January 2019

Immunoglobulin D Undergoes Placental Transfer To The Fetus

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IMMUNOGLOBULIN D UNDERGOES PLACENTAL TRANSFER TO THE FETUS

by

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THESIS

Submitted to the Graduate School

of Wayne State University,

Detroit, Michigan

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

2019

MAJOR: IMMUNOLOGY & MICROBIOLOGY

Approved By:

_____

Advisor Date

ACKNOWLEDGMENTS

There are plenty individuals in my life that deserve to be acknowledged for their support during the pursuit of my graduate studies. First and foremost, I would like to thank my mentor, Dr. Kang Chen, who has been a superlative role model to me. He has provided me with phenomenal insight throughout the entirety of my research project. I cannot thank him enough for all his time and effort that was devoted towards my graduate studies. Additionally, I would like to thank all members of the Chen lab, past and present, who have also taught me and helped me along the way to become a better researcher. Thanks to Dr. Azure Faucette and Dr. Bo Pei, who provided me with an exceptional base of research knowledge that allowed me to progress my project to completion. Also, I would like to sincerely thank Dr. Bihui Huang who played a crucial role in teaching me and providing me with guidance in the lab during my graduate studies. He is a great friend and colleague who I could always go to with questions. I cannot thank him enough for all of his time and effort that was devoted towards my experiments. I would also like to thank my fellow graduate students in the Biochemistry, Immunology, and Microbiology department for their friendship.

Last but certainly not least, I would like to thank my family who has provided me with tremendous support during this wonderful experience. My parents, David and Sandra Pawlitz, have continuously provided me with encouragement throughout my entire life to follow my heart and pursue my dream of becoming a scientist. Not only have they supported me with encouragement, but they have also provided a roof over my head and an endless supply of food for me to eat, which I will be eternally grateful

for. My brother and sister, Sean and Carly Pawlitz, also deserve an acknowledgement for constantly pushing me to be my best.

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GENERAL INTRODUCTION

History of IgD

Dr. David Rowe, MD and Dr. John Fahey, MD were studying the disease multiple myeloma in humans in 1964 when they made a fantastic discovery. During analysis they were able to identify and characterize an atypical myeloma protein that displayed no cross reactivity to the anti-serum of the currently known human immunoglobulin classes (IgM, IgG, IgA). This atypical myeloma protein had unique metabolic and electrophoretic qualities in comparison to the other known Ig classes (1). Following up on their initial discovery, an antigenically related protein in the serum of healthy individuals was identified in lower concentration than the myeloma patients that did not share the antigenic characteristics of the other Ig classes. This suggested to Rowe and Fahey that the newly discovered protein was not a subclass of the currently known Ig classes or abnormal product of the myeloma cells but was in fact a novel Ig class that displayed distinct properties in the heavy polypeptide chains. Because these uniquely distinctive properties were enough evidence to differentiate the novel protein from the currently known Ig classes both Rowe and Fahey thought it was appropriate that the protein should be named IgD (2). While Rowe and Fahey did have limited options for picking the name of the new Ig class, the name IgD was a good choice for this antibody because in addition to being distinctive from the other Ig classes, this protein also displays a large amount of diversity in glycosylation between groups of individuals as well as within single individuals.

Following its initial discovery, further studies were conducted during the late 1970's and early 1980's to identify the other species that also contained IgD. Initially the research teams were able to detect IgD in primates, mice, rats, dogs, guinea pigs and

rabbits but were not able to locate any IgD in birds or pigs (3-6). However, due to the technological advances that have accompanied the course of time, scientists have been able to identify a wide variety of additional species with IgD including: pigs, sheep, cattle, amphibians, reptiles, birds, bony fish, cartilaginous fish. The oldest of the species that express IgD are the cartilaginous fish which first appeared on earth about 470 million years ago. The continued preservation of IgD throughout evolution solidifies that its presence plays a role in critical biological functions and provides survival advantages to the host (7-24).

Induction and Expression of IgD

Immunoglobulin D (IgD) is an enigmatic protein that is solely produced by the B cells of the adaptive immune system. IgD is not produced throughout the entirety of B cell development and our B cells utilize multiple strategies to induce its expression. During the early stages of B cell development, the only immunoglobulin that is expressed is IgM. B cells first begin to express IgD when the immature B cells begin to exit the bone marrow in search of the secondary lymphoid organs. Upon egression from the bone marrow into the periphery, B cells utilize alternative RNA splicing to co-express both IgM and IgD on the cell surface with IgD being expressed at a higher level than IgM (25). This co-expression of IgM and IgD is maintained in both transitional and mature B cells until the cells experience antigenic stimulation. Following antigenic stimulation, most mature B cells will begin to quickly downregulate their surface IgD expression and undergo a process known as class switch recombination (CSR) in order to solely express either IgG, IgA or IgE depending on the antigenic signals they received. While most of the mature B cells will undergo CSR to express IgG, IgA and

IgE, some of the mature B cells will in fact undergo CSR to only express IgD. These class switched B cells will eventually differentiate into cells known as plasmablasts. During differentiation into plasmablasts, the class switched B cells will down regulate their respective membrane bound immunoglobulins from the cell surface. After differentiation into plasmablasts is completed, the cells will begin to secrete large quantities of their respective immunoglobulins into circulation, where the antibodies will carry out various effector functions (26).

The co-expression of IgM and IgD by alternative RNA splicing is a strictly monitored event by the B cell. Initially, a long primary mRNA transcript will be generated that contains the rearranged VDJ exons, the C_μ exons and the C_δ exons. If the long primary mRNA transcript is spliced to the first C_μ exon, it will eventually result in the production of IgM. If the long primary mRNA transcript is spliced to the first C_δ exon, it will eventually result in the production of IgD (27). The amount of μ and δ exon transcription does not always correlate to the cell surface expression levels of IgM and IgD. It has been observed in some types of B cells that even though there are more μ mRNA transcripts than there are δ mRNA transcripts, there is still higher protein expression of IgD at the cell surface than the IgM protein. This is thought to be the result of a higher turnover rate of membrane bound IgM compared to the membrane bound IgD turnover rate. Additionally, it is thought that there is an increase in the δ mRNA stability and an increase in the efficiency of δ mRNA translation when compared to μ mRNA (25, 28, 29). Conversely, in B cells that are experiencing antigenic stimulation, μ exon transcription and alternative RNA splicing to the C_μ exon are specifically upregulated while δ exon transcription and IgD expression is significantly reduced. This can readily be observed by the lack of IgD expression canonically found

in the germinal centers of secondary lymphoid tissues. Additionally there are publications that have identified a unique region between the μ and δ genes which has the ability to impair the transcription complex before reaching the δ exons, thus specifically promoting the production of μ mRNA, completely halting the production of δ mRNA and ultimately leading to an exclusive increase in membrane bound IgM expression (30, 31).

The expression of IgD by CSR is unique in comparison to the other antibody isotypes. There is not a typical switch region located 5' to the C_δ exons like there is for the other antibody classes which originally led to the belief that IgD CSR was a rare event, however a study utilizing normal human tonsil B cells was able to identify a G-rich pentameric repeat region between the C_μ exons and the C_δ exons that can act as an atypical switch region to facilitate IgD CSR and C_μ exon deletion (32). These results provided the information necessary to contradict the previous belief that IgD CSR was a rare event, however the overall regulation of IgD CSR was still poorly understood until Chen et al. was able to shed a huge amount of light on the mystery (33).

They were able to identify both T-cell dependent and T-cell independent pathways for B cell IgM to IgD CSR in the human upper respiratory mucosa by showing that B cells required CD40L, B-cell activating factor (BAFF), or a proliferation-inducing ligand (APRIL) signals, along with the cytokine signal combination of IL-15 and IL-21 or IL-2 and IL-21 to undergo IgM to IgD CSR. Furthermore, they demonstrated that the B-cell protein activation-induced cytidine deaminase (AID) was required for IgM to IgD CSR to occur and that AID activity resulted in the production of both mucosal and circulating IgD plasmablasts that secrete IgD (33). These were ground breaking results for the research field of immunology.

Structural Characteristics of IgD

IgD has several unique characteristics that differ from the typical antibody structure of other Ig isotypes. Human IgD consists of 2 light chains and 2 heavy chains with there being 3 constant (C_δ) domains in the heavy chains, while mouse IgD contains one less C_δ domain than humans. The human $C_\delta1$ and the $C_\delta2$ domains are very similar to the C domains of other Ig classes, however $C_\delta3$ is distinctive from the others because it contains two noteworthy N-linked glycosylation sites: Asn445 and Asn496 (34). The $C_\delta3$ domain also does not contain several prolines that are typically crucial for turns in the polypeptide backbone. It has been proposed by multiple groups that these features contribute to the unique biological properties of IgD (34-36). Another unique characteristic of IgD is its hinge region. The human IgD hinge region is the longest hinge region of all the human antibody classes. When characterizing the IgD hinge region it can be divided into 2 sections. The first section is a threonine- and alanine-rich section that has a large amount of O-linked glycosylations that contain N-acetylgalactosamine groups within the O-linked glycans. Both the O-linked glycosylations of the hinge region and the N-linked glycosylations of the $C_\delta3$ domain can account for up to 15% of the total molecular weight of IgD. The second section of the hinge region contains a plethora of charged amino acids including serine (Ser), threonine (Thr), glutamatic acid (Glu), and lysine (Lys). This densely charged section of the antibody hinge region is only found in IgD and is largely responsible for the hydrophilic nature of the protein (37-41). Additional IgD hinge region features were elucidated after performing constrained X-ray scattering on the protein. The constrained X-ray scattering revealed that, while the other antibody isotypes adopt a Y-shaped structure, the extended hinge region of IgD promotes IgD to adopt a highly flexible T-

shaped structure. This T-shaped structure allows the Fab fragments of IgD to be extremely mobile/flexible, and the associated motion of this Fab flexibility can shield/hide the Fc fragment (42). These structural characteristics provide valuable insight to the functional capabilities of IgD.

Functions of IgD

For the most part, the functions of IgD largely remain a mystery but some of the functions carried out by IgD have been defined by previous studies. Originally IgD was thought to only function as a transmembrane B-cell antigen receptor. Utilizing transgenic mice, it was demonstrated by Lutz et al. that IgD could largely substitute for the loss of IgM function in B cells and vice versa (43). Although IgD-deficient mice had no observable defect in B-cell function, aside from a slightly delayed antibody response in comparison to wild type mice, they did however have a 30-50% reduction in peripheral mature B cells, indicating that IgD had a large impact on B cell homeostasis (44, 45). Furthermore, while transmembrane IgD and IgM have the same 3 cytoplasmic amino acids they are affiliated with different signaling molecules which may lead to different functions based on different signaling combinations between IgD and IgM (40). Additionally, it has been shown that IgD can be post-translationally linked to transmembrane lipids by a glycosylphosphatidylinositol (GPI) anchor, which ultimately initiate different signaling pathways than transmembrane IgD without the GPI anchor. The GPI-anchored IgD specifically activates cAMP signaling pathways and works together with transmembrane IgM and IgD to commence Ca^{2+}-dependent signaling (46, 47). The functional advantage to having both IgD and IgM with the same antigen specificity on the surface of the same B-cell has yet to be determined, but Loset et al.

has proposed a model that may provide some explanation. Their model suggests that IgD and IgM are preferentially utilized for capturing antigen at different antigen concentrations and this assertion is supported by the solved protein structure of the T-shaped IgD, showing that IgD is better suited to bind antigen at lower concentrations because the Fab fragments have a large amount of flexibility due to the uniquely long IgD hinge region (42, 48).

