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New Biological Functions of Human Serum IgA

QUANG L. NGUYEN, RENATA S. LEITE AND ROBERT J. BOACKLE
Medical University of South Carolina, Charleston, USA

MUSC MEDICAL UNIVERSITY OF SOUTH CAROLINA

Abstract

Human IgA does not effectively activate the Classical Complement Pathway in undiluted serum. However, serum IgA and IgG antibodies do co-deposit on suspected periodontal pathogens. Interestingly, only negative-functions are known for serum IgA affects on IgG in complement-mediated elimination of antigens.

Objective: To determine a positive complement-mediated function for human serum IgA antibodies, which co-deposit alongside human IgG antibodies.

Methods: Dansylated human serum albumin (DHSA) was used as an immobilized antigen in ELISA. Increasing doses of IgA1 antibody with constant IgG1 antibody (both were human-mouse chimeric antibodies with identical mouse Fv) were added to the immobilized antigens. As a comparison, doses of myeloma IgA1 were directly (irreversibly) co-deposited onto microtiter plates with a constant pre-titrated dose of myeloma IgG1. In both cases, relatively undiluted fresh normal human serum (neat serum or 1:3 in barbital buffered saline, BBS^{++}) as a source of human complement was applied for 30 minutes at 37ºC. Complement deposition (C4b and C3b) and IgA1 and IgG1 deposition were quantified.

Results: In the directly immobilized approach, myeloma IgA1 functioned as a strong bystander acceptor of C4b and C3b. As the amount of IgA1 increased, the level of C4b and C3b deposition also increased. However, in experiments using antibodies and immobilized antigens (immune complexes), the level of C4b deposition decreased as the level of co-deposited IgA1 antibodies increased. Probing the residual (bound) IgA1 and IgG1 confirmed that after IgA1 accepted the C4b and C3b, the complement-coated IgA1 departed from the immobilized antigen (DHSA). In addition, by intercepting the C4b and C3b (generated via IgG1-mediated complement activation), the bound IgA1 antibodies protected the antigen-binding function of IgG1 and allowed IgG1 antibodies to remain bound to the immobilized antigen.

Conclusions: Co-deposited IgA1 functioned as an excellent bystander interceptor for C4b and C3b, then departed from the antigen. Even though IgA1 did not effectively activate the Classical Complement Pathway, it prolonged the antigen-binding function IgG1 by slowing C4b and downstream C3b deposition on IgG1 and on the antigen.

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Goals

1. Long range goal: to develop an active and passive immunization against the periodontal disease.

2. Immediate goal: to understand the functions of serum IgA when it co-deposits with IgG.

Overall Objective

To study functions of human serum IgA when it co-deposits with IgG onto antigens (e.g., in periodontal disease and other mucosal infections) using two different approaches:

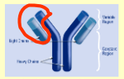
1. Irreversibly Bound, e.g., Directly Immobilized: Different ratios of IgA1 to IgG1 antibodies were directly co-immobilized on microtiter plates.

2. Reversibly Bound, e.g., Immune Complexes: Different ratios of IgA1 to IgG1 antibodies were co-deposited onto immobilized antigen, DHSA.

Definitions

Antigen: Dansyl on human serum albumin (DHSA)

Antibodies: Human-mouse chimeric IgA1 and IgG1 with identical Fv for Dansyl (DHSA) antigen.



Complement: Inactive components that exist in the serum. When these components are converted to their active form, a sequential, rapid, cascading sequence ensues. Use of relatively undiluted serum reduces or prevents the background deposition by antigen alone.

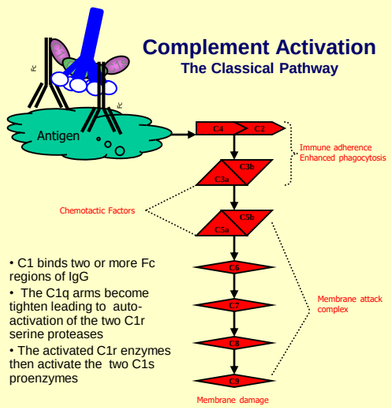
The Classical Pathway

The Lectin Pathway

The Alternative Pathway

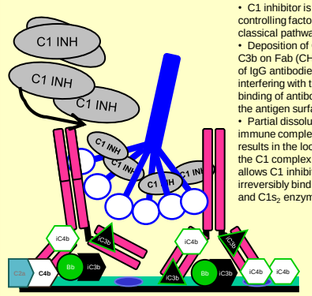
Complement Activation

The Classical Pathway



• C1 binds two or more Fc regions of IgG
• The C1q arms become tighten leading to auto-activation of the two C1r serine proteases
• The activated C1r enzymes then activate the two C1s proenzymes

Possible Control Mechanism



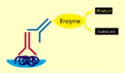
• C1 inhibitor is the major controlling factor for the classical pathway.
• Deposition of C4b and C3b on Fab (CH1) region of IgG antibodies interfering with the binding of antibodies to the antigen surface.
• Partial dissolution of the immune complexes results in the loosening of the C1 complex and allows C1 inhibitor to irreversibly bind to $C1r_2$ and $C1s_2$ enzymes

Methods

Directly Immobilized immunoglobulins: Different ratios of human IgA1 to IgG1 were directly immobilized to microtiter plates. Directly Immobilized immunoglobulins can not be released.

