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Research article

Possible molecular basis for macromolecular antigen attachment to host cells: their immune complex with plasma antibodies have unoccupied binding sites enabling binding to smaller ligands

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Abstract: Macromolecules such as lipoprotein(a) and antigens of *Streptococcus mutans* that cause dental infections accumulate in perivascular cells in inflammatory vascular disorders though they do not bind host cells directly. Suspecting a role for cognate circulating antibodies in the molecular mechanisms of macromolecular antigen deposition in tissues we investigated the ligand binding properties of *de novo* immune complexes (IC) formed between these two entities. Negatively charged *Streptococcus mutans* antigens (NSMAg) from cultured bacteria and apo(a) subunit separated from human plasma lipoprotein (a) were used as antigens to interact with anti β-glucan (ABG) and anti-α-galactoside (anti-Gal) antibodies respectively of normal plasma. Binding of antigen to antibody was measured in terms of the enhancement of fluorescence of FITC-labeled antibody that accompanies antigen binding. Unoccupied binding sites on macromolecular antigen-antibody ICs were demonstrated by capturing them on smaller ligands immobilized on microplates. Biotin-labeled macromolecular antigens were detected in ICs using HRP-labeled avidin. While total *Streptococcus mutans* antigens and a synthetic glycoprotein in which an ABG-specific disaccharide (cellobiose) had been covalently attached were equal in avidity towards ABG, NSMAg was superior. When binding of antibody to plate-coated ligands was monitored using HRP-labeled anti-immunoglobulin preincubation of ABG or anti-Gal with respective macromolecular antigens significantly enhanced binding response whereas antibodies preincubated with small sugars were fully inhibited from binding, indicating that macromolecular antigen binding had activated the Fc part of antibodies and that the resulting ICs in turn bound to other ligands using binding sites in the antibody spared by the large antigens. Presence of biotinylated macromolecular antigen in IC bound

to immobilized ligands was confirmed using avidin-HRP probe. Unoccupied binding sites in the antibodies involved may enable homing of macromolecule ICs on vascular cells possessing smaller ligands. This along with an activated Fc could enhance their inflammatory potential.

Keywords: immune complex; anti-β-glucan antibody; anti-α-galactoside antibody; *Streptococcus mutans*

1. Introduction

Infection-mediated inflammatory damage has been implicated in several vascular diseases affecting cardiovascular system [1], brain [2] and kidney [3]. Plasma concentration as well as phenotypic variant of endogenous macromolecules such as lipoprotein(a) [Lp(a)] are also known to determine vascular pathology leading to atherosclerosis [4,5]. However molecular mechanism of immune inflammatory damage caused at vessel walls by microbial antigens or Lp(a) are unclear. Two features common to microbe- and Lp(a) phenotype-dependent vascular damages are: (i) dependence of inflammatory potential on surface antigenic determinants of these macromolecules and (ii) accumulation of the macromolecular antigens in perivascular cells following atherosclerosis [6-8]. Since recognition of vascular cell surface epitopes by the above antigens hardly occurs involvement of circulating antibodies that recognize the antigens was suspected. Several naturally occurring anti-carbohydrate antibodies in normal individuals are known to recognize corresponding saccharide or surrogate antigens presented by dietary, infused, pathogen-born or autologous macromolecules, leading to formation of circulating immune complexes (IC).These include the dextran-binding immunoglobulin (DIg) that bind α-linked glucose in dextrans [9], anti-β-glucan (ABG) antibody that recognizes β-glucans including those in *Streptococcus mutans* (*S. mutans*) [10,11], lactose-binding immunoglobulin (LIg) that recognizes desialylated lipoprotein(a) [12] and the anti-α-galactoside antibody (anti-Gal) that also recognizes serine- and threonine-rich peptide sequences (STPS) in lipoprotein(a) [13,14]. Having been recently detected the toxic potentials of the above ICs are yet to be ascertained. Two such ICs namely anti-Gal-unmodified-Lp(a) and LIg-desialylated-Lp(a) had been found to bind to affinity matrix and desialylated human RBC respectively [12,15]. However the rationale or molecular mechanisms of binding of macromolecule-derived circulating ICs in turn to other ligands including those on host cells has not been investigated. Accommodation of secondary ligands by macromolecule-containing ICs has potential bearing on the fate of ICs, vascular damage inflicted by ICs and transport of macromolecules across cell membranes. In this communication we demonstrate that macromolecules occupy only a fraction of binding site in immunoglobulins enabling the resulting ICs to utilize their unoccupied binding sites to bind along with the macromolecule to smaller ligands. Two circulating naturally occurring human antibodies namely the anti-β-glucan antibody (ABG) and anti-α-galactoside antibody (anti-Gal) were used as models.

