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Hyper-Ag-specific Ab Production in NC/Nga Mice is not associated with Deletion Polymorphism in the Promotor Region of the FcγRIIB Gene

Tohru Sakai, Mariko Nakamoto and Emi Suto
Department of Public Health and Applied Nutrition, Institution of Health Bioscience,
University of Tokushima Graduate School, Tokushima, Tokushima 770-8503, Japan

Abstract: Problem statement: NC/Nga (NC) mice produced high levels of ovalbumin (OVA)-specific IgG, IgG1 and IgG2a. We previously found deletion polymorphisms in the promoter region of fcgr2b in NC mice. To determine whether this mutation causes a hyper-humoral immune response, we generated congenic BALB/c mice carrying the NC-type fcgr2b allele (NC fcgr2b) and analyzed humoral immune response and FcγRIIB on germinal center (GC) B cells. **Approach:** BALB/c, NC and BALB/c- NC fcgr2b congenic mice were immunized with OVA 2 times at a 2-week-interval. Levels of OVA-specific IgG, IgG1 and IgG2a in serum were determined by ELISA. Four-color (anti-B220, anti-IgD, 2.4G2 and PNA) flow cytometry analysis was performed on splenocytes obtained from OVA-immunized mice and levels of FcγRIIB in GC (IgD-PNA^{high}) and non-GC (IgD+PNA^{low}) B cells were analyzed. **Results:** Although perturbed up-regulation of FcγRIIB on GC B was observed in congenic mice, levels of OVA-specific Abs were comparable to those in BALB/c mice. **Conclusion:** NC fcgr2b affects the level of FcγRIIB in GC B cells but that the reduced FcγRIIB expression is not related to enhanced Ag-specific Ab responses in NC mice.

Key words: NC/Nga mice, FcγRIIB, antibody, congenic mice

INTRODUCTION

FcγRIIB, a low-affinity FcR for IgG, acts as a negative feedback regulator by inhibiting B Cell Receptor (BCR)-mediated activation signal through an immunotyrosine-based inhibition motif when these two receptors are co-cross-linked by Ags and IgG-containing immune complex[1-3]. NC/Nga (NC) mice have been shown to develop human atopic-like skin lesions with elevated serum IgE level when kept in conventional conditions[4,5]. In the course of investigating these mechanisms, we found that NC mice produced a higher level of Ag-specifc IgG2a than did BALB/c mice and that NC mice have three deletion sites in regulatory regions of the FcγRIIB gene, two in the promoter region and one in the third intron[6]. We investigated the role of the NC-type fcgr2b allele (NC fcgr2b) in humoral immune response in (BALB/c x NC) x BALB/c or (BALB/c x NC) x NC backcross mice. Results of analysis showed that hyper Ag-specific IgG2a is not controlled by NC fcgr2b[6]. However, the role of NC fcgr2b in Ab response is not fully understood. Establishment of congenic mice for NC fcgr2b made it feasible to examine the *in vivo* effect of fcgr2b allele polymorphism. In this study, we examined the effect of NC fcgr2b on Ag-specific Ab response in a congenic mouse strain.

MATERIALS AND METHODS

Mice: Specific pathogen-free female NC and BALB/c mice were purchased from SLC (Hamamatsu, Japan). Congenic BALB/c mice carrying NC fcgr2b were generated by backcrossing ten times (BALB/c × NC)F1 mice × BALB/c mice. Genotyping for NC fcgr2b was done using anti-Ly 17.2 mAb. Anti-Ly 17.2 mAb reacts with BALB/c-type FcγRIIB but not with that of NC. Backcross mice showing reduced levels of Ly 17.2 staining were judged as being positive for NC fcgr2b.

Immunization and flow cytometric analysis: Mice were intraperitoneally immunized with 10 μg of Ovalubumin (OVA) (Sigma Chemical Co., MO, USA) absorbed in 1 mg of Aluminium Hydroxide Gel Adjuvant (HCI Biosector, Denmark) 2 times at a 2-week interval. Serum OVA-specific Ab levels were measured by the standard method. Spleen cells from OVA-immunized mice were stained FITC-anti-IgD

Corresponding Author: Tohru Sakai, Department of Public Health and Applied Nutrition, Institution of Health Bioscience, University of Tokushima Graduate School, Tokushima, Tokushima 770-8503, Japan
Tel: +81-88-633-7096 Fax: +81-88-633-9427

mAb, PE-anti-FcγRIIB/FcgRII mAb (clone 2.4G2), APC-anti-B220 mAb and biotin-peanut lectin (aggutinin) (PNA), followed by PerCP-streptavidin. Stained cells were analyzed by FACSCalibur and CellQuest software (BD Biosciences, Mountain View, CA).

