A Protein in the Cell Wall of *Helicobacter Pylori* Binds Human IgG Deprived of Sugar Chains

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MASUDA, Y. and SUGIYAMA, T. *A Protein in the Cell Wall of Helicobacter Pylori Binds Human IgG Deprived of Sugar Chains*. Tohoku J. Exp. Med., 2001, **194** (1), 65-69 — We found a protein binding non-antibody human IgG in *Helicobacter pylori* cell wall, when the IgG was enzymically deglycosylated. The protein had the amino acid sequence AEDGAHVAFM. IgG of *H. pylori* patients also bound to the protein and the IgG lost the sugar chain structure reactivities. These findings indicate that *H. pylori* binds human IgG without the sugar chains on its cell wall and suggest that *H. pylori* infection has a relation to the human IgG deglycosylation. ——— *Helicobacter pylori*; human IgG; glycosylation; a protein of the cell wall

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It is well known that *Staphylococcus aureus* and *Escherichia coli*, common bacteria living in human body, have protein A and protom K to bind human IgG on the cell wall (Stalenheim et al. 1973; Paakkanen et al. 1979). *Helicobacter pylori* (*Hp*) is also found in the patient gastric mucosal layer. *Hp* may also bind non-antibody IgG on the cell wall. Although Fc-binding protein like as protein A and protein K has not been known in *Hp*, sugar chains of glycoproteins have a relation with cell-adhesion (Liubimov and Bannikov 1978) and human IgG is an N-glycosylated glycoprotein. In this paper, sugar chains of human IgG are investigated in aspect of binding to *Hp* cell wall.

MATERIALS AND METHODS

Preparation of Hp cell wall

Cultured *Hp* was given from Animal Facilities for Experimental Medicine, Akita University School of Medicine. The *Hp* was packed with centrifugation of $3000 \times g$ for 10 minutes at room temperature (RT) and was three times washed with physiological saline (PS). The washed *Hp* was sustained in water and left for 12 hours at RT. The suspension of *Hp* was centrifuged at $10\,000 \times g$ for one hour at RT and the supernatant was obtained.

The fragments of the cell wall in the supernatant was separated to the proteins and the lipids using chloroform and methanol as previously described (Masuda and Sugiyama 2000).

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The proteins were also fractioned as previously described (Masuda et al. 2000). Briefly, the protein solution was desalted using PD-10 column (Pharmacia Co., Uppsala, Sweden). The desalted solution was applied on an ion-exchanger DE-52 (Whatman Co., Maidstone, UK) previously saturated with 10 mM NaHCO₃, pH 8.3, and eluted with the buffer, 50, 100, 150, 200, 250 and 300 mM NaCl. These were, furthermore, separated to above 50, 50–30, 30–10, 10–3 and below 3 kDa with the use of an ultra-filtration membrane (Centricon, Amicon Co., Tokyo). Chloroform of the lipid fraction was evaporated and the remaining glycolipids were re-solved in water.

IgG preparations

Serum was obtained from five healthy male volunteers (Age 23–43) who had not suffered from *Hp*, and from five patients suffering from *Hp* (one female and 4 male, age 32–68). The infection or non-infection with *Hp* was confirmed by a C¹³-labeled urea breath test, which is a harmless and highly precise methods for detecting *Hp* infections (Megraud 1995).

The IgG was purified from serum with the use of an Econo-Pac protein A kit (Bio-Rad Co., Richmond, LA, USA). A hundred mg of the healthy volunteers IgG was prepared in the 100 μ l of 50 mM citrate-phosphate buffer, pH 5.0, and was incubated with 1 micro unit of Aminoglycosidase-A (EC 3, 5, 1, 52, Seikagaku Co., Tokyo), which cuts asparagine-sugar chain connection of N-glycosylated protein, for 16 hours. The treated IgG was prepared with PS, refined with the use of the Econo-Pac protein A kit and diluted to 50 μ g/ml with PS. The deglycosylation was detected in the lectin-ELISA, using three different biotinized lectins recognizing the sugar chain structure (DSA; Gal β 1-4GlcNAc, Lotus; Fuc α 1-2Gal, SSA; Sial α 2-6Gal) and peroxidase-conjugated avidin (Hohnen Co., Tokyo), as previously described (Masuda and Hishikawa 1993). Briefly, the intact IgG was also prepared to 50 μ g/ml with

