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Periodontitis and Inflammation: Plasma High Titer Naturally Occurring Anti-Glucan Antibodies Form Immune Complex with *Streptococcus mutans* Antigens

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Abstract

Atheromatous plaques usually contain antigens of the periodontitis-causing bacteria *Streptococcus mutans* though molecular mechanism of this incorporation remains unknown. Since vascular adhesion and inflammatory potential of Immune Complexes (IC) are known we investigated the naturally occurring plasma antibodies that recognize major antigens from *S. mutans*. *S. mutans*-binding plasma proteins (SMBP) prepared by affinity chromatography on a column of heat-killed *S. mutans* could recognize *α*- and *β*-linked glucose in dextran and yeast respectively but not galactose in glycoproteins. SMBP contained only three proteins, each corresponding in electrophoretic mobility to standard plasma IgG, IgA or IgM. The major positively and negatively charged protein antigens (PSMAg and NSMAg) isolated from *S. mutans* by electrophoresis and ion exchange chromatography respectively were recognized sugar-reversibly by the anti-*β*-glucan antibody (ABG) and though less avidly, by the dextran-binding immunoglobulin (DIg) in normal plasma. NSMAg addition resulted in near doubling of IC-bound immunoglobulins in immunoglobulin-rich fraction of plasma. IC isolated from above fraction after NSMAg addition had substantially more IgA and IgM content than total plasma immunoglobulins. IC formation by NSMAg was significantly inhibited by ABG- and DIg-specific sugars or by selective withdrawal of ABG or DIg from plasma. ABG and DIg being relatively high titer plasma antibodies IC formation with them suggested a possible route for

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vascular adhesion and damage by *S. mutans* and its antigens. Further, high IgA content of these ICs indicated their susceptibility to tissue uptake through cell surface galectin-1 for which IgA is the lone immunoglobulin ligand.

Keywords

***Streptococcus mutans*, Anti-*β*-Glucan Antibody (ABG), Dextran Binding Immunoglobulins (DIg), Immune Complexes**

1. Introduction

Besides causing dental caries and infective endocarditis [1] the oral bacteria *Streptococcus mutans* contribute to inflammation in cardiovascular diseases [2], diabetes [3] and ulcerative colitis [4]. Since only half of the cardiovascular disease incidence could be explained by classical risk factors including hyperlipidemia, redundant lifestyle and hypertension [5] contribution of pathogen-mediated inflammatory processes that may injure the vascular wall assumed importance. Enhanced synthesis of pro-inflammatory mediators during dental caries and periodontitis supports the above possibility [6]. *Streptococcus mutans* was one of the most abundant bacterial species isolated from extirpated heart valve (69%) and atheromatous plaque samples (74%), while other periodontal bacteria including *Porphyromonas gingivalis* were found at a much lower frequency [2]. However the mechanism of tissue deposition of *S. mutans* remains unclear.

In the present study we demonstrate that naturally occurring anti-carbohydrate antibodies, anti-*β*-glucan antibody (ABG) and dextran binding immunoglobulins (DIg) recognize the cell wall antigens of *S. mutans* to form immune complexes (ICs) in plasma. This offered a possible mechanism for the organism or its antigens to adhere to vascular tissue since complement receptors and Fc*γ* receptor at the cell surface bind to Fc of antigen-bound antibodies in ICs rather than to Fc of free antibodies to produce pro-inflammatory reactions.

2. Materials and Methods

2.1. Materials

Polystyrene 96-well microplates (MAXISORP) were purchased from Nunc, Roskilde, Denmark. Rabbit antibodies to human IgA, IgM and IgG were obtained from Dako, Denmark. All fine chemicals used were purchased from Sigma-Aldrich, Bengaluru, India. Outdated human plasma was obtained from the Department of Blood Transfusion Services of this Institute with Institutional Ethics Committee approval (IEC-511). *Streptococcus mutans* (MTCC-890) was from IMTECH, Chandigarh, India.

