

IgG2 subclass isotype antibody and intrauterine infections

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The foetus is dependent on its mother for passive immunity involving receptor-mediated specific transport of antibodies. IgG antibody is present in highest concentration in serum and is the only antibody type that can cross the placenta efficiently, except for its IgG2 subclass. Most of the pathogenic manifestations affecting the foetus involve capsular antigens and polysaccharides of pathogens and it is known that immune response to these antigens is primed to the predominant production of IgG2 type of antibody. Paradoxically, the IgG2 subclass cannot cross the placenta and neutralize such antigens; therefore, infections related to these antigens may persist and can lead to serious conditions like miscarriage and stillbirth. This article describes in brief the properties of IgG subclasses, intrauterine infections seen during pregnancy and discusses possible IgG-based strategies to manage infections to afford protection to the foetus.

Keywords: Class switching, clinical approaches, foetal immunity, IgG subclasses, intrauterine infections.

ACCORDING to a *Lancet* study¹, more than 3 million stillbirths occur each year across the globe. Risk factors for stillbirths and other pathogenic manifestations include genetic defects, obesity, advanced child-bearing age and most importantly, intrauterine infections. Intrauterine infections caused predominantly by bacteria and viruses are considered as the major maternal insults during pregnancy and could lead to permanent damage/fatality of the developing foetus². Viruses known for their pathogenic manifestations in the foetus include cytomegalovirus, herpes simplex, mumps, Western equine encephalitis, chicken pox shingles, smallpox, vaccinia, rubeola, influenza, polio, coxsackie, hepatitis and rubella virus³. Bacterial infestation in the foetus involves *Streptococcus agalactiae* (*Strep B*), *Listeria monocytogenes*, *Treponema pallidum*, *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

Humans have a range of mechanisms for combating infections. The breakdown of the innate immunity barrier by pathogens is followed by confrontation by the adaptive immune system. An adaptive immune response can be classified into humoral and cell-mediated. Humoral immunity involves the detection of soluble antigens, lead-

ing to antibody production. Based on their heavy chains, antibodies are categorized into five types – IgA, IgE, IgD, IgM and IgG. Each antibody class has a unique role apart from specific recognition of antigenic epitopes, for example, complement activation, antigen neutralization, opsonization, induction of phagocytosis, etc. IgG is present in highest concentration (13–15 mg/ml) in circulation and therefore can be regarded as physiologically most important. IgG can be further grouped into IgG1, IgG2, IgG3 and IgG4 (Table 1). Each IgG subtype heavy chain contains a separate constant region coding gene segment that is endowed with unique biological and functional properties. There are nine such gene segments⁴ localized on chromosome 14. All IgG subclass antibodies can pass through the placenta efficiently, except IgG2. The IgG2 antibody is known to be produced mainly against polysaccharides and capsular antigens^{5,6}. Interestingly, most of the pathogens responsible for intrauterine infections possess capsule/polysaccharide content in their envelope and IgG2 response has been reported in many of these infections^{6–8}. But IgG2 response in a mother cannot be passed through the placenta efficiently. We discuss here on the role of IgG in intrauterine infections and propose clinical approaches that could be tested and explored for treating such infections.

Class-switching

Vertebrates have the capacity to switch the expression of different constant heavy-chain genes by a DNA recombination mechanism. This allows the choice of switching to one of the several different constant heavy-chain genes depending on the requirement of the immune system to combat a specific pathogen effectively. Class-switching is directed by the type of antigen and cytokines, which also determines the production of a specific class of immunoglobulin. The isotype switch is influenced in both a positive and negative manner by the cytokines and B-cell activators due to their ability to regulate germline (GL) transcription. IL-4, IFN- γ and TGF- β are the cytokines which have been found to be significant in affecting germline transcription. In the case of human B-cells stimulated with IL-4 and phorbol 12-myristate-13-acetate or IL-4 plus CD40L, switching is directed to IgG1, IgG3, IgG4 and IgA. It has also been well demonstrated that IFN- γ acts synergistically with IL-6 to induce IgG2 in

