation site [8]. Drugs targeting the α_4 integrins and the ICAM-1 have been developed and already tested in clinical trials (Table **5**).

5.1. Natalizumab

Natalizumab (Tysabri, Antegren) is a humanized (95% human-derived) IgG_4 monoclonal antibody against the human α_4-subunit of integrins. It inhibits both the VCAM-$1/\alpha_4\beta_1$ and MAdCAM-$1/\alpha_4\beta_7$ pathways of transendothelial migration by specific immunoblockade [49].

In the first randomized, double-blind, placebo-controlled study, enrolling 30 patients with mild to moderately active CD, natalizumab showed short-term efficacy after a single intravenous dose [50].

In the second phase II, randomized, double-blind, placebo-controlled study on 248 patients with moderate to severe active CD [51], natalizumab failed to achieve a statistically significant higher remission rate at week 6 in patients receiving two infusions at the dose of 6 mg/Kg (31%) vs the placebo group (27%) (primary endpoint). The high placebo remission rate could have influenced this failure; conversely, all patients in the two infusions treatment groups (both at 3 and 6 mg/Kg dose) achieved significance in remission and response rate vs placebo group at all other time points (from 4 to 12 weeks). Safety profile was very good: no differences versus placebo and a low immunogenicity (only 7% of anti-natalizumab antibody formation) was observed.

Table 5. Adhesion Molecules Inhibitors

Drugs	Target molecule	Adhesion system inhibited (endothelial cell/leukocyte)
Natalizumab	α_4-subunit of integrins	VCAM-1/$\alpha_4\beta_1$ and MAdCAM-1/$\alpha_4\beta_7$
LDP-02(MLN-02)	$\alpha_4\beta_1$ integrin	VCAM-1/$\alpha_4\beta_1$
Alicaforsen (ISIS 2302)	ICAM-1	ICAM-1/$\alpha_L\beta_2$ integrin (LFA1)

After these encouraging results a large phase III study (known as ENACT-1: Evaluation of Natalizumab in Active Crohn's disease Therapy-1) on 905 active CD patients treated with 300 mg of natalizumab infusions at 4-week intervals for 3 doses failed to show a statistically significant superiority on placebo in achieving response or remission at week 10 (primary endpoint). Likewise, also in this case, an unexpected high response rate in the placebo group could have influenced the results. Finally, it should be emphasized that about 40% of patients in this study had previously been treated with infliximab, suggesting a possible efficacy of anti-adhesion molecule drugs in anti-TNF-α resistant patients [52].

Natalizumab-responders of ENACT-1 (339 patients) were subsequently assigned to a maintenance program, receiving placebo or 300 mg of natalizumab at a 4-weeks interval throughout 6 months (ENACT-2 study): 61% of patients in the natalizumab-treated group vs 28% in the placebo group ($p<0.001$) maintained response (sustained CDAI score less than 220), supporting the idea that patients initially responsive to natalizumab will continue to do it [53].

Natalizumab also showed to have short-term benefit in UC in an uncontrolled trial on 10 patients [54].

Unexpectedly, natalizumab has recently been suspended (March 2005) [55] because of some serious adverse events consisting in progressive multifocal leukoencephalopathy (PML) in patients affected by multiple sclerosis and treated with the combination of natalizumab and interferon-β-1a [56,57]. It has initially been thought that only the combination of these two drugs could have been dangerous, but another case of PML has been signalled in a CD patient treated with natalizumab-monotherapy [58]. Natalizumab's producers actually are investigating the possible role of the drug in determining PML.

5.2. LDP-02 (MLN-02)

The humanized IgG1 monoclonal antibody LDP-02 or MLN-02 is a biological drug targeting the $\alpha_4\beta_7$ integrin, thus specifically inhibiting its interaction with Mad-CAM-1. A double-blind, phase II, multi-center trial on 181 patients with mild to moderately active ulcerative colitis [59] has shown significantly higher remission rate in the group of patients treated with 2 administrations (at days 1 and 29) of LDP-02 vs the placebo group (at week 6: 33% vs 14%; $p<0.03$).

