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In vitro analysis of IGN-gamma and IL-12 production and their effects in ileal Crohn's disease



Abstract

Crohn's disease is an inflammatory disease of the gut in which tumour necrosis factor (TNF) and T helper 1 (Th1) cytokines (interleukin (IL)-12, interferon (IFN)- γ) are thought to play a major role. After the successes obtained with neutralisation of TNF, interest is now growing for therapy aiming at neutralisation of Th1-associated cytokines. Since cytokines are linked in a delicate network, in vitro cultures of ileal lamina propria mononuclear cells (LPMC) were set up for evaluation of a) IFN- γ and IL-12 production, b) effects of rhIFN- γ and rhIL-12 and c) effects of anti-IFN- γ and anti-IL-12 on pro-inflammatory cytokines and IL-10 production. LPMC

were isolated from surgical specimens of a total of 27 Crohn's disease and 17 caecum carcinoma (control) patients. Cells were stimulated with CD40L (which triggers myeloid CD40-expressing cells) or anti-CD3 +CD80 (which triggers T cells). LPMC from involved ileal, Crohn's disease produced, in both non-stimulated and stimulated conditions, more IFN-g and IL-12p70 than LPMC from non-involved tissue or from control patients. rhIFN-g significantly enhanced TNF production in both controls and in ileal Crohn's disease patients, while rhIL-12 enhanced IFN-g but not TNF production. LPMC from involved tissue were more sensitive to IL-12 than control LPMC. LP-T cell-dependent activation of monocytes was then studied by co-culture of anti-CD3/CD80-stimulated LPMC with fresh monocytes, which resulted in high IL-12, IFN-g, TNF and IL-10 production. The data show that neutralisation of either IL-12 or IFN-g with mAb in these cultures also affects secretion of the reciprocal cytokine and (in the case of anti-IL-12) also that of the anti-inflammatory cytokine IL-10. However, no effect of anti-IL-12 or anti-IFN-g on production of TNF, a cytokine with an important pathogenic role in Crohn's disease, could be found. Therapies aiming at neutralisation of IFN-g or IL-12 are therefore unlikely to replace anti-TNF, but they might provide an additive or synergistic effect.

Full text

Introduction

Crohn's disease is an inflammatory disorder of the intestinal tract, with a predilection for the terminal ileum. Both intestinal and extra-intestinal manifestations of the disease are currently thought to be the result of abnormal permeation of luminal antigenic material from the bowel lumen into the intestinal wall. TNF plays a pivotal role for recruitment of inflammatory cells [1]. Intricate pathways of innate and acquired immunity are involved, with the latter response believed to be largely T helper (Th)1-polarised.

One of the central cytokines in Th1-polarised responses in general, and in Crohn's disease in particular, is IFN-g [2-10]. IFN-g is primarily produced by Th1 and NK cells. It is believed to be a major regulator of antigen-specific immune and inflammatory responses [11]. CD4⁺ CD45RB^{high} T cell transfer from IFN-g-knockout (KO) to severe combined immuno-deficiency (SCID) syngeneic mice fails to induce wasting disease [12]. The major cytokine eliciting T and natural killer (NK) cells to produce IFN-g is dendritic cell- (DC) and macrophage-derived IL-12 [13-14]. These two cytokines, similar to TNF, are also believed to induce down-stream release of tissue-destructive enzymes such as matrix-metalloproteinases [1, 15].

In recent years, anti-IFN-g and anti-IL-12 antibodies have been used both *in vitro* and *in vivo* in various Th 1-polarised IBD model settings. *In vitro*, anti-IFN-g has been shown to largely inhibit the development of CD4 effectors producing IFN-g [16-17]. Its *in vivo* application in the CD4+ CD45RB^{high} transfer to SCID model led, if administered soon after T cell transfer, to prevention of colitis for up to 12 weeks [18]. Anti-IFN-g therapy in the tri-nitrobenzene sulfonic acid (TNBS) model was however not convincingly effective, with incomplete *ex vivo* suppression of murine colitic lamina propria T cell IFN-g production [19].

Interest has also grown for administering anti-IL-12 in Crohn's disease. Although anti-IL-12 cannot inhibit IFN-g production by activated NK or T cells in the absence of IL-12 producing cells [20], Monteleone *et al.* have shown that anti-IL-12 dose-dependently down-regulates IFN-g production by non-stimulated Crohn's disease lamina propria mononuclear cells [21]. This latter finding was also confirmed with LPMC from a Crohn's disease patient in whom a neutralising anti-IL-12 antibody down-regulated the *in vitro* development of IFN-gamma-producing CD4+ T cells from gut biopsy specimens [7]. *In vivo*, anti-IL-12 therapy has been effective in preventing colitis in both the TNBS model [18, 22], the bone marrow transfer to Tgε26 mice model [23], and the IL-10-deficient mice [24]. Our group administered anti-IL-12 in the CD4+ CD45RB^{high} transfer to SCID model. We documented diminished colonic leukocyte infiltration (both for CD4 T cells and F4/80 positive macrophages), diminished intracellular adhesion molecule (ICAM)-1 expression and diminished *ex vivo* TNF, IL-2 and IFN-g production by colonic lamina propria T cells [25].

