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Viewpoint

The urgent need for more basic research on SARS-Cov2 infection and vaccines in assessing potential psychoneurological effects using maternal immune activation (MIA) and other preclinical modeling

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ABSTRACT

The rapid development and application of different SARS-Cov2 vaccines world-wide has resulted in impressive efficacy and protection from this deadly pandemic. However, the existence of different and continuously developing vaccine candidates coupled with the likelihood of continued application due to both waning immune responses and emergence of viral mutants, means that more basic research regarding their efficacy and continued application are needed. This is particularly true with use of preclinical models involving effects when given during pregnancy. The substantial body of data on the impact of maternal immune activation (MIA) on neurologic development and behavior in the progeny necessitates the need to have all vaccine candidates, particularly when inducing strong toll receptor (TLR) responses, involving these models. Use of other preclinical models involving autoimmunity and allergy coupled with incorporation of human modifying variables of aging and obesity should also be applied to better reflect the heterogeneity of the general population and potential off-target effects that may arise. Additionally, the use of human ACE2 receptor transgenic mouse models can shed insights given the differential tissues expression at different stages in development. However, to foster these types of basic research studies involving different vaccine products, initiatives must first be implemented and supported at the governmental level even while clinical data still accumulates.

The extraordinary nature of the SARS-Cov2 pandemic has resulted in an unprecedented accelerated development of multiple vaccine candidates (RNA, DNA and protein-based) demonstrating considerable efficacy and broad application to not only adults and children but also in pregnancy. The importance of having vaccines available in these at-risk populations is unquestionable with SARS-Cov2 given the extreme severity and morbidity of the pandemic at a global level. The impact of SARS-Cov2 infection on both the mother and fetus could also be significant, especially with recent reports of infection resulting in maternal immune activation (MIA) at the placental interface (Lu-Culligan, 2021). However, basic science questions related to the use of these newer RNA-based and adenoviral-based gene delivery vaccine approaches on these and other at-risk population cohorts remain understudied. Coupled with the existence of multiple vaccine products in use with even more still being developed and evaluated world-wide, this adds complications as it dilutes thorough preclinical vetting for each one. Additionally, the emergence of viral variants as well as evidence of waning immune protection (Cromer, 2021) indicates that it is likely that further administration of these and/or further modified vaccines will be necessary for the foreseeable future. Surprisingly though, robust preclinical investigations pertaining to these issues has been seriously lacking. This is especially true now as there has not been resources made available for independent assessment of different vaccines on possible long-term off-target effects or the risk factors that may impact them using all available appropriate models. Simply because clinical application has been initiated should not preclude doing such assessments now, not only for being proactive, but also for potentially optimizing efficacy and guiding future use.

Different preclinical models have a huge impact on their ability to detect potential short or long-term effects and what can be safely used in an adult should not be automatically extrapolated to be safe in all stages of life and development. Case in point, a substantial body of literature in multiple preclinical models, from laboratory mice to non-human primates (NHP), demonstrate that administering toll receptor (TLR) agonists such as poly I:C, a synthetic double-stranded RNA (dsRNA) analog which signals via TLR3, and TLR4 agonists such as LPS, during pregnancy can result in maternal immune activation (MIA) culminating with the manifestation of abnormal behaviors in the progeny resembling

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autism and schizophrenia (Careaga et al., 2017; Bauman, 2014). These neurological effects are not observed when such stimuli are applied postnatally. The behavioral alterations in the progeny are also associated with marked transcriptomic changes in neuronal gene and immune pathways (Tsivion-Visbord, 2020). While these preclinical models do have limitations and the ability to directly extrapolate to humans is still unclear, it is extremely fortunate such preclinical models ranging from mouse to NHP exist to even attempt to mirror human neuropsychiatric conditions. Therefore, the effects of all SARS-Cov2 vaccines given during pregnancy and potential neuropsychiatric effects on their offspring merits closer attention using these models.

