Treatment of Crohn's disease with recombinant human interleukin 10 induces the proinflammatory cytokine interferon gamma


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**INFLAMMATION AND INFLAMMATORY BOWEL DISEASE**

**Treatment of Crohn’s disease with recombinant human interleukin 10 induces the proinflammatory cytokine interferon γ**


**Background:** Interleukin 10 (IL-10) exerts anti-inflammatory actions by counteracting many biological effects of interferon γ (IFN-γ).

**Aims:** To investigate this in humans, we studied the effects of human recombinant IL-10 administration on IFN-γ production by patient leucocytes. Furthermore, we assessed the IFN-γ inducible molecule neopterin and nitrite/nitrate serum levels, which are indicative of endogenous nitric oxide formation.

**Methods:** As part of two placebo controlled double blind studies, we analysed patients with chronic active Crohn’s disease (CACD) who received either subcutaneous recombinant human IL-10 (n=44) or placebo (n=10) daily for 28 days, and patients with mild to moderate Crohn’s disease (MCD) treated with either subcutaneous IL-10 (n=52) or placebo (n=16) daily for 28 days. Neopterin and nitrite/nitrate concentrations were measured in serum, and ex vivo IFN-γ formation by lipopolysaccharide or phytohaemagglutinin (PHA) stimulated whole blood cells were investigated before, during, and after IL-10 therapy.

**Results:** In patients with CACD, the highest dose of 20 µg/kg IL-10 caused a significant increase in serum neopterin on days +15 and +29 of therapy compared with pretreatment levels. No changes were observed for nitrite/nitrate levels under either condition. In MCD, treatment with 20 µg/kg IL-10 resulted in a significant increase in PHA induced IFN-γ production.

**Conclusions:** High doses of IL-10 upregulate the production of IFN-γ and neopterin. This phenomenon may be responsible for the lack of efficacy of high doses of IL-10 in the treatment of CACD and MCD.

Interleukin (IL)-10 is secreted by several cell populations including T helper cells (Th), monocytes/macrophages, dendritic cells, B cells, and keratinocytes. This cytokine suppresses inflammation by various mechanisms, including reduction of HLA class II expression, decreasing T cell secretion of IL-2, and diminishing the production of IL-1α, IL-1β, tumour necrosis factor α (TNF-α), and IL-8 by activated monocytes/macrophages. Gene targeted IL-10 deficient mice develop transmural inflammation of the small and large bowel, reminiscent of Crohn’s disease (CD). This type of inflammation is aggravated by the presence of bacteria within the gut lumen, and can be prevented by administration of IL-10. Administration of IL-10 ameliorates inflammation in several other animal models not only limited to the gut. These data together suggested that IL-10 is a promising cytokine for treatment of inflammatory diseases. However, the clinical efficacy of recombinant human IL-10 (rHuIL-10) in the treatment of CD has been disappointing. In both animal models and endotoxin challenged volunteers, administration of recombinant IL-10 caused a significant reduction in TNF-α production. However, we have reported that in contrast with results obtained in various animal models, administration of rHuIL-10 in endotoxin challenged volunteers caused an increase in the production of interferon γ (IFN-γ).

Circulating concentrations of IFN-γ and neopterin are increased in patients with active CD, and correlate with clinical disease activity, as measured by the CD activity index (CDAI). Neopterin, a pyrazino-pyrimidino derivate, is mainly produced by monocytes/macrophages under the control of IFN-γ, and thus is a valuable in vitro and in vivo marker for monitoring cell mediated immune function and IFN-γ activity. In addition, IFN-γ, in combination with proinflammatory cytokines such as TNF-α and IL-1β, stimulates synthesis of inducible nitric oxide synthase (iNOS), thereby increasing nitric oxide (NO) production.

The present studies were designed to assess the impact of IL-10 on monocyte and T lymphocyte activity by (i) investigating serum levels of neopterin and nitrite/nitrate, the stable end products of NO in serum, in patients with chronic active steroid unresponsive CD (CACD) and by (ii) studying ex vivo production of TNF-α and IFN-γ in patients with mild to moderately active CD (MCD). The analysed patients were part of two large, double blind, randomised, multicentre collaborative trials studying the therapeutic effects of IL-10 in CACD and MCD.