All of these cytokines are linked to each other in a delicate network and/or cascade. Addition or neutralisation of one cytokine might have unexpected effects on other cytokines. In an attempt to study this network, and to predict effects of IFN-g and/or IL-12 neutralisation, we performed an *in vitro* study with lamina propria mononuclear cells (LPMC) derived from non-involved or non-lesional (NL) and involved or lesional (L) ileal resection specimens from Crohn's disease patients. In these cultures, the extent of IFN-g, IL-12, anti-IFN-g and anti-IL-12 to affect cytokine production was compared by means of ELISA measurement of IFN-g, IL-12p70, TNF and IL-10 production. Specific aims were (a) to measure IFN-g and IL-12p70 production levels in control, non-involved (NL) and involved (L) ileal LPMC cultures, (b) to examine IFN-g and IL-12 effects in the same cultures and (c) to compare these results with the effect of anti-IFN-g and anti-IL-12 on cytokine production in co-cultures of anti-CD3-stimulated LPMC with fresh monocytes.

Methods

Surgical specimens

Surgical ileal specimens from a total of 27 Crohn's disease patients and a total of 17 caecum carcinoma patients (control) (the latter not staged beyond T2N1M0) were used. Specimens from ileal mucosa (at the utmost distance of the tumour) from control, and from non involved or non lesional (NL) and involved or lesional (L) ileal mucosa of Crohn's disease patients were collected and quickly transferred to the laboratory in ice-chilled medium, consisting of glutamin-enriched RPMI 1640 with Penicillin (100 U/ml), Streptomycin (100 mg/ml) (Bio-Wittacker, Heidelberg, Germany) and Polymixin B sulphate (4 U/ml) (ServaGmbH, Heidelberg, Germany).

Cell preparation

Lamina propria mononuclear cells (LPMC) were prepared as recently described [26]. After chemical digestion and further purification, the resulting LPMC were resuspended at 1×10^6 viable cells/ml in medium containing 10% iron-supplemented BCS and Amphotericin B (2.5 mg/ml) (Sigma Chemicals, St-Louis, MO, USA). A portion of the LPMC were then analysed phenotypically, using mouse anti-human antibodies to CD3, CD16-56, CD19 and CD33 (Pharmingen, San Diego, CA, USA), each time compared with isotype matched controls. All staining procedures were performed after addition of inactivated human plasma in order to prevent non-specific adhesion.

Peripheral blood purified monocytes were obtained from a single healthy donor using anti-CD33 magnetic microbeads (Miltenyi-Biotec, Köln, Germany), with a MACS cell sorter as described by the manufacturer. The CD33 positive monocyte cell population obtained was > 99% pure.

In vitro incubation

The remaining LPMC were then incubated at a final concentration of 5×10^5 /ml.

In 11 control and 12 Crohn's disease patients, the LPMC were cultured at a 2:1 ratio, with human CD80-bearing, mitomycin- (50 mg/ml) inactivated P815 cells (gift from L. Lanier (DNAX, Palo Alto, CA, USA)) and/or anti-human-CD3 monoclonal antibody (mAb) (UCHT-1 1 mg/ml) (gift from P. Beverly (Jenner Institute for Vaccine Research, Compton, UK)) and/or IL-12 0.5 ng/ml (Genetics Institute, MA, USA). IFN-g and TNF production was measured by ELISA after 36 hours of incubation.

In another 6 control and 8 Crohn's disease patients, the LPMC were stimulated with IFN-g 1000 U/ml (Boehringer, Mannheim, Germany) or mitomycin- (50 mg/ml) inactivated CD40 Ligand- (L) transfected 3T6 mouse fibroblasts (gift from K Thielemans, Free University, Brussels, Belgium) at a 4:1 ratio, or a combination of both. Supernatants were collected after 36 hours of incubation. IL-12p70 and TNF levels were assessed by ELISA.

In a last group of 7 Crohn's disease patients, LPMC from both non-involved and involved ileum were cultured with CD80 and anti-CD3 mAb, in co-culture with IFN-g-primed monocytes (at a 4:1 ratio) from a single healthy donor. For IFN-g priming, CD33+ monocytes were washed and re-suspended in medium containing 2000 U IFN-g/10⁶ monocytes (Boehringer, Mannheim, Germany). After 6 hours incubation, the cells were washed 3 times. The remaining primed monocyte suspension did not contain any free IFN-g as ascertained by ELISA. Co-cultures of LPMC + IFN-g-primed monocytes were set up for 36 hours to evaluate cytokine production. Anti-IFN-g antibody (mIgG1, gift from M. De Ley, Rega Institute, Leuven, Belgium [27]) or anti-IL-12 (HulG1, clone J695, lot ALP903, a gift from Genetics Institute, Cambridge, MA, USA) were added at a concentration of 10 mg/ml. IFN-g, IL-12p70, TNF and IL-10 levels were assessed by ELISA.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism 2.0. Data were paired wherever possible and analyzed with Wilcoxon's matched pairs test. For unpaired data, a Mann-Whitney U test was used. A Chi-square test, using classical contingency tables, was used for proportions. All tests were 2-sided and at a 95% significance level.