Statistical analysis: Statistical analysis was done by one-way ANOVA followed by Sheffe's multiple comparison test. Values in the text are means ± SD. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

NC mice produced significantly higher levels of Ag-specific IgG, IgG1 and IgG2a Ab than did BLAB/c mice when immunized with OVA. Quantitative analysis using serially diluted serum revealed that the amounts of Ag-specific IgG2a Ab in NC/Nga mice were ⁓100-times greater than those in BALB/c mice (data not shown). In congenic mice carrying NC fcgr2b, levels of OVA-specific Ab response were comparable to those in BALB/c mice (Fig. 1). These results indicate that NC fcgr2b does not cause hyper IgG production and that genetic factors other than NC fcgr2b contribute to the hyper Ag-specific Ab response in NC mice.

The Germinal Center (GC) is a microenvironment formed in the follicles of secondary lymphoid organs by highly proliferative B cells responding to T cell-dependent Ags. It has been shown that FcγRIIB on GC B cell regulates Ab-Forming Cells (AFC)[7,8]. BCR and FcγRIIB are cross-linked when GC B cells interact with Ag, resulting in inhibition of AFC differentiation[9]. We evaluated the expression of FcγRIIB in GC B cells because down-regulation of FcγRIIB in the GC has been reported in NZB mice, which have deletion mutation of fcg2b similar to that in NC mice[10-12]. Figure 2 shows FcγRIIB expression levels in GC (PNA^{high} IgD^{-}) and non-GC (PNA^{low} IgD^{+}) B cells in BALB/c and congenic mice at the time of primary immune response by OVA immunization. Expression of FcγRIIB was up-regulated in GC B cells compared to that in non-GC B cells in BALB/c mice, whereas expression of FcγRIIB in congenic mouse GC B cells was two-fold lower than that in BALB/c mice. Therefore, NC fcgr2b affects the level of FcγRIIB expression in GC B cells but does not affect Ag-specific Ab levels. Previous studies showed that expression of FcγRIIB in GC B cells is down-regulated in NZB mice[11,12]. However, a recent study by Rahman showed that expression of FcγRIIB in GC B cells is unchanged in autoimmune-prone mice by using multiple GC B cell maker Ags[13]. Our results showing increased expression of FcγRIIB in GC B cells are consistent with Rahman's report.

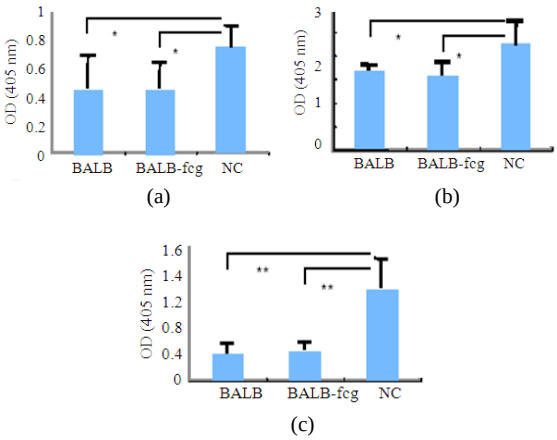

Fig. 1: OVA-specific Ab responses in BALB/c, NC/Nga and NC fcgr2b congenic BALB/c mice. Mice were immunized with OVA 2 times at a 2 week-interval. Levels of OVA-specific IgG; (a): IgG;1 (b): and IgG2a; (c): In serum were determined by ELISA. Data are presented as means ± SD. *$p<0.05$. **$p<0.01$[6]