**MATERIALS AND METHODS**

**Patients**

Two multicentre, randomised, double blind, placebo controlled studies were conducted in patients with CACD and MCD (for details see Fedorak and colleagues and Schreiber and colleagues). In the CACD trial, patients were included if they had active steroid resistant CD involving the colon or both the ileum and colon, with or without external fistula. Active...
Measurement of neopterin and nitrite/nitrate in CACD patients

The analysed patients were part of the international collaborative trial described above. Serum levels of neopterin and nitrite/nitrate were analysed in 10 patients treated with placebo, 12 patients treated with 1 µg/kg, 12 patients treated with 4 µg/kg, 10 patients treated with 8 µg/kg, and 10 patients treated with 20 µg/kg body weight 1L-10 subcutaneously. Serum levels of neopterin and nitrite/nitrate were determined on day –1, day +15, and day +29 of therapy and after four weeks of follow up. Neopterin levels were assessed by a specific ELISA (Brahms, Berlin, Germany). The detection limit for neopterin was 3 nmol/l and normal values are below 8 nmol/l. For determination of nitrite/nitrate levels, samples were deproteinised by sulphasalicylic acid and neutralised with NaOH. After enzymatic reduction of nitrate to nitrite using nitrate reductase (Sigma, Munich, Germany), total nitrite concentration was determined spectrophotometrically after addition of the Griess Ilosvay’s reagent (Merck, Darmstadt, Germany). Sodium nitrite served as standard. The detection limit for nitrite/nitrate was 1 µmol/l.

Assessment of whole blood cell synthesis of IFN-γ and TNF-α in MCD patients

A total of 68 patients who all completed the trial were studied (placebo, n=16; 1 µg/kg, n=15; 5 µg/kg, n=12; 10 µg/kg, n=12; and 20 µg/kg, n=13). Venous blood was aseptically collected in endotoxin free heparinised tubes (final heparin concentration 10 U/ml whole blood) and immediately aliquots of 2.5 ml of blood were mixed with 25 µl phytohaemagglutinin (PHA) (final concentration 5 µg/ml; Murex Diagnostics Ltd, Dartford, UK) or with 25 µl of lipopolysaccharide (LPS) (final concentration 10 ng/ml; E coli type 055-B5). As a negative control, 2.5 ml of blood were added to 25 µl polymyxin B (1 mg/ml endotoxin free buffered saline). All samples were incubated for 24 hours at 37°C. After incubation, samples were centrifuged at 1000 g for 30 minutes at 4°C and platelet poor plasma was stored at −70°C until analysis. TNF-α and IFN-γ levels were assessed by specific ELISAs (CLB, Amsterdam, the Netherlands). The detection limit for TNF-α was 1 pg/ml and for IFN-γ 7.4 pg/ml; normal values (heparinised plasma) are below 5 pg/ml and 10 pg/ml, respectively. TNF-α and IFN-γ were determined prior to administration of IL-10 on day +1, on day +8 (only TNF-α levels assessed), and 24 hours after the last dose of study medication on day +29.

Statistics

Statistical analysis was performed using the non-parametric comparison according to Kruskal-Wallis and Friedman. ANOVA analysis and the Friedman test were used for comparison within groups for the CACD and MCD studies, respectively. The Wilcoxon signed rank test was used for comparison within groups. Correlation analysis between neopterin/cytokine values and clinical response was performed by applying the Pearson correlation test. Data are presented as mean (SEM).