The efficacy of MLN-02 in inducing remission of mild to moderately active CD has been evaluated in a placebo-controlled study on 185 patients [60]. Patients were treated at days 1 and 29 with 0,5 mg/Kg or 2 mg/Kg of MLN-02 and were followed for 6-months. The primary endpoint (statistically significant decrease of >70 points in CDAI score at 8 weeks vs placebo) was not achieved, but statistically significant clinical remission was observed in the higher dose group (36.9% vs 20.7% of placebo; $p<0.05$). Subgroups analysis found a relation between $\alpha_4\beta_7$-saturation on peripheral blood lymphocytes and clinical response, suggesting that a suboptimal dose could be the cause of unresposiveness. Moreover, MLN02 showed a very good safety profile in both CD and UC patients.

5.3. Anti-ICAM-1 Therapy

Alicaforsen (ISIS 2302) is a 20 base phosphorothioate oligodeoxy nucleotide that is able to hybridise to a sequence in the 3'-untranslated region of the human ICAM-1 mRNA. It causes the activation of the nuclease RNase-H with the cleavage of the heterodimer that results in a reduction of ICAM synthesis.

The first placebo-controlled pilot study on 20 patients with chronically active CD (intravenously treated with ISIS-2302 at different doses or with placebo) showed a rapid and persistent effect of the drug [61]. However, these positive findings were not confirmed by following larger trials. In fact a large multicenter placebo-controlled trial on patients with moderately active steroid-resistant CD (treated with subcutaneous formula of ISIS-2302) was stopped during patients enrollment because of manifest inefficacy of the drug [62]. Another multicenter placebo-controlled study [63] on 299 moderately active steroid-dependent CD patients failed to show efficacy; anyway the finding of a relationship between plasma levels of ISIS-2302 and the response rate suggested that suboptimal doses had been used. Therefore, an open label high-dose ISIS-2302 study [64] has been conducted on 22 active CD patients. The intravenous administration of the drug (three weekly for 4 weeks) permitted clinical remission in 9 patients (41%) and an overall response rate of 41% at week 8 and of 47% at week 12.

ISIS-2302 has been relatively safe in all these trials: more common side effects consisted in injection side reactions and less frequently in fever, chills, headache, nausea, emesis or arthralgias; they were more frequent at the highest dosage and were usually prevented by corticosteroids; also a moderate, not clinically relevant, increase in aPTT was observed. On the basis of these results phase III trials on high dose i.v. ISIS-2302 in patients with active CD are underway.

Furthermore, topically administered (enema formulation) ISIS-2302 has been recently tested in an open-label trial on 12 patients with unremitting pouchitis [65]. After 6 weeks of

nightly treatment a statistically significant remission rate of 58% was observed in association with a reduction of endoscopic score.

The enema formulation of ISIS-2302 has been also tested in a small randomized, placebo-controlled, double-blind study on 40 patients with mild to moderately active distal ulcerative colitis [66]. At day 29 of treatment DAI score significantly ameliorated (p=0.003 vs placebo) permitting to avoid other surgical or medical treatments.

6. IMMUNOSTIMULATORS

It has always been thought that CD results from an overactive immune system, justifying the the often successful use of the immunosuppressant treatment. Alternative hypotheses suggest that CD immune system dysregulation primarily consists in an impairment of the first line innate immune system reactivity to microbes that leads to a compensatory and persistent overactivation of the specific immune system with a chronic inflammation [4,5]. The occurrence of CD-like gastrointestinal lesions in some forms of genetically-dependent immunodeficiency (chronic granulomatous disease, glycogen storage disease Ib, leucocyte adhesion deficiency, congenital cyclic neutropenia) [67,68, 69,70,71], the association between NOD2/CARD15 mutation and CD [5,6] and the response of CD patients to antibiotic therapy [9] further support the hypothesis of an underlying defect in the innate immune system.