2. Materials and Methods

2.1. Materials

Soybean trypsin inhibitor, bovine thyroglobulin (Tg), horse radish peroxidase (HRP),

methyl-α-D-glucopyranoside, methyl-α-D-galactopyranoside, methyl-α-D-mannoside, cellobiose, melibiose, sulpho-NHS-biotin, avidin-HRP, fluorescein isothiocyanate (FITC), Biogel P-4, soluble guar gum, AMICON ultracentrifugal filter units (MW cut-off 10kDa) and streptavidin were purchased from Sigma-Aldrich, Bangalore, India. Polystyrene 96-well microplates (MAXISORP) were purchased from Nunc, Roskilde, Denmark. Polystyrene 96-well microplates used for fluorescence measurement was from Dynex Technologies, USA. Antibodies to human IgA, IgM, IgG and apo(a) raised in rabbit were obtained from Dako, Denmark. Outdated human plasma was obtained from the Department of Blood Transfusion Services of this Institute with Institutional Ethics Committee approval (IEC-511). *S. mutans* strain (MTCC-890) was from Institute of Microbial Technology (IMTECH), Chandigarh, India.

ABG from normal human plasma was prepared by affinity chromatography on cellulose-celite column [10]. Cross-linked guar galactomannan (CLGG) for the preparation of anti-Gal was prepared by procedure described earlier [16]. Anti-Gal free from lipoprotein(a) was prepared by affinity chromatography on CLGG [15]. Biotin labeling of proteins was done by the method described by Paul et al. [17]. Disaccharide ligands of anti-Gal and ABG (melibiose and cellobiose respectively) covalently attached to the non-glycosylated protein soybean trypsin inhibitor and termed trypsin inhibitor-melibiose and trypsin inhibitor-cellobiose (TIC) respectively, were prepared by reductive amination using sodium cyanoborohydride [18]. Proteins were conjugated to horse radish peroxide (HRP) using protein and HRP in the ratio of 3:2 by mass [19]. Proteins were estimated with bovine serum albumin as standard [20]. Albumin from normal human plasma was isolated by affinity chromatography on blue-Sepharose as described by Travis et al. [21].

2.2. Methods

2.2.1. Isolation of apo(a) from plasma lipoprotein(a)

Apo(a) was prepared essentially as described by Geetha et al. [14]. Briefly plasma (8 mL) was brought to a density of 1.24 g/cc with potassium bromide, subjected to ultracentrifugation in 1 mL tubes at 535,000 g for 4 h at 4 °C and top 20% layer containing lipoproteins (L1) was collected and dialysed against 20 mM potassium phosphate buffer containing 150 mM NaCl (PBS), pH 7.4. L1wasreduced by treating with 4 mM DTT at 37 °C for 15 minutes, density brought to 1.24 g/cc with potassium bromide and ultracentrifugation repeated as above. Bottom 20% volume containing apo(a) was collected and dialysed in PBS.

2.2.2. FITC conjugation of antibodies

FITC labeling of antibodies was done essentially as described by Hudson and Hay [22]. Antibody samples were concentrated to 1 mg/mL, dialysed against 0.25 M carbonate-bicarbonate buffer (pH 9.0) and pre-incubated for 2 h with respective specific sugars (25 mM) to protect the antigen binding site from labeling. FITC (0.15 mg/mg protein) was then added and mixture kept overnight at 4 °C. Gel permeation chromatography using 25 mL Biogel-P4 column in PBS medium was used to separate the FITC-labeled antibody from reagents.

2.2.3. Effect of specific ligands on antigen-induced increase in fluorescence of FITC-labeled antibody

Antigen-induced increase in fluorescence of FITC-labeled antibody was measured by adapting a protocol reported by George et al. [23]. FITC-labeled ABG (6.5 μg) was treated with 15 μg each of TIC, *S. mutans* antigen (SMAg), negatively charged protein antigens from *S. mutans* (NSMAg), human serum albumin (HSA) or cellobiose (50 mM) in 40 μL PBS overnight at 4 °C. After diluting to 300 μL with PBS, fluorescence was measured in BIOTEK fluorescence reader using excitation at 485 nm and emission at 520 nm.

2.2.4. Preparation of crude antigens (SMAg) from *S. mutans* cell wall

The cell harvest from cultured *S. mutans* was sedimented and washed thrice with PBS by centrifugation at 17,400 g for 30 min. 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 were collected in supernatant following centrifugation as above and dialysed against PBS.

2.2.5. Preparation of negatively charged protein antigens from *S. mutans* (NSMAg)

NSMAg from *S. mutans* was isolated by ion exchange chromatography by a protocol recently developed by George et al. [24]. Briefly the crude cell wall antigen (2 mg) prepared by freeze thawing and sonication was passed through CM-Sephadex A-50 (15 mL) in 100 mM sodium acetate-acetic acid buffer pH 5.0 for the removal of 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.

