Original article **P43**

Correlation of FcγRIIIa polymorphisms and the response to rituximab in Thai population

Chayapol Somboonyosdech*, Supeecha Wittayalertpunya, Wacharee Limpanasithigul

Department of Pharmacology, Faculty of Medicine, Chulalongkorn University, Thailand.
* Presenting Author

Abstract

It has been reported that polymorphism in Fc gamma IIIa receptor (FcγRIIIa) associates with antibody-dependent cellular cytotoxicity (ADCC) activity of rituximab, the chimeric IgG_1 monoclonal antibody against CD20. This antibody has been used for treatment of several non-Hodgkin's lymphomas originate from B cells and has ADCC as an important mechanism of action. It is also known that genotype frequencies of FcγR polymorphisms depend on race and ethnicity. In this study, we investigated Fc gamma IIIa receptor (FcγRIIIa) genetic polymorphism at amino acid position 158 in Thai population. The nested polymerase chain reaction-restriction fragment length polymorphism (nested PCR-RFLP) was used to identify the FcγRIIIa genotypes of 60 healthy Thai male volunteers with informed consent. The distributions of FcγRIIIa-158 polymorphisms in these subjects were as follows: high binding genotypes (VV and VF), 55%; and low binding genotypes (FF), 45%. The different response of these genotypes to ADCC activity of rituximab was also evaluated. Rituximab-induced ADCC was performed by using PBMCs from 60 volunteers mentioned before as effect cells and ramos cells which express CD20 as target cells in the present of rituximab. High rituximab-induced Ramos cell cytotoxicity (mean rank 37.8%) was observed in the present of PBMCs from subjects with VV and VF genotypes while lower cytotoxicity (mean rank 21.6%) was determined in the present of PBMCs from subjects with FF genotypes. Our results provide the distribution of FcγRIIIa polymorphisms in Thai population which seems to differ from other Asian countries. This information should be useful for considering to use many IgG therapeutic antibodies in Thai population.

Keywords: Anti CD20, FcγRIIIa polymorphisms, Non-Hodgkin's lymphoma, Rituximab.

Introduction

Several monoclonal antibodies have been used for treatment cancers during the past decade. Most of them are IgG_1 cytotoxic antibody specific to tumor antigen on tumor cells. Rituximab, the chimeric mouse/human IgG_1 monoclonal antibody has been approved either alone or in combination with chemotherapeutic agents to treat several non-Hodgkin's lymphomas (NHLs) originate from B-cell since 1997 (1). This antibody targets CD20 which specifically express on B lymphoma cells. ADCC by rituximab-activated NK cells has been suggested to be an important mechanism of rituximab (2). This antibody uses its Fab part for recognizing CD20 on the surface of B lymphoma cells and its Fc part for binding to its receptors, Fc gamma IIIa receptor (FcγRIIIa), on natural killer (NK) cells. This leads to NK cell activation and follows by destruction of the CD20 positive cancer cells by ADCC mechanism. FcγRIIIa on macrophages and NK cells has two expressed alleles that differ at amino acid position 158 in the extracellular domain, valine (V158) and phenylalanine (F158) (3). These allelic variants have been demonstrated to differ in IgG_1 binding and ADCC. VV homozygotes and V/F homozygotes bind IgG high affinity than FF homozygotes (2). Genotype frequencies of FcγRIIIa polymorphisms depend on race and ethinicity. The low binding FF allele is high among Asian population (68%) than Europes or Africa population

(58%) (4, 5). In this study, we identified the distribution of FcγRIIIa polymorphisms in Thai population and correlated these polymorphisms to rituximab-induced ADCC activity of NK cells.

Materials and Methods

Materials:

Human peripheral blood mononuclear cells (PBMCs) were used as effector cells in ADCC assay. The PBMCs were isolated by Ficoll gradient centrifugation of buffy coats from 8 ml-whole blood of healthy male blood donors with informed consent from the National Blood Bank, Thai Red Cross Society. The cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin, at 37°c in 5% CO_2/ 95% air. One ml whole blood from each donor was used for FcγRIIIa genotyping.