Results

Phenotypic analysis of ileal lymphoid and myeloid lamina propria cells

As shown in Table 1, no significant differences in the composition of LPMC preparations isolated from involved, non-involved or control tissues were observed.

IFN-g production in ileal LPMC cultures from Crohn's disease patients

Under both non-stimulated or anti-CD3/CD80 triggering conditions, increased IFN-g production in cultures of Crohn's disease lamina propria cells could be

documented (Figure 1). Spontaneous production was low but detectable, with values of 64 ± 8 pg/ml in LPMC cultures from controls, 120 ± 34 pg/ml in non-involved Crohn's disease LPMC and 273 ± 85 pg/ml in involved Crohn's disease LPMC cultures ($p = 0.03$ for comparison of control versus non-involved and $p = 0.01$ for comparison of control versus involved Crohn's disease LPMC cultures). Anti-CD3/CD80 triggering resulted in high IFN-g production: 1352 ± 175 pg/ml in LPMC cultures from controls, 1942 ± 328 pg/ml in non-involved Crohn's disease and 2434 ± 275 pg/ml in involved Crohn's disease LPMC cultures ($p = 0.004$ for comparison control versus involved Crohn's disease LPMC cultures).

IL-12p70 production in ileal LPMC cultures

Data concerning IL-12p70 production levels are represented in Figure 2. In only about half of the LPMC cultures was IL-12p70 produced above the detection limit ($= 10$ pg/ml), with values never exceeding 100 pg/ml. In 2 out of 8 Crohn's disease patients, minute amounts of spontaneously produced IL-12p70 could be seen in LPMC cultures from involved tissue (16 ± 3 pg/ml), while it was completely absent in non-involved and control LPMC. Triggering with IFN-g could provoke these two spontaneous producers to produce more IL-12p70, with values being 12 ± 1 pg/ml in non-involved ileal LPMC cultures, and 18 ± 5 pg/ml in the involved counterpart. Two additional LPMC cultures from involved ileal Crohn's disease did respond to CD40L triggering (hence 4/8 responders) (30 ± 10 pg/ml), while no response was observed in non-involved and control LPMC. More noticeable IL-12p70 production could be achieved by dual stimulation with CD40L + IFN-g. This was also the case in control ileal LPMC cultures, where dual stimulation was revealed as being a prerequisite for IL-12p70 production, resulting in production of 23 ± 4 pg/ml IL-12p70 (5/6 positive) triggering. Crohn's disease LPMC also produced IL-12 in response to CD40L + IFN-g stimulation: values were 37 ± 10 pg/ml (5/8 positive) in non-involved LPMC and 48 ± 14 pg/ml (4/8 positive) in involved LPMC.

Effect of IFN-g on TNF production in ileal LPMC cultures

The data above already indicated that IFN-g could enhance IL-12 production, although the number of responders was low. We next assessed the effect of adding IFN-g to the bowel LPMC cultures in terms of TNF production by myeloid cells (Table 2). TNF production levels were, although still low, more substantial. From Table 2, it can be seen that IFN-g is a TNF-inducing cytokine. In control LPMC, this effect is synergistic with CD40L-triggering ($p = 0.0331$ for comparison of relative IFN-g effect under CD40L triggered versus non-stimulated control LPMC). Surprisingly,

such a synergistic effect could not be found in LPMC derived from Crohn's disease patients.

Effect of IL-12 on cytokine production in ileal LPMC cultures

From Table 3, it can be seen that IL-12, as expected, can up-regulate IFN- γ . By comparing stimulation indices obtained by dividing the value with by the value without addition of IL-12, we could assess differences in IL-12 efficacy for IFN- γ production amongst the three different cell sources. This analysis revealed that IL-12 is more potent in up-regulating IFN- γ in involved Crohn's disease LPMC as compared to non-involved Crohn's disease LPMC, with confidence intervals for stimulation indices being [2.534-6.615] in non-involved LPMC and [5.357-15.68] in involved LPMC ($p = 0.0015$). TNF production on the other hand was not affected by IL-12.