Therefore, stimulators of the innate immune system such as recombinant human granulocyte-colony stimulating factors (rhuG-CSF, filgrastim) and recombinant human granulocyte monocyte-colony stimulating factors (rhuGM-CSF, sargramostim) have been tested in CD patients.

Initial positive results with rhuG-CSF have been observed in uncontrolled conditions. A first evidence suggesting positive effects in fistulizing and luminal active CD patients [70,72] have been recently confirmed in a 12 weeks open-label study on 20 patients with active luminal CD treated with daily subcutaneous rhuG-CSF administrations at the dose of 300 mcg [73]. Clinical response was rapid (within 1-3 weeks) and 25% of patients achieved clinical remission; a decrease of at least 70 and 100 points of the CDAI score was observed respectively in 55% and 35% of patients at week 12; moreover 3 of 4 patients with fistulae responded. A dose reduction for excessive neutrophils count (more than 35 $x10^9$/L) was necessary in 2 patients, while the most common reported side effect was a mild transient bone pain. Further investigation are certainly necessary to confirm these preliminary results and better explore side effects profile.

RhuGM-CSF has been firstly tested in an open label dose-escalation trial on 15 patients with moderate to severe CD daily treated for 8 weeks. Mean CDAI score decreased of 190 points, more than 50% of patients achieved clinical remission and about 80% had a decrease in the CDAI score of more than 100 points; furthermore the healing of a chronic recto-vaginal fistula [74].

Recently a phase II placebo-controlled trial on RhuGM-CSF in patients with moderate to severe CD has been published [75]. In this study patients were randomly assigned to receive 6μg/Kg of rhuGM-CSF subcutaneously daily or placebo for 56 days. Even if the primary endpoint (statistically significant decrease of CDAI score of at least 70 points in the treatment group vs placebo at day 57) was not achieved (54% vs 44% of placebo), secondary endpoints, consisting in a significant remission rate and in a decrease of at least 100 points of the CDAI score during treatment (at days 15, 29 and 57) and through the 30 days of follow up, were achieved. The best response rate was of 53% (vs 28% of placebo) and was obtained at day 29. The high placebo remission rate probably contributed to the failure in achieving the primary end-point. Moreover, the rapid and persistent clinical response to the drug was accompanied by the observation of mucosal healing.

RhuGM-CSF showed a good safety profile: the most common adverse events were injection-side reaction (90% of patients) and bone pain (37% of patients). Severe adverse events were observed in 3 cases (migraine, demyelinating syndrome, ischemic heart disease).

7. INHIBITOR OF TH1 POLARIZATION

IL-12, IFN-γ and IL-18 are key cytokines in promoting Th1-dependent immune response [76]. Macrophages and dendritic cells produce IL-12 that is able to induce differentiation of IFN-γ-activated naïve T cells into Th1 cells that produce in their turn IFN-γ [76,14]. Same capability is shared by IL-18, produced by macrophages and epithelial cells. Evidence of the involvement of these molecules in CD intestinal inflammation stimulated the elaboration of cytokine-blocking compounds. After preli-minary demonstration of efficacy in animal models of colitis [14], anti-IL-12 and anti-IFNγ antibodies have been tested in CD patients.

7.1. Anti IL-12

A recent early phase II placebo-controlled study [77] on 78 CD patients evaluated the safety (primary end-point) and efficacy of an IgG1 full-human monoclonal antibody directed to IL-12. Patients were divided in three groups respectively treated with seven weekly subcutaneous injection of 1 mg, 3 mg of anti-IL-12 or placebo with a four week interval between the first and the second injection (Cohort 1) or with only 1 week interval (Cohort 2). Patients were evaluated at the end of the treatment and through an adjunctive 18-week follow up period.