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Table 1. Subclasses of IgG

Property	IgG1	IgG2	IgG3	IgG4
Normal serum level (mg/ml)	9	3	1	0.5
<i>In vivo</i> serum half-life (days)	23	23	9	23
Fc γ R receptors	Fc γ RI, Fc γ RII and Fc γ RIII	Fc γ RII	Fc γ RI, Fc γ RII and Fc γ RIII	Fc γ RI
Complement activation	Y	Minimal	Y	N
Crossing the placenta	Y	Not efficiently	Y	Y
Antipolysaccharide immune response	Little	Predominant	Little	N

human B-cells. Clinical trials have demonstrated enhanced production of IgG2 (10–100%) in pokeweed mitogen (PWM)-activated peripheral blood B-cells isolated from normal controls treated with IFN- γ in six out of seven individuals. Though the study comprised of a small sample size, the observations are relevant to direct clinical applications⁴. A study, involving IgG profiling in response to anti-measles vaccine, revealed the complete absence of IgG2 in children below 3 years of age and its predominant presence in children of age 4 years and above. Up to the age of 12, IgG2 levels are found to be not more than 50% that of the adults⁹.

Inability of IgG2 to cross the placenta

In 1958, Franklin and Kunkel showed that only the IgG class of antibodies is transported across the placenta, which was later shown to be transported through receptor-mediated endocytosis by receptors specific for the Fc portion of the molecule^{10–13}. Any transfer of IgG antibody molecule involves the crossing of two cellular barriers: the syncytiotrophoblast epithelium and the endothelium of foetal capillaries. It has been shown that IgG2 is transported into the syncytiotrophoblast, but obstructed at the foetal capillary endothelium¹². Discrimination of the IgG2 subclass against other subclasses has been proved beyond any doubt^{14,15}.

In humans, there are three major classes of Fc γ R which include Fc γ RI (CD64), Fc γ RII (CD32) and Fc γ RIII (CD16). Fc γ RI is a high-affinity receptor capable of binding monomeric human IgG1, IgG3 and IgG4. Fc γ RII and Fc γ RIII are low-affinity receptors, interacting only with IgG in complexed or aggregated form. Both Fc γ RII and Fc γ RIII interact with human IgG1 and IgG3, whereas Fc γ RII is the sole Fc γ R class capable of binding human IgG2 complexes^{6,16,17}. It has been shown that placental villous trophoblasts could be stained intensely by anti-Fc γ RIII MAb 3G8, while both anti-Fc γ RI (MAb 32) and anti-Fc γ RII (MAbsIV3, KU79, CIKM5, 2E1, KB61, and 41H16) antibodies did not react with these cells^{18,19}.

Unique features of IgG2

IgG2 antibody consists of γ type 2 heavy chains and light chains which could be κ or λ . IgG2 antibody is present in

variable amounts depending on the age, pathogenicity and special conditions like pregnancy and nutritional deficiency. Its titre varies during the different phases of life. Its relative titre in comparison to other subtypes in adults is²⁰: IgG1 > IgG2 > IgG3 \approx IgG4, but as mentioned earlier⁹, children below 4 years of age are deficient in IgG2.

The flexibility of antibodies varies in accordance with the number of disulphide bonds, the length and type of amino acids present in the hinge region. In IgG2, the hinge region is constituted by 12 amino acids and four inter-disulphide bonds. It consists of a polyproline double helix and lacks glycine residues, making IgG2 highly rigid.

IgG2 requires Fc γ RII (CD 32) receptor for transport across the placental membrane and absence of these receptors on the placental barrier is solely responsible for their poor transport across the placenta. This antibody is the most predominant antibody in an anti-polysaccharide immune response. It contributes little to the immune response against proteins²¹.

IgG2 antibodies have been found to play a key role in immunity against infection with encapsulated bacteria. IgG2 effector function mainly involves phagocytosis by neutrophil granulocytes. It is a poor complement activator, especially when the epitope density is low²².

Characteristics of intrauterine infections

Microorganisms can infect amniotic cavity and the foetus through the following ways: hematogenous dissemination through the placenta; ascending from the vagina and the cervix; retrograde seeding from the peritoneal cavity through the fallopian tubes and rarely accidental introduction at the time of invasive procedures. The most common pathway for intrauterine infection is the ascending route^{23–25}. Both bacterial and viral infections can cause lethal/irreversible damage to foetus. Effects of these pathogens on foetus have been summarized in Table 2. Most of the bacterial pathogens possess capsular antigens as mentioned in Table 2. It is known that capsular antigens give predominant IgG2-type response. In many of these bacterial and viral pathogens, predominant IgG2 responses have been documented^{6–8}.

