LACK OF CROSS-REACTION OF ANTIBODIES AGAINST CELL-ASSOCIATED GLUCOSYLTRANSFERASE FROM *STREPTOCOCCUS MUTANS* WITH HUMAN HEART TISSUE

Takashi Iwase,[1] Jun-ichiro Hiraoka,[2] Toshio Horikoshi,[2] Shigeyuki Hamada,[3] and Itaru Moro[1]

[1]Department of Pathology, Nihon University School of Dentistry, Tokyo, Japan; [2]Cosmetics Laboratory, Kanebo, Ltd., Odawara, Japan; [3]Department of Oral Microbiology, Osaka University Faculty of Dentistry, Osaka, Japan

INTRODUCTION

Production of glucan from sucrose by glucosyltransferases (GTases) is essential for adherence of *Streptococcus mutans* to the tooth surface, and may lead to the development of dental caries. We recently isolated and characterized cell-associated (CA)-GTase of *S. mutans* MT8148 (serotype *c*) that synthesizes insoluble glucan from sucrose.[1] Furthermore, we have found that oral passive immunization with the egg yolk antibody specific for CA-GTase resulted in a reduced severity of dental caries in *S. mutans*-infected rats.[2]

It is well known that strains of *S. pyogenes*, the causative agent of rheumatic heart disease, possess antigens that cross-react with heart tissue.[3] Often reports[4,5] indicated that rabbit antisera against *S. mutans* whole cells may cross-react with human and monkey heart tissue. However, different findings of immunofluorescence stainings were reported by various investigators[6, 7] and this cross-reactivity induced by *S. mutans*-related antigens has not been elucidated.

In this study, we investigated the ability of yolk antibody against CA-GTase to cross-react with human heart tissue by ELISA and immunohistochemical method.

MATERIALS AND METHODS

Preparation of Antigens

CA-GTase was isolated from whole cells of *S. mutans* MT8148 (serotype *c*) as described previously.[1] *S. pyogenes* were grown in tryptic soy broth medium for 18 h at 37°C and killed by 0.075% (wt/vol) formaldehyde. Human heart tissue was obtained at autopsy from an 81-year-old individual without heart disease. Small pieces of the heart tissue were homogenized in 4 volumes of 0.01M phosphate-buffered saline pH 7.4 (PBS) containing 1mM phenyl-methylsulfonyl fluoride at 0 °C, filtered though cotton gauze, and sonicated for 1 min at 0°C.

Advances in Mucosal Immunology, Edited by
J. Mestecky *et al.*, Plenum Press, New York, 1995

Yolk Antibodies

White Leghorn hens (18 weeks old) were immunized intramuscularly with the above antigens emulsified in Freund's complete adjuvant (Difco Laboratories, Detroit, Mich.). Booster injections were given 3 times at interval of 2 weeks with Freund's incomplete adjuvant (Difco). Yolk antibodies were purified from egg yolk by the modified method of Aulisio and Shelokov.[8] Briefly, egg yolks were separated from eggs of immunized or sham-immunized hens, delipidated by chloroform, and the water-soluble fraction containing IgG was obtained. The IgG fractions were further purified by precipitation with 40% and 30% saturated $(NH_4)_2SO_4$ to obtain the purified yolk IgG (yIgG) fraction (>90% pure).

The purified yIgGs were conjugated with FITC isomer I (Sigma) for 6 h at 4°C. The FITC-conjugated IgGs were purified by G-25 Superfine (Pharmacia-LKB Biotechology) column to remove the free FITC and DEAE-Sepharose Fast Flow (Pharmacia-LKB Biotechology) to obtain the IgGs having a suitable F/P ratio.

Assays of Yolk Antibodies

Specific activity to the immunizing antigens was estimated by ELISA or agglutination. ELISA titer was measured at a starting concentration of yIgG (10 mg/ml) and defined as the maximum dilution giving $A_{492} \geq 0.2$. Specific activity to *S. pyogenes* was expressed as the minimum agglutinating concentration (MAC) of yIgG in the reaction mixture [*S. pyogenes* whole cells (2mg dry wt/ml) and yIgG]. CA-GTase activity was estimated by measuring the amount of insoluble glucan synthesized from sucrose by CA-GTase preincubated with yIgGs (1 mg/ml), and was expressed relative to the activity in the absence of yIgG.