Only the 3 mg/Kg group in the Cohort 2 obtained a statistically significant response rate at week 7 (75% vs 25% of placebo; p=0.03), but significance was not maintained (p=0.08) at 18 weeks of follow up. Also differences in remission rate were not significant at the end of the treatment and through the follow up (38% at both times vs 0% of placebo). Even with these uncertain effects on clinical response, anti-IL-12 showed to improve the histologic intestinal abnormalities and to reduce IL-12, IFN-γ and TNF-α secretion by mononuclear cells of the colonic mucosa. Moreover, the drug showed a good safety profile: no difference in adverse events were observed, apart for injection site reactions; anti-drug antibody were detected only in 3 patients [77]. All these results do not allow to draw any firm conclusion on the efficacy and safety of this drug, but certainly encourage further clinical evaluation.

7.2. Anti-IFN-γ Antibody (Fontolizumab)

Fontolizumab (HuZAF) is a subcutaneously administered humanized monoclonal antibody against IFN-γ. Preliminary results, coming from a small phase IIa study on 28 CD patients (Rutgeerts P 2002) [78] and from a larger phase II study on 196 patients treated with increasing doses (up to 4 mg/Kg) of fontolizumab failed to demonstrate efficacy [79]. Later on, an high-dose phase II trial on 133 patients with active CD has been conducted [80]. Patients were randomized to receive 1 or 2 infusions (days 0 and 28) of fontolizumab at 4 or 10 mg/Kg dose. Results in remission and response rates were not significant but the mean CDAI change in the double-infusions groups was significant from baseline at any time. Furthermore, post-hoc analysis showed that patients in the double-infusions groups with a CRP higher or equal to 10 mg/dL achieved statistically significant higher remission and response rates than placebo: the best results consisted in a remission rate of 57% (vs 0% of the placebo) in the 10 mg/Kg group and in a response rate of 54% (vs 0% of placebo) in the 4 mg/Kg group at week 28. Also considering the good safety and tolerability shown by the drug, larger and trials should be encouraged.

8. ANTI-INFLAMMATORY CYTOKINES

8.1. IL-10

IL-10 is an anti-inflammatory cytokines with potential positive effect on IBD since it can interfere with IL-2, IFNγ and IL-12 production [81].

After encouraging, results on mice-model of IBD [82], recombinant human IL-10 (rHuIL10) has been tested on CD patients.

Initial results, suggesting benefits of intravenous administration of the drug (van Deventer SJ 1997) [83], were not confirmed by a phase II trial testing intravenous rHuIL10 on 62 CD patients with previous ileal or ileo-colonic resection. In fact this trial failed to show any efficacy of the drug in preventing endoscopic relapse [84]. Also three phase III trials that tested subcutaneous rHuIL10 respectively on 95 patients with mild to moderate CD [85], 329 patients with steroid-resistant CD [86] and 373 patients with steroid-dependent CD [87] failed to show any efficacy of the drug. Furthermore, IL-10 failed to demonstrate efficacy in a phase II trial on 94 patients with mild to moderately active UC [88].

The possible existence of a pharmacokinetic mechanism at the basis of the failure and the evidence of IL-10 receptors existence on intestinal epithelial cells [89] led to topical delivery formulations of the drug. Because of that, oral administration of genetically engineered lactococcus bacteria secreting IL-10 [90], rectal administration of adenovirus able to produce IL-10 [91] and gelatin microspheres [92] of IL-10 have been tested and obtained good results in murine genetic and chemical model of colitis, but at present no studies on humans exist.

8.2. IL-11

IL-11, an interleukin produced by mesenchimal cells, has thrombocytopoietic, anti-inflammatory and mucosal protective effects [93]. All these activities suggested a potential benefit in the treatment of IBD and led to test a recombinant human IL-11 (rhIL11). This drug has, at present, been evaluated in two placebo-controlled trials [94,95], involving a total of 224 patients with active CD: the best result consisted in 36.7% of remission rate vs 16.3% of placebo when the drug was administered weekly at the dose of 15μg/Kg. Evidence of a dose-related thrombocytosis, could be matter of concern and imposes a further careful safety evaluation also considering the increased prothrombotic risk of IBD patients. An oral formulation of rhIL-11, without systemic absorption and thus without systemic effects, is under evaluation in a phase 2 trial on CD patients.

