Increase in neutrophil Fc γ receptor I expression following interferon gamma treatment in rheumatoid arthritis

N J Goulding, S M Knight, J L Godolphin, P M Guyre

Abstract

The therapeutic potential of interferon γ (IFN γ) in a number of disease states is still being explored, but progress is hampered by the lack of a suitable measure of in vivo biological activity. To assess the in vivo biological effects of recombinant human IFN γ (rhIFN γ), 14 patients were studied in a randomised, prospective, double blind, placebo controlled trial of this cytokine for the treatment of rheumatoid arthritis. The levels of Fc γ receptors on peripheral blood neutrophils were measured at baseline and after 21 days of once daily, subcutaneous injections of rhIFN γ or placebo. An induction of neutrophil Fc γ receptor type I (Fc γ RI) was seen in the group of patients receiving recombinant human rhIFN γ but not in those receiving placebo. No change in the expression of Fc γ RII or Fc γ RIII was detected. The amount of induction of Fc γ RI detected on the neutrophils of patients receiving rhIFN γ did not correlate with clinical measures of response at either 21 days or at the end of the study (24 weeks). No significant clinical responses were observed in the rhIFN γ group at these times. These data confirm that the reported in vitro effect of IFN γ on human neutrophil Fc receptor expression can be reproduced in vivo.

Interferon gamma (IFN γ) is a low molecular weight dimeric glycoprotein with potent immunoregulatory activities (reviewed by Trinchieri and Perussia¹). Reports of its in vitro action on monocytes and B lymphocytes expose paradoxical pro- and anti-inflammatory effects: classical in vitro actions of interferon gamma include the upregulation of class II major histocompatibility complex (MHC) molecules on antigen presenting cells and stimulation of the production of the proinflammatory cytokines interleukin 1 (IL 1) and tumour necrosis factor by macrophages. It has also been reported that IFN γ stimulates hyperplasia of cultured synovial fibroblasts. In contrast, other actions of IFN γ include the inhibition of prostanoid production by monocytes, suppression of the bone resorbing activities of IL 1 and tumour necrosis factor, and also the inhibition of B lymphocyte MHC class II antigen expression, proliferation, and antibody secretion.² These latter actions suggest that IFN γ might play a part in controlling the rate of B lymphocyte proliferation and differentiation. Indeed, evidence now exists that the in vivo administration of rhIFN γ in rheumatoid arthritis decreases circulating B lymphocyte numbers.³ These

observations suggest that rhIFN γ might beneficially influence autoantibody production in diseases such as rheumatoid arthritis.⁴ The part played by endogenous IFN γ in rheumatoid arthritis is uncertain. The finding by some workers of only low levels of detectable IFN γ in rheumatoid synovial fluid and the inability of neutralising antibodies to impair synovial MHC class II antigen expression in vitro suggests that IFN γ is not the primary macrophage activating factor in the chronically inflamed joint.⁵

Subcutaneous administration of IFN γ has recently been shown to reduce infections in patients with chronic granulomatous disease, but did not cause statistically significant changes in laboratory measurements of phagocyte activation.⁶ A number of clinical trials have also been undertaken to assess the potential of IFN γ as a disease modifying drug in rheumatoid arthritis. Early uncontrolled pilot trials suggested that it might be effective. Earlier randomised, double blind, prospective, placebo controlled studies include those by Veys *et al*⁷ and Cannon *et al*.⁸ Veys *et al* studied 26 patients in a 24 week trial of daily injections for five days, then twice weekly injections for the remainder of the study period.⁷ The dose of rhIFN γ used was 100 μ g. They observed no significant improvement in the active treatment group compared with the placebo group, but their intragroup comparisons showed a significant improvement in the joint score after 24 weeks of IFN γ treatment. Several other clinical variables showed a trend (not reaching statistical significance) towards improvement. Cannon *et al* studied 105 patients in a 12 week trial during which five injections were given each week.⁸ The dose of interferon used was 100 μ g. In their study concomitant use of certain disease modifying antirheumatic drugs was allowed providing that the dose remained stable throughout the study period. Evaluation of intragroup changes again showed that a number of clinical parameters tended to improve during IFN γ treatment but none of the differences reached statistical significance due to the small number of subjects studied. Intergroup comparisons at the end of the study showed no significant differences. The findings of these and previous studies indicated the need for the large multicentre trial with adequate statistical power which has been completed and in which the patients described in this paper were participants.