2.2. Methods

2.2.1. Preparation of *Streptococcus mutans* Binding Proteins (SMBP) from Plasma

All operations were at 4˚C. Out-dated plasma (40 ml) was dialysed twice against 20 mM potassium phosphate buffer pH 7.4 containing 150 mM NaCl (PBS). After centrifugation at 12,000 rpm for 20 minutes, the plasma was applied to a column of a mixture of Biogel P-4 (25 ml) and *Streptococcus mutans* cells (2 ml) cultured in Brain Heart Infusion Broth. After thorough washing to remove the unbound proteins the bound antibodies were eluted with a mixture of 250 mM dextrose and 150 mM galactose in PBS. The eluted proteins were then dialysed and concentrated using CENTRICON (Millipore) membrane concentrator (10,000 Da cut off).

2.2.2. Specificity of SMBP

Dextran, yeast-*β*-glucan and melibiose-conjugated soybean trypsin inhibitor (TIM) were coated on polystyrene wells by incubating 2 µg of each in 200 µl PBS on polystyrene wells overnight at 4˚C. The wells were washed with PBS containing 0.05% Tween20 (PBST), blocked by incubation with PBS containing 0.5% Tween20 for 30 min and again washed with PBST. After adding SMBP (2 µg in 200 µl PBS) pre-incubated with or without specific sugars (25 mM each of methyl-*α*-D-glucoside, cellobiose or methyl-*α*-D-galactoside for dextran-, yeast *β*-glucan- and TIM-coated wells respectively) wells were incubated for 2 h at 4˚C. Following further washing

with PBST, bound SMBP was probed with a mixture of HRP-conjugated anti-human IgA, IgG and IgM (1.5 µg/ml each in PBST). After washing thrice with PBST bound HRP was assayed by incubating with 200 µl OPD (0.5 mg/ml) in 0.1 M citrate-phosphate buffer, pH 5.0, containing 0.03% H_2O_2 for 15 min, followed by addition of 50 µl 12.5% H_2SO_4 and the absorbance read at 490 nm in BIOTEK (Winooski, VT, USA) microplate reader.

2.2.3. Preparation of Positively Charged (PSMAg) and Negatively Charged (NSMAg) Protein Antigens from *Streptococcus mutans* Cell Wall

The cell harvest was sedimented and washed thrice with PBS by centrifugation at 12,000 rpm. Cell suspension in PBS was subjected to three cycles of freezing and thawing and later to ultrasonication in probe sonicator (six 30 second treatments) at 25˚C. The antigens released to the supernatant were collected by centrifugation at 12,000 rpm for 20 min, dialysed against PBS 7.4 and concentrated using CENTRICON before subjecting to electrophoresis in 7.5% polyacrylamide gel at pH 4.5 [7]. The lone protein that moved into the gel in 4 h run was PSMAg and was electroeluted using the method described by Ogden and Adams [8]. Alternatively the crude extract was passed through CM-Sephadex A-50 (15 ml) in 100 mM sodium acetate-acetic acid buffer pH 5.0 to remove positively charged proteins. The unbound antigen collected from above were dialysed against 10 mM Tris-HCl buffer pH 8.0 and passed through a 10 ml column of DEAE-Sephadex A-50 equilibrated in the same buffer. The bound protein (NSMAg) was then eluted using 200 mM NaCl, dialysed against water and concentrated by lyophilization.

2.2.4. Anti-Carbohydrate Antibody Binding to *S. mutans* Antigens

Purified antigen from *S. mutans* (PSMAg or NSMAg; 2 µg in 200 µl PBS) was coated on polystyrene wells. After washing wells were probed with anti-carbohydrate antibodies (ABG, DIg and anti-*α*-galactoside antibody [anti-Gal] from human plasma; 2 µg in 200 µl PBS-T) pre-incubated for 1 h with or without specific sugars (25 mM each of cellobiose, 1-O-methyl-*α*-D-glucoside, and melibose respectively for ABG, DIg and anti-Gal). The bound antibody was detected using mixture of HRP-conjugated rabbit antibodies to human IgA, IgG and IgM (1.5 µg/ml each in 200 µl PBS-T). The bound HRP was then assayed as described earlier.