9. INHIBITORS OF T CELLS ACTIVATION (ANTI-CD40 L)

CD40/CD40 ligand is a pivotal cell-cell interaction system in immune and non-immune response. CD40 ligand (CD40L) is expressed on T cells after their interaction with an APC and enables T cells activation [96]. Also activated platelets can express CD40L and in IBD patients are the major source of CD40L and of its soluble form (sCD40L) [97]. Furthermore, both forms of CD40L can activate mesenchimal cells (endothelial cells, fibroblasts) leading to cytokines production and adhesion molecules expression [98,99]: all these events participate to the maintenance of mucosal inflammation in IBD. Since inhibition of this intercellular system could be useful for treatment of IBD patients, a humanized anti CD40L antibody (IDEC-131) has been tested in a phase II trial; unfortunately, the occurrence of thromboembolic events imposed the interruption of the trial [100].

10. ANTI-CD4 THERAPY

The observation of CD4-positive T cells (T-helper cells) activation in IBD patients and the observation of complete remission of CD in a patient infected with the human immunodeficiency virus suggested the possible usefulness of monoclonal antibodies against CD4 [101]. Three monoclonal antibodies have been developed: cM-T412, MAX.16H5 and BF-5. They have been tested in four phase I studies on UC and CD patients [102,103,104,105]. In these studies a total of 14 UC patients and 35 CD patients have been treated for short term periods (less than 15 days). Results have been encouraging, with response rates ranging from 36% to 100% in CD and 57% to 75% in UC patients. A persistent response was observed in some cases: the longest remission (12 months) was observed in 4 UC patients treated with cM-T412.

Even if no study showed significant increase in opportunistic infection, concern coming by the significant reduction of blood values in CD4-positive cells (in reality short-lasting for MAX.16H5 and not observed for BF-5) led to abandon further investigation on these drugs.

11. GROWTH FACTORS

Growth factors have been shown to decrease mucosal permeability of the bowel, to maintain mucosal trophism and to favour mucosal healing after a damage [106]. Some of these growth factors (GH, KGF, EGF) have been evaluated in IBD patients with uncertain results.

Keratynocyte growth factor-2 (KGF-2, repifermin) has not shown superiority versus placebo in a phase II dose finding trial on 88 patients with UC: anyway the possible under dosage and the too short treatment-period of this study could have affected the results [107]. Epidermal growth factor (EGF) has been evaluated in a little placebo-controlled study on 24 patients with mild to moderate left-sided UC or proctitis, treated with daily EGF-enemas [108]. Even with the considerable limits of the study (non equivalence among patients in concomitant therapies assumption, small number of patients and use of the unvalidated activity score) the drug showed a good effectiveness at short- (remission rate of 83% at week 2 vs 8% of placebo) and medium-term evaluation. Further investigations are need to confirm these preliminary results and contemporary to better define the potential neoplastic risk related to the proliferative stimulus induced by this compound.

A limited placebo-controlled trial on 19 patients with moderate-severe CD [109] showed a certain effectiveness of growth hormone (GH) but this trial has not been followed by other ones.

12. IMMUNOMODULATORS

Interferons (IFN) are a class of cytokines with immunomodulatory action. These compounds are largely used in infective (hepatitis C and B), neoplastic (melanoma, Kaposi's sarcoma) and autoimmune (multiple sclerosis) diseases [110] and have recently been tested also in IBD patients. Even with the low strength of evidence, coming from the uncontrolled trials conducted in CD patients, a response rate up to 50% has been obtained using both IFN-γ-2a [111,112] and IFN-γ-2b [113,114,115]; in a little series of 5 CD patients IFN-γ showed even better results, showing clinical response in 80% of patients [116]. After these preliminary findings, further trials on IFNs in CD patients have not been published.