Resting polymorphonuclear leucocytes (neutrophils) express relatively high levels of two receptors for the Fc portion of IgG, namely Fc γ RII (CD32) and the glycan linked form of

Bath Institute for
Rheumatic Diseases,
Trim Bridge,
Bath BA1 1HD, UK
N J Goulding
S M Knight
J L Godolphin
Department of
Physiology,
Dartmouth Medical
School, Hanover,
NH 03756, USA
P M Guyre

Correspondence to:
Dr Goulding.

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Fc γ RIII (CD16).⁹ Neutrophils normally express low or undetectable levels of Fc γ RI, the only Fc γ receptor that binds monovalent IgG with high affinity.¹⁰ Constitutive expression of Fc γ RI appears to be restricted to mononuclear phagocytes.

The *in vitro* induction of Fc γ RI on human neutrophils by IFN γ has been shown.¹¹ This induction can be inhibited by IL 1. IFN γ is the only cytokine reported to be capable of this induction in neutrophils and it has no effect on the expression of Fc γ RII or Fc γ RIII on these cells.¹²

The objective of this study was to find out whether the subcutaneous injection of a low dose of IFN γ can induce a significant increase in neutrophil expression of Fc γ RI. Our results show that this biological effect can serve as a marker of the *in vivo* bioactivity of rhIFN γ given to patients with rheumatoid arthritis.

Patients and methods

Fourteen patients were recruited into a prospective, randomised, double blind, placebo controlled study of rhIFN γ . Treatment consisted of rhIFN γ (Biogen, Cambridge, MA, USA) or placebo seven days a week for three weeks, three days a week for five weeks, and two days a week for 16 weeks, administered by subcutaneous injection in the abdominal area. All patients gave written informed consent before entry. The dose of rhIFN γ used was 50 μ g. All patients were between 18 and 75 years of age and had at least a six month history of definite or classical rheumatoid arthritis with onset of rheumatoid arthritis after 16 years of age. At the time of enrolment all patients had active disease with at least six joints swollen and tender on pressure or painful on motion (distal interphalangeal joints excluded), or both, and two of the following: nine or more joints tender on pressure or painful in motion (distal interphalangeal joints excluded), or both; early morning stiffness lasting 45 minutes or more; an erythrocyte sedimentation rate of at least 40 mm/h. They were all receiving a stable therapeutic dose of non-steroidal anti-inflammatory drugs (NSAIDs) for at least one month before the study; this was not altered during the study. One patient (7) in the treatment group was receiving prednisolone 5 mg per day; this dose was stable and was unchanged during the study.

No injection of corticosteroids was given during the study or in the preceding three months. Concomitant second line drugs were not allowed during the study and were discontinued (if previously given) at least three months before entry to the study.

Before the study began and 21 days into the study period, venous blood was collected into preservative free heparin. Polymorphonuclear leucocytes were isolated by single step density centrifugation over a Ficoll-meglumine M85 gradient.¹³ After erythrocyte contamination had been removed by three seconds of haemolysis in distilled water followed by washing in phosphate buffered saline, the cells were resuspended in RPMI 1640 (buffered with 25 mM HEPES containing 2% bovine serum albumin) at a final concentration of 5×10^7 cells/ml. A 20 μ l volume of cells was reacted with an equal volume of 60 μ g/ml monoclonal antibody (mAb) (obtained from Medarex, West Lebanon, NH, USA). The antibodies used were mAb 32.2 recognising Fc γ RI (CD64), mAb IV.3 specific for Fc γ RII (CD32), or mAb 3G8 raised against Fc γ RIII (CD16). Potential non-specific binding of antibody to Fc receptors was blocked by the addition of 20 μ l of human IgG at a concentration of 12 mg/ml. The neutrophils were incubated on ice for one hour. After washing in ice cold phosphate buffered saline plus 2% bovine serum albumin, these cells were incubated with 40 μ l F(ab')₂ fragment of goat antimouse IgG conjugated to fluorescein isothiocyanate (FITC) for 45 minutes. Antibody isotype, second antibody and autofluorescence controls were run concurrently. After further washing steps, 200 μ l of these cells in phosphate buffered saline plus 2% bovine serum albumin was added to an equal volume of phosphate buffered saline containing 2% paraformaldehyde as a fixative. Fixed cells were sealed in polycarbonate tubes and sent to Dartmouth College (NH, USA) for flow cytometric analysis.