Over the years, the functions of membrane bound IgD have gained most of the research attention, while the functions of secreted IgD have been largely overlooked. Soon after IgD's discovery the literature suggested that there were some fascinating associations between secreted IgD and the myeloid cell lineage (49-53). Furthermore, an increase in serum concentration of secreted IgD was typically observed in patients suffering from autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (54). Several years later it was shown that not only was their autoreactive IgD in autoimmune diseases, but that autoreactive IgD secreted by IgD class switched B-cells was regularly detectable in healthy individuals (55). This information laid the necessary frame work for Chen and colleagues to discover a major function of secreted IgD. The monumental discovery they made was that circulating secreted IgD enhanced immune system surveillance by binding to a calcium-mobilizing receptor on the surface of basophils. The binding of IgD to basophils enhanced immune system surveillance by inducing basophils to secrete antimicrobial, opsonizing, proinflammatory and B-cell stimulating factors such as: cathelicidin, interleukin 1(IL-1), IL-4, and BAFF. Not only did they show that secreted IgD was capable of actively binding to and communicating with basophils, but they also showed that IgD was capable of binding to and possibly neutralizing the respiratory pathogens: *Moraxella*

catarrhalis and *Haemophilus influenzae* (33). These data combined suggest that secreted IgD may be able to educate basophils on the pathogenic and commensal composition of the respiratory tract. Following the initial monumental discovery that secreted IgD enhanced immune system surveillance by binding to a receptor on the surface of basophils, it was later determined by mass spectrometry that the basophil surface receptor responsible for binding IgD was in fact galectin-9 (56).

Moreover, upon careful examination of the previously published literature it appears that an additional function of secreted IgD was speculated back in 1985. Salonen and colleagues were studying the kinetics of specific IgA, IgD, IgE, IgG and IgM antibody responses to rubella infection when they came across some interesting data. Upon analyzing the serum from a mother who had previously suffered a rubella infection they were able to detect rubella specific IgG and rubella specific IgD in both maternal and umbilical cord blood serum samples (57). This observation suggested that IgD may be capable of crossing the placenta, but Salonen and colleagues did not provide any other data to support this claim and they were never able to definitively prove this speculation.

Maternal Vaccination

Infectious disease continues to be a leading cause of death for children aged 0 to 5years old. The World Health Organization reported in 2014 that almost 30 percent of all deaths worldwide by children under the age of 5 were caused by vaccine preventable disease (58). Vaccination prior to infection is a highly effective way of preventing these tragically unnecessary deaths, however newborn infants do not efficiently mount a protective immune response to many vaccines. This inefficient immune response to

immunization leaves newborns extremely vulnerable until their immune system is developed enough to begin receiving vaccinations (59). As of 2011, the Center for Disease Control began recommending the strategy of maternal vaccination to confront the vulnerability of the neonatal immune system (60). The ideology of maternal vaccination is that by immunizing the mother during gestation, she will effectively mount a vaccine-induced humoral immune response generating vaccine-specific IgG. This vaccine-specific IgG will then be transferred to the fetus in utero, as well as after birth during breastfeeding, thus providing the necessary immune protection to the vulnerable newborn child. Currently the Advisory Committee on Immunization Practices (ACIP) at the CDC highly recommends two immunizations for all pregnant women including the inactivated influenza vaccine (IIV) as well as the Tetanus-diptheria-pertussis (Tdap) vaccine. The maternal immunization of Tdap is universally recommended to all pregnant women because children younger than 6 months old are at the utmost risk for hospitalization and death by *Bordetella pertussis* (61). In the United States it is recommended that mothers be vaccinated for pertussis between 27 and 36 weeks of gestation and there are no reported negative outcomes to the neonate regardless of maternal vaccination schedule (62).

B-cell Changes During Pregnancy

Pregnancy has a profound effect on humoral immunity both systemically and locally within the reproductive tract. Many of these alterations to the humoral immune system have been shown to be facilitated by pregnancy steroid hormones, more specifically estrogen and human chorionic gonadotropin (hCG). Initially it was demonstrated using pregnant mice that the number of early B-cell progenitors within the

bone marrow are significantly reduced, presumably due to the increase in estrogen associated with pregnancy (63-67). However, the mature B cells of these pregnant mice had normal or even slightly elevated amounts of mature B cells, suggesting that these mature B cells are exempt from estrogen sensitivity (63, 64). Later, it was shown by multiple research groups that not only did estrogen result in a significant reduction of B cell precursors but that estrogen had the opposite effect on the mature B cell population, with one group showing that estrogen causes a significant increase in the development of mature B cells into plasmablasts, and another group demonstrating that estrogen caused a significant expansion of both marginal zone and follicular B cells (68-70). However, estrogen is not the only pregnancy hormone that has been shown to have modulatory effects on humoral immunity. Recently, Fettke and colleagues showed that hCG, but not estrogen or progesterone, could induce the development of IL-10 producing B cells (B_{reg}) and it has been indicated that these B_{reg} cells play a crucial role in pregnancy protection from preterm birth (PTB) (71, 72). However, there are other mechanisms utilized by B cells, independent of IL-10 production, that protect against PTB. For instance, the CD5+ B-cell population is significantly reduced during normal healthy pregnancy and a lack of reduction in CD5+ B-cells is associated with not only PTB but also with pre-eclampsia and recurring spontaneous abortions (RSA) (72-76). The CD5+ B-cell population contains enhanced autoreactivity and the mechanism for cell reduction during pregnancy is still unknown. Furthermore, Huang and colleagues recently discovered a novel protective role of B-cells during pregnancy. They showed that decidual B-cells produced a large amount of progesterone induced blocking factor 1 (PIBF1) which was extremely critical for the prevention of PTB and this PTB prevention was completely independent of IL-10 production by B cells (77).

Placental Transfer of Maternal Immunity

The cornerstone of maternal vaccination is that vaccine-induced maternal IgG will be transferred across the placenta providing passive immune protection to the fetus prior to birth. It is transferred across the placenta through a receptor-mediated transcytosis mechanism. The receptor utilized by IgG to effectively cross the placenta is the neonatal Fc receptor (FcRn) that is expressed by trophoblasts at the maternofetal interface. The pathway for placental transfer of maternal IgG has been widely studied and well defined (78-80). The pathway begins with the syncytiotrophoblasts internalizing maternal IgG into early endosomes. The maternal IgG remains unbound to FcRn until they both become completely encapsulated by the early endosome. Once completely encapsulated, the pH of the early endosome significantly drops and it is the acidic pH that is responsible for allowing IgG to bind to FcRn. If there is any unbound IgG located within the acidic pH of the early endosome it will eventually be targeted to a lysosome for degradation. The bound IgG-FcRn complex within the early endosome can then be either recycled back to the maternal blood or can undergo transcytosis to the fetal side of the syncytiotrophoblast. This transcytosed IgG is then released by FcRn under neutral pH where it then enters the fetal villous stroma and ultimately makes its way into fetal circulation through a mechanism that is still unclear. The FcRn located on the fetal side of the syncytiotrophoblast can also be retrieved and recycled back to the maternal side of the synctiotrophoblast for additional IgG transcytosis (81). There is a direct correlation between the maternal concentration of IgG and the amount of IgG that is transferred to the fetus. Furthermore, there are preferences for the transcytosis of IgG based on its subclass, with IgG1 and IgG4 being preferentially transported over IgG2

and IgG3 (82).Consequently, it is no surprise that inactivated protein antigen vaccines such as Tdap, which generally elicit a more pronounced induction of IgG1 and IgG3, are considered more effective for maternal vaccination that polysaccharide vaccines which mainly induce an IgG2 response (83). The largest amount of IgG transfer from mother to fetus has been shown to occur in the third trimester of pregnancy, however maternal IgG can be transferred to the fetus as early as gestational week 13 (84, 85).

Goal of This Study

The goal of this work is to change the widespread ideology of antibody transfer across the placenta. Currently it is universally believed that IgG is the only antibody capable of crossing the placenta, but this work includes experimental data that suggests otherwise. This thesis shows that secreted IgD undergoes placental transcytosis.

CHAPTER ONE

Immunoglobulin D undergoes placental transfer to the fetus

ABSTRACT

Immunoglobulin D is a mysterious protein that has largely been overlooked since its discovery. It is uniquely distinct from all the other antibody classes in: expression, induction, structure and function. Within the last few years IgD has made its way back into the spotlight and novel functions of the protein have been discovered. These recent discoveries have provided the necessary information for us to uncover an additional and important function of IgD during pregnancy. It is a long-held belief among scientists that during pregnancy the only antibody class that can cross the placenta is IgG, however our data shows that this common place ideology is not correct. We find that IgD is fully capable of crossing the placenta and that vaccine-specific IgD induced by maternal vaccination undergoes placental transfer to the fetus.

INTRODUCTION

Immunoglobulin D (IgD) is an enigmatic protein that is solely produced by the B cells of the adaptive immune system. While IgM is the only antibody expressed on the surface of B cells during the early stages of development within the bone marrow, IgD surface expression is also induced when immature B cells egress from the bone marrow in search of secondary lymphoid organs to finish maturation. The co-expression of IgM and IgD on the surface of transitional and mature B cells is controlled by the process of alternative RNA splicing and is tightly regulated by the B cell (25, 26). However, once mature B cells experience antigenic stimulation, the expression of IgD is also controlled

by the B cell process of CSR through both T-cell independent and T-cell dependent pathways (33). After CSR occurs, B cells can further differentiate into plasmablasts that begin to secrete large amounts of their respective class switched immunoglobulin isotype. These secreted antibodies then enter circulation to carry out their various effector functions. Secreted IgD is distinctive from the other antibody classes and therefore carries out distinctive effector functions. It is known that secreted IgD is found in high concentration within the human respiratory mucosa and one of its functions is binding to respiratory pathogens such as: *Moraxella catarrhalis* and *Haemophilus influenzae*, however the most well-known function of secreted IgD is to bind to galectin-9 on basophils, thus amplifying humoral T helper 2 cell responses by activating basophil release of antimicrobial, opsonizing, proinflammatory and B-cell stimulating factors such as: cathelicidin, interleukin 1(IL-1), IL-4, and BAFF (33, 56). The combination of these known functions suggests that secreted IgD may be able to educate basophils on the microbial composition of the respiratory tract, thus providing a critical component of immune system surveillance for both adults and children.

Pathogenic microbes within the respiratory tract pose a major health risk to children who do not have a fully developed immune system and infectious disease continues to be a leading cause of death for children aged 0 to 5 years old (58). Vaccination is highly effective at preventing these deadly infections, however newborn children do not mount efficient immune responses to most vaccines and are left particularly vulnerable (59). This neonate vulnerability to infectious disease was the driving force behind the development of maternal vaccination which is a safe and effective strategy that utilizes the immunization of pregnant woman to boost maternal immunity which will then be transferred to the fetus prior to birth. The alterations to the

humoral immune system during pregnancy are essential to the success of maternal vaccination. Efficient vaccine responses rely on the generation of plasmablasts and the secretion protective antibodies in large quantities. It has also been previously shown that plasmablasts specifically expand during pregnancy (68). Utilizing the expansion of plasmablasts during pregnancy is what makes maternal vaccination such an effective strategy in combating neonatal infection. Currently, plasmablast secretion of IgG is the cornerstone of maternal vaccination because it is the only antibody class that has been extensively shown to cross the placenta. However after taking previous speculation by Salonen and colleagues into consideration (57), we then hypothesized that secreted IgD was capable of crossing the placenta and transferring immunity to the fetus.