These drugs have been experimented also in UC patients. In two uncontrolled trials on patients with left-sided UC, IFNγ2a obtained a remission rate of 82% that was equivalent to corticosteroids enemas [117,118]. The pegylated-IFNγ-2b, in a placebo-controlled trial on 60 UC patients, obtained at 0.5 μg/Kg dose a response rate only of 18% better than placebo, with the counterpart of very frequent side effects [119]. Even if preliminary data on IFN-γ-1 suggested efficacy [120] in steroid-dependent UC patients, two successive placebo-controlled studies have not confirmed the results [121,122].

13. INHIBITORS OF TRANSCRIPTION FACTORS

A group of transcriptional factors indicated as NF-kB (nuclear factor-kB) are involved in the intestinal immune system function; they mediate the synthesis of intracellular proinflammatory cytokines, transcription factors, adhesion molecules and cell surface receptors [123]. Inhibition of these factors could result in a beneficial effect at many levels in IBD inflammatory cascade. Moreover, it is worth to remember that sulphasalazine and mesalazine, two drugs effective on IBD patients, are non selective inhibitors of NF-kB [14].

A selective inhibitor of the p65 (a transcriptional factor belonging to NF-kB group) has been recently tested in IBD patients with positive results. In this trial, 11 patients with steroid-resistant UC or colonic CD were treated with a single dose of a topical formulation of an antisense NF-kB oligonucleotide, while continuing mesalazine, antibiotics/ azathioprine therapy. Clinical, endoscopic and histologic improvement have been reported in 71% of patients in the treatment group vs 25% of the placebo group [124]. These findings need further evaluation to be confirmed.

14. INHIBITORS OF PROINFLAMMAORY CYTOKINES RECEPTORS

Interleukin-6 (IL-6) is a pleiotropic cytokine involved in the regulation of inflammation and immunity. Its signal transduction is mediated by IL-6 receptors (IL-6R). The involvement in IBD pathogenesis has been well documented in animal models and in humans [125,126,127]. The blockade of IL-6 signalling pathway has been recently tested in IBD patients using a humanized anti-IL-6 receptor monoclonal antibody (MRA). In a little phase II, 12-week lasting, placebo-controlled trial on 36 patients with CD resistant to conventional therapies, the biweekly administration of MRA at the dosage of 8 mg/Kg resulted in a response rate of 80% (vs 31% of placebo) and in a striking improvement in the acute phase reactants. However, the drug did not show to improve endoscopy and histology. Safety and immunogenicity were very good [128]. Studies on larger population of patients could further clarify the efficacy and safety of this drug.

15. INHIBITORS OF T CELLS PROLIFERATION

Interleukin-2 (IL-2) is produced by Th1 activated cells and through its receptor (CD25) induces Th cells activation and proliferation of [4].

The efficacy in UC patients of cyclosporine (an immunosuppressant inhibiting IL-2 synthesis) suggested the potential therapeutic effect of IL-2 blockade in UC and stimulated research on selective inhibitors. Daclizumab (a full-human antibody) and basiliximab (a chimeric antibody), specifically block IL2R preventing its interaction with IL-2 [14].

The preliminary observation of IL-2 role to favour steroid-resistance [129] suggested to test IL-2 inhibitors on steroid-resistant UC patients. In a little open-label study on 10 steroid-resistant UC patients, Daclizumab (administered in two infusions 4 weeks apart), permitted clinical remission in 80% and clinical response in 50% of patients. Contemporary decrease in CRP serum level and in CD25+ mucosal T cells number, while no effect on mucosal healing were observed [130].

Basiliximab, administered to 10 steroid-resistant UC patients (single dose of 40 mg), showed very interesting short-term results, with clinical remission at 8 weeks obtained in 90% of patients, but with the limit of a rapid relapse (median time of 9 weeks). Furthermore, it was observed that basiliximab was a steroid-sensitiver, suggesting the opportunity of a combination with steroids [131]. Finally, the rapid onset of symptoms relief after anti-IL-2

administration suggested the possibility to utilize these drugs as bridge therapies to a maintenance immunomodulator therapy. Also in this case larger and controlled trials are necessary to confirm these initial findings and identify a possible clinical role of these drugs.

