Project Immune networks of vaccination during pregnancy and beyond

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Background and preliminary data:

Vaccination in Pregnancy: mechanisms of protection

Vaccination during pregnancy plays an important role in protecting pregnant women and developing fetuses and infant. In the last two decades, the pace and focus of maternal vaccination has accelerated most significantly since the influenza pandemic 2009 and the ongoing SARS-CoV-2 pandemic. However, a number of challenges and key knowledge gaps remain including acceptance of an increasing number of vaccines with an indication for use in pregnancy, optimal timing of vaccination, the effect of antigen type on transplacental transfer of antibody, correlates of protection against key pathogens, and the effect on subsequent infant immune responses to vaccination. Therefore, it is crucial to decipher the mechanisms of protection aiming to improve safety, efficacy and ultimately uptake and acceptance of vaccines in pregnancy.

Within the immune mechanisms of protection by vaccination in pregnancy, the Immunoglobulin G (IgG) remains as one of the predominant. For instance, IgG is the only antibody isotype actively transferred across the placenta from around 13 weeks of gestation and increases exponentially during the 3rd trimester such that the concentration of IgG in the newborn infant is similar to, or exceeds, that in the mother. IgG provides passive immunity to the infant in the first months of life. The neonatal Fc receptor facilitates transcytosis of maternal IgG. IgG is transferred from maternal blood across the syncytiotrophoblast (STB) layer of the placenta, initially by endocytosis of IgG. Within the acidic endosome, IgG binds to membrane-bound neonatal Fc receptor, which is then released on the fetal side of the STB as the pH returns to physiologic pH. Neonatal Fc receptor is then recycled back to the maternal side of the STB to bind further IgG. There are a number of factors that may affect the efficiency of IgG transcytosis, including gestation, IgG subclass and maternal infection. While the predominant mechanism of protection afforded by maternal vaccination is transplacental transfer of IgG, there is potential for additional protection conferred by antibody in breast milk. Secretory IgA (sIgA) is thought to protect against diarrheal and respiratory pathogens through a variety of mechanisms, including immobilization, prevention of adhesion or by neutralization of toxins or virulence factors.

Glycans are an integral part of the IgG molecule

IgG is a highly abundant glycoprotein and the most abundant immunoglobulin in the human plasma. It exists in four subclasses (IgG1 to 4), differing in the amino acid sequence of the heavy chain and the number of interchain disulfide bridges. IgG is involved in, amongst others, antigen binding, activation of the complement system and antibody-dependent cell-mediated cytotoxicity (ADCC), and its appearance and biological activity are highly influenced by the N-glycan structure on the fragment crystallizable (Fc)-region. Changes in the abundances of different glycan features on the Fc-region are reported to associate with pregnancy. For example, galactosylation increases during pregnancy. Alterations in attached glycan structures change the conformation of the IgG Fc-region and with that the possible interactions with Fc-receptors (FcγRs) expressed on leukocytes. A notable example is the difference in binding affinity of IgG1 to FcγRIIIa when carrying a glycan that is either fucosylated or nonfucosylated, resulting in enhanced ADCC for antibodies carrying nonfucosylated Fc-glycans. The topic of healthy human IgG Fc-glycosylation during pregnancy is for a large part covered by studies describing levels of glycosylation features in healthy pregnant women, however, the Fc-glycosylation of vaccinated pregnant women is yet not comprehensively covered. In addition, not all studies into the modulating effects of glycans are performed for all subclasses of IgG, but often only for IgG1. Our own ongoing study shown reduced levels of neutralizing IgG in pregnant vaccinees compared with non-

pregnant vaccinees and a significantly lover cell-mediated immunity, as analyzed 2 weeks after the second vaccine application. This underpins the urgent need to understand the specific features of the IgG glycosylation in respond to vaccination during pregnancy. Moreover, the risk for neonatal infections can be considerably reduced by a passive transfer of pathogen-specific antibodies from mother to fetus during pregnancy. In this context, our own data highlighted that the transfer of IgG antibodies from mother to fetus is also dependent on placenta-specific features including placental vascularization.

Hypothesis: We hypothesise that antigen-specific IgG glycosylation triggered by "pregnancy" during vaccination are causative factor that influence IgG effector functions.

Aims and Work Programme:
1. Investigate the glycosylation patterns of IgG in pregnancy compared to non-pregnant populations upon vaccination.
2. Identify the IgG effector functions during vaccination in pregnant and non-pregnant populations.

In Aim #1,
2.1 Under this aim, we will analyze IgG glycosylation (Fc-glycopeptides) which is responsible for effector functions using matrix-assisted laser desorption/ionization (MALDI)-time of flighty (TOF)-mass spectrometry (MS) method. Both total IgG and pathogen specific IgG-class of pregnant and non-pregnant samples (maternal serum) will be included.

In Aim #2,
2.1 Once we have determined the glycosylation patterns of the pathogen specific IgG in non-pregnant patients, these findings will be compared to the pregnant population, specifically considering the immunogenicity and reactogenicity of vaccination.

Project-related publications: (max. 5)

Fathi A, *et al.* … **Addo MM**. Increased neutralization and IgG epitope identification after MVA-MERS-S booster vaccination against Middle East respiratory syndrome. Nat Commun. 2022 Jul 19;13(1):4182. doi: 10.1038/s41467-022-31557-0. PMID: 35853863; PMCID: PMC9295877

Rechtien A, *et al.* …**Addo MM**. Systems Vaccinology Identifies an Early Innate Immune Signature as a Correlate of Antibody Responses to the Ebola Vaccine rVSV-ZEBOV. Cell Rep. 2017 Aug 29;20(9):2251-2261. doi: 10.1016/j.celrep.2017.08.023. PMID: 28854372; PMCID: PMC5583508

Blois SM *et al.* Role of galectin-glycan circuits in reproduction: from healthy pregnancy to preterm birth (PTB). Semin Immunopathol. 2020 Aug;42(4):469-486. doi: 10.1007/s00281-020-00801-4. Epub 2020 Jun 29. PMID: 32601855; PMCID: PMC7508936.

Olearo F, *et al.* **Diemert A** ... Tallarek AC. Anti-SARS-CoV-2 antibodies in breast milk during lactation after infection or vaccination: A cohort study. J Reprod Immunol. 2022 Sep;153:103685. doi: 10.1016/j.jri.2022.103685. Epub 2022 Aug 4. PMID: 36029724; PMCID: PMC9349337.

Tallarek AC *et al.* **Diemert A**, Arck PC. Inefficient Placental Virus Replication and Absence of Neonatal Cell-Specific Immunity Upon Sars-CoV-2 Infection During Pregnancy. Front Immunol. 2021 Jun 3;12:698578. doi: 10.3389/fimmu.2021.698578. PMID: 34149740; PMCID: PMC8211452.