Table 2. Intrauterine infections

Intrauterine Infections	Effects on foetus
Bacterial infections [Presence of capsule has been indicated by (+) and absence by (-).]	
<i>Chlamidia trachomatis</i> (+)	Neonatal conjunctivitis, pneumonia, Ectopic pregnancy, stillbirth
<i>Listeria monocytogenes</i> (-)	Septicemia, meningitis, abortion
<i>Neisseria gonorrhoeae</i> (+)	Ophthalmia neonatorum, systemic neonatal infection
<i>Streptococcus agalactiae</i> (+)	GBS neonatal infection, morbidity
<i>Treponema pallidum</i> (+)	Foetal syphilis, hydrops, prematurity, neonatal death, late sequelae
Viral infections	
Chicken pox-shingles	Chicken pox or shingles, increased abortions and stillbirths
Coxsackie B viruses	Myocarditis, congenital heart disease
Cytomegalovirus	Microcephaly, chorioretinitis, deafness and mental retardation, cerebral calcifications, seizures, blindness, hepatosplenomegaly and lethal damage to the foetus
Hepatitis	Hepatitis
Herpes simplex	Generalized herpes, encephalitis, death
Influenza	Malformations
Mumps	Premature birth rate, foetal death, endocardial fibroelastosis and cardiac malformations.
Polio	Spinal or bulbar polio
Rubella	German measles and congenital rubella syndrome involving malformation of heart, cataract, deafness, microcephaly, mental retardation. During newborn period : bleeding, hepatosplenomegaly, pneumonitis, hepatitis, encephalitis, etc.
Rubeola	Increased abortions and stillbirths
Small pox	Small pox, increased abortions and stillbirths
Vaccinia	Generalized vaccinia, increased abortions
Western equine encephalitis	Encephalitis

Immune response in foetus

The foetus relies mainly on passive immunity acquired from the mother and therefore to all those antigens to which the mother is exposed. As mentioned earlier, passive immunity involves transport of immunoglobulins that could neutralize the antigen in the foetus. Greater susceptibility of the embryonic tissue and the relative immaturity of immunological responses of the developing foetus may play significant roles in the pathogenicity of the infection²⁶. The developing foetal immune system involves immune tolerance after exposure to foreign antigens. It has been reported that tolerance induction in the human foetus is in part mediated by an abundant population of foetal regulatory T-cells (T-regs), which is significantly greater in percentage (~15) of the total peripheral CD4⁺ T-cells in the developing human foetus than is found in healthy infants and adults (~5). Foetal T-cells undergo enhanced proliferation after exposure to allo-antigens and are poised to become T-regs upon stimulation^{27,28}, a process dependent on TGF- β . Thus, the foetus has a tendency of becoming tolerant to antigens on exposure.

Antibody responses are not detected in human foetus up to the second trimester of pregnancy. After six weeks, both passive and early active antibodies have been found against certain congenital infections, including rubella and cytomegalic inclusion disease³. Before the mid-trimester there is a deficiency of IgG2 due to poor transport across the placenta. Thus, the foetus is vulnerable to pathogens.

Possible clinical approach for the management of infections in pregnancy

It has been observed that people deficient in IgG2 have infections with *Haemophilus influenzae*, *Neisseria meningitides* and *Streptococcus pneumoniae*, thus proving the tolerance of IgG2 against such pathogens⁴. Though the predominant subclass isotype antibody produced in response to polysaccharide and capsular antigens is IgG2, because of lack of its corresponding receptors on the placental cells, this antibody cannot cross the placenta to provide protection to the foetus. The question that arises here is, why the human immune system which appears to have evolved so optimally in parallel with increasing complexities of pathogens did not evolve in case of the IgG2 subclass antibody? Protection of the foetus from bacteria should have been evolutionarily significant as IgG2 type of response to polysaccharide antigens is the most predominant one in adults, and the foetus would look toward the maternal immune system to protect itself. Yet the placenta is deficient in transporter receptors to IgG2.