Neutrophils were examined by flow cytometry, gated by forward and 90° light scatter, and specific surface monoclonal antibody markers. Analysis was performed on an Ortho 50H cytofluorograph with 488 nm argon ion laser and 2150 computer. Cell associated mean fluorescence intensity was converted to FITC equivalent second antibody molecules bound per cell according to a calibration graph using standard fluorescence beads from Flow Cytometry Standards (Research Triangle Park, NC, USA).¹⁴

Results

Clinical assessments were made before and regularly during the study by a single observer. Table 1 summarises the major entry variables for the two treatment groups. The placebo (n=7) and active treatment (n=7) groups did not differ significantly at baseline. Entry values were: mean (SD) erythrocyte sedimentation rate (mm/h) IFN group 32 (7), placebo group 43 (39); mean (SD) visual analogue pain score (cm) IFN group 6.8 (3.4), placebo group 7.1 (1.7); and mean joint score IFN group 83 (27), placebo group 92 (38). In view of earlier studies

Table 1 Patient entry variables. The first 14 patients were recruited from a double blind, randomised, placebo controlled trial of interferon γ (IFN) treatment in rheumatoid arthritis

Patient No	Group	Sex	Age (years)	Rheumatoid factor status	Erythrocyte sedimentation rate (mm/h)	Visual analogue score (cm)
1	IFN	F	37	+	32	2.3
2	IFN	F	46	+	38	6.0
3	IFN	F	29	+	22	3.8
4	IFN	F	48	+	28	11.3
5	IFN	F	70	+	42	9.8
6	IFN	F	51	—	28	5.4
7	IFN	F	31	+	36	9.3
8	Placebo	F	41	+	11	6.3
9	Placebo	F	53	+	28	10.3
10	Placebo	F	57	—	115	5.3
11	Placebo	F	66	+	80	8.0
12	Placebo	F	59	+	25	7.1
13	Placebo	F	50	+	12	6.2
14	Placebo	M	60	+	30	6.3

Expression of Fcγ receptors on human neutrophils as measured by flow cytometry following either recombinant human interferon γ or placebo treatment for 21 days. The two bars on the left represent patients treated with interferon γ; the bars on the right represent those receiving placebo. (A) Fcγ receptor type I (CD64) expression measured by monoclonal antibody 32·2; (B) Fcγ receptor type II (CD32) expression measured by monoclonal antibody IV·3; (C) Fcγ receptor type III (CD16) expression measured by monoclonal antibody 3G8. All results given as mean (SD) fluorescein isothiocyanate (FITC) equivalent sites per cell. *Significantly higher at day 21 in the interferon treated but not placebo group ($p<0·01$), Wilcoxon signed rank test.

we anticipated that this small group of patients would not have the statistical power necessary to show significant inter- or intragroup changes in clinical parameters in response to rhIFN γ. This was indeed so and no significant changes were observed. The combined results of the participating centres are not yet available, but a more meaningful clinical picture should emerge from the multicentre study.

Flow cytometric analysis was shown to be a sensitive method of measuring changes in the surface expression of Fcγ receptors on human neutrophils from patients receiving IFN γ treatment for rheumatoid arthritis. Fcγ RI was barely detectable on neutrophils before the beginning of the trial (mean (SD) 210 (70) sites per cell). Initial mean (SD) levels of the two highly expressed Fcγ receptor types II and III were 18 358 (1452) and 56 748 (6985) sites per cell respectively.