2.2.5. Incorporation of Biotinylated Antigen into IC Fraction

Negatively charged *Streptococcus mutans* antigen (NSMAg) was biotinylated by method proposed by Paul *et al.* [9] and used for the preparation of *in vitro* immune complex. From 20 ml normal plasma high molecular weight components such as immune complexes were precipitated with 20% ammonium sulphate. Supernatant was dialysed against PBS and subjected to ultracentrifugation at 202,000 g in 1 ml tubes. Bottom 30% volume was dialysed against PBS and made up to original plasma volume (plasma immunoglobulin-rich [Ig-rich] fraction). Biotinylated antigen (50 µg) in 200 µl was added to 800 µl of the Ig-rich fraction, mixture incubated for 2 h and immune complexes formed were precipitated with 2% PEG, collected by centrifugation at 6000 rpm and dissolved in 500 µl PBS. Plasma Ig-rich fraction incubated with a mixture of 25 mM each of methyl-*α*-D-glucoside and 25 mM cellobiose (S1) or of methyl-*α*-D-mannoside and melibiose (S2) was used as control. To isolate IC formed between biotinylated NSMAg and plasma antibodies Ig-rich fraction for the above protocol was prepared from plasma made ABG- and DIg-free by passing through half its volume of a mixture consisting of cellulose, celite and Sephadex G-200 (1:1:2 by volume) in PBS. Ig-rich fraction from plasma treated with cross-linked guar galactomannan gel (CLGG) which does not offer ligands for ABG or DIg was used as control.

2.2.6. Immunoglobulin Composition of SMBP and NSMAg IC

SMBP (25 µg) was subjected to electrophoresis in SDS-polyacrylamide (7.5%) gel [10] and mobility compared with those of standard IgG, IgA and IgM. Streptavidin was coated on polystyrene wells by incubating the wells with its solution (1 µg in 200 µl of PBS) overnight at 4˚C. The wells were washed and blocked as described earlier. After incubation with 200 µl of 10× dilution of IC prepared using biotinylated NSMAg wells were washed with PBS-T and probed with 200 µl HRP-conjugated anti-human IgA, IgG or IgM (1.5 µg/ ml in PBS-T) for 2 h at 4˚C. After washing thrice again with PBS-T bound HRP was assayed as described earlier.

2.2.7. Determination of Immunoglobulins in NSMAg IC

IC (50 ng) obtained by treating non-labeled NSMAg with plasma Ig-rich fraction as described earlier was coated on polystyrene wells (50 ng per well), wells washed with PBS-T and probed with 200 µl HRP conjugates of an-

ti-human IgG, IgA or IgM (1.5 µg/ml in PBS-T) for 2 h at 4˚C. After washing thrice with PBS-T, bound HRP was assayed as described earlier.

2.2.8. Other Preparations

DIg and ABG from normal human plasma were prepared by affinity chromatography on Sephadex G-200 [11] and cellulose celite column respectively [12]. Anti-Gal free from lipoprotein (a) was prepared as described earlier [13]. Disaccharide ligands of anti-Gal and ABG (melibiose and cellobiose respectively) covalently attached to the non-glycosylated protein soybean trypsin inhibitor and termed TIM and TIC respectively were prepared by reductive amination using sodium cyanoborohydride [14]. Proteins were conjugated to HRP using protein and HRP in the ratio of 3:2 by mass [15]. Proteins were estimated with bovine serum albumin as standard [16]. Polyacrylamide gel electrophoresis in SDS was done by the protocol described by Laemmli [10].

2.2.9. Statistical Analysis

Statistical analysis (Student's *t test*) was done using Microsoft excel and Graphpad Prism. P value of <0.05 was considered significant.

3. Results

3.1. Anti-Carbohydrate Antibodies in Normal Plasma Recognize *S. mutans* Cell Wall Antigens

Heat-treated unbroken cells of *S. mutans* mixed with Biogel-P4 for better porosity were used as affinity matrix to isolate plasma antibodies that recognize surface antigens of the bacteria. Solubilized or electro-transferred antigens from the organism were not used in affinity matrix since these fractions might not carry the polysaccharides and highly glycosylated protein antigens [17]. *S. mutan*-binding normal plasma (SMBP) proteins that bound to the above matrix and were eluted with high concentrations of dextrose and galactose consisted solely of immunoglobulin since SDS-polyacrylamide gel electrophoresis of SMBP (**Figure 1(a)**) showed only three bands each with mobility identical with those of standard IgA, IgG or IgM. Probing of microplate-coated SMBP