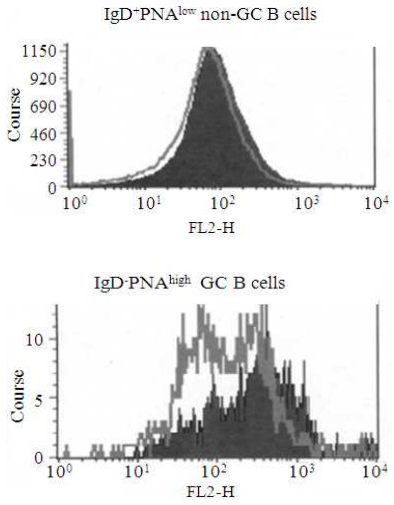

Fig. 2: Levels of FcγRIIB in GC (IgD^{-} PNA^{high}) and non-GC (IgD^{+} PNA^{low}) B cells in BALB/c and NC fcgr2b congenic BALB/c mice. Four-color (anti-B220, anti-IgD, 2.4G2 and PNA) flow cytometry analysis was performed on splenocytes obtained from OVA-immunized each mice on day 10 of primary immune response. Closed histograms indicate the profile for BALB/c mice. Open histograms indicate the profile for NC fcgr2b congenic BALB/c mice. The data are representative of two independent experiments of pooled samples from two to three mice[6]

Xiu examined the effect of NZB-type deletion polymorphism on transcriptional regulation of the fcgr2b gene and showed defective transcription activity in an NZB-derived segment due to absence of transcription by AP4, which binds to the polymorphic 13-nucleotide deletion site [12]. Since NC fcgr2b has the same mutation, this mutation might affect induction of FcγRIIB level. The finding that NC fcgr2b does not induce hyper Ab production is unexpected because NZB-type fcgr2b congenic B6 mice show a stronger anti-KLH Ab response than do B6 mice[12]. Polymorphisms in putative regulatory regions of the FcγRIIB gene in NZB and NC mice are identical. There are several possible reasons for the different results. First, genetic background might influence the effect of NC fcgr2b on Ab response since BALB/c mice are known to be a mouse strain that induce Th2 response and show strong Ab response compare to B6 mice[14,15]. FcγRIIB-deficient mice develop autoantibodies and glomerulonephritis with a pathology resembling that of human lupus on B6 background. The same mutation on the BALB/c background does not lead to spontaneous disease, suggesting that the effect of FcγRIIB on susceptibility to the disease is different in BALB/c and B6 strains[16]. Several candidate genes for regulating autoantibody production proximal to fcgr2b have been reported[17-19]. Thereby, contribution of these genes on Ag-specific Ab production is not excluded at present stage.

CONCLUSION

The present study showed that NC fcgr2b affects the level of FcγRIIB in GC B cells but that the reduced FcγRIIB expression is not related to enhanced Ag-specific Ab responses in NC mice. In addition, the results showed the contribution of the deletion mutation in the promoter region of fcgr2b to the humoral immune response might be limited.

