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Description and characterization of a unique human immunoglobulin G1 kappa idiotype found in placental tissue

Craig D. Scoville [a, *], Devon Rasmussen [b]

[a] Institute of Arthritis Research, 2220 East 25th St, Idaho Falls, ID 83404, USA
[b] Idaho State University, Department of Biology, 921 South 8th Avenue, Pocatello, ID 83201, USA

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ABSTRACT

Does maternal IgG found in placental tissue provide the fetus with more than just humoral immunity? To address this question, the IgGs from twelve placentas were studied and four of these samples were examined using mass spectrometry which revealed an IgG1k idiotype. A special dodecapeptide portion of the 3rd framework region of the VH chain sequence was identified as an idiotypic determinant in these placental- IgG1k (p-IgG1k) and referred to as peptideX2 and found to have biological activity. Antiserum to peptideX2 was made and then used with Western Immunoblotting to show that this unique H chain (containing peptideX2) appears to be present in all p-IgG tested and in all subjects tested. It appears that the placenta contains not only conventional polyclonal maternal IgGs but also an idiotypic population of maternal IgG1k which binds to TLR2>TLR4 via the epitope "peptideX2″ and promotes IL-6, TNFα, and IL-10 production and may play a role in maternal-fetal tolerance.

1. Introduction

The maternal-fetal relationship during pregnancy continues to challenge and enlarge our understanding of the immune system. Several placental factors play a role in this immune-tolerant condition inclusive of HLA-G [1–3], a bias toward type 2 over type 1 cytokines via TH2/TH1 cells [4,5] and NK cells [6,7], the possible role of IL-17 [8], the role of Treg cells [9,10], and the humoral immune response [11]. The work of Liu and Lu [11] demonstrated that placenta eluted gamma globulins (PEGG) have a potent effect against graft versus host reactions *in vitro* and *in vivo*. Although the exact nature of these immunoglobulins was not identified in their work, it is proposed that a subset of placental immunoglobulins may play an important role in this maternal-fetal interface.

The placenta shows preferential transport of maternal immunoglobulins allowing IgG1> IgG4/IgG3>IgG2, and not the other immunoglobulin classes [12–14]. This active transport occurs by selective binding of the maternal IgG to specific neonatal Fc receptors (FcRn) on the surface of syncytiotrophoblasts. The maternal antibodies are internalized and transported within coated vesicles to protect from proteolysis, and then released into the fetal circulation [14–15]. There appears to be preferential escorting of certain antibodies noting that antibodies reactive to viral proteins and anti-toxins tend to be IgG1, whereas antibodies to encapsulated bacteria tend to be IgG2 [14,15]. This selective transport of IgG1 antibodies over other subclasses of IgG may be driven by natural selection for something unique about IgG1 more than just the antigen binding potential of the antibodies.

To better understand the nature of these placental IgG proteins, this work analyzed the primary structure of these antibodies in selected placentas and discovered a unique placental- IgG1k (p-IgG1k) idiotype. This report describes a special dodecapeptide sequence found in the 3rd framework area of the VH (variable heavy) chain, called peptideX2, which represents an idiotypic determinant of p-IgG1k, and shows biological activity. This report suggests that the preferential polyclonal IgG1 transport may be due to selecting a unique maternal IgG1k idiotype in order to possibly contribute to the maternal-fetal tolerance during pregnancy.

2. Materials and methods

2.1. Placental derived IgG preparation

Twelve placentas were obtained with appropriate informed consent. Of the twelve subjects, nine were healthy women (identified as MP, KP, CB, MC, CH, SB, JC, BC, RA), two (MM and KS) had

* Corresponding author.
E-mail addresses: cdscoville@yahoo.com (C.D. Scoville), rasmdevo@isu.edu (D. Rasmussen).

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rheumatoid arthritis, and one (BV) had gestational diabetes. All subjects delivered at term except for BV who delivered at 35.2 weeks. Preparing and processing each placenta has already been described in detail [16]. Briefly, each placenta was washed, homogenized, centrifuged, and then the tissue component obtained, prepared in a hypotonic solution, sonicated, centrifuged, and then the cell lysate made 1:10,000 merthiolate (v/v) and stored at −20 °C. Then each placental lysate sample was thawed and made 30 mM EDTA, treated with DNase/RNase, centrifuged, filtered, and then applied to HiPrep 26/60 Sephacryl S-200 HR column at flow rate 1.0 ml/min in 0.05 M phosphate, 0.15M NaCl buffered saline, pH 7.8, and then collected fractions from 132 to 150 min representing the placental IgG (p-IgG) fractions, then treated to protein A column, the IgG eluted, and desalted and concentrated. These procedures were performed on all twelve placental samples.

2.2. SDS-PAGE and Western Immunoblotting and protein identification

In preparation for SDS-PAGE, the final p-IgG samples were made 1:5 HES buffer (10 mM HEPES, 10 mM EDTA, 250 mM sucrose), then added reducing lane marker solution (Thermo Scientific Pierce). Samples were boiled, and then aliquots layered onto 8% or 10% precast Precise Tris-HEPES gels (Thermo Scientific Pierce) for performing SDS-PAGE, then stained with Imperial Protein Stain (Thermo Scientific Pierce) to show H (heavy) and L (light) chains. The H and L bands of four out of the twelve placental samples (KS, RA, MP, and BC) were carefully excised and sent for proteomic analysis by ProtTech Inc (Norristown, PA) using the proprietary technique and mass spectrometry which has been previously reported [16].