2.2.6. Increase in anti-Ig response of antibody (ABG and Anti-Gal) in presence of a ligand

TIC or Tg for ABG and anti-Gal respectively was coated on polystyrene wells by incubating their solutions in PBS (2 μg in 200 μL) in the wells at 37 °C for 3 h followed by blocking of wells by incubation with PBS containing 0.5 M Tween 20 for 30 min. After washing with PBS containing 0.05 M Tween 20 (PBS-T) wells were treated with ABG (250 ng) or anti-Gal (100 ng) pre-incubated with or without ligand [NSMAg (2 μg) for ABG and apo(a) (500 ng) for anti-Gal] in 200 μL PBS-T. After 2 h incubation at 4 °C and washing bound ABG or anti-Gal was measured by probing with a mixture of HRP conjugates of anti-human IgG, IgA and IgM (1.5 μg/mL). 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 to stop the reaction and the absorbance read at 490 nm in BIOTEK (Winooski, VT, USA) microplate reader.

2.2.7. The presence of free binding sites on ABG-NSMAg complex and anti-Gal-apo(a) complex

ABG or anti-Gal (100 ng in 200 μL PBS-T) preincubated for 2 h with 25 mM of specific (S2) or non-specific (S1) sugar or no sugar was allowed to interact for 4 h with biotin-labeled antigenic ligand

(500 ng NSMAg in 200 µL PBS-T for ABG and 500 ng apo(a) in 200 µL PBS-T for anti-Gal) and added to TIC- or TG-coated wells (2 µg in 200 µL PBS). Biotinylated antigen alone without antibody served as control. The presence of biotinylated antigen on coated wells was detected using avidin-HRP (1.5 µg HRP per mL) and the bound HRP determined as described earlier.

2.2.8. Statistical analysis

Statistical analysis was done using Microsoft Excel 2000 version and GraphPad Prism 5. Comparison between groups was done using Students t-test. P value of < 0.05 was considered significant.

3. Results

3.1. Macromolecular antigens produce conformational shift in ABG

Trypsin inhibitor, a non-glycosylated protein was derivatized with β-glucan substitutions by treatment with excess of the disaccharide cellobiose (Glc β1→4Glc) in presence of sodium cyanoborohydride in alkaline medium. The reducing end of the disaccharide undergoes reductive amination at the primary amino groups on the protein resulting in a protein derivative with a non-reducing β-Glc moiety attached to each of the available primary amino groups. This synthetic glycoprotein (TIC) rich in β-glucosides was compared to SMAg and NSMAg as ligands for ABG using increase in fluorescence of FITC-labeled ABG as a measure of binding affinity of the antigen. FITC ligand covalently attached to ABG in presence of a sugar ligand specific for the antibody had been shown to reside mostly on its Fc region [23]. Further, upon treatment with macromolecular ligands fluorescence of FITC-labeled antibody, measured in terms of emission at 528 nm after excitation at 485 nm, increased directly with the affinity of the ligand added [23], apparently since fluorescence increase resulted from conformational shift in Fc region consequent to ligand binding. Results in Figure 1 show that while total *S. mutans* antigens were comparable with TIC in affinity for ABG, NSMAg alone was a ligand with significantly higher affinity for the antibody. HSA was inactive as an antigen indicating requirement of specific sugar moiety on the antigen for antibody binding. Notably the disaccharide ligand cellobiose also could not increase fluorescence of FITC-ABG apparently because its binding strength is too low to affect the conformation of Fc part of the antibody [23].

3.2. ABG-macromolecule IC has unoccupied binding site(s) that enable its binding to smaller ligands

Results in Figure 2 show the differential effect of preincubation with macromolecular or small ligands in solution on ABG binding to polystyrene plate-coated TIC, determined by ELISA. TIC contains the smallest ABG-binding ligand (β-glucoside) linked covalently to a protein. The amount of ABG bound to TIC coated on microplate was determined by probing with HRP-conjugated anti-immunoglobulins. The disaccharide cellobiose that contains a β-linked glucose fully inhibited ABG binding to plate-coated TIC indicating that small antigens bind at all binding sites of the antibody. In contrast, in presence of NSMAg anti-immunoglobulin-HRP binding showed an increase over what was observed in case of uninhibited ABG. Failure of NSMAg to inhibit binding of ABG showed that all the NSMAg-bound ABG molecules could bind in turn to plate-coated TIC. Sparing of binding sites in the antibody could not be attributed to paucity of antigen since 2 µg NSMAg was provided to

