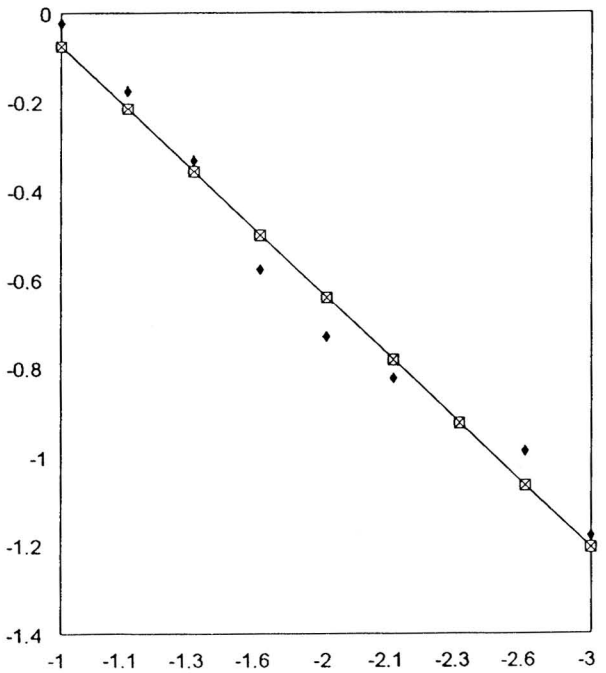

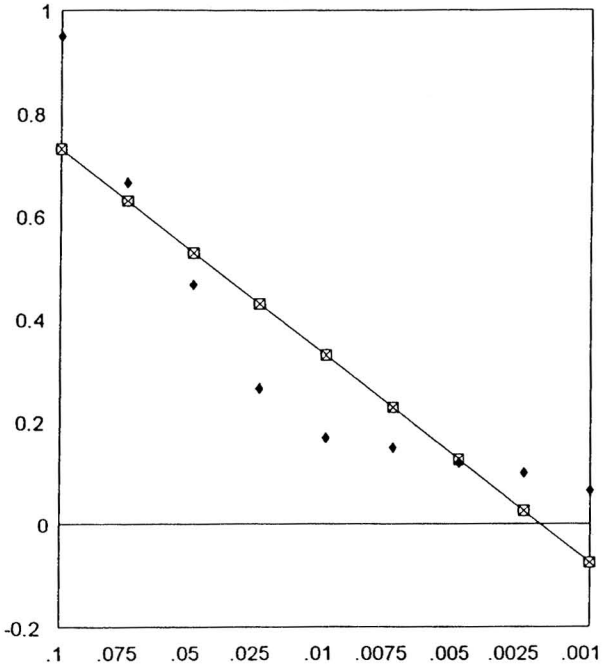

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THE IFNγ PATHOPHYSIOLOGY. THE ROLE OF SOLUBLE IFNγR α CHAIN

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INTRODUCTION

IFNγ is a lymphokine produced by T cells and NK cells. IFNγ exerts its activities by binding to specific cell surface receptor (R).

IFNγ acts as a potent immunomodulator and is a powerful macrophage activating factor. In the basis of its properties the IFNγ could play the principal role in the antigen specific immune response and has been considered the master key to the inflammatory response (1).

Some experimental findings point to a disease-promoting role of IFNγ in several pathological status as multiple sclerosis, systemic lupus erithematosus, type I diabetes, septic shock and others (2). In the case of rheumatoid arthritis (RA) specially the route of administration are of critical importance in determining the effects of IFNγ.

It is possible that at sites of inflammation the pro-inflammatory properties of IFNγ predominate, whereas critical concentration of circulating IFNγ are anti-inflammatory.

An aberrant regulation of the IFNγ action at site of inflammation could contribute to the development and/or exacerbation of some autoimmune and inflammatory disorders.The control of IFNγ function at the

receptor level could be an important place to elucidate the possible disregulation of IFNγ system in IFNγ promoting diseases.

We showed for the first time the altered levels of the IFNγR α chain in patients with AR and some RFLP of genomic DNA. The IFNγR α chain soluble fragment was cloned and expressed in *E. coli*.

EXPERIMENTAL PROCEDURES

Soluble IFNγR α chain was isolated from plasma of RA patients and controls using minichromatographies on IFNγ coupled to affigel-10 columns. Messenger RNA and genomic DNA were isolated from PBL after CsCl gradient, and processed for Northern Blot and RFLP analysis respectively.

The recombinant IFNγR α chain soluble fragment was isolated by cDNA-PCR using specific primers. The cDNA was inserted in a pIL-2 expression vector. The recombinant protein was extracted with Guanidium chloride 7M and then refolded by gel filtration. The protein was purified sequentially by anion exchange chromatography and ligand affinity.

RESULTS

Soluble forms of IFNγR α chain were identified in human plasma by dot blot with $^{(125)}$I-IFNγ. The soluble receptor migrated as 68kDa in reduced conditions and as 60 kDa in non-reduced conditions.

The levels of soluble receptor in RA patients and controls detected by dot blot is showed in table.

Table 1

% of relative high levels of IFNγR α chain

Populations	Control	Suspected	Patients
Swiss	14.3% (1/7)	60% (3/5)	35% (5/14)
Cuban	11.7% (2/17)	-	20% (1/5)

A possible RFLP was identified affecting the 5' coding region for IFNγ binding site in the IFNγR α chain. The recombinant IFNγR α chain soluble fragment was expressed at high level in *E. coli*. The maximum of expression was at 8 h after induction by starvation of tryptophan. The recombinant protein recognized $^{(125)}$I-IFNγ in a dot blot and Western Blot and non-labeled IFNγ in an ELISA system.

CONCLUSIONS

An altered soluble receptor molecule with high affinity or high levels of the soluble molecule could generate an aberrant regulation of IFNγ function during the primary events that contribute to the development of RA. The use of recombinant IFNγR α chain soluble fragment is a good candidate as antagonist to IFNγ at site of inflammation and will be helpful in counteract the proinflammatory action of the IFNγ.

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