Polymeric Plasma IgA Recovered from Cohn Fraction III Precipitate Binds Recombinant Human Secretory Component

Estelle F. Chiari, Ph.D.[1], Nadia Petlakh, Ph.D. [1] Stephen C. Brown, Ph.D.[1], Michael R. Simon, M.D.[1,2,3]

1.Secretory IgA, Inc., Ann Arbor, Michigan
2. Allergy and Immunology Section, William Beaumont Hospital, Royal Oak, Michigan
3. Departments of Internal Medicine and Pediatrics, Wayne State University School of Medicine, Detroit, Michigan.

Abstract

Rationale: Cohn fraction III precipitate (CFxIII-ppt) is a discarded byproduct of the recovery of IgG from pooled donor plasma using cold ethanol fractionation. We propose that recovered IgA has the potential to be a new orally administered immunoglobulin therapy.
Methods: Water content of CFxIII-ppt was determined by weighing wet and then dried material. IgA was recovered from rehydrated CFxIII-ppt by jacalin affinity chromatography. Concentration of IgA was measured using a spectrophotometer. Proportions of monomer, dimer and polymer in IgA were determined by analyzing size exclusion chromatograms. ELISA demonstrated the association of J chain with IgA dimer and polymer. Immunoblotting showed dimer and polymer binding to recombinant human secretory component (rhSC). Antigenic specificity was determined by ELISA.
Results: : CFxIII-ppt is ~65% water. Up to ~15 mg of IgA is obtained per g of CFxIII-ppt. Recovered plasma IgA is 50%(+/-5.4 SD] monomer, 50%(+/-5.3 SD) dimer and higher polymers (n=7). The IgA dimer and higher polymer fractions contain J chain and readily combine with rhSC to form dimeric and polymeric semisynthetic secretory IgA. We previously reported that IgA recovered from CFxIII-ppt binds to *Clostridium difficile* toxins A and B as well as peanut extract. We now extend the known antigenic specificity of recovered IgA to include Campylobacter.
Conclusion: : Plasma IgA from CFxIII-ppt is slightly less than 50% polymeric IgA (dimer plus larger polymers). Higher IgA polymer as well as dimer can be converted into secretory IgA. Pooled plasma contains IgA with reactivity to Campylobactor.

Rationale

Cohn fraction III precipitate (CFxIII-ppt)(1) is a discarded byproduct of the recovery of IgG from pooled donor plasma using cold ethanol fractionation. Tons are discarded annually.

The antigenic specificity of plasma IgA is similar to that of circulating IgG. Plasma IgA binds to bacterial antigens *(e.g. C. difficile toxins* and *Shigella)*(2-3), and can neutralize food antigens and prevent the initiation of food antigen-induced mast cell and basophil activation(4-6).

Recombinant human secretory component combines with IgA dimers (3). Naturally occurring secretory IgA formed from higher polymers has recently been described (7) but the in vitro combination of secretory component with higher polymers has not been reported.

We propose that recovered IgA has the potential to be a new orally administered immunoglobulin therapy.

Objectives

- **Demonstrate the proportions of monomer, dimer and higher polymers in human plasma derived IgA recovered from frozen Cohn fraction III precipitate.**
- **Demonstrate that recombinant human secretory component combines with higher IgA polymers to form secretory IgA.**
- **Extend the known antigenic specificities of pooled plasma IgA.**

Methods I

- **IgA from Cohn fraction III precipitate (1), a byproduct of IVIG production from the pooled plasma of up to 5000 donors.**
- **Suspended in PBS; Viral inactivation by solvent—detergent (1% tri (N butyl) phosphate, 1% Triton X-100) treatment (2).**
- **IgA was isolated by jacalin affinity chromatography (2).**
- **Separation of IgA monomer, dimer and polymer by size exclusion chromatography using a GE HiLoad 16-600 Superdex 200 column on an FPLC (BioCAD Workstation, Applied Biosystems) system.**
- **Recombinant human secretory component (University of Michigan High Throughput Protein Lab) is added to IgA polymer in PBS which then forms polymeric secretory IgA.**