The figure shows the changes in the levels of expression of all three Fc receptor types from day 0 to day 21. A significant increase was seen in the expression of Fcγ RI in the active treatment group but not the placebo group ($p=0·01$, Wilcoxon signed rank test). No significant alteration was observed in Fcγ RII or Fcγ RIII expression in either group.

Table 2 shows that no correlation was found between Fcγ RI induction at 21 days or the overall expression of any of the three classes of Fc receptor and joint score, visual analogue score or erythrocyte sedimentation rate at this time. Overall, the degree of Fcγ RI receptor induction seen in patients receiving IFN γ did not correlate with the end of study outcome (as assessed by visual analogue score, joint score, or erythrocyte sedimentation rate). It is noteworthy that the one patient who showed the least Fcγ RI receptor induction following rhIFN γ treatment was withdrawn from the study at 17 weeks because of inefficacy of treatment. All other patients receiving active treatment completed the study.

Discussion

This paper reports a study of the in vivo effect of rhIFN γ on the induction of expression of neutrophil Fcγ RI in patients with rheumatoid arthritis. Changes in the level of expression were easily detectable by flow cytometry but were not as dramatic as those reported for the in

Table 2 Changes in clinical indices of disease activity and expression of neutrophil Fcγ receptors, three weeks after active interferon γ (IFN γ) treatment or placebo

Variable	IFN γ		Placebo		Significance
	Mean change day 0 to day 21	Fcγ R change (%)	Mean change day 0 to day 21	Fcγ R change (%)	
Erythrocyte sedimentation rate (mm/h)	-3·86		+6·43		<0·05*
Joint score	-13·0		-25·9		NS
Visual analogue score (cm)	+0·71		+0·09		NS
Fcγ receptor I (sites per cell)	+2309	2199	+387	113	<0·01*
Fcγ receptor II (sites per cell)	+1236	4·6	-332	-2·0	NS
Fcγ receptor III (sites per cell)	+28 475	43·6	+18 346	22·4	NS

*Statistical differences placebo v IFN γ assessed using the Mann Whitney U test. NS=not significant.

vitro culture of neutrophils from healthy donors with rhIFN γ .¹¹ This could reflect the dose and availability of the cytokine to the cells or, potentially, an impairment of response in this disease. This study only considered levels of Fc receptor expression at two time points. A further longitudinal study would give information about the time of optimum neutrophil responsiveness. At present the optimum dose of rhIFN γ for the treatment of rheumatoid arthritis is unknown. This assay could have potential value in giving an objective assessment of bioavailability and bioactivity of the drug as well as in determining patient compliance. In this study, no correlation was found between the increase in expression of Fc γ RI and clinical response (as measured by visual analogue score, joint score and erythrocyte sedimentation rate). This could be due to the small number of patients investigated and the cross sectional nature of the study.

It is unclear what effect the observed increase in neutrophil Fc γ RI expression might have on the rheumatoid process. It has been shown that there was an increase in the expression of this receptor following streptococcal pharyngitis,¹⁵ presumably due to increased endogenous production of IFN γ , but no indication was found of increased IFN γ levels in the peripheral blood of these patients. It is possible that induction of Fc γ RI is an important step in neutrophil activation pathways leading to phagocytosis or antibody dependent cellular cytotoxicity of antibody coated target cells. IFN γ can enhance the Fc γ RI and Fc γ RII mediated cytotoxicity,¹⁶ and is able to enhance oxidative metabolism of human neutrophils.¹⁷ There appears to be a functional correlation between Fc γ receptor mediated activation events and IFN γ treatment in human neutrophils.¹⁸ Further longitudinal studies on larger groups of patients are required to address some of these issues. This study shows clearly that the effects of rhIFN γ on neutrophils observed in vitro are also present in vivo and suggests that monitoring changes in the expression of Fc γ RI is a valuable means of assessing its biological activity in the treatment of rheumatoid arthritis.

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