IBD

Interfering with interferons in inflammatory bowel disease

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Early experience of fontolizumab, a humanised anti-interferon γ antibody, in active Crohn's disease has shown that the drug caused a significant decrease in endoscopic severity scores and CRP and was reasonably well tolerated. Fontolizumab may be worthy of further clinical trials

Interferons (IFN) are cytokines produced by immune cells in response to virus, bacteria, parasites, and tumour cells. Interferons exhibit immunomodulatory and antitumour activity in addition to the antiviral activity which defined the interferons. Three types of interferons are recognised in humans. Type I interferons consist of 14 alpha isoforms and beta, epsilon, omega, and kappa isoforms. Type I interferon receptor is composed of two chains, IFNAR-1 and IFNAR-2, and the signal transduction pathway involves constitutively expressed Janus family kinases Jak 1 and Tyk 2 with activation of Stat1, Stat2, Stat3, and Stat5. Interferon γ (IFN- γ) is classified as a type II interferon. Type II interferon receptor is composed of IFNGR-1 and IFNGR-2 subunits and the signal transduction pathway involves Jak 1 and Jak 2 kinases with phosphorylation and activation of Stat1 (fig 1). The third type consists of interferon lambda with three isoforms.

IFN- γ drives expression of MHC class II on antigen presenting cells, increases chemokine secretion, and activates macrophages, lymphocytes, and endothelial cells. In addition to activation of Stat1, IFN- γ also activates Raf-1 and β -Raf kinases with resulting activation of p42 mitogen activated protein kinase. A further signalling pathway activated by interferons is the ubiquitously expressed phosphatidylinositol 3' kinase enzymes. In inflammatory bowel disease, both Crohn's disease (CD) and ulcerative colitis (UC), plasma and tissue concentrations of proinflammatory cytokines such as tumour necrosis factor TNF, IFN- γ , interleukin (IL)-1 β , IL-6, and IL-8 are increased. It is customary to consider CD as a T_H1 lymphocyte mediated disease with lamina propria T lymphocytes producing large amounts of IFN- γ and small amounts of IL-13, while UC resembles an atypical T_H2 lymphocyte mediated disease with little IFN- γ but marked

IL-13 secretion from natural killer T cells.¹ However, other researchers have described high expression of IFN- γ and TNF in the mucosa of at least a proportion of patients with active UC.² Immunoregulatory therapy with type I interferons such as IFN- α or IFN- β can inhibit production of TNF and IFN- γ , antagonise IFN- γ signalling pathway, and increase production of anti-inflammatory cytokine IL-10, although most studies have been in multiple sclerosis.³⁻⁴ IFN- β has also been shown to be immunoregulatory by enhancing regulatory T lymphocyte and natural killer cell activity.⁵⁻⁶ Pilot studies with natural or recombinant IFN- β in 25 refractory UC patients showed high response rates in an open study.⁷ Subcutaneous recombinant IFN- β administered three times per week in a randomised dose escalation study in moderately active UC showed some beneficial anti-inflammatory effect with remission induced in three of 10 IFN- β treated patients

compared with 0/7 placebo treated patients ($p = 0.02$).⁸ However, a further and larger randomised controlled trial ($n = 91$) of recombinant IFN- β in active steroid refractory UC was negative for clinical efficacy end points.⁹ Subcutaneous IFN- α 2A significantly improved Powell-Tuck disease activity index, inflammatory bowel disease questionnaire (IBDQ) score, and histology score compared with baseline in 16 patients with active left sided UC.¹⁰ However, in this randomised open label study, the 16 patients who received prednisolone enemas were not different in terms of clinical disease activity index, IBDQ, and histology score from IFN- α 2A treated patients. The largest randomized controlled trial of pegylated IFN- α in 60 patients with active UC however failed to show clinical efficacy, although the higher dose of 1 μ g/kg subcutaneously showed biological efficacy with a significant decrease in C reactive protein (CRP) concentrations.¹¹ Overall, the evidence for the efficacy of type I interferons in IBD is therefore unconvincing.