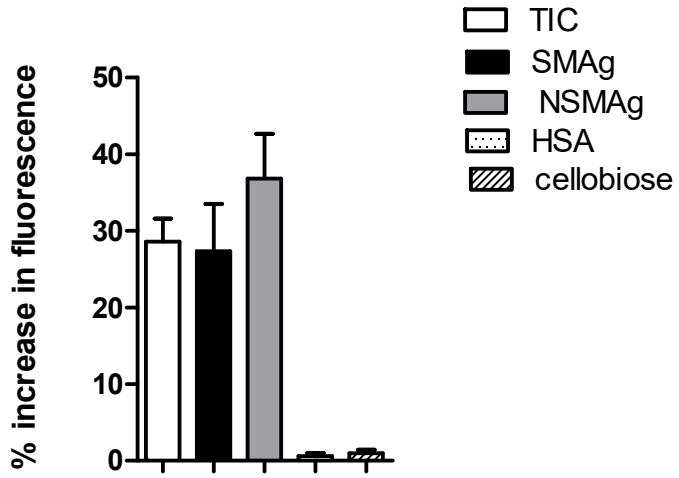

Figure 1. Percentage increase in fluorescence of FITC-labeled ABG with different ligands. FITC-labeled ABG (6.5 μg) was treated with 15 μg each of TIC, *S. mutans* antigen (SMAg), NSMAg, human serum albumin (HSA) or cellobiose (50 mM) in 40 μL PBS overnight at 4 °C. The solution was diluted to 300 μL with PBS and fluorescence measured in BIOTEK fluorescence reader using excitation at 485 nm and emission at 520 nm. Percentage increase in fluorescence produced by various ligands over that of untreated FITC-labeled ABG was compared. Values are mean ± SD of five ABG samples.

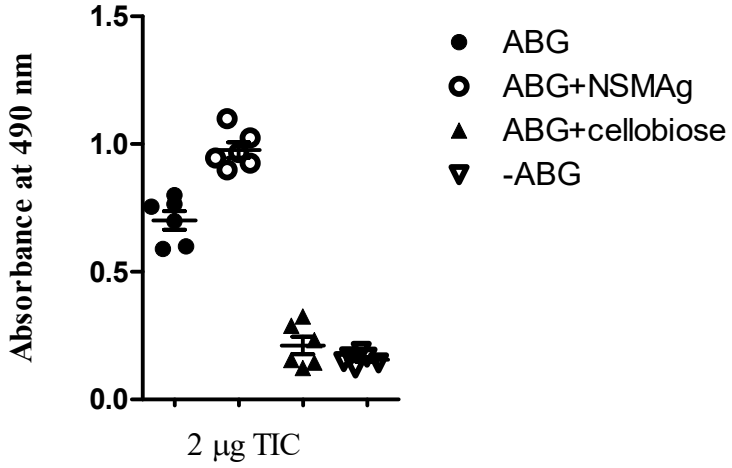

Figure 2. Pre-incubation with macromolecular antigen does not affect binding of ABG to smaller ligands, but increases anti-Ig response to the antibody. TIC (2 μg in 200 μL PBS) was coated on polystyrene wells and ABG (250 ng in 200 μL PBS-T) pre-incubated with or without NSMAg (2 μg) or cellobiose (50 mM) was added. After 2 h incubation at 4 °C and washing bound ABG 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 in the text. Values are mean ± SD of values using six ABG samples.

interact with 250 ng antibody. Further, a response to anti-immunoglobulins greater than in the case of untreated antibody alone could be attributed to the conformational changes that occurred on Fc part of NSMAg-bound ABG, since the anti-immunoglobulins recognize the Fc part. This conclusion is supported by results in Figure1 that show even large excess of small antigens could not produce the conformational changes produced by macromolecular antigens in the Fc part of the antibody. Results in Figure 2 also show that presence of low molecular weight ligand cellobiose could totally abolish antibody binding suggesting absence of nonspecific binding of ABG to TIC.

3.3. Apo(a)-anti-Gal IC binds to smaller anti-Gal ligands

Bovine thyroglobulin (Tg) is rich in terminal α-galactoside moieties that are natural small ligands for anti-Gal [25]. Apo(a) subunit of Lp(a) has been shown to offer a surrogate ligand namely STPS for anti-Gal [13] and occupy the α-galactoside-binding sites of the antibody resulting in formation of circulating Lp(a)-anti-Gal ICs [14]. Availability of free binding site in anti-Gal after occupation of the latter by macromolecular antigen was also examined using apo(a) as a macromolecular antigen. In ELISA using micro-plate coated Tg for capturing anti-Gal and measurement of bound antibody using HRP-conjugated anti-immunoglobulins as probe (Figure 3), anti-Gal pre-incubated with apo(a) samples produced a significantly greater response showing that retention of binding sites for smaller ligands is a general feature of all macromolecule-antibody ICs. Here again melibiose could reduce ELISA response to the level obtained without antibody indicating that antibody binding to coated Tg was entirely binding site-specific. Further, unoccupied binding sites on anti-Gal-apo(a) immune complex could not be a result of limited apo(a) availability since 500 ng of the latter were treated with 100 ng antibody.