N-terminal amino acid sequencing of specific H chains was done by first doing SDS-PAGE under reducing conditions on a given p-IgG, identifying the H band, transferring the H chain to PVDF, staining with MemCode Reversible Protein Stain Kit for PVDF membrane (Thermo Scientific Pierce), and then the H bands were cut out and sent to Alphalyze (Palo Alto,CA) for N-terminal amino acid analysis.

2.3. Peptide preparation

Two different dodecapeptide sequences from the reference H chain AAH90938.1 were commissioned to be made as lyophilized products (GenScript USA, Inc, Piscataway, NJ) to test for biological activity. One dodecapeptide, called peptideX2, represents a portion of the 3rd framework region of the reference protein AAH90938.1 and consists of the sequence KSIAYLQMNSLK (sequence 97–108). Another dodecapeptide, called peptideX3, represents a portion of the 2nd framework region of the reference protein AAH90938.1 and consists of the sequence RQAPGKGLEWVG (sequence 57–68).

A modification of peptideX2 was prepared by adding a cysteine to the COOH terminus of peptideX2 and then this product was conjugated via the cysteine either to keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA). The KLH-peptideX2 was tested in a cytokine assay to determine if the biological activity was destroyed by conjugating peptideX2 at the COOH end. The product peptideX2 and any modification of this product are proprietary and patent protected (US App. Pub. No. US2014/271,652).

2.4. Cytokine analysis

Samples of solubilized peptideX2 (0.1M Tris, 1% acetic acid, pH 7) were sent to Viracor-IBT (Lee's Summit, MO) for cytokine analysis using human peripheral whole blood cells. This procedure involved incubating the product with the whole blood cells of a given donor (commissioned by Viracor-IBT) for 22–24 h and then tested for cytokines; or incubated for 18 h and then stimulated with 1 μg/ml of PHA (phytohemagglutinin) for four more hours and then tested for cytokines. Samples were tested in duplicates for IL-2, IL-4, IL-6, IL-10, IL-12, and TNFα using Meso Scale Discovery (MSD) panel. Viracor-IBT used three different human donors known as #67, #68, and #85 in performing these experiments. Four other donors (#1–4) were also recruited to donate peripheral white blood cells to be incubated for 24 h in the presence of various concentrations of peptideX2 and peptideX3 (solubilized in 0.2% acetic acid, Dulbecco's phosphate buffered saline (DPBS), pH 6) and tested for cytokine production at the Institute of Arthritis Research.

2.5. Human TLR-receptor testing

PeptideX2 (at 200 μg/ml in 0.2% acetic acid, DBPS, pH 6) was sent to an outside laboratory, InvivoGen, San Diego, California, for human Toll-like receptor (TLR) ligand screening. The procedure used by InvivoGen consists of testing for NF-kB activation in HEK293 cells expressing a given TLR. The samples were tested in duplicates against seven different human TLRs (TLR2, 3, 4, 5, 7, 8, and 9). The media added to the wells is designed for the detection of NF-kB induced SEAP (secreted embryonic alkaline phosphatase) expression. After 16–24 h of incubation, the optical density (OD) is read at 650 nm on a Molecular Devices SpectraMax 340PC absorbance detector.

2.6. Rabbit immunization with KLH-peptideX2

Two New Zealand white rabbits were immunized with 2 mg of KLH-peptideX2 mixed with TiterMax (Sigma Aldrich) and followed by booster injections of 2 mg of KLH-peptideX2 with TiterMax 30 days later. Serial bleedings occurred over sixty days and the serum was collected and pooled, and then passed over a protein A column, the rabbit polyclonal IgG collected, concentrated, and stored at −70° C.

3. Results regarding the H and L chains of a placental immunoglobulin G

3.1. Characterization of placental-IgG1k

Placental IgGs (p-IgG) were obtained from all twelve of the placental donors as described in Methods. In four of the placental donors (RA, BC, MP, and KS) the respective H and L bands were excised and sent for mass spectrometry and protein identification and cross matching with other protein sequences in the NIH protein data bank (Table 1a,b). The H chain sequencing data obtained by ProtTech referenced the following BLAST accessions numbers (showing the most “hits”) for the following H chains: KS (AAH69016.1), MP (AAH41037.1), BC (AAH41037.1), RA (AAH69016.1). However when these reference proteins were compared with other IgG H chains in the protein databank (especially in the sequence range 20–38, and 134–140), the best matching with the H chains tested was found to be AAH90938.1 – an IgG1 H chain (see Table 2). The sequences found on mass spectrometry are nearly identical to this reference protein's sequences 20–38, 134–157, 298–312, 325–344, 341–348, 369–384, and 395–433. Also when comparing AAH69016.1 and AAH41037.1, these reference proteins were identical (100% homology) to the H chain sequence of AAH90938.1 (IgGH1) from sequence 139–471 – meaning all these reference proteins are IgG1 heavy chains. The L chains were also tested and only kappa chains were identified (see Table 1b) – even as L chain contaminants in the H chains tested. These results showed an IgG1k subpopulation.