In this study we show that IgD is fully capable of placental transcytosis both *in vitro*. Additionally, we demonstrate that placental transcytosis of IgD is significantly altered by cytokine signals, hormone signals, and post-translational protein glycosylation. Furthermore, we report that IgD co-localizes with cellular trafficking proteins in microscopy images of human placenta. Finally, while there was a reagent-related issue in our mouse model for placental transcytosis of vaccine-specific IgD, we were able to utilize both maternal and cord blood serum samples to demonstrate that vaccine-specific IgD does undergo placental transcytosis in humans.

MATERIALS AND METHODS

***In vitro* placental transcytosis assays.** The human villous trophoblast-derived BeWo cell line has been well established as a particularly useful model for studying placental transcytosis (86, 87). We followed the previously described protocol for this established *in vitro* placenta transcytosis assay and coated 0.2μm Transwell inserts with 80μL of 250μg/mL cell culture grade placental collagen purchased from Sigma. After coating the Trasnwell inserts with placental collagen, BeWo cells were then seeded onto the Transwell membrane at a subconfluent concentration. The BeWo cells were then allowed to grow in culture for 5-7 days until Transwell confluency was reached and the cells became an intact polarized monolayer. After an intact confluent monolayer was observed, then various purified human antibodies, depending on experimental conditions, would be added into the apical chamber of the Transwell insert at a concentration of 50μg/mL. After addition of the antibody into the apical chamber of the Transwell insert, a 50μL sample would then be collected from the basolateral chamber of the cell culture wells at the 0 minute (min), 10 min, 30 min, 1 hour (hr), 2hr, and 4 hr time intervals. The 50μL collected samples would later be analyzed by ELISA to show the amount of antibody transferred from the apical chamber to the basolateral chamber. The capture antibody and detection antibody for human IgD used in these ELISAs were both purchased from Bethyl Labratories. The goat anti human IgD capture antibody was diluted 100-fold before coating the ELISA plate and the goat anti human IgD-HRP conjugated antibody was diluted 50,000-fold for detection. The purified human antibodies that were utilized in the apical chamber of all the BeWo transcytosis assays in this thesis were purchased from Athens Research & Technology.

Enzymatic deglycosylation of human IgD. Purified human IgD was enzymatically stripped of its post-translational N-linked and O-linked glycans by following the manufacturer specifications for the PNGase F, neuraminidase, and o-glycosyidase enzymes purchased from New England Biolabs. After deglycosylation of the IgD heavy chain was confirmed by SYPRO Ruby stain of an SDS-PAGE gel, the deglycosylated human IgD was then utilized in a BeWo transcytosis assay as previously described.

Tdap maternal vaccination mouse model. BALB/c mice were purchased from Jackson laboratory and IgD -/- mice were graciously provided to us by the Cerutti lab at Cornell university. Female mice were either unimmunized/not vaccinated (control/NV) or received 3 separate 100µL doses of intramuscularly administered Boostrix (aka Tdap) 7, 14, and 21 days before pregnancy. A 4th 100µL dose of Tdap was then administered on either gestational day 4.5 or 14.5 to the previously immunized mothers. Immediately following delivery, maternal and pup blood samples were collected, and all mice were euthanized. Serum from the collected blood samples was then used in an ELISA to determine the presence of Tdap-specific IgD. The ELISA plate was coated with 10-fold diluted Tdap and all serum samples were diluted 10-fold for measurement. The detection antibody used in this ELISA was diluted 50,000-fold and is a goat anti mouse IgD-HRP conjugated antibody purchased from American Research Products. It is currently the only anti mouse IgD-HRP conjugated detection antibody on the market available for purchase. All mice protocols were approved by the Wayne State University IACUC.

Tdap-specific IgD placental transcytosis in humans. Pregnant DMC hospital patients were informed of our research project and signed medical consent forms giving us permission to collect their blood, cord blood and placenta tissues. The medical consent forms were approved by the Wayne State University IRB. The first maternal blood sample was collected immediately prior to the pregnant mother receiving her Tdap vaccination to establish her baseline antibody levels. The second maternal blood sample was collected 2-4 weeks later at her next healthcare visit to show that Tdap-specific IgD had been induced by vaccination. The third maternal blood sample as well as the cord blood sample was collected upon delivery of the baby to determine the Tdap-specific IgD levels at birth. Following full term delivery, the placenta was also collected and stored for immunofluorescent confocal images. The collected human blood samples were analyzed by ELISA to show their Tdap-specific IgD antibody titers. The ELISA plate was coated with 10-fold diluted Tdap, the human serum samples were diluted 10-fold and the goat anti human IgD-HRP antibody was diluted 50,000-fold for Tdap-specific IgD detection.

RESULTS

IgD undergoes placental transcytosis *in vitro*. By utilizing a well-established human villous trophoblast-derived cell line (BeWo) transcytosis assay (86, 87), we found that both human IgG and IgD undergo apical-to-basolateral transcytosis while IgM and IgA do not (**Figure 1A**). The lack of IgM and IgA placental transcytosis *in vitro* is consistent with their inability to cross the placenta *in vivo*. In order to confirm the integrity of the cellular monolayer, confocal images were taken of the cells following the BeWo transcytosis assay and their integrity was confirmed with the expression of the tight junction protein zonular occludin-1 (ZO-1) (**Figure 1B**). Additionally, in the confocal images IgD can be seen in what appears to be intracellular vesicles and perinuclear structures that bear a resemblance to cellular trafficking organelles. Furthermore, the cellular monolayer integrity was confirmed by the lack of detectable basolateral immunoglobulin within the first 10 minutes of the assay.

Cytokine and hormone signals modulate placental transcytosis of IgD *in vitro*. It has been demonstrated that both placental infection and placental inflammation have the ability to compromise the transfer of antibodies and metabolites to the fetus (88-91). Our results show that T_H1, T_H17, and T_{reg} cytokine signals significantly inhibit the placental transcytosis of IgD *in vitro* (**Figure 2A, C, D**) where as T_H2 cytokine signals had no effect on IgD placental transcytosis (**Figure 2B**). Furthermore, our results show that adrenocorticotropic hormone (ACTH) and 17β-estradiol hormone signals significantly inhibit placental transcytosis of IgD *in vitro* while progesterone signals significantly increase IgD placental transcytosis (**Figure 2E**).

Post-translational glycosylation of IgD is required for efficient placental transcytosis *in vitro*. The glycosylation of IgD has been shown to be distinct from the glycosylation of other antibody subclasses and it has been speculated that the unique structural characteristics of IgD contribute to its unique biological properties (34-42). Treatment of IgD with neuraminidase and o-glycosidase to enzymatically remove all O-linked glycans and treatment of IgD with PNGase F to enzymatically remove all N-linked glycans resulted in the expected reduction of IgD heavy chain molecular weight (**Figure 3A**). After enzymatic removal of N-linked and O-linked glycans from IgD was confirmed, the carbohydrate-less IgD was then used in an *in vitro* BeWo transcytosis assay where it demonstrated that a lack of O-linked glycans and a lack of N-linked glycans both resulted in a significant reduction in IgD placental transcytosis (**Figure 3B**).

IgD binding to BeWo cells is enhanced by galectin-9. The binding of IgD to galectin-9 on the surface of basophils has already been shown (56), however the binding of IgD to galectin-9 on the surface placental cells has not been demonstrated until now. We show using flow cytometry that IgD binding to BeWo cells was noticeably increased by an incubation with galectin-9 prior to cell exposure, but not with a galectin-1 or galectin-3 incubation prior to cell exposure (**Figure 4**). These results suggest that galectin-9 may be directly involved in the transcytosis of IgD across BeWo cells.

Cellular trafficking of IgD in human placenta. Progressing forward from our cell line-based transcytosis assays, we then used primary human placenta from full term deliveries to detect the presence of IgD. Indeed, confocal images show IgD colocalizing with the early endosome marker EEA-1, the trans-golgi network marker TGN-46, and the basolaterally targeting cellular trafficking protein CD44 (**Figure 5A-C**). These images indicate that IgD is undergoing apical to basolateral transcytosis within the

human placenta and further corroborate our earlier data from the BeWo transcytosis assays where we observed IgD in what appeared to be intracellular vesicles and perinuclear structures (**Figure 1B**).

Reagent issue in Tdap maternal vaccination mouse model. We were able to show by ELISA that wild type (WT) BALB/c mothers will significantly generate Tdap-specific IgD in response to immunization, and that this Tdap-specific IgD is easily detectable in the serum of pups whose mothers had received a Tdap vaccination on either gestational day 4.5 or gestational day 14.5 (**Figure 6A**). Furthermore, the vaccination series that the WT mothers received had no significant effect on the gestational length of the pregnancy or on the birth weight of the pups, suggesting that this maternal vaccination mouse model is safe and has no adverse effects to the fetus (**Figure 6B**). However, after applying the previously mentioned Tdap maternal vaccination series to $IgD^{-/-}$ female mice, an issue was observed in the data. We observed by ELISA that $IgD^{-/-}$ mothers who had received the Tdap vaccine supposedly display significant levels of "Tdap-specific IgD" in their serum and both the $IgD^{+/-}$ and $IgD^{-/-}$ pups of these vaccinated mothers also contained "Tdap-specific IgD" in their serum (**Figure 6C**). When comparing serum from entire litters of $IgD^{+/-}$ pups to serum from entire litters of $IgD^{-/-}$ pups, we noticed that $IgD^{+/-}$ pups from vaccinated $IgD^{-/-}$ mothers displayed slightly higher levels of "Tdap-specific IgD" than the $IgD^{-/-}$ pups from vaccinated $IgD^{-/-}$ mothers, suggesting that the antigens from the Tdap vaccine may possibly be crossing the placenta and thus priming the fetal immune system to generate its own "Tdap-specific IgD". After this observation, we designed a mating scheme that allowed for the generation of $IgD^{+/-}$ pups and $IgD^{-/-}$ pups within the same litter and administered the previously described Tdap maternal vaccination series to the $IgD^{-/-}$

mother. We found that “Tdap-specific IgD” was detectable in both $IgD^{+/-}$ pups and $IgD^{-/-}$ pups but there was no significant difference in “Tdap-specific IgD” serum levels of $IgD^{+/-}$ pups and $IgD^{-/-}$ pups from the same litter. These results suggest that fetal immune priming is not taking place within this Tdap maternal vaccination mouse model (**Figure 6D**). After confirming that IgD is in fact removed from the genome of the $IgD^{-/-}$ mice used throughout Figure 6 and the presence of Tdap-specific IgD within the $IgD^{-/-}$ mice is biologically impossible, we conclude that the detection antibody used in the ELISAs is nonspecifically binding to Tdap-specific IgG and this nonspecific binding is the sole reason for our observed data issue.

Placental transcytosis of maternally induced Tdap-specific IgD in humans. We wanted to determine whether or not placental transcytosis of IgD could also be readily observed within pregnant humans. By obtaining blood samples from pregnant women prior to Tdap vaccination and blood samples 2-4 weeks following their Tdap maternal vaccination, we were able to demonstrate by ELISA that there was a marked increase in total antibody titer of Tdap-specific IgD following vaccination, thus showing that Tdap-specific IgD was readily induced in humans by maternal vaccination (**Figure 7A**). Additionally, maternal blood samples and cord blood samples were obtained immediately following birth for ELISA. We observed that there is a substantially higher Tdap-specific IgD antibody titer in cord blood samples than there is in maternal blood samples at delivery, thus suggesting that maternally induced Tdap-specific IgD does in fact undergo placental transcytosis in humans (**Figure 7A**).