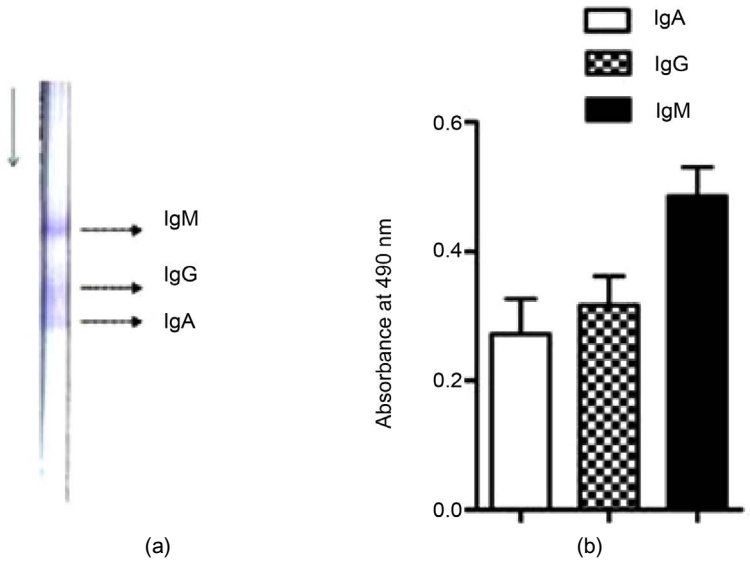

Figure 1. Plasma proteins that recognize *S. mutans*: (a) SMBPconstituents have identical electrophoretic mobility as immunoglobulins. SMBP (25 µg) was subjected to electrophoresis in SDS-polyacrylamide (7.5%) gel [10] and stained wth Coomassie brilliant blue stain. Horizontal arrows indicate positions of standard human immunoglobulin samples. (b) Immunoglobulin distribution in IC precipitated by NSMAg. IC obtained upon addition of biotin-labelled NSMAg to plasma Ig-rich fraction as described under "Methods" was diluted 10× in PBS-T and added to streptavidin-coated wells (1 µg). Following incubation for 2 h at 4˚C and washing with PBS-T immunoglobulins in bound IC were detected using HRP-conjugates of anti-human IgG, IgA and IgM separately (1.5 µg/ml) as described in "Methods". Values are mean ± SD of six trials.

with peroxidase-labeled anti-immunoglobulins also showed presence of the above immunoglobulins (data not shown). SMBP was captured by polystyrene microwell-coated forms of the β-glucan polymer from yeast and the α-glucan polymer dextran. This capture was significantly inhibited by the respective methyl glucoside anomers (**Figure 2**). The α-galactoside-bearing synthetic glycoprotein TIM, however was poor in capturing SMBP. Results indicated that ABG and DIg are prominent plasma antibodies that recognize *S. mutans* antigens while anti-Gal may spare these antigens.

3.2. Protein Antigens of *S. mutans* Bear Ligands for Normal Plasma Anti-Carbohydrate Antibodies

Positively charged proteins (PSMAg) of *S. mutans* extract moved as a single band in acid pH polyacrylamide gel electrophoresis (**Figure 3(a)**; 1). PSMAg electroeluted from the gel and coated on microwells was recognized sugar-dependently by ABG and to a lesser extent by DIg (**Figure 3(b)**). Negatively charged antigen (NSMAg) in *S. mutans* captured by DEAE-Sephadex also moved as a single peak in SDS-polyacrylamide gel electrophoresis (**Figure 3(a)**; 2). This protein in turn was also recognized both by ABG and DIg (**Figure 3(b)**). PSMAg or NSMAg recognition by ABG or DIg was poorly inhibitable by specific mono- or disaccharide sugars. One reason may be that the low molecular weight mono- or disaccharides used for inhibition were far different in structure from the antigenic epitopes in commensal or invading bacteria that induced production of these antibodies. In addition, secondary interactions between protein components of the *S. mutans* antigens and those on the antibody may render the antigen-antibody recognition harder for small sugar ligands to compete with.