Effect of anti-IFN- γ and anti-IL-12 in cultures of ileal LPMC from Crohn's disease patients

Activated T cells interact with monocytes/macrophages, resulting in cytokine induction. Monocytes themselves might enhance cytokine production by T cells. To study this phenomenon, we co-cultured LPMC with fresh monocytes. T cells among LPMC were stimulated with anti-CD3/CD80, and cytokine production was evaluated. Figure 3 shows that high amounts of IFN- γ , IL-12, TNF and IL-10 were produced in these cultures (much higher than when LPMC were cultured alone). Values for involved Crohn's disease anti-CD3/CD80-triggered LPMC, without the addition of exogenous monocytes, were 2351 ± 366 for IFN- γ , below detection limits for IL-12p70, 279 ± 98 for TNF and 418 ± 101 pg/ml for IL-10 production levels. Values with the addition of exogenous monocytes were 9723 ± 3549 for IFN- γ , 191 ± 46 for IL-12p70, 1710 ± 426 for TNF and 847 ± 240 pg/ml for IL-10 production levels. To study the role of IFN- γ and IL-12 in this increased production, the effect of anti-IFN- γ and anti-IL-12 was then analyzed. It can be seen that both antibodies were functional in reducing respectively IL-12p70 production (from 191 ± 46 without to 126 ± 41 pg/ml with anti-IFN- γ) and IFN- γ production (from 9723 ± 3549 without to 1408 ± 903 pg/ml with anti-IL-12). This shows that IL-12 production by monocytes induced by co-culture with activated T cells, is largely responsible for increased T cell interferon- γ production. On the other hand, interferon- γ contributes to monocyte IL-12 production, but the effect of anti-IFN- γ was limited, although significant. Importantly, anti-IFN- γ could not significantly down-regulate TNF production (from 1710 ± 426 without to 1495 ± 502 pg/ml with anti-IFN- γ), nor did it

up-regulate IL-10 production (from 847 ± 240 without to 794 ± 178 pg/ml with anti-IFN- γ). Anti-IL-12 similarly had no influence on TNF production (from 1710 ± 426 without to 1759 ± 476 pg/ml with anti-IL-12), although it significantly down-regulated IL-10 production (from 847 ± 240 without to 542 ± 178 pg/ml with anti-IL-12).

These results were largely comparable when LPMC from the non-involved ileal area of Crohn's disease were used in the same co-culture system. This non-involved tissue, although not having any disease characteristics, can be regarded as, in fact, being rather early lesional tissue. Anti-IFN- γ and anti-IL-12 could down-regulate respectively IL-12p70 and IFN- γ production in this non-involved LPMC/monocyte co-culture system ($p = 0.0156$ for both comparisons). Again, neither anti-IFN- γ nor anti-IL-12 had an influence on TNF production ($p = 0.5781$ and 0.8501 respectively) though.

Discussion

In this study, we could demonstrate increased spontaneous and induced secretion of IL-12 and IFN- γ in ileal Crohn's disease, and provide evidence that neutralisation of these cytokines will affect the secretion of the reciprocal cytokine, but without affecting TNF production. We worked with ileal LPMC in order to study production and especially the effect of Th1 cytokines, the latter being of potential importance for the pathogenesis of Crohn's disease. Three different stimulation systems were used, each with their own rationale: T cell stimulation with anti-CD3/CD80; myeloid cell stimulation with CD40L/IFN- γ ; and T cell-dependent monocyte activation in a co-culture of LPMC with fresh monocytes.

Firstly, T cell-directed anti-CD3/CD80 stimulation was used to assess IFN- γ production, and the effects of exogenous IL-12 on it. TCR triggering and an adequate second co-stimulatory signal such as CD80 are a prerequisite for optimal T cell activation [28]. As CD80 positivity has been reported in the bowel [29], we believe this stimulus to be close to the physiological *in vivo* situation. We confirmed increased IFN- γ production in Crohn's disease LPMC cultures compared to control LPMC cultures. This has previously been reported in the medical literature, both as a result of ELISA and techniques such as ELISPOT, rt-PCR, immunohistochemistry and intracellular staining [2-9]. Secondly, CD40L triggering was used in order to assess IL-12p70 production by myeloid cells and the influence of exogenous IFN- γ on IL-12 and TNF production. CD40L interacts with CD40-bearing cells: B cells, activated macrophages, and dendritic cells (DC). The interaction has been