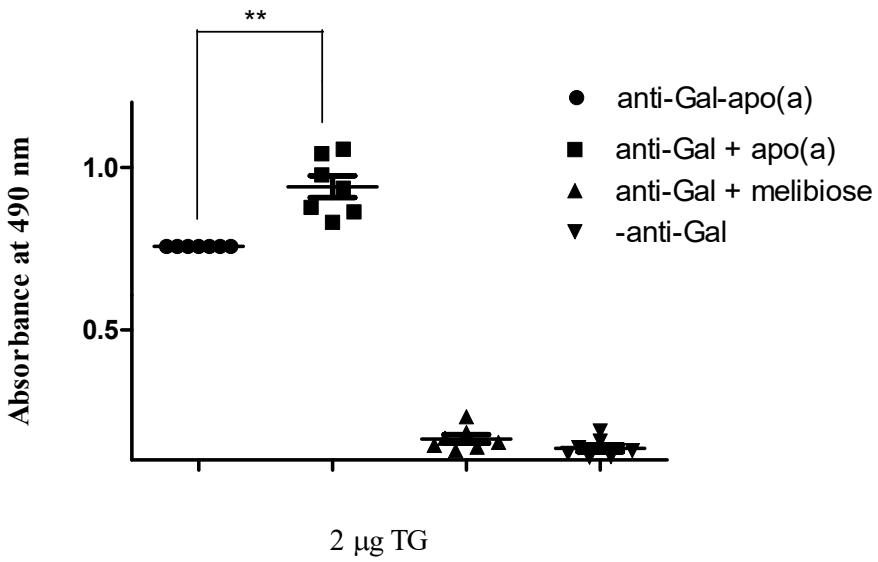

Figure 3. Effect of pre-incubation with apo(a) on anti-Gal binding to small ligands detected using HRP-conjugated anti-immunoglobulins. Tg (2 μg in 200 μL PBS) was coated on polystyrene wells and anti-Gal (100 ng in 200 μL PBS-T) pre-incubated with or without apo(a) (500 ng) or melibiose (50 mM) was added. After 2 h incubation at 4 °C and washing bound anti-Gal 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 7 samples of apo(a). P value for the increase in anti-Ig response with apo(a) = 0.0015 (**).

3.4. Immunoglobulins bind to small ligands along with pre-bound macromolecular antigens

In order to examine the integrity of the macromolecular antigen ICs after they bind in turn to smaller ligands, biotin-labeled macromolecular antigens were employed. Anti-Gal pre-incubated with biotin-labeled apo(a) was added to microplate-coated Tg and presence of labeled apo(a) in the bound anti-Gal complex was detected using avidin-HRP. Non-specific response in the avidin-biotin recognition utilized in this assay was ascertained by providing anti-Gal-specific or non-specific monosaccharide derivative during incubation of apo(a) with anti-Gal. Result (Figure 4) showed a significantly higher amount of bound apo(a) in the absence of inhibitory sugar indicating that apo(a) remained with the anti-Gal even after the latter recognized the plate-coated smaller ligand. Though biotinylated apo(a) alone bound non-specifically to plate-coated Tg to a limited extent, binding was significantly enhanced after preincubation with anti-Gal. Similar result was observed when ABG-biotinylated-NSMAg immune complex formed in presence of melibiose (non-specific) or cellobiose (specific) respectively were compared for their binding to microplate-coated TIC (Figure 5).