3.3. α- and β-Glucoside Specific Antibodies Are Involved in IC Formation with Major Protein Antigens of *S. mutans* in Plasma

ICs formed upon addition of biotinylated NSMAg to plasma Ig-rich fraction were captured on streptavidin-coated wells and their immunoglobulin contents quantitated by probing with HRP-labeled anti-immunoglobulins. Results (**Figure 1(b)**) show IgM to be the leading antibody involved in IC formation with NSMAg in plasma, closely followed by nearly equal amounts of IgA and IgG. This protocol was found to measure exclusively the NSMAg-specific IC since IC from NSMAg-free plasma produced negligible response ($P < 0.0001$) (**Figure 4**).

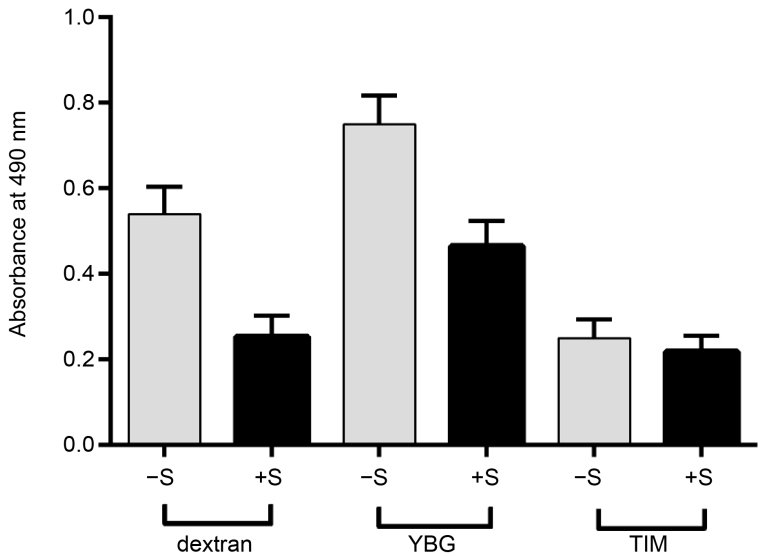

Figure 2. Sugar specificity of SMBP. Dextran, yeast-β-glucan (YBG) and TIM (2 µg each) was coated on polystyrene wells and SMBP (2 µg in 200 µl PBS-T) pre-incubated with or without respective specific sugar (25 mM of methyl α-D-glucopyranoside for dextran, cellobiose for YBG and methyl α-D-galactopyranoside for TIM) was added. After 2 h incubation at 4˚C and washing bound SMBP was probed with a mixture of HRP conjugates of anti-human IgG, IgA and IgM (1.5 µg/ml). Bound HRP was assayed as described. Values are mean ± SD of six trials. P values for inhibition were: <0.0001, <0.0001 and 0.2034 respectively for the above three inhibitors.

