Clinical Evaluation of Interferon-Gamma Treatment to Chronic Granulomatous Disease Patients with Splice Site Mutations

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SUMMARY: IFN-γ dependent increase of superoxide production by neutrophils was observed in three patients with Chronic Granulomatous disease from one family. The patients have the gp91-phox defect due to a splicing abnormality derived from a silent mutation adjacent to the third intron of CYBB gene. Apparent differences of splicing pattern of CYBB gene transcripts in patients’ neutrophils were detected between 1 and 25 days after administration of IFN-γ. Furthermore, the transcript containing all missing exons could be detected in all specimens after the treatment. The changes of splicing pattern in the transcripts and prolonged effect on superoxide generating ability of patients’ neutrophils indicate that IFN-γ induced an ability to correct abnormal splicing of CYBB gene transcripts in progenitor cells at least in part.

Chronic Granulomatous disease (CGD) is an inherited disorder of defense mechanism against microbial infections caused by defective activity of the phagocyte oxidase. Promoted by the findings of Ezekowitz et al. (1), who showed increase of the neutrophil superoxide generating ability in response to IFN-γ in a CGD patient, multi-centered group studies were performed to know effect of prophylactic medicated IFN-γ. However, no apparent increase of the phagocyte superoxide generation was recognized (2).

In our case, a great increased superoxide generation and changes of splicing pattern of gp-91-phox transcripts including the full-length transcripts by IFN-γ were observed. These findings indicated another possible mechanism of IFN-γ effect that it might correct abnormal splicing of CYBB gene transcripts in progenitor cells (3). In this paper, we also discuss about the effect of IFN-γ on the other patients with the splice mutations in gp91-phox.

Patients and their family: Among the patients enrolled in our IFN-γ study, three patients, MF (13 years old), YF (17 years old) and HM (16 years old), who responded well to IFN-γ treatment, were studied. They are members of the same kindred. The patients have been comparably well, though they occasionally suffered from liver abscess, pneumonia, lymphadenitis and cystitis. HM was once suffered from liver abscess at 12 years of his age. Except that, he has no other severe infections. In this family, 5 healthy carriers and 5 X-CGD patients were identified by flow cytometric analysis. Among the descendents, three had died of severe bacterial infection in their early life, suggesting that they were CGD patients. Their informed consents on IFN-γ studies were obtained. In this study, IFN-γ was administered subcutaneously once at a dosage of 25 × 10^6 JRU/m^2 (SHIONOGI CO. & LTD., Osaka, Japan). The samples were drawn and subjected to the following studies. Only YF developed a high-grade fever on the first day of the treatment. An intractable acne vulgaris on the face of MF and YF disappeared after the treatment.

RESULTS

1. A superoxide-generating ability was apparently increased during 2-4 weeks (DHR-123) (Fig. 1) but gp91-phox expression did not change (7D5) after a single IFN-γ treatment in all the patients with the same mutation.

2. Molecular analysis revealed a single base substitution adjacent to intron 3 in CYBB gene in the patients.

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3. Splicing patterns of \textit{CYBB} gene transcripts had been changed before and after IFN-\(\gamma\) treatment (0th day vs. 25th day) (Fig. 2A). The transcript containing all missing exons could be detected in all specimens after the treatment (Fig. 2B and 2C). The full length transcript of gp-91-\(\text{phox}\) was indicated by the arrow (Fig. 2).

**DISCUSSION**

We observed a great increased superoxide generating (Fig. 1) and changes of splicing pattern of gp91-\(\text{phox}\) transcripts including those of full length transcripts by IFN-\(\gamma\) (Fig. 2) in our CGD patients. IFN-\(\gamma\) was supposed to correct apart of splicing of transcripts at the progenitor cell, and induce a prolonged effect on superoxide generating ability in the present cases (Fig. 3) because of the following IFN-\(\gamma\) function reported; i) IFN-\(\gamma\) induces alternate splicing patterns of RNA transcripts in tryptophanyl-tRNA, nitric-oxide synthase mRNA and HLA Class I mRNA. ii) IFN-\(\gamma\) had the similar prolonged effect in the CGD patient (4). iii) IFN-\(\gamma\) stimulated the early stage of myelopoiesis by enhancing the frequency of growth factor-responding cells.

We also observed the discrepancy between the superoxide generating ability and a gp91-\(\text{phox}\) expression after IFN-\(\gamma\) treatment. Several unfunctional chimera gp91-\(\text{phox}\) proteins expressing the epitope against 7D5 monoclonal antibody were supposed to be induced on neutrophils of the patients before and after IFN-\(\gamma\) treatment (Fig. 3). Because the epitope was confirmed on the protein sequence corded between exon 5 and 6 of \textit{CYBB} gene (5) and such chimera gp91-\(\text{phox}\) transcripts were confirmed by RT-PCR analysis (Fig. 2C).

We expect that it is very likely that the other CGD patients with the same mutation would receive clinical benefit of IFN-\(\gamma\) treatment.

**REFERENCES**