Human heart homogenates were coated on microplates with 0.25% glutaraldehyde, and the antibody titer to heart tissue antigen was examined by ELISA. ELISA titer was measured as described above.

Immunofluorescence Staining

Nine heart tissues were obtained from individuals who died of non-heart diseases. They were embedded in O.C.T compound (Miles Inc, Elkhart, U.S.A) and snap frozen in dry ice-acetone. Sections were cut and fixed in cold acetone for 10 min.

Indirect Staining. Acetone-fixed frozen sections were incubated with 10% normal rabbit serum for 10 min to block non-specific staining. Sections were incubated with diluted yIgG (100 μg/ml, 75 μg/ml, 50 μg/ml, 25 μg/ml, 10 μg/ml in 1% BSA-PBS) for 1 h. After washing 3 times with PBS for 1 h, sections were incubated with FITC-conjugated rabbit anti-chicken IgG (Zymed laboratories, San Francisco, CA) for 1 h. Sections were washed with PBS for 1 h and then embedded in PBS-glycerine.

Direct Staining. Frozen sections pretreated with 10% normal rabbit serum were incubated with diluted FITC-conjugated yIgG (1000 μg/ml, 100 μg/ml, 75 μg/ml, 50 μg/ml, 25 μg/ml, 10 μg/ml in 1% BSA-PBS) for 1 h. Sections were washed with PBS for 1 h and then embedded in PBS-glycerine.

The sections stained by indirect and direct immunofluorescent histochemical methods were observed with a Nikon fluorescence microscope.

The Characteristics of Yolk Antibodies Against the Homologous Antigen

The characteristics of these antibodies are summarized in Table 1. yIgG to CA-GTase bound strongly to CA-GTase (ELISA titer 12.5 x10 ^{4}), but not other *S. mutans*-related antigen, e.g., PAc, cell-free GTase (data not shown), and it markedly inhibited CA-GTase activity. In addition, this antibody inhibited the adherence of cells of S. mutans to the glass surface in the presence of sucrose.[2] yIgG to *S. pyogenes* M4 and M6 reacted strongly with the homologous antigen, respectively.

ELISA Titer to Human Heart Tissue Homogenate

yIgG to CA-GTase did not react with human heart tissue homogenate (hHTH) (Table 1). yIgG to *S. pyogenes* M4 and M6 did not react with hHTH either. In contrast, yIgG to hHTH bound strongly to the hHTH.

Table 1. Profile of yolk antibodies.

yIgG to	Specific activity to the immunized Ag		ELISA titer to heart tissue	CA-GTase activity
	ELISA	MAC		(%)
CA-GTase	12.5×10^4	N.T.*	$<1.0 \times 10^3$	11.0
S. pyogenes M4 whole cells	N.T.*	0.020	$<1.0 \times 10^3$	N.T.*
S. pyogenes M6 whole cells	N.T.*	0.156	$<1.0 \times 10^3$	N.T.*
human heart homogenate	3.1×10^4	N.T.*	31.1×10^3	N.T.*
sham-immunized	N.D.**	N.T.*	$<1.0 \times 10^3$	131.0

*N.T.: Not Tested., **N.D.: Not Detected.

Table 2. Indirect immunofluorescence staining.

yIgG to	yIgG concentration (μg/ml)				
	100	75	50	25	10
CA-GTase	–	–	–	–	–
S. pyogenes M4 whole cells	+	+	+	+	+
S. pyogenes M6 whole cells	+	+	+	+	+
human heart homogenate	+	+	+	+	+
sham-immunized	–	–	–	–	–

+ denotes positive staining in sarcolemmal sheath of all sections (nine cases).
– denotes negative staining.