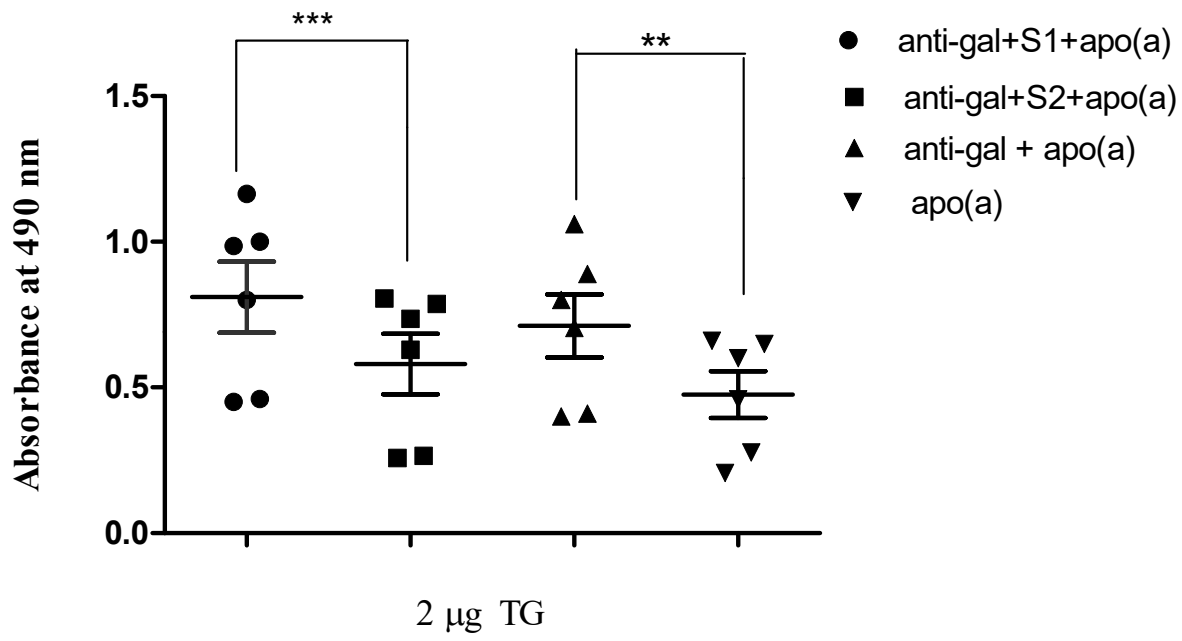

Figure 4. The presence of free binding sites on anti-Gal-apo(a) immune complex. Anti-Gal preincubated with 25 mM of specific (S2) or non-specific (S1) sugar or no sugar at all at 4 °C was allowed to interact with biotin-labeled apo(a) and added to Tg-coated wells (2 µg in 200 µL PBS). Details are under Methods (2.2.7). Binding of biotin-labeled apo(a) alone was also tested under same conditions. Biotin-labeled apo(a)bound to Tg-coated wells was detected using avidin-HRP (0.75 µg avidin per mL) and assaying bound HRP as described. Values are mean ± SD of six trials. P values were = 0.0005 (***) between S1 and S2 and 0.0022 (**) for biotin-labeled apo(a) with and without anti-Gal. S1 = 25 mM methyl-α-D-glucoside; S2 = 25 mM methyl-α-D-galactoside.

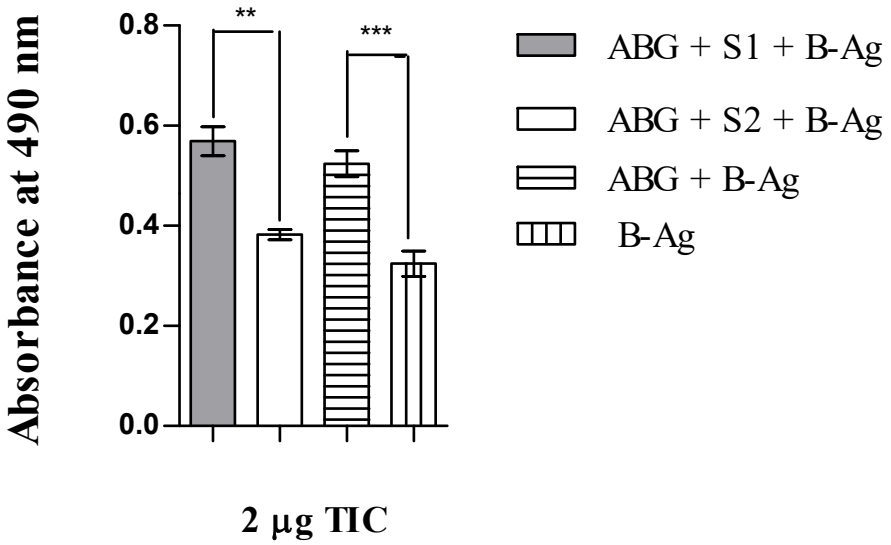

Figure 5. The presence of free binding sites on ABG-NSMAg complex. ABG preincubated with 25 mM of specific (S2) or non-specific (S1) sugar or no sugar at all was allowed to interact with biotin-labelled NSMAg (B-Ag) and added to TIC-coated wells (2 µg in 200 µL PBS). Details are under Methods (2.2.7). Binding of biotin-labeled NSMAg alone was also tested under same conditions. B-Ag attached to TIC-coated wells were detected using avidin-HRP (0.75 µg avidin per mL) and the bound HRP determined as described. Values are mean ± SD of four trials. P values were 0.0040 (**) between S1 and S2 and < 0.0001 (***) for B-Ag with and without ABG. S1: 25 mM cellobiose; S2: 25 mM melibiose.

4. Discussion

Using two prominent anti-carbohydrate antibodies in circulation, one specific towards α-linked galactose and the other towards β-linked glucose, it was shown above that their ICs with autologous or pathogen-derived macromolecular antigens have unoccupied binding sites with which they can bind in turn to specific ligands or smaller molecules. The potential of this phenomenon in precipitating vascular immune inflammatory events is obvious. Firstly the above results explain a recently reported observation that circulating LIg recognize desialylated versions of the primate-specific lipoprotein Lp(a) to form ICs which in turn agglutinate autologous desialylated RBCs in vitro [12]. LIg had been reported to recognize N-acetyl lactosamine (LacNAc) disaccharide groups that get exposed after terminal sialic acid residues in complex type N-glycan chains of glycoconjugates are released by desialylation [12]. Sialylated LacNAc groups are widely distributed on circulating glycoproteins, cells and endothelial cells [26, 27].While desialylated RBCs were used as an easily accessible model for demonstrating binding of LIg ICs to host cells, desialylation of all glycoconjugates including lipoprotein(a) and vascular endothelial cell surface glycoprotein is known to accompany diabetes [28]. It follows that a major contributor towards diabetic vasculopathy may be LIg-desialylated-Lp(a) IC. In support of this conclusion, chronic diabetes is major predisposing factor for atherosclerosis [29]. Significantly Lp(a) far outnumbers LDL in atherosclerotic plaques, though the reverse is true in plasma [6,7].