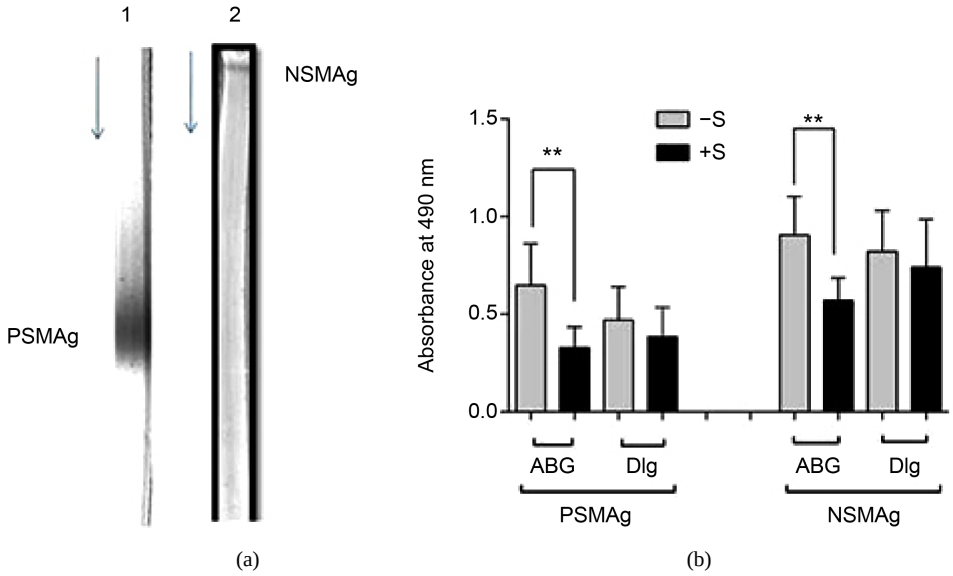

Figure 3. Properties of major *S. mutans* antigens. (a) Polyacrylamide gel electrophoresis Acid pH (pH 4.5) electrophoresis of solubilised *S. mutans* antigens (100 µg) in 7.5% gel (1); SDS electrophoresis in 7.5% gel of NSMAg (50 µg) eluted from DEAE-Sephadex A-50 (2). (b) Anti-carbohydrate antibody binding to PSMAg and NSMAg: Polystyrene wells coated with PSMAg or NSMAg (2 µg per well) were blocked and probed with anti-carbohydrate antibodies (2 µg of ABG and DIg) pre-treated with (+S) or without (−S) 25 mM specific sugar (cellobiose for ABG and methyl-*α*-D-glucopyranoside for DIg). The antibody bound to coated antigen was detected using mixture of HRP-conjugated anti-human IgG, IgA or IgM (1.5 µg/ml). Bound HRP was assayed as described. Values are mean ± SD of four trials for PSMAg and six trials for NSMAg. P values for inhibition of ABG and DIg binding to PSMAg were: 0.0050 (**≤0.005) and 0.2767 respectively. Corresponding P value for NSMAg were 0.0038 and 0.0436.

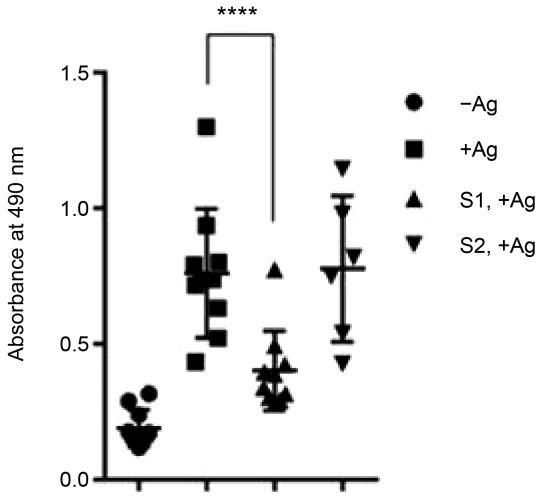

Figure 4. Specificity of plasma antibodies that form IC with NSMAg. Fifty µg biotin-labeled NSMAg in 200 µl PBS was mixed with 800 µl human plasma Ig-rich fraction pre-incubated with specific sugars (S1, +Ag), non-specific sugars (S2, +Ag) or no sugar (+Ag) and IC formed was isolated as described (Methods). IC without antigen addition (−Ag) served as control. IC was dissolved in 500 µl PBS and 10× dilution (200 µl in PBS-T) was added to streptavidin-coated (2 µg per well) polystyrene wells. After washing bound IC was probed with a mixture of HRP-conjugated antibodies to human IgG, IgA and IgM. Bound HRP was determined as described. Values are mean ± SD of 10 samples except for S2, +Ag (n = 6); P values were: <0.0001 (****) for both +Ag vs −Ag and S1, +Ag vs +Ag. S1: mixture of 25 mM cellobiose and 25 mM methyl *α*-D-glucopyranoside. S2: mixture of 25 mM melibiose and 25 mM methyl-*α*-D-mannopyranoside.

Result in **Figure 4** further suggests that plasma antibodies contributing to IC formation with NSMAg are almost totally contributed by α- or β-glucoside-specific antibodies such as ABG and DIg since sugars specific to these antibodies, unlike galactosides or mannosides were very efficient inhibitors of IC formation ($P < 0.0001$). In support of this conclusion selective removal of ABG and DIg from plasma by prior treatment with a column containing cellulose and Sephadex G-200 (dextran) resulted in significant reduction in IC formed by NSMAg in Ig-rich fraction (**Figure 5**).