Human B lymphoma cells, Ramos, which express CD20 from American Type Culture Collection (ATCC) were used as target cells. The cells were stained with 5μM carboxyfluorescein succinimidyl ester (CFSE) for 5 min and then washed with complete RPMI 1640 medium. The CFSE-stained cells were maintained in complete RPMI 1640 medium for 24h before ADCC assay

Rituximab, chimeric mouse-human IgG_1 monoclonal antibody against human CD20, was obtained from Chulalongkorn memorial hospital.

FcγRIIIa genotyping:

The genomic DNA was isolated from one ml whole blood by a blood DNA extraction kits (VIvantis, Malaysia). The FcγRIIIa G559T genotype was determined by nested PCR-RFLP with specific primers for FcγRIIIa gene and *Nla*III restriction enzyme. The PCR-RFLP products were identifies by 3% agarose gel electrophoresis, ethidium bromide staining and UV exposure. PCR products from G (V158) allele were digested by to 2 fragments by the restriction enzyme, *Nla*III, while the products from T (F158) allele were not digested by *Nla*III. The data of genotypes were presented as the percentage of high binding- (VV, VF) and low binding- (FF) genotypes.

FcγRIIIa-induced NK cell activation:

Rituximab (IgG_1 monoclonal antibody) was used as an antibody to activate NK cells in PBMCs via binding to FcγRIIIa on NK cell surface. Activated NK cells can release perforin and granzyme to kill their target cells, Ramos cells, which specifically recognized by rituximab.

Ramos cells, as target cells, were distinguished from PBMCs effector cells by staining with CFSE. CFSE-stained Ramos cells were treated with 10 μg/ml of rituximab for 1 h and then co-cultured with human PBMCs, at PBMCs:Ramos cells ratio of 10:1, for 4 h. These cella were washed with PBS and stained with 1 μg/ml propidium iodide (PI). Rituximab-mediated Ramos cell cytotoxicity ($CFSE^+/PI^+$ cells) was analyzed by flow cytometer. The percentage of rituximab-mediated Ramos cell cytotoxicity was calculated.

The correlation between FcγRIIIa genotype and ADCC response were presented as individual dot plot with mean rank values. The difference of rituximab-mediated cytotoxicity in each genotyping group was compared by using the Mann-Whitney u test to determine.

Results

The frequencies of FcγRIIIa -158 polymorphisms from 60 healthy Thai male volunteers were 55% V carrier (VV, VF) and 45% homozygous FF (Table 1). Polymorphism

at position 158 of FcγRIIIa have been reported influence human IgG1 binding and ADCC. It has been demonstrated that patients with follicular-NHL who were VV homozygous and VF heterozygous had higher response to rituximab than FF homozygous (6). We investigated the correlation of FcγRIIIa polymorphism among the volunteers in this study to FcγRIIIa-induced NK cells from these volunteers were used as effector cells and separated from target cells, Ramos cells, by staining Ramos cells with CFSE. Rituximab-mediated Ramos cell death was higher in VV and VF subjects than in FF subjects. The mean rank of the percentage of cytotoxicity in the former group (37.8%) was statistically different the latter group (21.6%), as shown in Fig 1.

Table 1: Distribution of FcγRIIIa genotyping from 60 normal volunteers

	High binding genotypes (VV, VF)	Low binding genotype (FF)
Normal volunteer (60n)	33	27
Percent (%)	55%	45%