DISCUSSION

Our findings indicate that the current widespread ideology of IgG being the only maternal antibody transferred to the fetus during pregnancy is not entirely correct, and that IgD is fully capable of crossing the placenta. The BeWo transcytosis assay has proven to be an excellent *in vitro* model to study placental transfer of IgD and the cytokine/hormone signal modulation of IgD placental transcytosis in BeWo cells has provided us with some very insightful information. Placental infection and placental inflammation can compromise the transfer of antibodies to the fetus (88-91), so it is expected that the proinflammatory T_H1 and T_H17 cytokines used in Figures 2A and 2D would result in a significant reduction in BeWo transcytosis of IgD. Additionally, it is not surprising that the T_H2 cytokines in Figure 2B have no effect on BeWo transcytosis of IgD because these cytokines are required for B cell CSR, which is a major pathway for the induction of secreted IgD. However, the idea of the T_{reg} cytokine TGF-β significantly reducing the BeWo transcytosis of IgD initially seemed slightly perplexing, but upon searching the literature further this finding corroborates the data we observed in our confocal images of human placental tissue in Figure 5C where IgD co-localizes with CD44. It has been demonstrated that TGF-β signaling directly regulates the alternative splicing of CD44, thus controlling which CD44 isoform is actually expressed by the cell (92), and it has also been shown that CD44 is involved with IgD binding to the surface of basophils (56). Therefore, treating the BeWo cells with TGF-β may have directly changed the normally expressed CD44 isoform to another isoform with different protein binding properties in comparison to the normal placental CD44 isoform, ultimately resulting in a significant reduction of BeWo IgD transcytosis from TGF-β treatment. It is noteworthy that additional experiments need to be conducted in order to completely

determine why the progesterone treatment in Figure 2E resulted in such a significant increase in BeWo transcytosis of IgD.

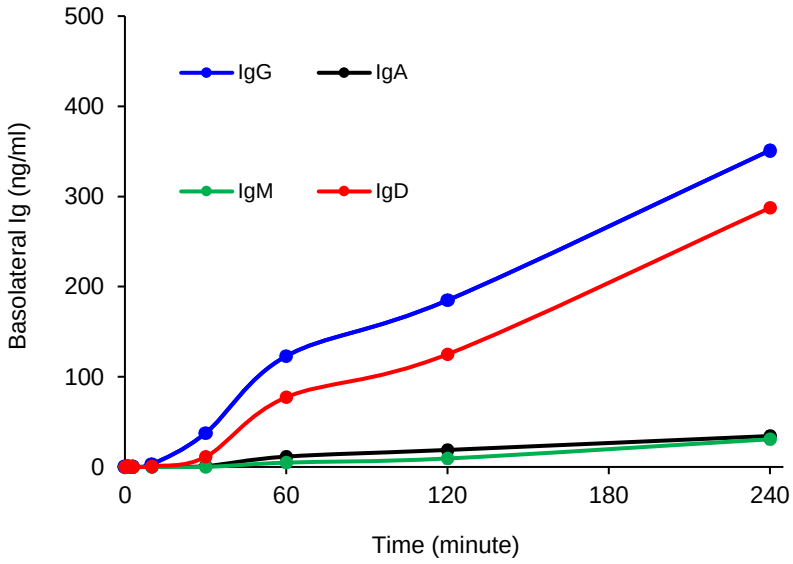
Furthermore, the use of deglycosylated IgD in a BeWo transcytosis assay has also provided us with some valuable information towards the future direction of researching IgD placental transcytosis. It has previously been shown that the effector functions of IgG, mediated through the FcγR, are severely compromised when IgG is deglycosylated due to a lack of FcγR binding (93). Our results show that a lack of N-linked glycans resulted in the biggest reduction of BeWo IgD transcytosis. These observations suggest that the two distinctive N-linked glycans in the $C_{\delta}3$ domain of IgD may be directly involved in the binding of an unidentified placental IgD receptor. This unidentified placental IgD receptor may possibly be galectin-9 but this has yet to be confirmed.

As previously stated, there was an issue observed in our Tdap maternal vaccination mouse model results, however we believe this issue is only reagent-related and the results do not prove our original hypothesis wrong. During the vaccination series of the $IgD^{-/-}$ mothers in Figures 6C and 6D it is impossible for them to actually generate a vaccine-specific IgD response because IgD has been removed from their genome, yet "vaccine-specific IgD" is readily observed in serum of $IgD^{-/-}$ mothers after performing a vaccine specific ELISA. While these vaccinated $IgD^{-/-}$ mothers are physically incapable of producing vaccine specific IgD they are still fully capable of producing a robust vaccine specific IgG response because IgG is still present in their genome. This vaccine specific IgG generated within the vaccinated $IgD^{-/-}$ mothers is still readily transferred across the placenta to her $IgD^{-/-}$ pups. This means that the only plausible explanation for the observed vaccine specific IgD signals within the $IgD^{-/-}$

mothers and pups is that the antibody we used for detection of mouse IgD in the ELISA, not only binds to mouse IgD but also nonspecifically binds to mouse IgG. If there was another company that sold a different anti-mouse IgD-HRP conjugated antibody this technical discrepancy could be easily mediated, unfortunately this is not the case and the antibody we used is currently the only one of its kind. This Tdap maternal vaccination mouse model will not be able to provide conclusive results of IgD placental transcytosis until an anti-mouse IgD-HRP conjugated antibody with much less nonspecific binding is developed.

This study shows that IgD is fully capable of placental transcytosis both *in vitro*. We demonstrated that placental transcytosis of IgD is significantly altered by cytokine signals, hormone signals, and post-translational protein glycosylation. Furthermore, we report that IgD co-localizes with cellular trafficking proteins in microscopy images of primary human placenta. Although our Tdap maternal vaccination mouse model remains inconclusive in determining the placental transcytosis of vaccine-specific IgD, we were able to utilize both maternal and cord blood serum samples to demonstrate that vaccine-specific IgD does undergo placental transcytosis in humans.

A)



B)

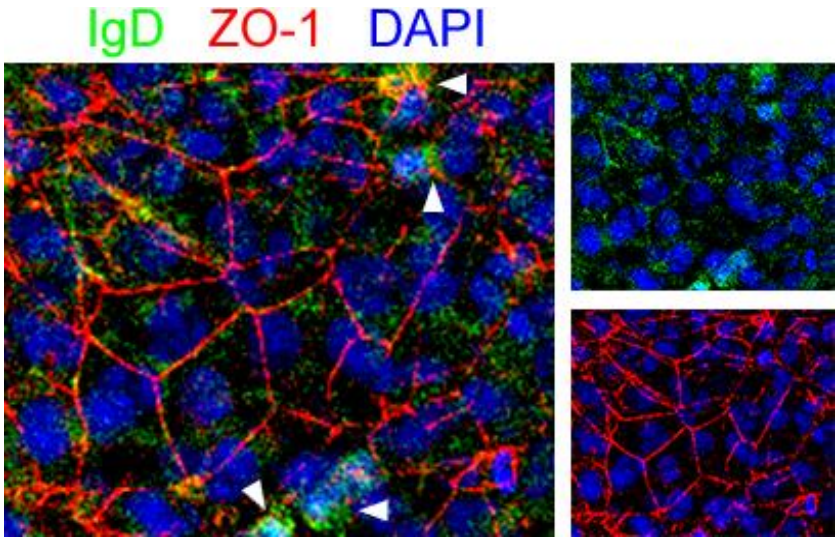


FIGURE 1. Placental transfer of IgD *in vitro*. (A) Kinetics of apical-to-basolateral transcytosis of human IgM, IgG, IgA, and IgD across intact confluent layer of BeWo cells, as determined by ELISA. 50ug/mL of each Ig class was added to the apical side at the beginning of the assay (B) Confocal image of IgD transcytosing BeWo cell layer expressing the tight junction protein zonular occludin-1 (ZO-1) captured following transcytosis assay. Arrowheads point to IgD staining visible in intracellular vesicles. Original magnification: x63

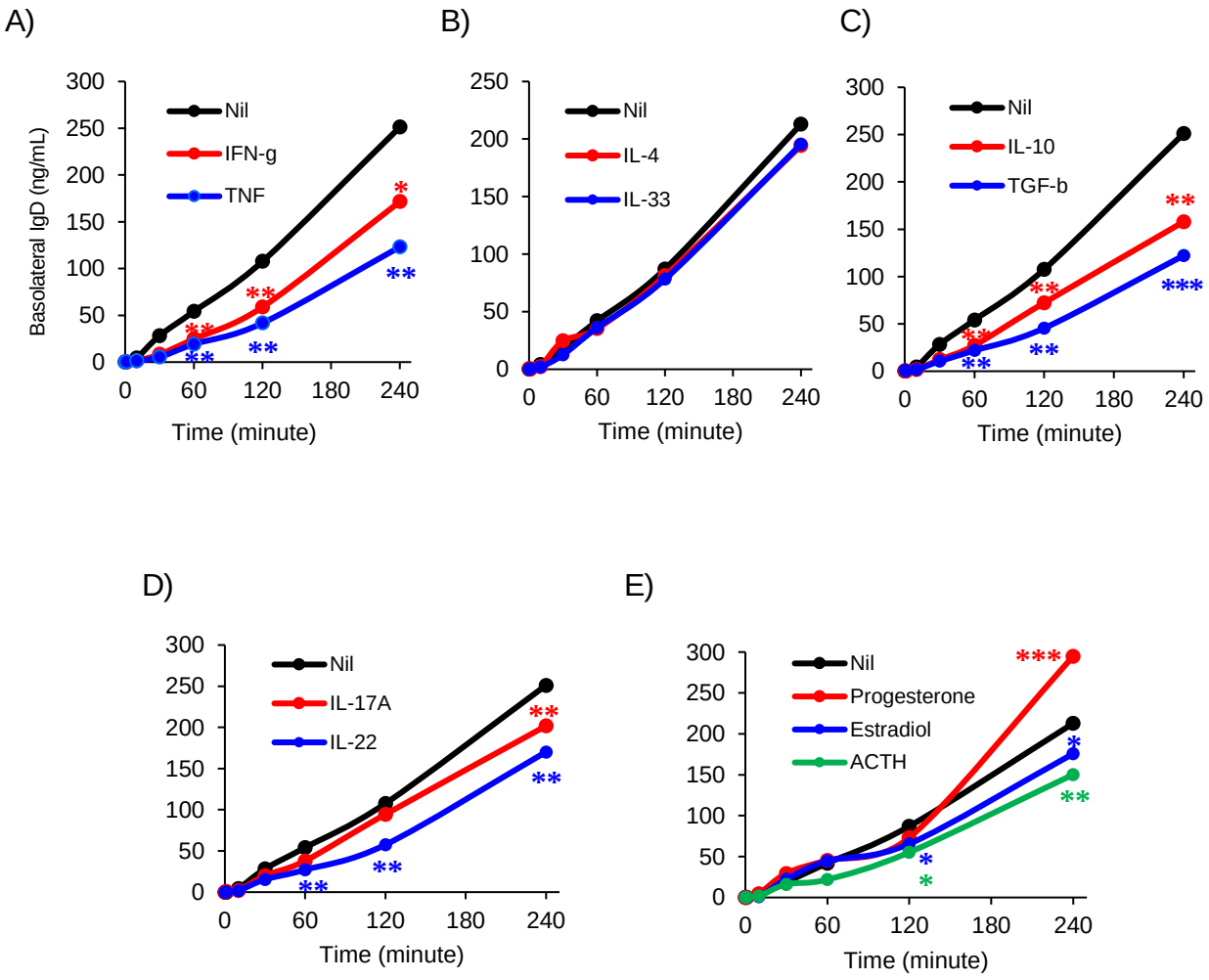
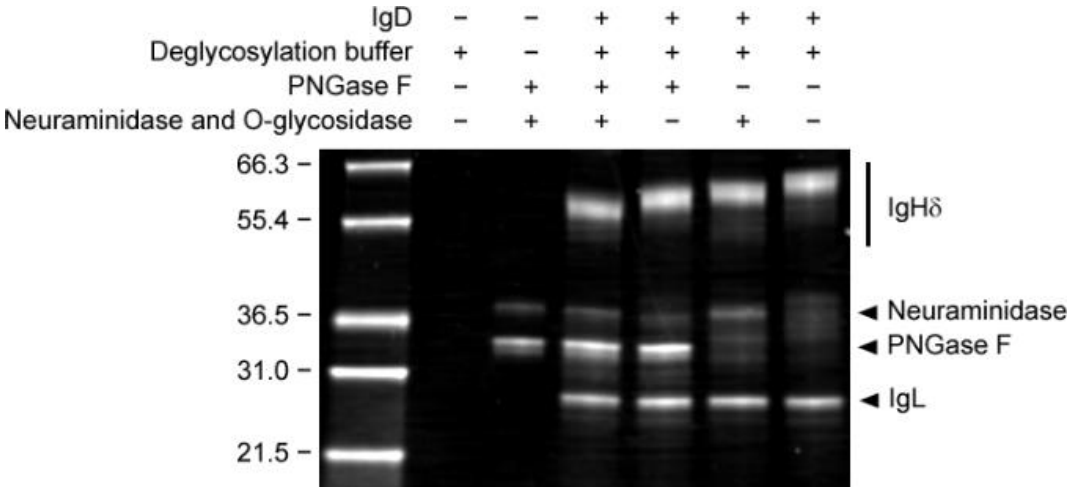