3.4. Elevated Plasma IC Content Following NSMAg Addition

Figure 6 shows the relative contents of immunoglobulin types IgA, IgG and IgM in same protein amount (50 ng) of total immunoglobulin fraction (TIg) from normal plasma and of IC precipitated by 2% PEG from normal plasma [IC(-Ag)] or from normal plasma to which NSMAg had been added in advance [IC(+Ag)]. IC obtained after addition of NSMAg, in comparison to naturally occurring IC contained significantly more of all three immunoglobulins despite part of the proteins in the former being the NSMAg itself, indicating the substantial contribution of this antigen towards IC formation.

4. Discussion

Multiple pathogens, both viral and bacterial, are causally related to atherosclerosis and several other disorders involving immune inflammatory reactions with the risk correlating with the aggregate pathogen load [18]. Chronic dental bacterial infections have been particularly implicated in atherosclerosis and other inflammatory diseases [19]. Though primarily a cause for periodontitis and dental plaques *S. mutans* is also associated with onset of various inflammatory reactions [19]. Since *S. mutans* infecting the oral cavity or their antigens can gain access into the circulation either through minor cuts or by routine brushing and flossing present results suggest that their recognition by circulating naturally occurring antibodies with primary specificities for α- and β-glucans will be a continuous event. These antibodies had earlier been identified as anti-β-glucan (ABG) [12] and dextran-binding (DIg) antibodies respectively and characterized [11].

Positively charged (PSMAg) and negatively charged (NSMAg) *S. mutans* antigens were both recognized by circulating ABG and to a lesser extent by DIg (**Figure 3(b)**). Since rhamnose-glucan polymers (RGP) with rhamnose backbone and $\beta(1 \rightarrow 2)$-, $\alpha(1 \rightarrow 2)$-, and $\alpha(1 \rightarrow 3)$-linked glucose side chains have been reported to

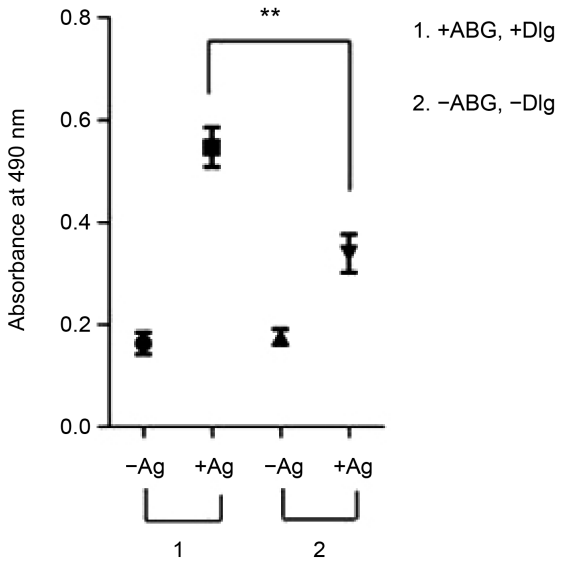

Figure 5. Effect of withdrawal of ABG and DIg on IC formation by NSMAg. Ig-rich fraction from normal plasma (+ABG, +DIg) or from plasma devoid of ABG and DIg (−ABG, −DIg) prepared as described under "Methods" was used to prepare IC with (+Ag) or without (−Ag) biotinylated NSMAg. The IC was analysed by ELISA as described under **Figure 4**. Values are mean ± SD of 6 trials. P value for IC formation by plasma samples (1 vs 2) with NSMAg = 0.0030 ($^{**}<0.005$).