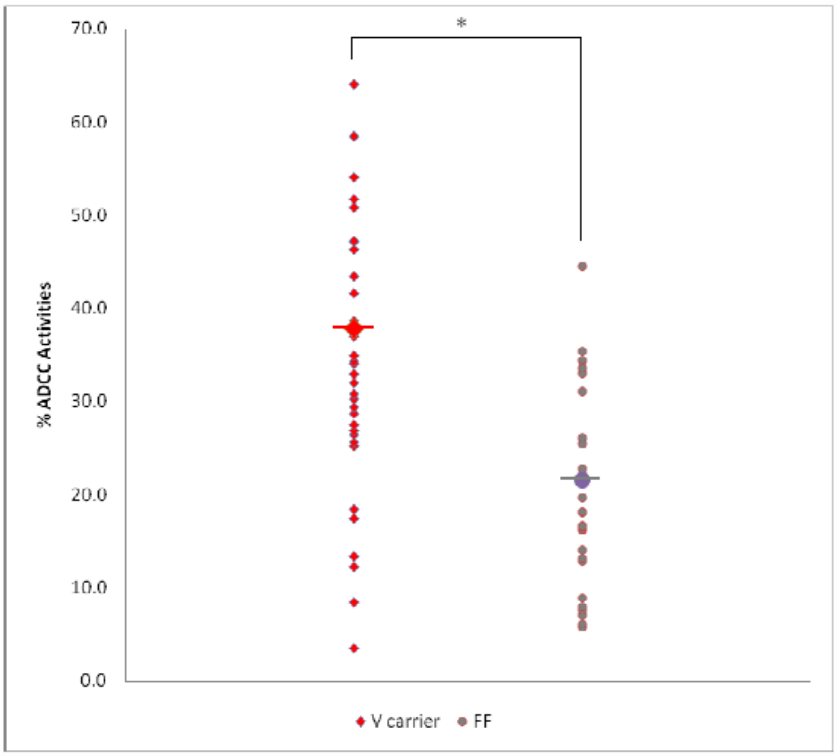

Figure 1: Correlation of the FcγRIIIa genotype to rituximab-induced ADC activity of NKc ells in PBMCs from 60 healthy Thai male volunteers. Data are presented as individual dot plot and the mean rank values of both polymorphisms.

Discussion

Rituximab is used for treatment of various B cell lymphomas. It has been demonstrated that this anti-CD20 antibody induces lymphoma cell death *in vitro* by ADCC, complement-mediated cytotoxicity or apoptosis (2). ADCC is an important rituximab cytotoxic mechanism against tumor cells. The implication of FcγRIIIa in the anti-tumor

effects of rituximab against human lymphoma cell lines has been demonstrated in murine models. Dimorphism of this antibody receptor at amino acid position 158 (V or F) has an in fluence on IgG binding and ADCC. Human IgG_1 binds more strongly to homozygous FcγRIIIa-158V NK cells than to homozygous FcγRIIIa-158F NK cells (5). It has been also reported that NHL patients with FcγRIIIa –FF have lower clinical response than patients with V homozygous (6). We indentified the distribution of FcγRIIIa polymorphisms in Thai population. Our results demonstrated that the percentage of FF homozygous in Thai populationis lower than Asian Population (68%) and Europes or Africa population (58%) (4, 5). The association between the FcγRIIIa genotype and the response to rituximab in Thai patients with NHL is ongoing. Because this polymorphism depends on race and ethinicity, these results may provide useful information to understand beneficial response of rituximab as well as other IgG_1 therapeutic antibody in Thai patients.

Acknowledgements

This study was supported by a grant from the Graduate School, Chulalongkorn University.

References

1. Glennie JM, French RR, Cragg MS, Taylor RP. Mechanism of killing by anti-CD20 monoclonal antibodies. Molecular immunology 2007; 44: 3823-37.
2. Cartron G, Dacheux L, Salles G, Solal-Celigny P, Bardos P, Colombat P, Watier H. Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcγRIIIa gene. Blood 2002; 99: 754-8.
3. Ravetch J and Perussia B. Alternative membrane forms of FcγRIII(CD16) on human natural killer cells and neutrophils cell type-specific expression of two genes that differ in single nucleotide substitutions. Journal of Experimental Medicine 1989; 170: 481-97.
4. Karassa FB, Trikalinos TA, Ioannidis PA. FcγRIIIA-SLE Meta-Amalysis Investigators. The FcγRIIIA-F158 allele is a risk factor for the development of lupus nephritis: a meta-analysis. Kidney Int 2003; 63: 1475–82.
5. Lehrnbecher T, Foster CB, Zhu S, Leitman SF, Goldin LR, Huppi K et al. Variant genotypes of the low-affinity Fcgamma receptors in two control populations and a review of low-affinity Fcgamma receptor polymorphisms in control and disease populations. Blood 1999; 94: 4220-32.
6. Weng WK and Levy R. Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. Journal of Clinical Oncology 2003; 21: 3940-7.