FIGURE 2. Placental transfer of IgD *in vitro* is modulated by cytokine and hormone signals. Confluent BeWo cell layers were either untreated (Nil) or treated for 24 hr with 50ng/mL of IFN-γ or TNF-α (A), IL-4 or IL-33 (B), IL-10 or TGF-β (C), IL-17A or IL-22 (D) or 100nM of progesterone or 17β-estradiol or 1μM of ACTH (E), prior to the IgD transcytosis assay. *:p<0.05; **:p<0.01; ***:p<0.001, as compared to the respective Nil sample

A)



B)

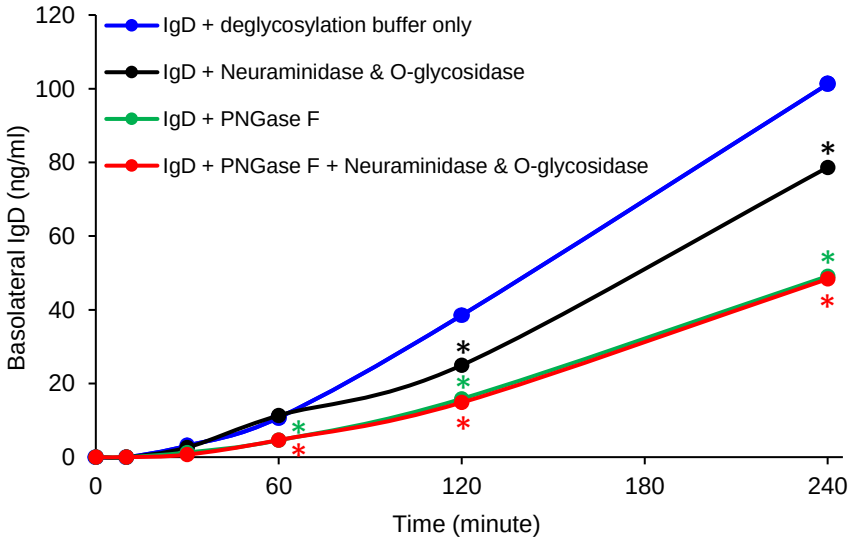


FIGURE 3. Placental transfer of deglycosylated IgD *in vitro*. (A) SYPRO Ruby stain of an SDS-PAGE gel of human IgD treated with deglycosylation enzymes and/or buffer, showing a reduction of IgD H chain size after N- and/or O-deglycosylation. PNGase F (36kDa) cleaves N-linked glycans, whereas neuraminidase (43 kda) and O-glycosidase (147kDa, Not shown in image) together remove O-linked glycans (B) Kinetics of transcytosis of IgD prepared in (a) across BeWo cells. *:p<0.05 as compared to IgD + deglycosylation buffer only.

A)

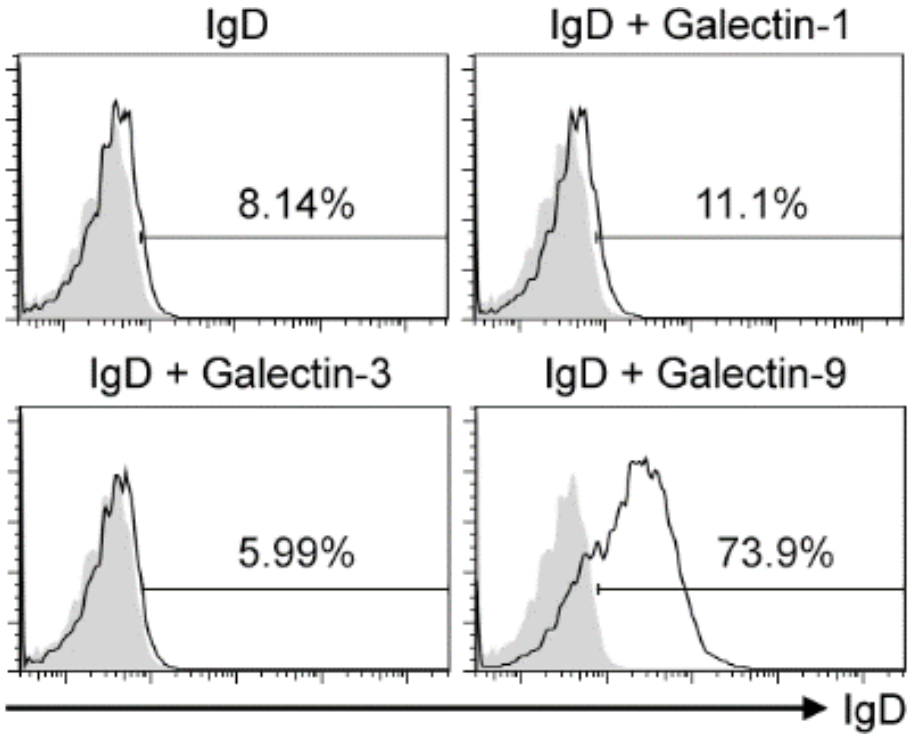


FIGURE 4. Galectin-9 enhances IgD binding to BeWo cells. (A) IgD staining on BeWo cell surface after incubation with 50µg/mL IgD with or without 2.5 µg/mL Galectin-1, Galectin-3, or Galectin-9 @ 4°C for 10 min. Shaded histogram indicates IgD staining without IgD incubation.

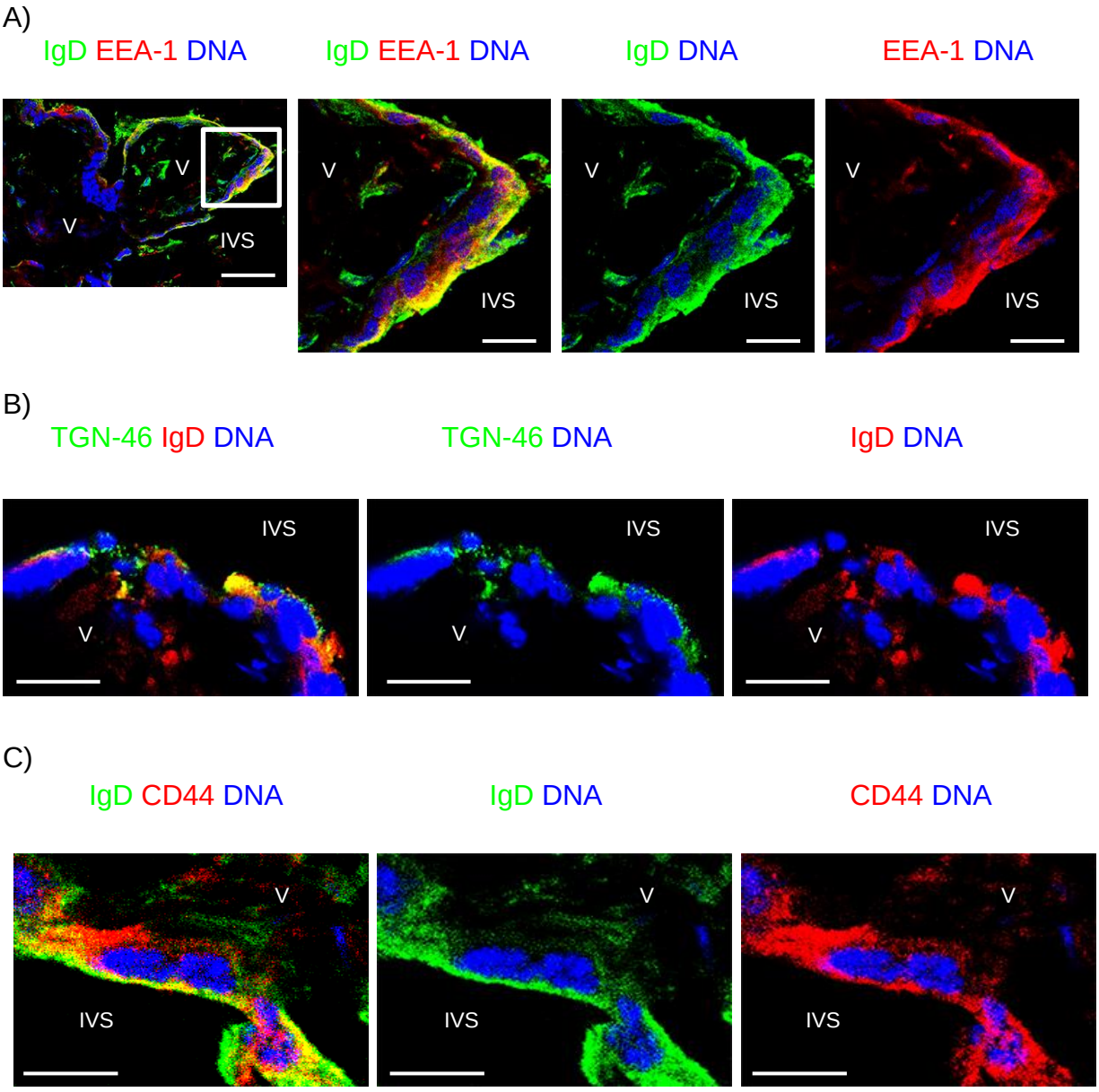


FIGURE 5. Confocal images of IgD Cellular Trafficking in Human Placenta. (A) Confocal immunofluorescence analysis of IgD (green) and EEA-1 (red) in term placenta villi. DNA stained with DAPI. Bars: 100 μm (left panel) or 30 μm (other panels). (B) Confocal immunofluorescence analysis of IgD (red) and TGN-46 (green) in term placenta villi. DNA stained with DAPI. Bars: 50 μm. (C) Confocal immunofluorescence analysis of IgD (green) and CD44 (red) in term placenta villi. DNA stained with DAPI. Bars: 50 μm. (A,B,C) V: villi, IVS: intervillious space. All images are representative of placental tissues of 3 term delivery donors