16. ANTI-CD3 THERAPY (VISILIZUMAB)

Visilizumab (HuM291), a humanized IgG2 antibody against the CD3 receptor (invariable chain of T-cell antigen receptor complex), induces T cells apoptosis [132]. It has been tested, with positive results, on acute graft-versus-host-disease, but it increases the risk of Epstein-Barr virus (EBV) reactivation and post-transplant lymphoproliferative disease [132].

Preliminary positive results come from a phase I study on severe steroid-resistant UC patients treated with 2 infusions of visilizumab at the dose of 15 mcg/Kg one day apart: all patients achieved clinical and endospopic remission [133].

The results from a following cohort study, exploring lower dose of the drug in 24 steroid-dependent UC patients, has been recently announced [79]: in the 20 patients evaluated at days 30 response rate was 85% and remission rate was 55%.

In all these studies a transient mild to moderate cytokines release syndrome and a transient T lymphocytes decrease (with a recovery time of about 3 weeks) was observed at each infusion.

No opportunistic infection were reported, but certainly further evaluation looking at the optimal dose in term of safety and efficacy are needed.

17. MAPK INHIBITORS

MAPK (mitogen activated protein kinases) are a class of proteins including the p38, the JNK, the ERK that can activate the transcription factor NF-kB leading to the production of TNF-α and other proinflammatory signalling molecules [14].

MAPK inhibitors are a series of little molecules that have been proven in clinical trials on IBD patients. BIRB-796, a specific inhibitor of the MAPK p38, failed to demonstrate efficacy in a phase II trial on active CD patients [134]. More promising is CNI-1493 (a guanylhydrazone small molecule inibithing p38 and JNK) that is able to suppress dendritic cells activation [135] and has showed positive results on 12 patients with severe CD but with the limit of liver toxicity [136]. An underway phase II study on CNI-1493 administered intravenously will probably give further information on efficacy and safety.

To overcome toxicity related to bioavaibility of CNI-1493 another MAPK inhibitor, RDP58, orally administered and not systemically bioavailable has been tested. RDP58 is a peptide consisting of 9 D-amino acids blocking P38 and JNK. Even if a phase II trial on CD patients failed to demonstrate efficacy [137], a multicenter parallel phase II trial on 127 patients with mild to moderate UC showed that the dose of 200 mg/day obtained significant higher remission rate (about 70% vs 40% of placebo) and histology score improvement than placebo, without manifesting notable adverse effects [138].

CONCLUSIONS

In the last few years the substantial knowledge of IBD pathogenesis has led to the development of biological compounds blocking specific steps of the inflammatory phenomenon. The most famous is infliximab. The rapid success of this drug in the treatment of IBD patients and, on the other hand, the limits related to immunogenicity of the drug encouraged further research in the field of "biologicals". Most of these drugs firstly have been studied on CD patients and then spread to UC.

However, some of these "biologicals" have been shown ineffective, others are under investigation with more or less confidence in their efficacy, others have shown effectiveness only in particular subgroups of patients, others, despite of initial positive results, have been abandoned because of unwanted side effects.

In the future, apart from waiting efficacy and safety results of biological agents currently under evaluation and to look for new biological drugs, it will be important to know the possible efficacy of combination therapies (e.g. biologic and immunosuppressant, more biologic agents). Moreover, clinical investigators should find strategies to reduce immunogenicity of the biologic agents, and attentively consider the safety profile of the drugs already used in clinical practice. The knowledge of predictors of clinical response should be increased and the utility of biologic drugs in relation to the clinical phenotype of IBD should be better clarified.

Far from finding an etiologic therapy for IBD patients, biological therapies will definitely continue to develop.

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