Indirect Immunofluorescence Staining

The results of indirect staining in all sections are summarized in Table 2. No specific fluorescence was found when sham-immunized yIgG was incubated with sections of all

nine human heart tissue specimens indicating that sham-immunized yIgG did not bind to heart tissue. yIgG to CA-GTase did not bind to any heart tissue speciman nor did sham-immune yIgG. In contrast, yIgG to hHTH bound to all heart tissue samples. These fluorescent staining reactions concentrated at the sarcolemmal sheath and cytoplasm of cardiac muscle, indicating that yIgG to hHTH has a selective affinity for these antigens. In addition, yIgG to *S. pyogenes* M4 and M6 cross-reacted with heart tissue in all of nine cases. These results are not in accordance with ELISA data described above, however, the fluorescent staining reactions concentrated at the sarcolemmal sheath and cytoplasm of cardiac muscle were observed in all specimens. yIgG to *S. pyogenes* M4 cross-reacted with sarcolemmal sheath from 100 μg/ml to 10 μg/ml and the intensity of fluorescence was dependent on yIgG concentration, but it did not cross-react with cytoplasm at the 10 μg/ml concentration. The cross-reactions of yIgG to *S. pyogenes* M4 and M6 were similar, but the intensity of fluorescence with yIgG to *S. pyogenes* M4 was stronger than that of yIgG to *S. pyogenes* M6.

Direct Immunofluorescence Staining

Direct immunofluorescent staining was done to remove possible artifacts of secondary antibody. yIgG to CA-GTase and hHTH, and sham-immunized yIgG were conjugated with FITC. The F/P ratio of yIgG to CA-GTase and hHTH, and sham-immunized yIgG was 1.51, 1.00, 1.01, respectively. In addition, ELISA titer to the immunizing antigen was 1.58×10^4, 2.90×10^3, respectively.

The results of direct immunofluorescent staining of all nine specimens are summarized in Table 3. FITC-conjugated yIgG to CA-GTase and sham-immunized yIgG did not bind to heart tissue at any IgG concentration. In contrast, FITC-conjugated yIgG to hHTH bound to sarcolemmal sheath and cytoplasm of cardiac muscle.

Table 3. Direct immunofluorescence staining.

yIgG to	yIgG concentration (μg/ml)					
	1000	100	75	50	25	10
CA-GTase	–	–	–	–	–	–
human heart homogenate	+	+	+	+	+	+
sham-immunized	–	–	–	–	–	–

Footnotes as in Table 2.

CONCLUSIONS

The results from ELISA and immunohistochemical study showed that antibody against CA-GTase from *S. mutans* (serotype *c*) lacks cross-reactivity to human heart tissues and suggest that the use of this antibody for oral passive immunization may not be pathogenic for human heart.

ACKNOWLEDGMENTS

We are grateful to Dr. H. Nagura, Tohoku university, for assistance in obtaining heart tissue, Mr. H. Yokoyama, Ghen Corporation, Gifu, Japan, for preparing yolk IgG to various antigens, and Dr. U. Murakami, Kanebo Ltd, Japan for their encouragement.

REFERENCES

1. S. Hamada, T. Horikoshi, T. Minami, N. Okahashi, and T. Koga, *J. Gen. Microbiol.* 135:335 (1989).
2. S. Hamada, T. Horikoshi, T. Minami, S. Kawabata, J. Hiraoka, T. Fujiwara, and T. Ooshima, *Infect. Immun.* 59:4161 (1991).
3. M. H. Kaplan and M. Meyeserian, *Lancet* 1:706 (1962).
4. I. Van de Rijn, A. S. Bleiweis, and J. B. Zabriskie, *J. Dent. Res.* 55:C59 (1976).
5. M. W. Stinson, R. J. Nisengard, and E. J. Bergey, *Infect. Immun.* 27:604 (1980).
6. M. W. Russell, *J. Oral Pathol.* 16:234 (1987).
7. R. J. Nisengard, M. W. Stinson, and L. Pelonero, *Ann. Acad. Sci.* 420:401 (1983).
8. C. G. Aulisio and A. Shelokov, *Proc. Soc. Exptl. Biol. Med.* 126:312 (1967).