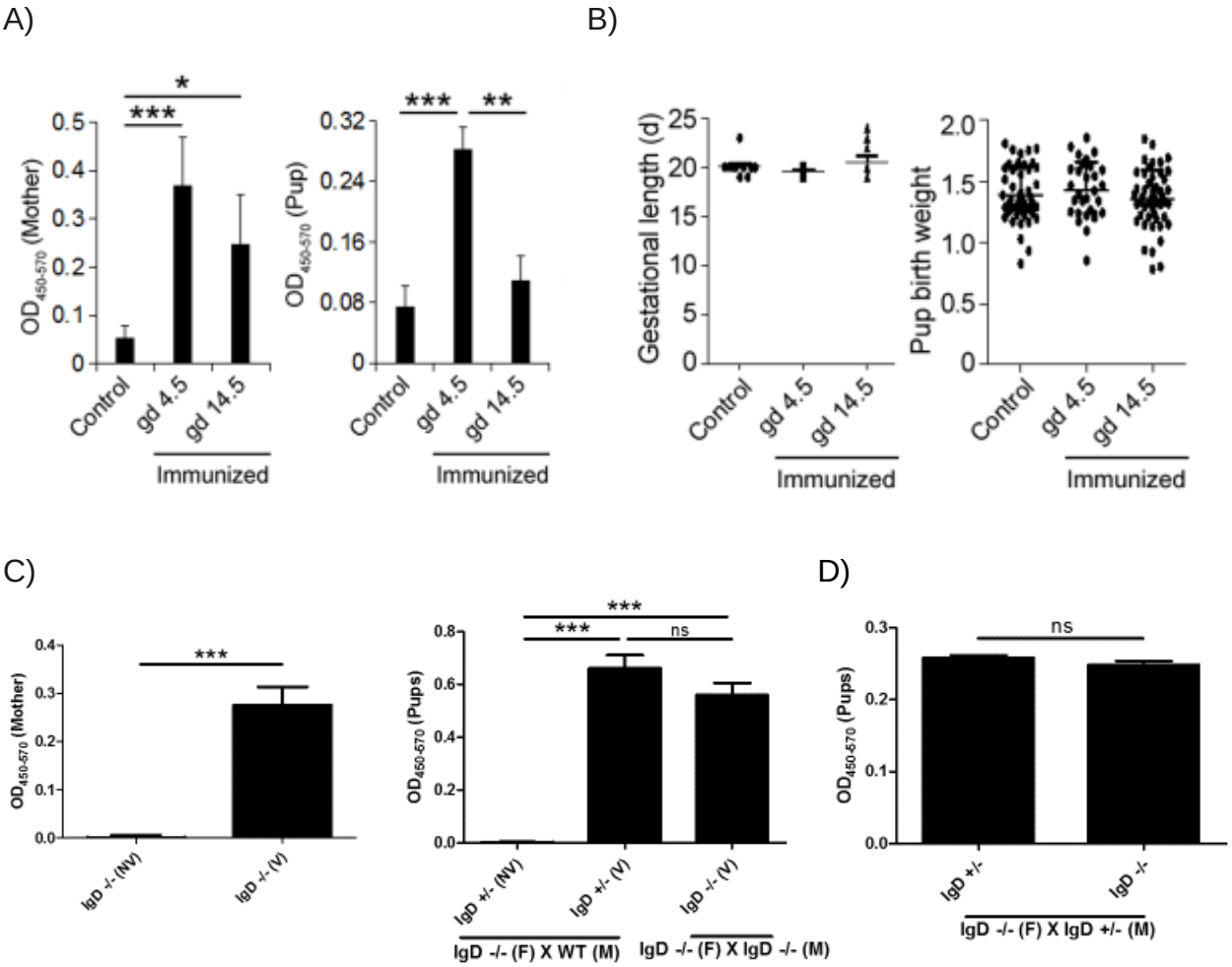


FIGURE 6. Reagent issue in the detection of maternally induced Tdap-specific IgD undergoing placental transfer in mice. (A) ELISA of Tdap-specific IgD in maternal and newborn pups' blood at delivery. The mothers were either unimmunized (control) or received 3 doses of intramuscular Tdap 7, 14, and 21 days before pregnancy and a 4th dose on gestational day (gd) 4.5 or 14.5. (B) Comparison of gestational length and pup birth weight of control and immunized mothers from (Figure 6A). (C) ELISA of Tdap-specific IgD in maternal (IgD$^{-/-}$) and newborn pups' (IgD$^{+/-}$ and IgD$^{-/-}$) blood at delivery. The IgD$^{-/-}$ females (F) were either not vaccinated (NV) or received 3 doses of intramuscular Tdap 7,14, and 21 days before pregnancy and a 4th dose on gestational day 4.5 (V) after being mated with either WT or IgD$^{-/-}$ males (M). (D) ELISA of Tdap-specific IgD in newborn pups' (IgD$^{+/-}$ and IgD$^{-/-}$) blood at delivery. The IgD$^{-/-}$ females (F) received 3 doses of intramuscular Tdap 7,14, and 21 days before pregnancy and a 4th dose on gestational day 4.5 after being mated with IgD$^{+/-}$ males (M). All serum samples were diluted 10-fold for measurement. *:p<0.05; **:p<0.01; ***:p<0.001 by 1-tailed unpaired t-test for (A, C, and D) and 2-tailed unpaired t-test for (B).

A)

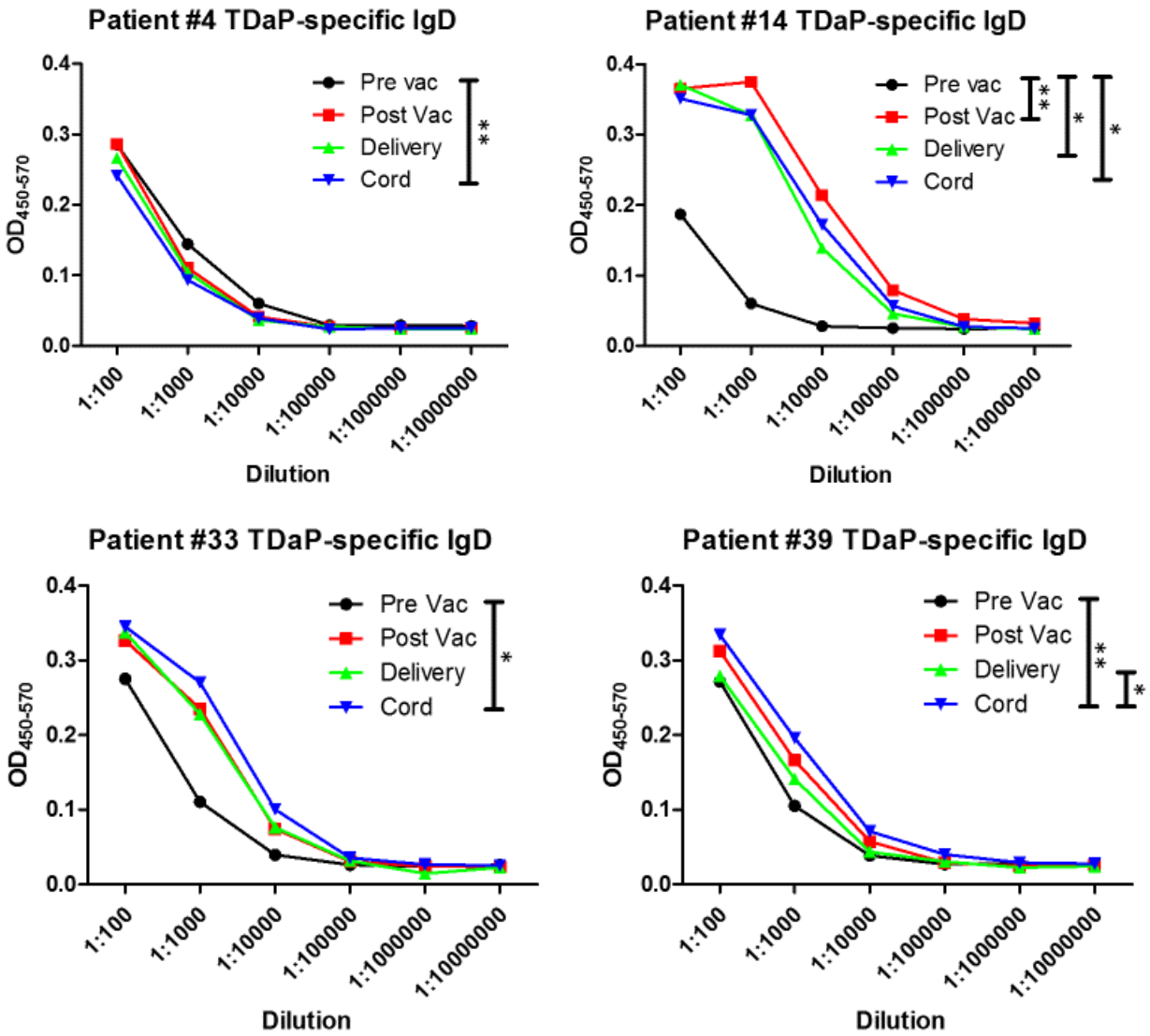


FIGURE 7. Maternally induced Tdap-specific IgD undergoes placental transfer in humans. (A) ELISA measurement of Tdap-specific IgD titers in maternal peripheral blood before vaccination (black lines, Pre Vac), 2-4 weeks after vaccination (red lines, Post Vac), at delivery (green lines) and in cord blood (blue lines) of 3 representative responders and 1 subject that already had a detectable antibody titer before vaccination.*:$p<0.05$; **:$p<0.01$ by 1-way repeated measures ANOVA with a Dunnett's Multiple Comparison Post Test.

REFERENCES

1. **Rowe DS, Fahey JL.** 1965. A NEW CLASS OF HUMAN IMMUNOGLOBULINS. I. A UNIQUE MYELOMA PROTEIN. J Exp Med **121:**171-184.
2. **Rowe DS, Fahey JL.** 1965. A NEW CLASS OF HUMAN IMMUNOGLOBULINS. II. NORMAL SERUM IGD. J Exp Med **121:**185-199.
3. **Finkelman FD, van Boxel JA, Asofsky R, Paul WE.** 1976. Cell membrane IgD: demonstration of IgD on human lymphocytes by enzyme-catalyzed iodination and comparison with cell surface Ig of mouse, guinea pig, and rabbit. J Immunol **116:**1173-1181.
4. **Finkelman FD, Woods VL, Berning A, Scher I.** 1979. Demonstration of mouse serum IgD. J Immunol **123:**1253-1259.
5. **Martin LN, Leslie GA, Hindes R.** 1976. Lymphocyte surface IgD and IgM in non-human primates. Int Arch Allergy Appl Immunol **51:**320-329.
6. **Chen CL, Lehmeyer JE, Cooper MD.** 1982. Evidence for an IgD homologue on chicken lymphocytes. J Immunol **129:**2580-2585.
7. **Ohta Y, Flajnik M.** 2006. IgD, like IgM, is a primordial immunoglobulin class perpetuated in most jawed vertebrates. Proc Natl Acad Sci U S A **103:**10723-10728.
8. **Choi DH, Jang HN, Ha DM, Kim JW, Oh CH, Choi SH.** 2007. Cloning and expression of partial Japanese flounder (Paralichthys olivaceus) IgD. J Biochem Mol Biol **40:**459-466.
9. **Gambon-Deza F, Espinel CS.** 2008. IgD in the reptile leopard gecko. Mol Immunol **45:**3470-3476.