reported to induce high IL-12 production levels [30]. Administration of anti-CD40L therapy resulted in alleviation of the disease in the TNBS colitis model and was reversed by injection of a recombinant IL-12p75 heterodimer, indicating the CD40L-CD40 interaction to be crucial for IL-12-dependent priming of Th1 T cells *in vivo* [31]. We recently demonstrated CD40L and CD40 expression in the IBD bowel and in the *in vitro* functionality of anti-CD40(L) to down-regulate TNF and IL-12p70 production by normal monocytes co-cultured with IBD lamina propria T cells [32]. IL-12 is primarily produced by antigen-presenting cells [33]. Its functional form is IL-12p70, composed of both a p40 and p35 sub-unit. Its production is readily inducible by intracellular parasites, bacteria and microbial products such as LPS or SEB. Increased IL-12 production has been reported in Crohn's disease mucosa, both by means of immunohistochemistry [7] and at the mRNA level [21]. Attempts to measure IL-12 at the secreted protein level have been so far less convincing, with spontaneous production values for IL-12p40 around 10 pg/ml [21] and stimulated values ranging from 5.8 pg/ml [34] to a maximum of 25 pg/ml [21]. Our study is the first to highlight IL-12p70 protein production levels. Our results were however hampered by over half the experiments not yielding a production value above our ELISA detection limit (= 10 pg/ml). It could be argued that these low production values are a reflection of LPS redundancy, which has been described in Crohn's disease lamina propria cells, both by others [35] and ourselves [36]. Moreover, production of IL-12 by IFN-g-primed monocytes was reported to be completely suppressed by pre-incubation with LPS for 6 to 24 hours before priming [37]. It is possible that this also happens with LPMC, *in vivo* or simply during the *in vitro* isolation procedure. IFN-g was revealed to be a stable IL-12 and TNF inducer in this system. The data maintain the role of IFN-g as a pro-inflammatory cytokine in ileal Crohn's disease lamina propria, and exclude a functional loss of the IFN-g receptor in Crohn's disease.

Thirdly, in order to assess LP-T cell-dependent cytokine secretion by myeloid cells also, anti-CD3/CD80-stimulated LPMC were co-cultured with IFN-g-primed monocytes from a single healthy donor. Heterogeneity of bowel macrophage behaviour, if functional, could, as such, be corrected for. Moreover, the IFN-g priming was revealed to be necessary to obtain sufficient IL-12p70 production, thus enabling us to evaluate the anti-IFN-g and also the anti-IL-12 effect. Anti-IFN-g down-regulated IL-12p70, but not TNF or IL-10 production. As indicated above, IFN-g up-regulates IL-12p70 and proved to be a stable TNF inducer. In this co-culture system, the values of IFN-g should have been sufficient to induce TNF production *in vitro*. It is possible that TNF production values were simply too high for anti-IFN-g to suppress, or that a bystander mechanism such as the CD40L-CD40 interaction was responsible for IFN-g-independent TNF up-regulation. The finding that IL-10

production was not affected is no surprise, as we have previously reported that IFN-g loses its potential to down-regulate IL-10 production in these involved ileal LPMC [26]. Blocking IL-12 activity by means of an anti-IL-12 mAb leads to down-regulation of IFN-g, but not TNF production. This result again underlines the aforementioned data, whereby IL-12 could up-regulate IFN-g but not TNF production. In contrast, anti-IL-12 was capable of significantly down-regulating IL-10 production. Again, this result is underscored by our previously reported data indicating that IL-12 is a potent IL-10 inducer in these same LPMC [26].

Increased functional IL-12R b 2 in active Crohn's disease has been reported [38]. *In vitro*, added IL-12 gave rise to increased IL-12R b2 expression [38]. This is completely in agreement with our findings that the effect of IL-12 on IFN-g production was more pronounced in involved as compared to non-involved Crohn's disease and control LPMC. The signaling pathway used by IL-12 to maintain Th1 differentiation hence seems reinforced in ileal Crohn's disease.

Antagonisation of cytokine activity in the treatment of inflammatory diseases has become an active field of research, and the success of anti-TNF mAb treatment in Crohn's disease has demonstrated the important role of TNF in the pathogenesis. When considering therapy against IFN-g or IL-12, it is important to consider our findings that neither anti-IFN-g nor anti-IL-12 can convincingly down-regulate TNF production in these Crohn's disease lamina propria cell cultures. In accordance with our findings, IL-12-induced intestinal inflammation was indeed recently demonstrated to be linked to TNF [39] and partially IFN-g- independent. [40] Thus, blocking IL-12 might have effects independent of TNF or IFN-g, by blocking ongoing Th 1 differentiation and activation. Blocking IL-12 was more effective than blocking IFN-g in the TNBS model [19]. Early and delayed anti-IL-12 treatment was effective in the CD4+ CD45RB^{high} transfer to SCID model [25]. Moreover, blockage of IL-10 production by anti-IL-12 might well be revealed to be beneficial in view of our previously reported data in which pro-inflammatory effects of IL-10 in involved ileal Crohn's disease lamina propria, possibly due to interference by IL-12 in the IL-10 effect, could be demonstrated [26]. Reasonable evidence in support of anti-IL-12 therapy in Crohn's disease is herein presented. As TNF production is not affected by anti-IFN-g or anti-IL-12, it seems unlikely that these might be useful as a single therapeutic agent. A potential use is to combine anti-IFN-g or anti-IL-12 with infliximab, in order to block several independent pathways of inflammation. Alternatively, infliximab (Remicade®) non-responders might well be revealed to be the preferential target patient population for therapy with anti-IL-12.