In the era of biological therapies with monoclonal antibodies, IFN- γ predictably has been identified as a target cytokine in chronic inflammatory diseases. In two studies published in this issue of the *Gut*,¹²⁻¹³ the early experience of anti-IFN- γ antibody in active CD has been reported (*see pages 1131 and 1138*). In a phase I/II dose ranging (0.1, 1.0, and 4.0 mg/kg) evaluation in active CD, fontolizumab, a humanised anti-IFN- γ antibody, was reasonably well tolerated with minimal immunogenicity. Flu-like symptoms, chills, and fever were noted in the active treatment groups but not in the placebo

Figure 1 Binding of interferon γ to type II interferon receptor composed of IFNGR-1 and IFNGR-2 subunits. This leads to activation of Janus family kinases Jak1 and Jak2 and phosphorylation of Stat1. Stat1 homodimers are translocated into the nucleus with initiation of gene transcription.

Table 1 Clinical uses of interferon modulation

Type of interferon	Established or potential clinical use
Interferon α	Chronic myelogenous leukaemia Hairy cell leukaemia Follicular lymphoma Renal cell carcinoma Bladder carcinoma Liver metastasis of carcinoid tumour Malignant melanoma AIDS related Kaposi's sarcoma Chronic hepatitis B Chronic hepatitis C
Interferon β	Multiple sclerosis ?Ulcerative colitis
Interferon γ	Chronic granulomatous disease Severe malignant osteopetrosis
Anti-interferon γ (Fontolizumab)	? Crohn's disease ? Rheumatoid arthritis

group.¹² Although in this small study ($n = 45$) with a high placebo response rate clinical efficacy end points did not reach statistical significance, a significant decrease in median CD endoscopic index of severity score was noted in the highest dose (4.0 mg/kg) subgroup compared with placebo. At day 29, evidence of biological efficacy was also noted with a significant decrease in CRP from baseline in the 1.0 and 4.0 mg/kg intravenous cohort. In addition to mucosal healing, aberrant HLA-DR, CD3, Stat-1, CXCR3, and Ki-67 expression decreased in mucosal biopsies in the higher dose (especially 4 mg/kg) group.¹² This would suggest that the 4.0 mg/kg dose of fontolizumab would

be worthy of further clinical trials, although even higher doses may need to be explored.

Therefore, in a larger phase II study involving 133 patients with active CD, fontolizumab was administered in a single dose of either 4 or 10 mg/kg compared with placebo.¹³ The study did not reach its primary efficacy end point, either clinical response (decrease in Crohn's disease activity index (CDAI) ≤ 100) or remission (CDAI < 150). However, the 91 patients who received two doses at 0 and 28 days showed a significant increase in clinical response rates at day 56 compared with the placebo group. There was no indication of any additional advantage of the

10 mg/kg dose compared with the 4 mg/kg dose.

A high placebo response was noted in both fontolizumab trials which could be diminished by stratification into elevated CRP at baseline. This has been noted in trials of other biological therapies such as certolizumab pegol phase II¹⁴ and natalizumab phase II and III.^{15 16} In these trials it is likely that elevated CRP identified a subgroup of recruited patients with genuine inflammatory activity, and therefore served as a quality control for the subjective nature of CDAI, which may be elevated with irritable bowel syndrome-like symptoms associated with IBD.¹⁷

Fontolizumab was generally well tolerated. Flu-like symptoms¹² are probably a consequence of action of type I interferons as a result of inhibition of IFN- γ . Type I interferons may induce transient chemokine release (such as CXCL10) which coincides with occurrence of flu-like symptoms.¹⁸ Immunogenicity was modest, but neutralising antibodies were detected in a proportion of patients.¹³