10. **Hordvik I, Thevarajan J, Samdal I, Bastani N, Krossoy B.** 1999. Molecular cloning and phylogenetic analysis of the Atlantic salmon immunoglobulin D gene. Scand J Immunol **50:**202-210.

11. **Saha NR, Suetake H, Kikuchi K, Suzuki Y.** 2004. Fugu immunoglobulin D: a highly unusual gene with unprecedented duplications in its constant region. Immunogenetics **56:**438-447.

12. **Wei Z, Wu Q, Ren L, Hu X, Guo Y, Warr GW, Hammarstrom L, Li N, Zhao Y.** 2009. Expression of IgM, IgD, and IgY in a reptile, Anolis carolinensis. J Immunol **183:**3858-3864.

13. **Wilson M, Bengten E, Miller NW, Clem LW, Du Pasquier L, Warr GW.** 1997. A novel chimeric Ig heavy chain from a teleost fish shares similarities to IgD. Proc Natl Acad Sci U S A **94:**4593-4597.

14. **Zhao Y, Pan-Hammarstrom Q, Yu S, Wertz N, Zhang X, Li N, Butler JE, Hammarstrom L.** 2006. Identification of IgF, a hinge-region-containing Ig class, and IgD in Xenopus tropicalis. Proc Natl Acad Sci U S A **103:**12087-12092.

15. **Hirono I, Nam BH, Enomoto J, Uchino K, Aoki T.** 2003. Cloning and characterisation of a cDNA encoding Japanese flounder Paralichthys olivaceus IgD. Fish Shellfish Immunol **15:**63-70.

16. **Zhao Y, Kacskovics I, Pan Q, Liberles DA, Geli J, Davis SK, Rabbani H, Hammarstrom L.** 2002. Artiodactyl IgD: the missing link. J Immunol **169:**4408-4416.

17. **Bengten E, Clem LW, Miller NW, Warr GW, Wilson M.** 2006. Channel catfish immunoglobulins: repertoire and expression. Dev Comp Immunol **30:**77-92.

18. **Bengten E, Quiniou SM, Stuge TB, Katagiri T, Miller NW, Clem LW, Warr GW, Wilson M.** 2002. The IgH locus of the channel catfish, Ictalurus punctatus, contains multiple constant region gene sequences: different genes encode heavy chains of membrane and secreted IgD. J Immunol **169:**2488-2497.

19. **Greenberg AS, Hughes AL, Guo J, Avila D, McKinney EC, Flajnik MF.** 1996. A novel "chimeric" antibody class in cartilaginous fish: IgM may not be the primordial immunoglobulin. Eur J Immunol **26:**1123-1129.

20. **Stenvik J, Schroder MB, Olsen K, Zapata A, Jorgensen TO.** 2001. Expression of immunoglobulin heavy chain transcripts (VH-families, IgM, and IgD) in head kidney and spleen of the Atlantic cod (Gadus morhua L.). Dev Comp Immunol **25:**291-302.

21. **Xu Z, Wang GL, Nie P.** 2009. IgM, IgD and IgY and their expression pattern in the Chinese soft-shelled turtle Pelodiscus sinensis. Mol Immunol **46:**2124-2132.

22. **Zhao Y, Hammarstrom L.** 2003. Cloning of the complete rat immunoglobulin delta gene: evolutionary implications. Immunology **108:**288-295.

23. **Zhao Y, Kacskovics I, Rabbani H, Hammarstrom L.** 2003. Physical mapping of the bovine immunoglobulin heavy chain constant region gene locus. J Biol Chem **278:**35024-35032.

24. **Han B, Yuan H, Wang T, Li B, Ma L, Yu S, Huang T, Li Y, Fang D, Chen X, Wang Y, Qiu S, Guo Y, Fei J, Ren L, Pan-Hammarstrom Q, Hammarstrom L, Wang J, Wang J, Hou Y, Pan Q, Xu X, Zhao Y.** 2016. Multiple IgH Isotypes Including IgD, Subclasses of IgM, and IgY Are Expressed in the Common Ancestors of Modern Birds. J Immunol **196:**5138-5147.

25. **Kerr WG, Hendershot LM, Burrows PD.** 1991. Regulation of IgM and IgD expression in human B-lineage cells. J Immunol **146:**3314-3321.

26. **Chen K, Cerutti A.** 2010. New insights into the enigma of immunoglobulin D. Immunol Rev **237:**160-179.

27. **Bengten E, Wilson M, Miller N, Clem LW, Pilstrom L, Warr GW.** 2000. Immunoglobulin isotypes: structure, function, and genetics. Curr Top Microbiol Immunol **248:**189-219.

28. **Weiss EA, Michael A, Yuan D.** 1989. Role of transcriptional termination in the regulation of mu mRNA expression in B lymphocytes. J Immunol **143:**1046-1052.

29. **Yuan D.** 1986. Regulation of immunoglobulin D synthesis in murine neonatal B lymphocytes. Mol Cell Biol **6:**1015-1022.

30. **Yuan D, Witte PL, Tan J, Hawley J, Dang T.** 1996. Regulation of IgM and IgD heavy chain gene expression: effect of abrogation of intergenic transcriptional termination. J Immunol **157:**2073-2081.

31. **Kim M, Qiu P, Abuodeh R, Chen J, Yuan D.** 1999. Differential regulation of transcription termination occurring at two different sites on the micro-delta gene complex. Int Immunol **11:**813-824.

32. **Kluin PM, Kayano H, Zani VJ, Kluin-Nelemans HC, Tucker PW, Satterwhite E, Dyer MJ.** 1995. IgD class switching: identification of a novel recombination site in neoplastic and normal B cells. Eur J Immunol **25:**3504-3508.

33. **Chen K, Xu W, Wilson M, He B, Miller NW, Bengten E, Edholm ES, Santini PA, Rath P, Chiu A, Cattalini M, Litzman J, J BB, Huang B, Meini A, Riesbeck K, Cunningham-Rundles C, Plebani A, Cerutti A.** 2009. Immunoglobulin D enhances immune surveillance by activating antimicrobial,

proinflammatory and B cell-stimulating programs in basophils. Nat Immunol **10:**889-898.

34. **Mellis SJ, Baenziger JU.** 1983. Structures of the oligosaccharides present at the three asparagine-linked glycosylation sites of human IgD. J Biol Chem **258:**11546-11556.

35. **Lin LC, Putnam FW.** 1981. Primary structure of the Fc region of human immunoglobulin D: implications for evolutionary origin and biological function. Proc Natl Acad Sci U S A **78:**504-508.

36. **Amin AR, Tamma SM, Swenson CD, Kieda CC, Oppenheim JD, Finkelman FD, Coico RF.** 1993. The immunoaugmenting properties of murine IgD reside in its C delta 1 and C delta 3 regions: potential role for IgD-associated glycans. Int Immunol **5:**607-614.

37. **Takahashi N, Tetaert D, Debuire B, Lin LC, Putnam FW.** 1982. Complete amino acid sequence of the delta heavy chain of human immunoglobulin D. Proc Natl Acad Sci U S A **79:**2850-2854.

38. **Gala FA, Morrison SL.** 2002. The role of constant region carbohydrate in the assembly and secretion of human IgD and IgA1. J Biol Chem **277:**29005-29011.

39. **Mellis SJ, Baenziger JU.** 1983. Structures of the O-glycosidically linked oligosaccharides of human IgD. J Biol Chem **258:**11557-11563.

40. **Preud'homme JL, Petit I, Barra A, Morel F, Lecron JC, Lelievre E.** 2000. Structural and functional properties of membrane and secreted IgD. Mol Immunol **37:**871-887.

41. **Smith AC, de Wolff JF, Molyneux K, Feehally J, Barratt J.** 2006. O-glycosylation of serum IgD in IgA nephropathy. J Am Soc Nephrol **17:**1192-1199.

42. **Sun Z, Almogren A, Furtado PB, Chowdhury B, Kerr MA, Perkins SJ.** 2005. Semi-extended solution structure of human myeloma immunoglobulin D determined by constrained X-ray scattering. J Mol Biol **353:**155-173.

43. **Lutz C, Ledermann B, Kosco-Vilbois MH, Ochsenbein AF, Zinkernagel RM, Kohler G, Brombacher F.** 1998. IgD can largely substitute for loss of IgM function in B cells. Nature **393:**797-801.

44. **Nitschke L, Kosco MH, Kohler G, Lamers MC.** 1993. Immunoglobulin D-deficient mice can mount normal immune responses to thymus-independent and -dependent antigens. Proc Natl Acad Sci U S A **90:**1887-1891.

45. **Roes J, Rajewsky K.** 1993. Immunoglobulin D (IgD)-deficient mice reveal an auxiliary receptor function for IgD in antigen-mediated recruitment of B cells. J Exp Med **177:**45-55.

46. **Wienands J, Reth M.** 1992. Glycosyl-phosphatidylinositol linkage as a mechanism for cell-surface expression of immunoglobulin D. Nature **356:**246-248.

47. **Chaturvedi A, Siddiqui Z, Bayiroglu F, Rao KV.** 2002. A GPI-linked isoform of the IgD receptor regulates resting B cell activation. Nat Immunol **3:**951-957.

48. **Loset GA, Roux KH, Zhu P, Michaelsen TE, Sandlie I.** 2004. Differential segmental flexibility and reach dictate the antigen binding mode of chimeric IgD and IgM: implications for the function of the B cell receptor. J Immunol **172:**2925-2934.

49. **Berretty PJ, Cormane RH.** 1979. Immunofluorescence studies on eosinophilic granulocytes. Br J Dermatol **101:**309-314.

50. **Walsh GM, Kay AB.** 1986. Binding of immunoglobulin classes and subclasses to human neutrophils and eosinophils. Clin Exp Immunol **63:**466-472.

51. **Lawrence DA, Weigle WO, Spiegelberg HL.** 1975. Immunoglobulins cytophilic for human lymphocytes, monocytes, and neutrophils. J Clin Invest **55:**368-376.

52. **Hunyadi J, Hamerlinck F, Cormane RH.** 1976. Immunoglobulin and complement bearing polymorphonuclear leukocytes in allergic contact dermatitis and psoriasis vulgaris. Br J Dermatol **94:**417-422.

53. **Dikeacou TC, van Joost T, Cormane RH.** 1979. The recruitment of inflammatory cells using the skin-window technique. Arch Dermatol Res **265:**1-7.

54. **Rostenberg I, Penaloza R.** 1978. Serum IgG and IgD and levels in some infectious and noninfectious diseases. Clin Chim Acta **85:**319-321.

55. **Koelsch K, Zheng NY, Zhang Q, Duty A, Helms C, Mathias MD, Jared M, Smith K, Capra JD, Wilson PC.** 2007. Mature B cells class switched to IgD are autoreactive in healthy individuals. J Clin Invest **117:**1558-1565.

56. **Shan M, Carrillo J, Yeste A, Gutzeit C, Segura-Garzon D, Walland AC, Pybus M, Grasset EK, Yeiser JR, Matthews DB, van de Veen W, Comerma L, He B, Boonpiyathad T, Lee H, Blanco J, Osborne LC, Siracusa MC, Akdis M, Artis D, Mehandru S, Sampson HA, Berin MC, Chen K, Cerutti A.** 2018. Secreted IgD Amplifies Humoral T Helper 2 Cell Responses by Binding Basophils via Galectin-9 and CD44. Immunity **49:**709-724.e708.