PS. A hundred μ l of these intact and enzymically treated IgG solutions were poured into a well of a 96 holes plastic plate (Sumitomo-Bakelite Co., Tokyo). Two hours after this, the wall was three times washed with washing solution (WS; PS containing 0.005% tween 20; Seikagaku Co.). After 30 minutes blocking with 5% bovine serum albumin (BSA; Sigma Co., St. Louis, MO, USA), 100 μ l of the biotinized lectins prepared to 2 μ g/ml were poured into the well. One hour after the incubation at RT, the well was three times washed with WS. A hundred μ l of peroxidase-conjugated avidine (Seikagaku Co.) prepared to 0.1 μ g/ml with PS was poured into the well. Fifteen minutes after the incubation at RT, the well was 4 times washed with PS. Coloring of the wall was performed with the coloring kit (Sumitomo-Bakelite Co.) and the optical absorbance was measured at the dual wavelength 455 and 650 nm.

Fraction of Hp cell wall to bind the IgG

The separated *Hp* cell wall protein solutions were also prepared to 1 μ g/ml with PS. A hundred μ l of these solutions was poured into a well of a 96 holes plastic plate (Sumitomo-Bakelite Co.). The glycolipid solution was prepared to 50% ethanol solution and was poured into a well of the plate (Masuda and Sugiyama 2000). Two hours after the incubation at RT, the well was three times washed with WS. Thirty minutes after the blocking with 5% BSA, 100 μ l of the *Hp* patients IgG, the intact volunteers IgG or the deglycosylated volunteers IgG solution, which was prepared to 50 μ g/ml with PS, was poured into the well. One hour after the incubation at RT, the wall was three times washed with WS, and the ELISA was performed with peroxidase-conjugated anti-human IgG rat IgG (Kokusai Co., Kobe) and the coloring kit.

Reactivities of sugar chain structures of the IgG binding the Hp protein fraction

The *Hp* cell wall protein fraction eluted 100–150 mM NaCl and divided to 50 kDa above was prepared to 1 μ g/ml with PS. The solution was poured into a well of a 96 holes plastic plate. Two hours after the incubation at RT, the well was three times washed with WS. A hundred μ l of the *Hp* patients IgG solution or the deglycosylated volunteers IgG solution was poured into the well. The successive lectin-ELISA was performed as described above.

Detection of the Hp cell wall protein

Amino acid sequence of the *Hp* cell wall protein described above was detected with a protein sequencer (Parkin-Elmer Co., Tokyo). The sequence was analyzed with the DBGET integrated database retrieval system Genome Net.

Statistical analysis

Mann-Whitney U-test was used.

RESULTS

The volunteers IgG was successfully deglycosylated using the enzyme (Table 1 upper).

The *Hp* patients IgG and the deglycosylated volunteers IgG bound to the *Hp* cell wall protein fraction eluted with 100–150 NaCl and divided to 50 kDa above, but the intact volunteers IgG did not (the optical absorbance in the protein fraction; the *Hp* patients IgG 0.275 ± 0.052 , the deglycosylated volunteers IgG 0.234 ± 0.041 , the intact volunteers IgG 0.038 ± 0.011 , the mean $\pm$ s.e. in the five preparations). These IgGs did not bind to the other protein fractions and the glycolipid fraction.

The *Hp* patients IgG and the deglycosylated volunteers IgG binding to the protein fraction lost the reactivities of the sugar chain structures (Table 1, lower).

The *Hp* cell wall protein in the fraction had the amino acid sequence AEPGAHVAFM. This sequence had 60% similarity with leukocyte relating IgG-like receptor 2, but was not completely identified.

TABLE 1. *Reactivities of the sugar chain structures in the IgG*

The volunteers IgG

	Sugar chain structure		
	Gal β 1-4GlcNAc	Fuc α 1-2Gal	Sial α 2-6Gal
Intact IgG	0.264 ± 0.011	0.078 ± 0.010	0.054 ± 0.008
Enzymically treated IgG	$0.047 \pm 0.010^*$	$0.030 \pm 0.008^*$	$0.021 \pm 0.011^*$
(Control) no IgG	0.028	0.021	0.010

IgG binding to the Hp protein fraction

	Sugar chain structure		
	Gal β 1-4GlcNAc	Fuc α 1-2Gal	Sial α 2-6Gal
The deglycosylated volunteers IgG	0.042 ± 0.009	0.022 ± 0.009	0.019 ± 0.008
The <i>Hp</i> patients IgG	0.045 ± 0.010	0.026 ± 0.018	0.022 ± 0.009
(Control) no IgG	0.040	0.025	0.021

Value indicates the mean $\pm$ s.e. of optical density (455 and 650 nm).