Secondly anti-Gal, the antibody that circulates only in man and advanced primates has been found to recognize serine- and threonine-rich peptide sequences (STPS) as surrogate ligand [13,14]. As shown above even though apo(a) is rich in STPS that are capable of binding to anti-Gal, the former is large enough to spare the other binding site of the largely IgG-containing anti-Gal. Our recent results [30] showed that anti-Gal could also recognize STPS-containing glycoproteins on human placental or macrophage cell surfaces. Notably, unlike apo(a) (Figure 3), solubilized human cell surface glycoproteins were small enough to occupy both binding sites of anti-Gal and thereby inhibit antibody binding to microwell-coated Tg [30]. This explained the observation that apo(a)-bound anti-Gal could in turn bind along with apo(a) to macrophages. In support of this conclusion pre-treatment of STPS-bearing cells and molecules with LDL considerably reduced their capacity to bind anti-Gal-apo(a) IC [30] probably because LDL binds to STPS-bearing glycoproteins by utilizing the negative charges contributed by sialic acid moieties of O-glycans abundant on these glycoproteins and block access for anti-Gal to STPS on the cells. The present results along with those referred to above may explain the significantly high incidence of atherosclerosis in individuals with high Lp(a) titer [31] since such individuals nearly always carry smaller Lp(a) phenotypes that form much less or no Lp(a)-LDL adduct in circulation than larger Lp(a) phenotypes [32]. Lp(a)-LDL adducts had been shown to contain one or several LDL molecules that are non-covalently attached to each Lp(a) molecule in addition to the LDL moiety to which apo(a) is covalently attached in the primary Lp(a) molecule. However the number of LDL molecules non-covalently attached to each Lp(a) molecule increased with the size of the Lp(a) phenotype [32]. Obviously smaller Lp(a) molecules with less adduct LDL on them would be more open for recognition by STPS-specific antibodies such as anti-Gal leading to formation of immune complexes.

5. Conclusion

Binding of endogenous or microbial origin antigens to naturally occurring anti-carbohydrate antibodies produces immune complexes with activated Fc, but leaves adjacent binding sites unoccupied, apparently for steric reasons. The balance binding site may be utilized to home the IC to immobilized smaller ligands. This offers a mechanism for macromolecule entry into vascular or macrophage cells.

Conflict of Interest

None declared.

References

1. Roivainen M, Viik-Kajander M, Palosuo T, et al. (2000) Infections, inflammation, and the risk of coronary heart disease. *Circulation* 101: 252-257.
2. Denes A, Humphreys N, Lane TE, et al. (2010) Chronic Systemic Infection Exacerbates Ischemic Brain Damage via a CCL5 (Regulated on Activation, Normal T-Cell Expressed and Secreted)-Mediated Proinflammatory Response in Mice. *J Neurosci* 30: 10086-10095.
3. Imig JD, Ryan MJ (2013) Immune and Inflammatory Role in Renal Disease. *Compr Physiol* 3: 957-976.