ABBREVIATIONS - rh: recombinant human / CD: cluster of differentiation / TCR: T cell receptor / RT-PCR: reverse transcriptase polymerase chain reaction / mRNA: messengerRNA / LPS: lipo-polysaccharide/ mAb: monoclonal antibody

CONCLUSION

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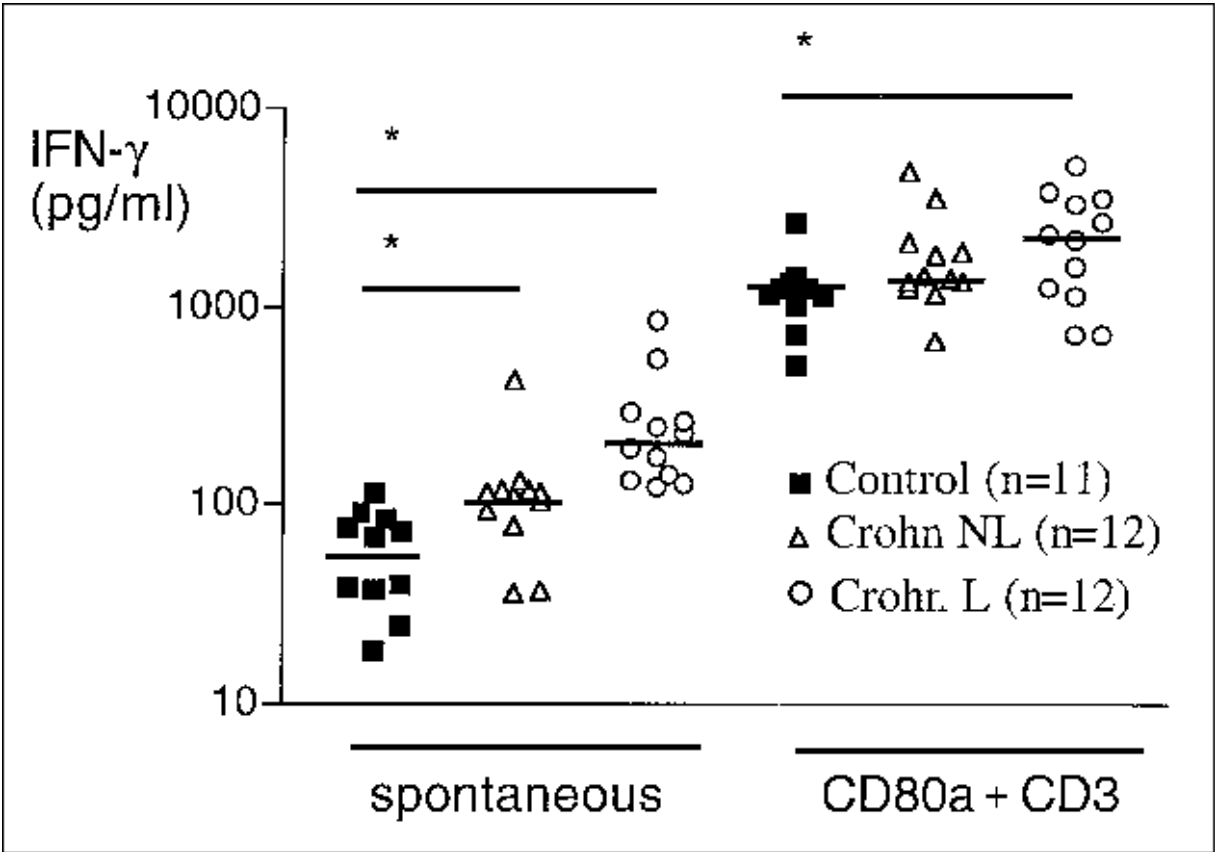
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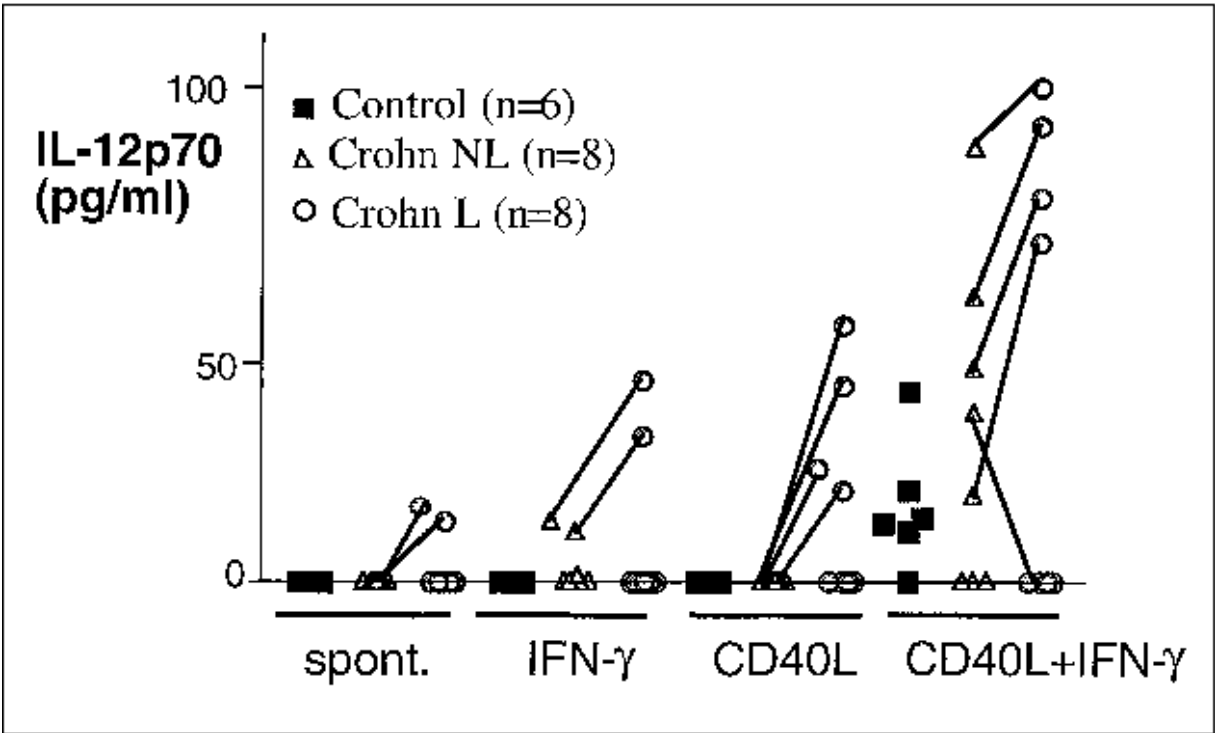
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Figures