Can we counteract this deficiency of nature? Though, presently in the experimental stages and plagued with problems, the following strategies may be considered for use in future, if refinement of the same becomes possible.

Vaccination

An expecting mother could be vaccinated with the pathogenic polysaccharides conjugated with a protein, thus

producing types of antibodies which could pass through the placenta and neutralize the infection. This type of vaccination has been suggested for IgG2-deficient patients⁵. Thus, response generated in the form of IgG1 and IgG3 could pass through placenta more efficiently and neutralize the infection. Titres would need to be monitored after immunization, to ensure protective amount of antibody.

In vitro antibody synthesis specific for intrauterine infections and administration

Techniques like chimerization, phage display and transgenic mice can be considered to produce specific antibodies (IgG1/IgG3). Chimerization involves joining of variable (V; antigen binding) domains of a specific mouse monoclonal antibody of interest to the constant domains of a human antibody and this process necessitated an appreciation of the structure and function of immunoglobulin domains^{29,30}. Transgenic mice and phage display involve human antibody genes, thus can give fully humanized IgG response. These techniques can overcome problems associated with chimeric antibodies like the high immunogenicity in humans and the weak interactions with human complement and FcγRs, resulting in improved effector functions³¹.

Specific peripheral blood lymphocytes (PBMcs) capable of giving IgG1/IgG3 response may be isolated from people diagnosed with relevant infections and immortalized with Epstein Barr Virus/hybridoma approach. Human–mouse hybrids are unstable³². However, once stable lines are obtained, the same can be used forever. The approach involving human–mice hybrid cells is preferred over immortalization by Epstein Barr Virus³³. Antibodies obtained thus can be checked for the isotype specificity and those that are of IgG1 and IgG3 type may be used for passive transfer of immunity.

Cytokines and future prospects

Cytokines are responsible for inducing class-switching to particular isotype/s. The combinations of cytokines could be tried *in vitro* first, followed by clinical trials on a larger population. If successful, the induced class-switching may increase the immunity in children against pathogens with capsular and polysaccharide antigens, by increasing the titre of IgG2. Therefore, cytokines may be injected *in vivo* that are reported to induce class-switching of IgM to IgG1 or/and IgG3, both of which have transporter receptors that can allow transport across the placental barrier. However, the problem that can be envisaged here is that cytokines have pleiotropic roles and administration with cytokines may result in unrelated and unwanted immunological reactions⁴.

Another treatment could be the passive transfer of the specific IgG2 type of antibodies by the external route

followed by purification and direct injection into the foetal circulation which, of course, would not be the method of choice.

The above-mentioned strategies have been proposed by us. None of them have been experimentally verified, and have lacunae. But, these have the potential to manage intrauterine infections.

Discussion

Majority of infections in pregnant women are localized and have no effects on foetus. But some infections can pass through placenta, leading to foetal health ailments like growth retardation and developmental defects. In developing countries like India, intrauterine infections are still important risk factors for stillbirths. Maternal or foetal infections are often associated with a range of adverse outcomes of pregnancy. These include stillbirth, preterm delivery, congenital malformations, intrauterine growth retardation and long-term neurological sequelae such as sensorineural hearing loss^{34,35}. Most of the important causative agents of intrauterine infections possess capsular and polysaccharide antigens which can elicit IgG2 response. But this response cannot be passively transported to the foetus in expecting mothers due to the inability of IgG2 to pass across placenta. This may be the reason for the deficit in neutralization of these pathogens and their persistence inside the uterus. Taking into account the adverse effects of invasive procedures for diagnosis and treatment therapies on the foetus, vaccination during pregnancy seems to be a promising alternative. Conjugate vaccination, as mentioned in the ‘clinical approach’ section, if developed, may help in decreasing the severity of infections. Other approaches for boosting immunity against pathogens in pregnancy as suggested here should be tested and improved by clinical research. Also, extensive research is required to be able to direct appropriate antibody responses to pathogens so as to optimize their immunological effects in curbing infections³⁶. Fatality in newborns is still high in many countries. It is even higher in poor and developing countries. Though efforts by international organizations like WHO are crucial in the prevention and cure of many diseases, there is much to be done. Intrauterine infections are much more complex than any other infections, therefore even more efforts towards care, diagnosis and treatment are needed.

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