REFERENCES

1. Amigorena, S., C. Bonnerot, J.R. Drake, D. Choquet and W. Hunziker *et al*., 1992. Cytoplasmic domain heterogeneity and functions of IgG Fc receptors in B lymphocytes. Science, 256: 1808-1812. http://cat.inist.fr/?aModele=afficheN&cpsidt=5433478
2. Bolland, S. and J.V. Revetch, 1999. Inhibitory pathways triggered by ITIM-containg receptors. Adv. Immunol., 72: 149-177. http://www.ncbi.nlm.nih.gov/pubmed/10361574
3. Takai, T., 2002. Roles of Fc receptors in autoimmunity. Nat. Rev. Immunol., 2: 580-592. http://www.ncbi.nlm.nih.gov/pubmed/12154377
4. Matsuda, H., N. Watanabe, G.P. Geba, J. Sperl and M. Tsudzuki *et al*., 1997. Development of atopic dermatitis-like skin lesion with hyperproduction in NC/Nga mice. Int. Immunol., 9: 461-466. http://www.ncbi.nlm.nih.gov/pubmed/9088984
5. Matsumoto, M., C. Ra, K. Kawamoto, H. Sato and A. Itakura *et al*., 1999. IgE hyperproduction through enhanced tyrosine phosphorylation of Janus kinase 3 in NC/Nga mice, a model for human atopic dermatitis. J. Immunol., 162: 1056-1063. http://cat.inist.fr/?aModele=afficheN&cpsidt=9913321
6. Sakai, T., M. Kogiso, K. Mitsuya, T. Komatsu and S. Yamamoto, 2006. Deletion polymorphisms in the promotor region of Fcg receptor IIB is not associated with antigen-specific IgG2a and IgG2b antibody responses in NC/Nga Mice. Microbiol. Immunol., 50: 475-480. http://cat.inist.fr/?aModele=afficheN&cpsidt=17952925
7. Berek, C., A. Berger and M. Apel, 1991. Maturation of the immune response in germinal centers. Cell, 90: 293-301. http://www.ncbi.nlm.nih.gov/pubmed/1760840
8. Jacob, J., G. Kelsoe, K. Rajewsky and U. Weiss, 1991. Intraclonal generation of antibody mutants in germinal centers. Nature, 354: 389-392. http://cat.inist.fr/?aModele=afficheN&cpsidt=5174446
9. Tarlinton, D.M. and K.G. Smith, 2000. Dissecting affinity maturation: A model explaining selection of antibody-forming cells and memory B cells in the germinal center. Immunol. Today, 21: 436-441. http://www.ncbi.nlm.nih.gov/pubmed/11738998
10. Luan, J.J., R.C. Monteiro, C. Sautes, G. Fluteau and L. Eloy *et al*., 1996. Defective FcγRII gene expression in macrophages of NOD mice. J. Immunol., 157: 4707-4716. http://www.jimmunol.org/cgi/content/abstract/157/10/4707
11. Jiang, Y., S. Hirose, M. Abe, R. Sanokawa-Akakura and M. Ohtsuji *et al*., 2000. Polymorphisms in IgG Fc receptor IIB regulatory regions associated with autoimmune susceptibility. Immunogenetics, 51: 429-435. http://www.ncbi.nlm.nih.gov/pubmed/10866109
12. Xiu, Y., K. Nakamura, M. Abe, N. Li and X.S. Wen *et al*., 2002. Transcriptional regulation of Fcgr2b by polymorphic promoter region and its contribution to humoral immune responses. J. Immunol., 169: 4340-4346. http://www.ncbi.nlm.nih.gov/pubmed/12370366

13. Rahman, Z.S.M. and T. Manser, 2005. Failed up-regulation of the inhibitory IgG Fc receptor FcγRIIB on germinal center B cells in autoimmune-prone mice is not associated with deletion polymorphisms in the promoter region of the FcγRIIB gene. J. Immunol., 175: 1440-1449. http://www.ncbi.nlm.nih.gov/pubmed/16034080
14. Reiner, S.L. and R.M. Locksley, 1995. The regulation of immunity to Leishmania major. Annu. Rev. Immunol., 12: 151-177. http://www.ncbi.nlm.nih.gov/pubmed/7612219
15. Sher, A. and R.L. Coffman, 1992. Regulation of immunity to parasites by T cells and T cell-derived cytokines. Annu. Rev. Immunol., 10: 385-409. http://www.ncbi.nlm.nih.gov/pubmed/1590992
16. Terasenko, T., H.K. Kole and S. Bolland, 2008. A lupus-suppressor BALB/c locus restricts IgG2 autoantibodies without altering intrinsic B cell-tolerance mechanisms. J. Immunol., 180: 3807-3814. http://www.ncbi.nlm.nih.gov/pubmed/18322187
17. Boackle, S.A., V.M. Holers, X. Chen, G. Szakonyi, D.R. Karp, E.K. Wakeland and L. Morel, 2001. Cr2, a candidate gene in the murine Sle1c lupus susceptibility locus, encodes a dysfunctional protein. Immunity, 15: 775-785. http://cat.inist.fr/?aModele=afficheN&cpsidt=13380515
18. Morel, L., K.R. Blenman, B.P. Croker and E.K. Wakeland, 2001. The major murine systemic lupus erythematosus susceptibility locus, Sle1, is a cluster of functionally related gene. Proc. Natl. Acad. Sci. USA., 98: 1787-1792. http://adsabs.harvard.edu/abs/2001PNAS...98.1787M
19. Rozzo, S., J.J.D. Allard, D. Choubey, T.J. Vyse, S. Izui, G. Peltz and B.L. Kotzin, 2001. Evidence for an interferon-inducible gene, ifi202, in the susceptibility to systemic lupus erythematosus. Immunity, 15: 435-443. http://www.ncbi.nlm.nih.gov/pubmed/11567633