The different vaccine modalities do differ from the stimuli used in experimental MIA preclinical modeling. RNA-based vaccines are single-stranded RNA (ssRNA), signaling primarily via TLR7/8, are not equivalent to poly I:C which, as dsRNA, primarily signal through TLR3. However, these signaling paradigms are not as restricted as once thought as even ssRNA has been also demonstrated to trigger via TLR3 (Marshall-Clarke, 2007; Tatematsu et al., 2013). Furthermore, recent reports suggest that TLR7 agonists can also induce MIA resulting in similar effects on the progeny (Missig, 2020). While RNA modifications, such as those applied in current vaccine formulations, have been shown to reduce these cytokine-inducing effects as demonstrated by *in vitro* assays suggesting possible lesser systemic immune reactions (Karikó et al., 2005), these *in vitro* immune assessments are severely limited in scope and are inadequate substitutes for complex *in vivo* immune responses which can vary greatly depending on the different stages in development. This also applies to different stages in neonatal development as the timing of MIA initiation likely effects on susceptibility to fetal neuronal perturbation and needs to be determined. Up to now, the paucity of *in vivo* studies that have been reported primarily focused on assessment of protective immune responses or evaluation of any early overt toxicities. However, RNA editing during neonatal brain development is a tightly regulated process, making these stages particularly vulnerable to perturbations that may not occur after birth and into adulthood and not even being revealed unless assessed by behavioral assays. Furthermore, assuming lesser immune responses are automatically less of a risk may not hold true as recent data using the poly I:C mouse model suggests that intermediate levels of mouse MIA confer the greatest risk for brain development and behavioral alterations in the progeny with higher levels appearing protective indicating the complexity of the phenomena (Estes, 2020). In this regard, understanding the impact of SARS-Cov2 infection itself during pregnancy using these models is also important considering the presence of neurological effects in some adults persisting long after infection, some of which may be due to autoreactive responses.

Other factors outside of the ssRNA or adenoviral DNA delivery itself can also potentially impact immune responses to vaccines. The nano-liposomal formulations used in the RNA vaccine delivery platform appear to elicit inflammatory responses (Ndeupen et al., 2021) which likely also contribute to the systemic effects observed as do effects resulting from adenoviral-based vaccines. The potential addition of adjuvants to augment immune responses can exert similar additive effects. The antigen encoded by the vaccines may also contribute to overall immune responses due to recent data demonstrating that the SARS-Cov2 spike protein which is the primary antigen targeted, can directly trigger TLR2 inflammatory responses by different cell-types (Khan et al., 2021). As this is the predominant antigen targeted with vaccines, this would suggest that vaccines using this determinant may also have potential effects depending on the stages in development and should be assessed. Other factors intrinsic to the host also dramatically impact immune responses and need to be incorporated in the models which often rely on young healthy laboratory animals. Heightened pro-inflammatory responses commonly observed with aging and obesity (Bouchlaka, 2013; Murphy, 2016) add yet other variables impacting immune responses. This has been demonstrated with some cancer immunotherapy toxicities being observed in aged and obese mouse models but not observed in young healthy mice. Additionally, given some of the reported SARS-Cov2 vaccine toxicities, while rare, involving the RNA-based vaccines with regard to allergic reactions and the thrombolytic events associated with the adenoviral-based vaccines, it would be important to also incorporate preclinical models where autoimmune and allergic reactions are also modeled combined with pregnancy. Models should also involve mice transgenic for the human ACE2 receptor which is the receptor for the Spike protein and now being used for assessing SARS-Cov2 infection effects. As ACE2 receptor expression also can vary in tissue expression and with age (Chen et al., 2020), these models can provide insights on other potential off-target effects possibly due to anti-idiotype responses following infection or vaccination. Thus, any new type of vaccine application in pregnancy coupled with obesity, increased age, or autoimmune predisposition should probably not be automatically assumed to be without risk to fetal brain development because it has been deemed safe in young, healthy pregnant preclinical models. The general application of these different vaccines to the general population and increasing pressure for compliance, immediate prioritization for evaluation of the vaccines using these preclinical neurodevelopmental models as well as incorporating human modifying variables such as age and obesity in pregnancy outcome should be initiated at the governmental level world-wide. Preclinical modeling, such as ones which involve behavioral studies, can be complex and need appropriate resources, time, and expertise to sufficiently address these questions. This is particularly important given the at times vocal public backlash regarding vaccines overall and attempts to link with autism fueled, in large part, by misinformation or misinterpretation of the scientific literature. However, it is vital that this adverse environment not also inhibit or adversely impact reasonable scientific discourse on hypothesis-driven concerns and research or be viewed as a condemnation of vaccines. There is little doubt on the critical need for vaccines to be applied to all aspects of the population and it is hoped that these vaccines are safe and effective in pregnancy mirroring the efficacy seen in adults. However, the safety of these newer vaccine products needs to be coincided with continuous and robust preclinical evaluation using appropriate models building off the extensive literature that is used to study such effects. It is very fortunate that different vaccines classes are potentially available for use as it may be possible to tailor a particular vaccine for different at-risk populations. However, preclinical research is first needed to guide such recommendations. Given the severity and impact of the pandemic, it is striking at the paucity of resources and research initiatives currently in place for such evaluations, particularly within the NIH and FDA, given the world-wide reliance on continuous vaccine success. Relying on clinical outcome data will likely take many years to accumulate and the landscape is continually changing. Without concurrent evaluation using relevant models and significant research investment, this can undermine the acceptance of future vaccine recommendations. Given the extreme severity of the pandemic on the global population and unknown effects infection has during pregnancy, the importance and benefits of vaccination are unquestionable. However, it is with extensive and independent basic science evaluation that more complete reassurances on safety as well as further optimization will also occur.

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