Interferons were the first recombinant DNA technology product to be widely used in cancer therapeutics and are still very valuable therapeutic agents in the treatment of chronic leukaemia, hairy cell leukaemia, lymphoma, renal carcinoma, bladder carcinoma, and melanoma.¹⁹ The antiviral efficacy of interferons have found established clinical use in chronic hepatitis B and hepatitis C (table 1). However, the use of interferons in chronic immune mediated inflammatory diseases is still limited, apart from relapsing and remitting multiple sclerosis. IFN- γ is the signature cytokine of T_H1 lymphocytes which characterise CD, suggesting that interfering with IFN- γ signalling may be an attractive therapeutic strategy in CD. Dogmatic classification of CD as a T_H1 mediated disease and UC as a T_H2 like disease has however hampered the progress of targeted therapy in both diseases. For example, recent evidence suggests that anti-TNF therapy is equally effective in both CD and UC, despite the initial reluctance to use anti-TNF therapy in a T_H2-like disease.²⁰ Classifying chronic inflammatory diseases into T_H1 (IFN- γ , IL-2) or T_H2 (IL-4, IL-5, IL-13) mediated diseases is an over simplification. IL-12 is crucial in the development of IFN- γ producing T_H1 lymphocytes and anti-IL-12p40 antibody has been shown to be effective in CD.²¹ However, the IL-12p40 chain is also a component of IL-23, a key cytokine for driving IL-17 production, which is a recently described potent proinflammatory cytokine which upregulates chemokine production in target cells.²² Both IL-12 and IL-23 interact with receptors that share the

Figure 2 Schematic diagram showing the development of naïve CD4⁺ T lymphocytes into T_H1, T_H2, and T_H17 effector cells. Interferon γ (IFN- γ) characterises the T_H1 lymphocytes. Interleukin (IL)-12 and IL-23 bind to receptors that share the IL-12R β 1 subunit. The putative blocking effects ($\perp$) of anti-IFN- γ , anti-tumour necrosis factor (TNF), and anti-IL12p40 are shown.

IL-12 receptor $\beta 1$ (IL12R $\beta 1$) subunit. IL-23 promotes the development of a pathogenic T lymphocyte population described as T_H-17, distinct from T_H1 and T_H2, which is characterized by IL-6, IL-17, and TNF production.²³ Therefore, it is possible that antagonism of IFN- γ signalling may have a more restricted effect compared with antagonism of IL-12p40 (antagonising both IL-12 and IL-23) and antagonism of TNF (fig 2). In addition, blockade of IFN- γ may enhance the development of T_H17 effector cells. Chronic inflammation is a nexus of pathways, and multipoint blockade may be necessary to increase clinical efficacy. Further clinical trial experience with anti-IL-12p40 and anti-IFN- γ is necessary to determine which one of these monoclonal antibodies prove to be more effective in the treatment of inflammatory bowel disease, including CD and UC. *Gut* 2006;**55**:1071–1073. doi: 10.1136/gut.2005.090134

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Liver disease

Human hepatic stellate cells are resistant to apoptosis: implications for human fibrogenic liver disease

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Resistance of hepatic stellate cells or myofibroblasts to proapoptotic stimuli is different between rodent and human cells. This may be important when looking for antifibrotic agents that can be used in human liver fibrosis

Hepatic stellate cells (HSCs) are one of the sinusoid constituent cells that play multiple roles in liver pathophysiology and, in particular, in liver fibrosis. In the intact liver, HSCs localise in the space between sinusoids and hepatocytes, so called space of Disse, embrace the sinusoids as liver specific pericytes to regulate sinusoidal blood flow

by their contractility, and store lipid droplets largely containing vitamin A.¹ When the liver parenchyma suffers from chronic injury caused by various disease aetiologies, such as iron overload, alcohol consumption, infection by hepatitis B virus or hepatitis C virus (HCV), non-alcoholic steatohepatitis, autoimmune hepatitis, and bile duct obstruction,

elimination of damaged hepatocytes causes HSCs to depart from the sinusoidal wall and become activated. This process is considered to be triggered by multiple peptide, lipid, and gaseous mediators that are released from hepatocytes, Kupffer cells, endothelial cells, and infiltrating inflammatory cells.^{2–5}

HSC activation accompanies their phenotypic transformation into myofibroblast (MFB)-like cells. The latter cell type exhibits expression of α smooth muscle actin and growth factor receptors, such as platelet derived growth factor receptor β (PDGF receptor β), production of contractile mediators, such as endothelin-1, and mitogenic mediators, such as PDGF, insulin-like growth factor, vascular endothelial growth factor, and chemokines, and production of extracellular matrix materials (that is, collagens, fibronectin, laminin, and proteoglycans), thereby playing major roles in the progression of fibrosis in chronically damaged livers. HSC activation is supported particularly by transforming growth factor β (TGF- β). Activated HSCs produce TGF- $\beta 1$ which