Role of Glycosylation Sites in the IgE Fc Molecule

M.Y.	Mignon Y.	Nettleton
J.P.	Jarema P.	Kochan

Department of Inflammation and Autoimmune Diseases, Hoffmann-La Roche, Nutley, N.J., USA

Key Words

Immunoglobulin F (IgF)
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Abstract

The Fc region of immunoglobulin E (IgE) comprising the C_ε3 and C_ε4 domains (residues 329–547) is sufficient for binding to the high-affinity IgE Fc receptor (FcεRIα). Three potential N-linked glycosylation sites are present within the C_ε3 domain. To determine the effect of the glycosylation sites on IgE Fc synthesis and on FcεRIα binding, site-directed mutagenesis was performed. Mutant IgE Fc constructs were expressed in COS cells and analyzed for protein synthesis and secretion, and FcεRIα binding activity. We find that only N³⁷¹ and N³⁹⁴ are glycosylated, and that the residues surrounding the glycosylation site at N³⁹⁴ are required for FcεRIα binding activity.

Correspondence to: Dr. J. P. Kochan, Department of IAD, Hoffmann-La Roche, 340 Kingsland St., Nutley, NJ 07110 (USA)

Introduction

The Fc region of immunoglobulin E (IgE) comprising the C_ε3 and C_ε4 domains (residues 329–547) is sufficient for binding to the high-affinity IgE Fc receptor (FcεRIα) [1–3]. Three potential N-linked glycosylation sites are present at positions 371, 383 and 394, within the C_ε3 domain. The role these glycosylation sites play in the synthesis and binding of the IgE Fc to FcεRIα has not been defined. We have utilized site-directed mutagenesis to mutate the relinked sites and determine the effects on synthesis and secretion, as well as the ability of the glycosylation-deficient IgE Fc to bind the FcεRIα subunit.

Results

The three N-linked glycosylation sites in the IgE Fc 315–547 polypeptide were mutated singly or in multiple combinations by changing the Asn residues to Gln [1]. Preparation of recombinant polypeptides from transfected COS cells, and analysis by Western blot, detected the synthesis of all the different proteins (fig. 1). Both N371 and N394 are N-link-

Fig. 1. Expression of mutant IgE Fc 315-547 polypeptides in transfected cells. COS cells were transfected with mutant IgE Fc 315-547 constructs, and extracts were Western blotted with an anti-IgE Fc polyclonal antibody. The numbers 0,1,2 on the right side represent the total number of glycosylation sites utilized, while the numbers on the left are the MW standards.

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Fig. 2. Ability of mutant IgE Fc 315-547 polypeptides to bind FcRI. COS cells were transfected with mutant IgE Fc 315-547 constructs, and extracts were tested for receptor binding activity using SPEBA [1].

ed glycosylated, while N383 is not glycosylated, at least in COS cells.

To determine if the recombinant IgE Fc polypeptides were functional, the same extracts were tested for their ability to bind FcεRIα using the SPEBA assay [1]. As seen in figure 2, all of the IgE Fc 315-547 mutant polypeptides which contained Gln-Asn substitutions in N371 and N383 could bind FcεRIα. However, substitution of N394 resulted in the synthesis of a protein which did not bind receptor.

Further analysis of the N394 site revealed that different amino acid substitutions for the Asn residue did not reconstitute receptor binding activity. Different amino acid substitutions for the Thr396 residue also resulted in a loss of FcεRIα binding activity. The latter results were especially surprising when substitution of the Thr by Ser did not affect the level of glycosylation, but resulted in an IgE Fc polypeptide which did not bind receptor (results not shown).

Discussion

We have demonstrated that N371 and N394 are N-linked glycosylated in mammalian cells, whereas N383 is not glycosylated in COS cells. Mutation of the N394 or the T3% results in the synthesis of a protein which does not bind to FcεRIα. These results demonstrate that N394 and T396 are essential for the IgE Fc molecule to be synthesized and to bind receptor. However, we cannot determine whether the glycosylation of the IgE Fc is essential for proper folding or for receptor binding activity. Alternatively, the Asn and Thr residues which comprise the N-linked glycosylation site at N394 are essential for receptor binding. The finding that enzymati-

deglycosylated IgE Fc can bind receptor [1] clearly indicates that glycosylation is not essential for receptor binding, although it may be important for correct protein folding.

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