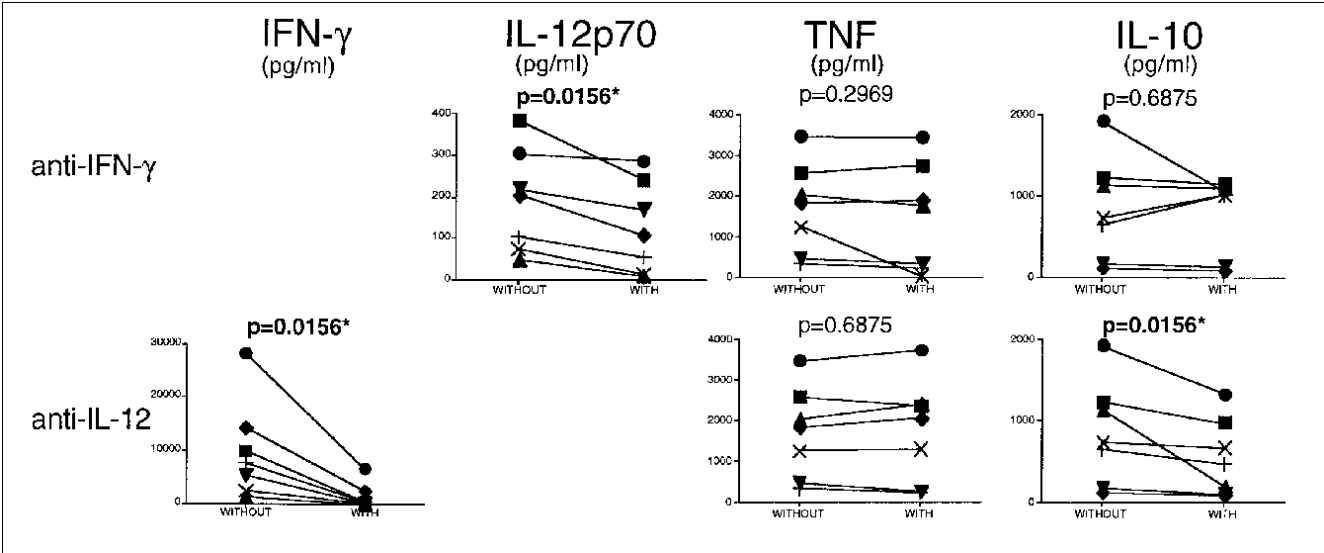
IFN-g production in ileal lamina propria mononuclear cell cultures.

Ileal lamina propria mononuclear cells (LPMC) from 11 caecum carcinoma (control) and LPMC from non-involved (NL) and involved (L) areas from 12 Crohn's disease (Crohn) patients were isolated and incubated at 5×10^5 /ml without (spontaneous) or with CD80 transfected, Fc receptor-bearing mouse P815 cells at a 2 to 1 ratio. Anti-CD3 (UCHT-11 mg/ml) was added to induce optimal stimulation and co-stimulation of T cells. Supernatants were taken after 36 hours of incubation. IFN-g was measured *via* ELISA. Data are expressed in pg/ml. Horizontal lines indicate median value. * * $p < 0.05$ for comparison between control and Crohn (Mann-Whitney U test).



IL-12p70 production in ileal lamina propria mononuclear cells.