4. Momiyama Y, Ohmori R, Fayad ZA, et al. (2012) Associations between serum lipoprotein(a) levels and the severity of coronary and aortic atherosclerosis. *Atherosclerosis* 222: 241-244.
5. Kalaivani V, Appukuttan PS (2015) Plasma lipoprotein(a) size polymorphism and function: apo(a) subunit size determines galectin-1 recognition and apoB subunit epitope masking. *Open J Biochem* 2015: 22-33.
6. Pepin JM, O'Neil JA, Hoff HF (1991) Quantification of apo[a] and apoB in human atherosclerotic lesions. *J Lipid Res* 32: 317-327.
7. van Dijk RA, Kolodgie F, Ravandi A, et al. (2012) Differential expression of oxidation-specific epitopes and apolipoprotein(a) in progressing and ruptured human coronary and carotid atherosclerotic lesions. *J Lipid Res* 53: 2773-2790.
8. Krebs P, Scandella E, Bolinger B, et al. (2007) Chronic Immune Reactivity Against Persisting Microbial Antigen in the Vasculature Exacerbates Atherosclerotic Lesion Formation. *Arterioscler Thromb Vasc Biol* 27: 2206-2213.
9. Kabat EA, Berg D (1953) Dextran; an antigen in man. *J Immunol* 70: 514-532.
10. Geetha M, Annamma KI, Mathai J, et al. (2007) Normal human plasma anti-beta-glucoside antibody has markedly elevated IgA content and binds fungal and yeast polysaccharides. *Immunol Invest* 36: 73-83.
11. Tsuda H, Yamashita Y, Toyoshima K, et al. (2000) Role of serotype-specific polysaccharide in the resistance of *Streptococcus mutans* to phagocytosis by human polymorphonuclear leukocytes. *Infect Immun* 68: 644-650.
12. Sabarinath PS, Appukuttan PS (2015) Immunopathology of desialylation: human plasma lipoprotein(a) and circulating anti-carbohydrate antibodies form immune complexes that recognize host cells. *Mol Cell Biochem* 403:13-23.
13. Sandrin MS, Vaughan HA, Xing PX, et al. (1997) Natural human anti-Gal alpha(1,3)Gal antibodies react with human mucin peptides. *Glycoconj J* 14: 97-105.
14. Geetha M, Kalaivani V, Sabarinath PS, et al. (2014) Plasma anti-α-galactoside antibody binds to serine- and threonine-rich peptide sequence of apo(a) subunit in Lp(a). *Glycoconj J* 31: 289-298.
15. Mandagini G, Subramanian SP, Vasantha K, et al. (2013) Human plasma anti-α-galactoside antibody forms immune complex with autologous lipoprotein(a). *Immunol Invest* 42: 324-340.
16. Appukuttan PS, Surolia A, Bachawat BK (1977) Isolation of two galactose-binding proteins from Ricinus communis by affinity chromatography. *Indian J Biochem Biophys* 14: 382-384.
17. Paul A, Antony M, Mathai J, et al. (2011) High polymeric IgA content facilitates recognition of microbial polysaccharide-natural serum antibody immune complexes by immobilized human galectin-1. *Immunol Lett* 136: 55-60.
18. Baues RJ, Gray GR (1977) Lectin purification on affinity columns containing reductively aminated disaccharides. *J Biol Chem* 252: 57-60.
19. Weir DM (1986) *Handbook of experimental immunology*. Blackwell Scientific Publications, Oxford; Boston.
20. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254.
21. Travis J, Bowen J, Tewksbury D, et al. (1976) Isolation of albumin from whole human plasma and fractionation of albumin-depleted plasma. *Biochem J* 157: 301-306.
22. Hudson L, Hay FC (1980) *Practical immunology*, 2. ed. Blackwell, Oxford.

23. George G, Geetha M, Appukuttan PS (2015) Antigen-Induced Activation of Antibody Measured by Fluorescence Enhancement of FITC Label at Fc. *J Fluoresc* 25: 1493-1499.
24. George G, Antony M, Mathai J, et al. (2016) Periodontitis and Inflammation: Plasma High Titer Naturally Occurring Anti-Glucan Antibodies Form Immune Complex with *Streptococcus mutans* Antigen. *Mod Res Inflamm* 05: 45-54.
25. Galili U, Korkesh A, Kahane I, et al. (1983) Demonstration of a natural antigalactosyl IgG antibody on thalassemic red blood cells. *Blood* 61: 1258-1264.
26. Gupta D, Kaltner H, Dong X, et al. (1996) Comparative cross-linking activities of lactose-specific plant and animal lectins and a natural lactose-binding immunoglobulin G fraction from human serum with asialofetuin. *Glycobiology* 6: 843-849.
27. Dong X, Andre S, Hofer B, et al. (1997) Disease type-associated increases of the plasma levels and ligand expression for natural alpha- or beta-galactoside-binding immunoglobulin G subfractions in patients with lung cancer. *Int J Oncol* 10: 709-719.
28. Roozbeh J, Merat A, Bodagkhan F, et al. (2011) Significance of serum and urine neuraminidase activity and serum and urine level of sialic acid in diabetic nephropathy. *Int Urol Nephrol* 43: 1143-1148.
29. Colwell JA, Lopes-Virella M, Halushka PV (1981) Pathogenesis of atherosclerosis in diabetes mellitus. *Diabetes Care* 4: 121-133.
30. Sheela B, George G, Mandagini G, et al. (2016) Plasma anti-α-galactoside antibody mediates lipoprotein(a) binding to macrophages. *Glycoconj J* 33: 953-961.
31. Kronenberg F, Kronenberg MF, Kiechl S, et al. (1999) Role of lipoprotein(a) and apolipoprotein(a) phenotype in atherogenesis:prospective results from the Bruneck study. *Circulation* 100: 1154-1160.
32. Kalaivani V, Appukuttan PS (2014) Circulating Lp(a):LDL complexes contain LDL molecules proportionate to Lp(a) size and bind to galectin-1: a possible route for LDL entry into cells. *Lipids* 49: 1101-1113.

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