Methods II

- **Chromatography: Size exclusion chromatography was accomplished using a GE HiLoad 16-600 Superdex 200 prepacked column on a BioCAD Workstation for perfusion chromatography (Applied Biosystems).**
- **ELISA: High binding ELISA plates (Microfluor 2) coated with 1 μg/mL anti-IgA or anti kappa, blocked with BSA block buffer or Superblock (Thermo); HRP-conjugated goat anti-human IgA (Zymed); fluorogenic Amplex Red (Invitrogen) used for detection. Plates were read on a fluorescent plate reader (F-Max, Molecular Devices). *Campylobacter jejuni* antigen (Clin Exp Immunol 2000;122:55-60) and rabbit hyperimmune serum was generously provided by Shaun Cawthraw (Animal and Plant Health Agency, Weybridge, UK).**
- **Immunoblot: Protein samples were mixed with 2× SDS-sample buffer (Nupage), warmed to 70ºC for 10 min before being subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred to 0.22 μm membranes. Membranes were then blocked in 5% nonfat dry milk and incubated with antibody against secretory component [ABIN, 457936, Antibodies On-Line] and secondary antibody [Anti-Mouse HRP, NA931V) or the directly conjugated goat-anti-human IgA-HRP [Zymed]. Chemiluminescence reagents were used for signal detection.**

Results

CFxIII-ppt is ~65% water. Up to ~15 mg of IgA is obtained per g of wet CFxIII-ppt (data not shown). Recovered plasma IgA is 50% (+/-5.36% SD] monomer, 50% (+/-5.3%SD) dimer and higher polymers (n=7).

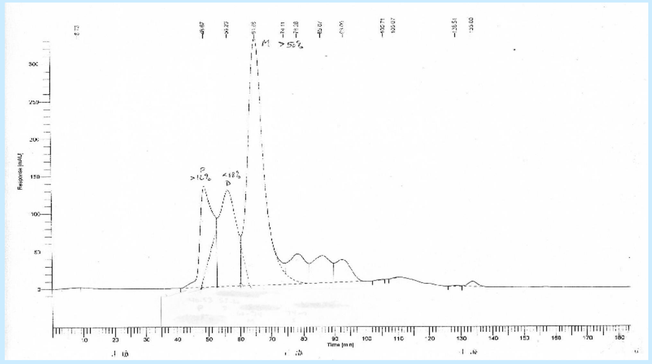

Fig 1. Chromatogram showing IgA polymer (P >16%), dimer (D <18%) and monomer (M >50%) peaks.

Results (continued)

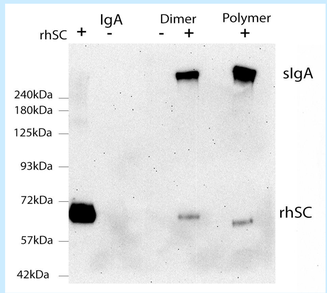

Figure 2. Immunoblot showing recombinant human secretory component binding to IgA dimer and polymer.

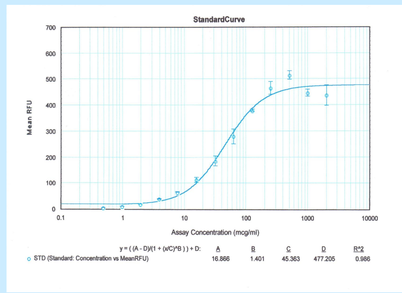

Figure 3. Polyclonal plasma IgA binds to Campylobacter antigen.

Discussion

- **Naturally occurring polymeric secretory IgA has been reported in human mucosa (7).**
- **Secretory IgA can be synthesized from dimeric plasma IgA and recombinant human secretory component (2, 3).**
- **We accomplished the synthesis of semi-synthetic polymeric secretory IgA using recombinant secretory component and IgA polymer in human plasma.**
- **We found that the proportion of IgA dimer and polymer recovered from a Cohn fraction III plasma precipitate is significantly greater than previously reported proportions in plasma.**
- **Secretory IgA_1 is resistant to digestion (3)**
- **We hypothesize that this semisynthetic secretory IgA can be used as an orally administered passive immunization.**

References

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