Table 1 Serum levels of nitrite/nitrate (µmol/l) in patients with chronic active Crohn’s disease treated with either placebo (n=10), or recombinant human interleukin 10 at 1 (n=12), 4 (n=12), 8 (n=10), or 20 (n=10) µg/kg body weight subcutaneously over 28 days

<table>
<thead>
<tr>
<th>Group</th>
<th>Day –1</th>
<th>Day +15</th>
<th>Day +29</th>
<th>p Value*</th>
<th>Follow up week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>31.6 (3.6)</td>
<td>32.4 (4.0)</td>
<td>30.4 (3.2)</td>
<td>0.79</td>
<td>36.4 (4.8)</td>
</tr>
<tr>
<td>1 µg/kg</td>
<td>29.6 (4.0)</td>
<td>38.4 (10.6)</td>
<td>22.8 (3.2)</td>
<td>0.27</td>
<td>28.0 (3.6)</td>
</tr>
<tr>
<td>4 µg/kg</td>
<td>46.0 (9.6)</td>
<td>46.8 (10.4)</td>
<td>43.6 (10.8)</td>
<td>0.79</td>
<td>38.0 (8.0)</td>
</tr>
<tr>
<td>8 µg/kg</td>
<td>38.0 (6.4)</td>
<td>38.8 (5.2)</td>
<td>47.6 (8.8)</td>
<td>0.5</td>
<td>40.0 (5.2)</td>
</tr>
<tr>
<td>20 µg/kg</td>
<td>34.4 (6.0)</td>
<td>39.6 (8.0)</td>
<td>46.0 (11.6)</td>
<td>0.29</td>
<td>32.8 (6.8)</td>
</tr>
</tbody>
</table>

Data are mean (SEM).

*Statistical analysis was performed using ANOVA.
higher dose groups (fig 3). Levels of LPS induced IFN-γ production were low on both day +1 and day +29 in all groups (data not shown). However, high IL-10 doses (20 µg/kg) significantly increased PHA induced IFN-γ production on day +29 (p=0.028) (fig 4).

When correlating PHA induced IFN-γ production and clinical response, we found that IFN-γ production was lower in responding patients (data not shown) but the effect was not statistically significant. There was however no correlation between LPS induced TNF-α values on day +8 and day +29 and clinical response.

**DISCUSSION**

IL-10 is an important negative regulator of cell mediated immunity. In many animal models, IL-10 has been shown to...
inhibit IFN-γ secretion from activated Th1 cells and natural killer cells and antagonise the effects of IFN-γ towards target cells such as macrophages. In mice, the immunoregulatory roles of IL-10 however appear to be complex. Murray et al showed that IL-10 transgenic mice are unable to clear mycobacterial infection. In this study, excess administration of IL-10 did not inhibit T cell responses to mycobacteria and IFN-γ production in these mice. This study suggested that IL-10 enhances IFN-γ production by antigen specific Th1 cells and/or non-specifically by natural killer cells under chronic inflammatory conditions. Furthermore, several studies indicate that IL-10 may act as an immunostimulatory agent. In mice with graft versus host disease (GVHD), IL-10 administration dose dependently decreased survival. Peritt et al demonstrated that proliferation of IL-2 activated natural killer cells is enhanced by IL-10, and the production of IFN-γ and TNF-α by IL-2 activated natural killer cells is significantly stimulated by IL-10. More importantly, Shibata et al showed that IL-10 may enhance IFN-γ production by natural killer cells.

In humans, IL-10 can also have a dual role. We have previously reported that in low dose endotoxaemia in human volunteers, a high dose of IL-10 (25 µg/kg) increases serum levels of IFN-γ. IL-10 treatment also enhanced the release of the IFN-γ dependent chemokines IFN-γ inducible protein 10 and monokine induced by IFN-γ in vivo. In addition, increased levels of soluble granzymes were measured after IL-10 treatment, reflecting activation of cytotoxic T lymphocytes and natural killer cells.

Whether the stimulatory or inhibitory effect of IL-10 on IFN-γ production may be predominant in a chronic inflammatory disease such as CD however is not yet known. In the current study, we decided to assess cytokine production by lymphocytes and monocytes separately, using specific stimuli (that is, PHA which directly stimulates lymphocytes and LPS which stimulates monocytes). Our data showed increased formation of neopterin and IFN-γ in response to high doses of IL-10. The observation that in CACD patients in vivo, probably reflecting endogenous formation of IFN-γ as well as neopterin production while viral complications as well as neopterin production while viral complications, and infectious diseases.

NITRITE/NITRATE LEVELS IN THE CACD STUDY

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Effects of human recombinant IL-10 on IFN-γ production in CD

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