Immune Complexes: Dansylated human albumin was used as the immobilized antigen. Different ratios of IgA1 to IgG1 antibodies were added to the immobilized antigen. Under these conditions, antibodies can be released from the antigen after complement deposition.

In both methods, human serum (1:3 dilution) served as a source of complement.

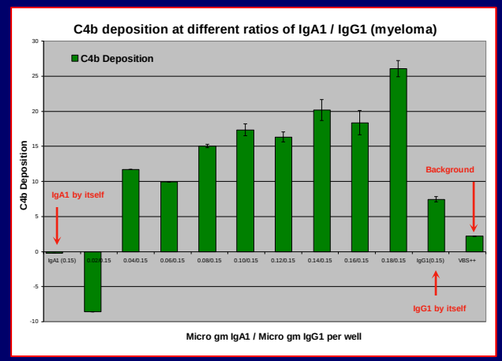


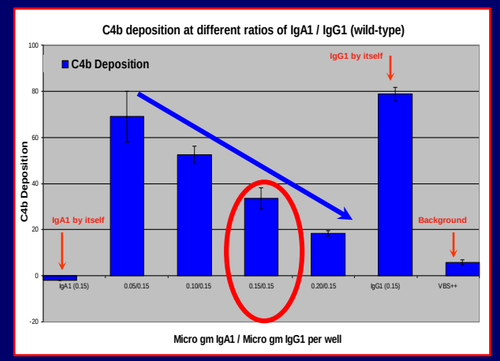
After 30 minutes incubation at 37ºC, levels of C4b and C3b deposition were probed using sheep anti-human C4 and C3 as the primary antibody and anti-sheep immunoglobulins (peroxidase labeled) as the secondary antibody in a kinetic ELISA.

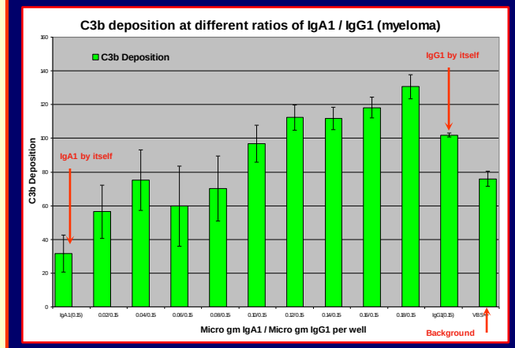
In addition, the residual chimeric IgA1 and IgG1 antibodies were simultaneously probed using sheep anti-human IgA-HRP-labeled (alpha-chain specific) and goat anti-human IgG-HRP-labeled (gamma-chain specific).

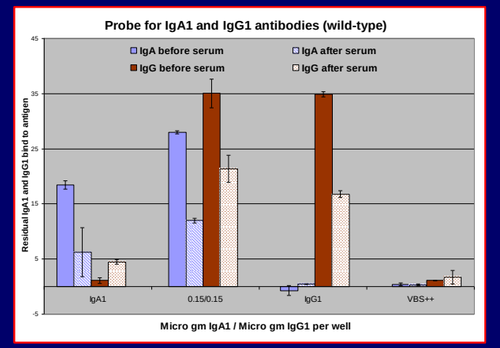
Results

Directly Immobilized Immunoglobulins



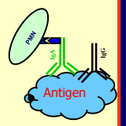




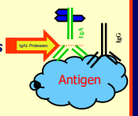


Discussion

1. Serum IgA1 antibodies functioned as excellent acceptors for C3b and C4b. Complement-coated IgA1 may adhere to PMNs and macrophages in infected tissues (PMNs and macrophages have receptors for complement).



2. Periopathogens like *Porphyromonas gingivalis* produce IgA1-specific proteases, which remove of the Fc region of serum IgA1



3. IgA1 regulated complement consumption. Serum IgA antibodies may help control the inflammatory process.

4. IgA1 protected the function of IgG1. It prolonged the binding of IgG1 to the antigenic surface.

Conclusions

1. Serum IgA1 functioned as an excellent acceptor for C3b and C4b. It slowed complement consumption.

2. After IgA1 accepted complement, it was released from the antigen surface.

3. IgA1 intercepted C3b and C4b and thereby prolonged the binding of co-deposited IgG on the antigen surface.

Future Plans

1. To investigate the function of complement coated IgA1 after it departs from the antigen surface.

2. To study the complement deposition (C3b & C4b), at different ratios of IgA1 to IgG1 in the presence of C1-Inhibitor (the main factor controlling of the level of C1-mediated C4 deposition).