57. **Salonen EM, Hovi T, Meurman O, Vesikari T, Vaheri A.** 1985. Kinetics of specific IgA, IgD, IgE, IgG, and IgM antibody responses in rubella. J Med Virol **16:**1-9.

58. **Organization WH.** 2014. Global Immunization Data. Accessed

59. **Lambert PH, Liu M, Siegrist CA.** 2005. Can successful vaccines teach us how to induce efficient protective immune responses? Nat Med **11:**S54-62.

60. **Anonymous.** 2011. Updated recommendations for use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis (Tdap) vaccine from the Advisory Committee on Immunization Practices, 2010. MMWR Morb Mortal Wkly Rep **60:**13-15.

61. **Munoz FM, Bond NH, Maccato M, Pinell P, Hammill HA, Swamy GK, Walter EB, Jackson LA, Englund JA, Edwards MS, Healy CM, Petrie CR, Ferreira J, Goll JB, Baker CJ.** 2014. Safety and immunogenicity of tetanus diphtheria and acellular pertussis (Tdap) immunization during pregnancy in mothers and infants: a randomized clinical trial. Jama **311:**1760-1769.

62. **Healy CM, Rench MA, Baker CJ.** 2013. Importance of timing of maternal combined tetanus, diphtheria, and acellular pertussis (Tdap) immunization and protection of young infants. Clin Infect Dis **56:**539-544.

63. **Medina KL, Smithson G, Kincade PW.** 1993. Suppression of B lymphopoiesis during normal pregnancy. J Exp Med **178:**1507-1515.

64. **Medina KL, Kincade PW.** 1994. Pregnancy-related steroids are potential negative regulators of B lymphopoiesis. Proc Natl Acad Sci U S A **91:**5382-5386.

65. **Medina KL, Strasser A, Kincade PW.** 2000. Estrogen influences the differentiation, proliferation, and survival of early B-lineage precursors. Blood **95:**2059-2067.

66. **Kincade PW, Medina KL, Payne KJ, Rossi MI, Tudor KS, Yamashita Y, Kouro T.** 2000. Early B-lymphocyte precursors and their regulation by sex steroids. Immunol Rev **175:**128-137.

67. **Medina KL, Garrett KP, Thompson LF, Rossi MI, Payne KJ, Kincade PW.** 2001. Identification of very early lymphoid precursors in bone marrow and their regulation by estrogen. Nat Immunol **2:**718-724.

68. **Verthelyi DI, Ahmed SA.** 1998. Estrogen increases the number of plasma cells and enhances their autoantibody production in nonautoimmune C57BL/6 mice. Cell Immunol **189:**125-134.

69. **Grimaldi CM, Michael DJ, Diamond B.** 2001. Cutting edge: expansion and activation of a population of autoreactive marginal zone B cells in a model of estrogen-induced lupus. J Immunol **167:**1886-1890.

70. **Grimaldi CM, Jeganathan V, Diamond B.** 2006. Hormonal regulation of B cell development: 17 beta-estradiol impairs negative selection of high-affinity DNA-reactive B cells at more than one developmental checkpoint. J Immunol **176:**2703-2710.

71. **Fettke F, Schumacher A, Canellada A, Toledo N, Bekeredjian-Ding I, Bondt A, Wuhrer M, Costa S-D, Zenclussen AC.** 2016. Maternal and Fetal Mechanisms of B Cell Regulation during Pregnancy: Human Chorionic Gonadotropin Stimulates B Cells to Produce IL-10 While Alpha-Fetoprotein Drives Them into Apoptosis. Frontiers in immunology **7:**495-495.

72. **Wang Z-Y, Mathiarasu A, Hong P, Xu Y, Li A, Bluth M, Lum L, Huang B, Romero R, Cerutti A, Chen K.** 2013. Defective induction of regulatory B cells by decidua stromal cells underlies the pathogenesis of preterm birth (P3213). The Journal of Immunology **190:**171.119-171.119.

73. **Bhat NM, Mithal A, Bieber MM, Herzenberg LA, Teng NN.** 1995. Human CD5+ B lymphocytes (B-1 cells) decrease in peripheral blood during pregnancy. J Reprod Immunol **28:**53-60.

74. **Kwak JY, Beaman KD, Gilman-Sachs A, Ruiz JE, Schewitz D, Beer AE.** 1995. Up-regulated expression of CD56+, CD56+/CD16+, and CD19+ cells in peripheral blood lymphocytes in pregnant women with recurrent pregnancy losses. Am J Reprod Immunol **34:**93-99.

75. **Tamiolakis D, Anastasiadis P, Hatzimichael A, Liberis B, Karamanidis D, Kotini A, Petrakis G, Romanidis K, Papadopoulos N.** 2001. Spontaneous abortions with increased CD5 positive cells in the placental tissue during the first trimester of gestation. Clin Exp Obstet Gynecol **28:**261-265.

76. **Jensen F, Wallukat G, Herse F, Budner O, El-Mousleh T, Costa SD, Dechend R, Zenclussen AC.** 2012. CD19+CD5+ cells as indicators of preeclampsia. Hypertension **59:**861-868.

77. **Huang B, Faucette AN, Pawlitz MD, Pei B, Goyert JW, Zhou JZ, El-Hage NG, Deng J, Lin J, Yao F, Dewar RS, 3rd, Jassal JS, Sandberg ML, Dai J, Cols M, Shen C, Polin LA, Nichols RA, Jones TB, Bluth MH, Puder KS, Gonik B, Nayak NR, Puscheck E, Wei WZ, Cerutti A, Colonna M, Chen K.** 2017. Interleukin-33-induced expression of PIBF1 by decidual B cells protects against preterm labor. Nat Med **23:**128-135.

78. **Raghavan M, Chen MY, Gastinel LN, Bjorkman PJ.** 1994. Investigation of the interaction between the class I MHC-related Fc receptor and its immunoglobulin G ligand. Immunity **1:**303-315.

79. **Kristoffersen EK.** 1996. Human placental Fc gamma-binding proteins in the maternofetal transfer of IgG. APMIS Suppl **64:**5-36.

80. **Vaughn DE, Bjorkman PJ.** 1998. Structural basis of pH-dependent antibody binding by the neonatal Fc receptor. Structure **6:**63-73.

81. **Faucette AN, Pawlitz MD, Pei B, Yao F, Chen K.** 2015. Immunization of pregnant women: Future of early infant protection. Hum Vaccin Immunother **11:**2549-2555.

82. **Costa-Carvalho BT, Vieria HM, Dimantas RB, Arslanian C, Naspitz CK, Sole D, Carneiro-Sampaio MM.** 1996. Transfer of IgG subclasses across placenta in term and preterm newborns. Braz J Med Biol Res **29:**201-204.

83. **van den Berg JP, Westerbeek EA, Berbers GA, van Gageldonk PG, van der Klis FR, van Elburg RM.** 2010. Transplacental transport of IgG antibodies specific for pertussis, diphtheria, tetanus, haemophilus influenzae type b, and Neisseria meningitidis serogroup C is lower in preterm compared with term infants. Pediatr Infect Dis J **29:**801-805.

84. **Saji F, Koyama M, Matsuzaki N.** 1994. Current topic: human placental Fc receptors. Placenta **15:**453-466.

85. **Saji F, Samejima Y, Kamiura S, Koyama M.** 1999. Dynamics of immunoglobulins at the feto-maternal interface. Rev Reprod **4:**81-89.

86. **Ellinger I, Schwab M, Stefanescu A, Hunziker W, Fuchs R.** 1999. IgG transport across trophoblast-derived BeWo cells: a model system to study IgG transport in the placenta. Eur J Immunol **29:**733-744.

87. **Bode CJ, Jin H, Rytting E, Silverstein PS, Young AM, Audus KL.** 2006. In vitro models for studying trophoblast transcellular transport. Methods Mol Med **122:**225-239.

88. **Cumberland P, Shulman CE, Maple PA, Bulmer JN, Dorman EK, Kawuondo K, Marsh K, Cutts FT.** 2007. Maternal HIV infection and placental malaria reduce transplacental antibody transfer and tetanus antibody levels in newborns in Kenya. J Infect Dis **196:**550-557.

89. **de Moraes-Pinto MI, Almeida AC, Kenj G, Filgueiras TE, Tobias W, Santos AM, Carneiro-Sampaio MM, Farhat CK, Milligan PJ, Johnson PM, Hart CA.** 1996. Placental transfer and maternally acquired neonatal IgG immunity in human immunodeficiency virus infection. J Infect Dis **173:**1077-1084.

90. **Palmeira P, Quinello C, Silveira-Lessa AL, Zago CA, Carneiro-Sampaio M.** 2012. IgG placental transfer in healthy and pathological pregnancies. Clin Dev Immunol **2012:**985646.

91. **Boeuf P, Aitken EH, Chandrasiri U, Chua CL, McInerney B, McQuade L, Duffy M, Molyneux M, Brown G, Glazier J, Rogerson SJ.** 2013. Plasmodium falciparum malaria elicits inflammatory responses that dysregulate placental amino acid transport. PLoS Pathog **9:**e1003153.

92. **Tripathi V, Sixt KM, Gao S, Xu X, Huang J, Weigert R, Zhou M, Zhang YE.** 2016. Direct Regulation of Alternative Splicing by SMAD3 through PCBP1 Is Essential to the Tumor-Promoting Role of TGF-beta. Mol Cell **64:**549-564.

93. **Jefferis R.** 2009. Recombinant antibody therapeutics: the impact of glycosylation on mechanisms of action. Trends Pharmacol Sci **30:**356-362.

ABSTRACT

IMMUNOGLOBULIN D UNDERGOES PLACENTAL TRANSFER TO THE FETUS

by

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MAY 2019

Advisor: Dr. Kang Chen

Major: Immunology & Microbiology

Degree: Master of Science

Immunoglobulin D is a mysterious protein that has largely been overlooked since its discovery. It is uniquely distinct from all the other antibody classes in: expression, induction, structure and function. Within the last few years IgD has made its way back into the spotlight and novel functions of the protein have been discovered. These recent discoveries have provided the necessary information for us to uncover an additional and important function of IgD during pregnancy. It is a long-held belief among scientists that during pregnancy the only antibody class that can cross the placenta is IgG, however our data shows that this common place ideology is not correct. We find that IgD is fully capable of crossing the placenta and that vaccine-specific IgD induced by maternal vaccination undergoes placental transfer to the fetus.

AUTOBIOGRAPHICAL STATEMENT

Being a purebred Michigander, I graduated from Chippewa Valley High School in 2010. Shortly after high school graduation, I began competing in NCAA Division III wrestling and began my undergraduate studies at Ohio Northern University. I majored in Biology and minored in Chemistry at Ohio Northern University and initially planned on attending medical school after graduation. Eventually I realized that this might not be the ideal career path for me and I began to become seriously interested in scientific research careers. Following my graduation from Ohio Northern University in 2014, I immediately began looking for employment opportunities in the medical research field. Not long after my graduation from Ohio Northern University, I was hired as a full-time research assistant at the Wayne State University School of Medicine by Dr. Kang Chen. After working in Dr. Chen's lab for a year I undoubtedly knew that I wanted to pursue a lifelong career in immunology research. I applied and was accepted into the Wayne State University School of Medicine Immunology and Microbiology department as a masters student in the Fall semester of 2016. I continued to stay in Dr. Chen's lab for my masters research project and all of my time spent in Dr. Chen's lab is where I truly fell in love with the immunology and microbiology field. After completion of my M.S., I plan on furthering my education and pursuing a PhD in immunology.