* $p < 0.01$ (Mann-Whitney U-test) compared to the intact volunteers IgG.

Hp, *Helicobacter pylori*.

DISCUSSION

The present findings in this study clearly demonstrate that *Hp* has a cell wall protein binding human IgG and the IgG loses the sugar chain structures.

Casein is a hydrophobic protein and it binds alkali-denaturated IgG (Whatley et al. 1983). As alkali-deraturation removes sugar chains from IgG, this and the present findings may suggest that the protein in *Hp* cell wall is hydrophobic, as well as casein, and it binds hydrophobic IgG which loses the hydrophilic sugar chains.

In this study, the *Hp* patients IgG binding the protein also lost the sugar chains. This strongly suggests that *Hp* infection induce the antibody IgG-deglycosylation. Cutler and Prasad (1995) reported that a large number of *Hp* patients show the high titer of anti-*Hp* IgG in the ELISA method even over two year after the successful *Hp* exception. This may suggest that *Hp* infection strongly induces the IgG-deglycosylation. Though chonic inflammatory diseases induce abnormal glycosylation of IgG (Adebajo and Hazelman 1995), the deglycosylation mechanism of *Hp* infection has not been clarified in the present time.

When non-antibody IgG binds to the protein with the deglycosylated Fc portion, *Hp* can avoid the bind of the antibody IgG, as well as *S. aureus* and *E. coli*. As it was reported that some bacilli in the human body bind the immunoglobulins on their cell walls (Forsgen and Grubb 1979), this phenomenon may be not rare in bacilli that live in the human body. The immunoglobulin-bind on the cell wall will be considered an adaptation of the bacilli in the human body (Masuda 1999), but the other functions of the *Hp* cell wall protein are unclear in the present time.

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References

- Adebajo, A.O. & Hazelman, B.C. (1955) IgG glycosylation is associated with tropical infections and rheumatoid arthritis in the tropics. *Clin. Exp. Rheumatol.*, **13**, 737-740.
- Cutler, A.F. & Prasad, V.M. (1996) Long-term follow-up of *Helicobacter pylori* serology after successful eradication. *Am. J. Gastroenterol.*, **1**, 85-88.
- Forsgen, A. & Grubb, A.O. (1979) Many bacterial species bind human IgD. *J. Immunol.*, **122**, 1468-1472.
- Liubimov, A.V. & Bannikov, G.A. (1978) Effect of substances that disrupt cell adhesion to the substrate on the glycoprotein spectrum of normal fibroblasts in culture. *Tsitologiya*, **20**, 1052-1059.
- Masuda, Y. & Hishikawa, Y. (1993) Sugar constituent of IgG changes in different immune responses. *Med. Sci. Res.*, **21**, 395-395.
- Masuda, Y. (1999) Mechanism of behavioral adaptation: analyses by a simple model. *Med. Hypotheses*, **52**, 115-117.
- Masuda, Y. & Sugiyama, T. (2000) The effect of globopentaosylceramide on a depression model, mouse forced swimming. *Tohoku J. Exp. Med.*, **191**, 47-54.
- Masuda, Y., Kawarada, Y. & Sugiyama, T. (2000) Behavioral effect of mouse fibrinopeptide A on mouse forced swimming. *Peptides*, **21**, 459-461.
- Megraud, F. (1995) Diagnosis of *Helicobacter pylori*. *Baillieres Clin. Gastroenterol.*, **9**, 507-518.
- Paakkanen, J., Gotschlich, E.C. & Makela, P.H. (1979) Protein K: a new major outer membrane protein found in encapsulated *Escherichia coli*. *J. Bacteriol.*, **139**, 835-841.
- Stalenheim, G, Gotze, O., Cooper, N.R., Sjoquist, J. & Muller-Eberhard, H.J. (1973) Consumption of human complement components by complexes of IgG with protein A of *Staphylococcus aureus*. *Immunochemistry*, **10**, 501-507.
- Whaley, K., Lappin, D., Macphaden, A.R., Riches, D. W., & Sandilands, G.P. (1983) A comparison

of the effects of heat-aggregated and chemically cross-linked IgG on monocytes C₂ pro-

duction. *Immunology*, **49**, 457-461.