ÎÎÎÎÎÎÎÎÎÎ Ileal lamina propria mononuclear cells (LPMC) were isolated from gut resection specimens from 6 caecum carcinoma (control) and 8 Crohn's disease (Crohn) patients for whom non-involved (NL) and involved (L) areas were used. Cells were incubated at 5×10^5 /ml either alone, or with 1000 U/ml IFN-g or with CD40L transfected 3T6 mouse fibroblasts, or a combination of both stimuli. "Spont." stands for the unstimulated condition. Supernatants were taken after 36 hours of incubation. IL-12p70 was measured using ELISA. Data are expressed in pg/ml. Connecting lines are for paired samples from non-involved and involved area of the same Crohn's disease patient.



Effects of anti-IFN-g and anti-IL-12 on cytokine production in involved ileal LPMC cultures.

ÎÎÎÎÎÎÎÎÎÎ LPMC from involved lamina propria from 7 Crohn's disease patients were incubated at 5×10^5 /ml, stimulated with anti-CD3 (1 mg/ml) and CD80 transfected P815 cells (2 to 1 ratio) in co-culture with purified monocytes (4 to 1 ratio) from a single healthy donor. The latter cells were primed with IFN-g (2000 U/ 1×10^6 monocytes/ml) for 6 hours. After thorough washing, the remaining primed monocyte suspension did not contain any free IFN-g as ascertained by ELISA. Neutralising anti-IFN-g or anti-IL-12 mAb were used at 10 mg/ml. Each symbol in the figure represents one patient in whom IFN-g, IL-12p70, TNF and IL-10 production was measured after 36 hours of incubation, each time without or with the anti-IFN-g or anti-IL-12 antibody. The p value shown refers to the "without/with" comparison (Wilcoxon matched pairs test) and is printed in bold whenever < 0.05 .

	CD3	NK	B	CD33
--	-----	----	---	------

control (n = 17)	58% ¹ (54-76) ²	2% (1-5)	33% (22-45)	6% (3-11)
non-involved Crohn's disease (n = 27)	68% (59-76)	3% (1-5)	19% (11-30)	6% (4-12)
involved Crohn's disease (n = 27)	73% (63-87)	3% (2-6)	11% (8-23)	11% (8-14)

		Phenotypic analysis of isolated ileal lamina propria cells
		¹ percentages indicate mean values;
		² interquartile ranges (also%) are given between brackets.

		spont. ¹		CD40L	CD40L
			+ IFN-g		+ IFN-g
TNF ³	Control ²	< 1 ⁴	4 ± 1*	5 ± 2	90 ± 29*
	Crohn NL	< 1	8 ± 3*	6 ± 2	14 ± 6*
	Crohn L	15 ± 6	28 ± 12*	16 ± 1	36 ± 16*

		Effect of IFN-g on TNF production by ileal LPMC
		¹ Cells were either unstimulated (spont.) or stimulated with CD40L (4 to 1 ratio) each time without or with IFN-g (1000 U/10 ⁵ cells/ml).
		² Cells were derived from ileal resection specimens from

	<p>carcinoma patients (control) (n = 6) and Crohn's disease (Crohn) non-involved (NL) and involved (L) area (n = 8).</p> <p>³ Production levels are expressed as mean ± s.e.m. (pg/ml).</p> <p>⁴ Detection limit for TNF production 1 pg/ml.</p> <p>* significant up-regulation by IFN-g (p < 0.05, Wilcoxon matched pairs text).</p>

	IFN-g			(p-value)	TNF			(p-value)
	CD80antiCD3 ²	+ IL-12	(s.i.)		CD80antiCD3	+ IL-12	(s.i.)	
control (n = 11) ³	497 ⁴	2960	5.64 ⁵	(0.0003) ⁶	115	118	1.09	(0.2166)
Crohn NL (n = 12)	1098	4634	4.52	(<i>< 0.0001</i>)	91	90	0.95	(0.3452)
Crohn L (n = 12)	1583	7396	7.25	(<i>< 0.0001</i>)	134	157	1.44	(0.2820)

	<p>Effect of IL-12 on production of IFN-g and TNF by CD80aCD3-stimulated ileal lamina propria cells¹</p> <p>¹ Cells were derived from ileal resection specimens from carcinoma patients (control) (n = 11) and Crohn's disease (Crohn) non-involved (NL) and involved (L) area (n = 12).</p> <p>² Cells were stimulated with an Fc receptor bearing P815 mouse mastocytoma cells, transfected with the human CD80 gene and with addition of anti-CD3 mAb.</p> <p>³ The number of patients in each group is given in italics in the first column.</p> <p>⁴ Data are expressed as median values (pg/ml).</p>

⁵ (S.I.) means median stimulation index, obtained through dividing the value with IL-12 by its respective value without IL-12.

⁶ a Wilcoxon paired rank test was used for comparison of the condition without and with IL-12, the respective p-value being given behind the corresponding median values. Values and p values are printed in bold whenever $p < 0.05$.

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