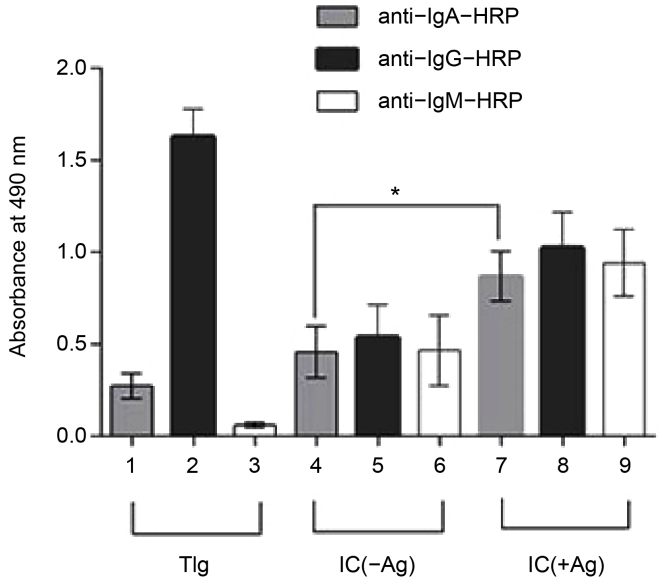

Figure 6. Increase in IC immunoglobulins upon NSMAg addition to plasma immunoglobulins. IC obtained by treating plasma Ig-rich fraction with (+Ag) or without (−Ag) unlabeled NSMAg as described under **Figure 4** and coated on polystyrene wells (50 µg protein) was probed with HRP conjugates of antibodies to human IgG, IgA or IgM as described earlier. Total plasma immunoglobulins (TIg) served as control. Values are mean ± SD of 3 samples. P value for increase of IgA per unit IC protein = 0.0215 (*<0.05).

be characteristic of *S.mutans* cell wall glycoproteins [20] binding of ABG and DIg to PSMAg and NSMAg could be attributed to possible presence of RGP structures in the latter. RGP-rich glycoproteins are known to induce platelet aggregation [20].

De novo IC formed between naturally occurring antibodies of normal plasma and biotin-labeled NSMAg contained IgM as leading antibody, closely followed by IgA and IgG in contrast to circulating free immunoglobulins which are predominantly IgG (**Figure 6**). However IC obtained upon addition of NSMAg to normal plasma immunoglobulins contained twice as much IgA, IgG and IgM as did IC precipitated without antigen addition (**Figure 6**). This indicated that *S. mutans* antigens contribute significantly towards circulating IC in infected patients.

Superiority of IgA over other immunoglobulins as ligand for galectin-1 had been demonstrated earlier [21]. Even in ICs in which IgA1 is partly engaged by anti-glycan antibody (eg. IC of plasma anti-T antibody with desialylated IgA), sufficient O-glycan chains recognizable by lectins remained unoccupied by anti-T [22]. Since IC formation with *S. mutans* antigen does not involve the Fc part of IgA that bear most of its glycan chains, the latter are fully available for lectin recognition in these ICs. Moreover ICs formed by microbial polysaccharides had been reported to be recognized by immobilized galectin-1 mainly due to their high polymeric IgA content [9]. DIg and ABG contain higher polymeric IgA content than does circulating immunoglobulins as a whole [11] [12], accounting for the higher IgA content in *S. mutans* antigen ICs observed here. Major cell types in the sub-endothelial region involved in inflammation- and/or infection-mediated pathology are activated monocytes (macrophages) in which expression of another lectin, viz. galectin-3 is several fold higher than in resting monocytes [23]. Incidentally affinity of galectin-3 for O-glycans of the type present in IgA (core 1) is about two orders of magnitude higher than that of galectin-1 [24]. This factor also underlines the pathological significance of IgA enrichment in bacterial antigen ICs. Increased serum IgA against several periodontal pathogens is recognized as risk factor for cardiovascular diseases [25].

While the contribution of IgA suggested above is deductive there is direct evidence that high circulating immune complexes cause vascular damage in several diseases including Henoch-Scholnein purpura and Dermatitis Herpetiformis [26] [27], glomerulonephritis, arthritis, transplantation rejection, rheumatic fever and cardiovascular disorders [28]. *S. mutans* antigens are ideal candidates for IC-mediated pathology both due to the affinity

of circulating natural antibodies for them and due to the relatively high levels of these antibodies in circulation (ABG: 2.5 - 6.4 mg per dL and DIg: 4.3 - 5.7 mg per dL [11] [12]. Continuous release of bacteria or their antigens into circulation being a feature of periodontic infections [29] IC generation by these antigens would last as long as the infection does. In summary the present results suggest that *S. mutans* antigens are poised for inducing marked IC-mediated vascular inflammatioin by virtue of the composition, specificity and concentrations of circulating antibodies that recognize them.

5. Conclusion

Two high titre plasma anti-β-glucan antibodies recognize antigens of *S. mutans* to form immune complexes. The latter are known to be mediators of vascular injury thus accounting for the significant contribution of periodontic infections towards vascular diseases. Abundance of the lone galectin-1-binding immunoglobulin IgA in these immune complexes may also facilitate their homing to vascular cells that are rich in this lectin.

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