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Maternal-fetal immune responses in pregnant women infected with SARS-CoV-2

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Abstract

- Pregnant women are a high-risk population for severe/critical COVID-19 and mortality
- Infection primarily induces unique inflammatory responses

01

Introduction

Relevance

>150K

Pregnant women in the United States have been infected with SARS-CoV-2.

Increased risk

Of hospitalization, mechanical ventilation, intensive care unit admission, and preterm birth.

Most neonates

Most neonates born to infected women test negative for COVID-19.

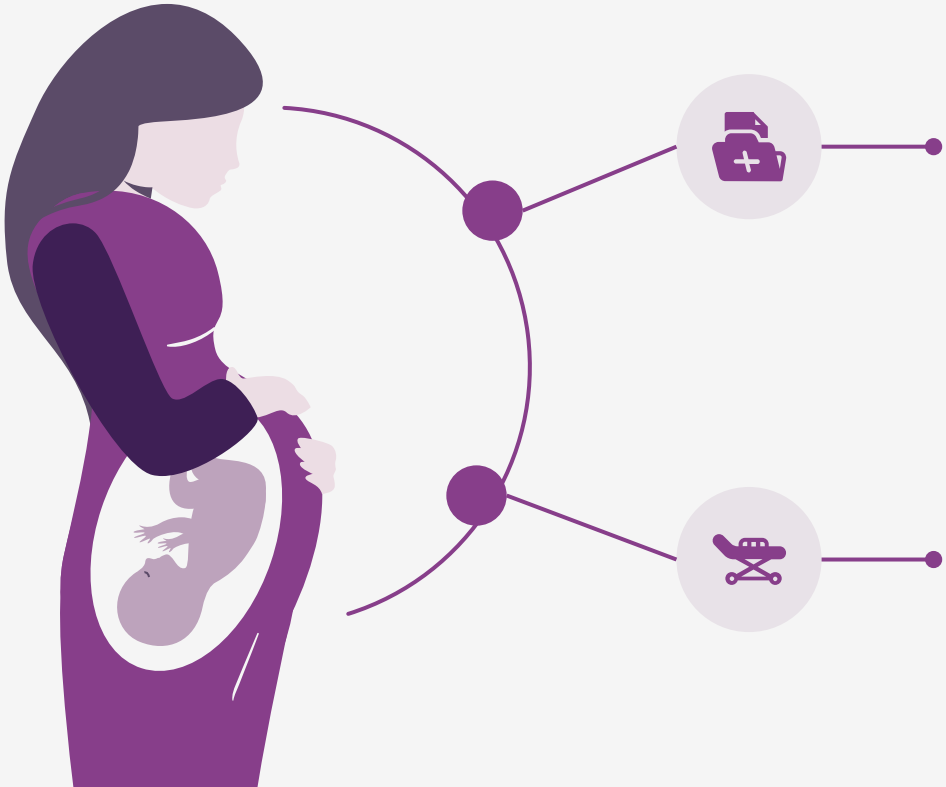
In utero

Vertical transmission is possible.

Infection

Associated with vascular damage in pregnant women.

Coronaviruses can enter host cells via two main canonical mechanisms:



Direct Pathway

Host cells are required to express both the angiotensin-converting enzyme 2 (ACE-2) receptor and the serine protease TMPRSS2

Endosomal Route

Cell entry can be mediated by ACE-2 alone

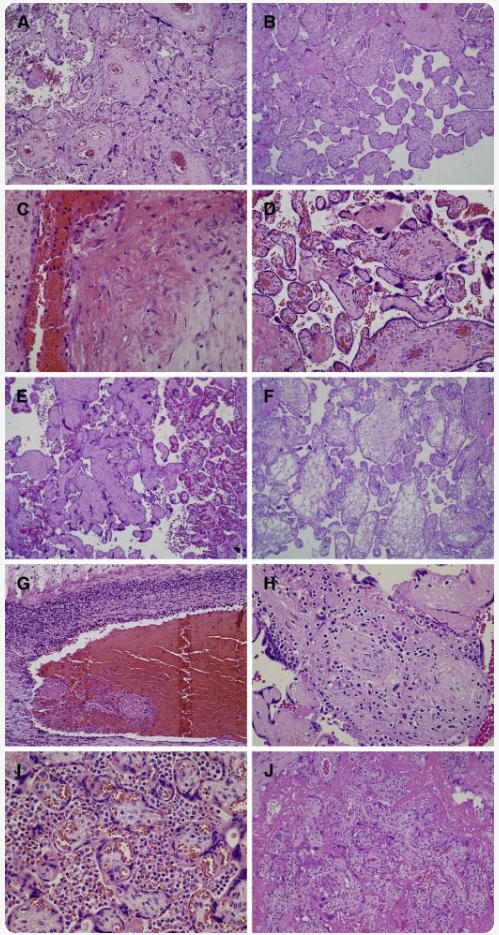
Methods

Human participants, clinical specimens, & definitions

- The Perinatology Research Branch, an intramural program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health, U.S. Department of Health and Human Services, Wayne State University, and the Detroit Medical Center obtained human maternal peripheral blood, umbilical cord blood, and placental tissues
- Pregnant women with positive RT-PCR results for SARS-CoV-2 and healthy gestational age-matched controls made up the study's two main groups

Placental histopathological examination

- Sections from the center of the placenta and placental disk were taken and histologically examined
- Lesions on the placenta samples were diagnosed according to certain standards



Immunoassays

Immunoglobulin

- Maternal and umbilical cord blood were collected and centrifuged
- Concentrations of IgM and IgG from the serums were then determined

Cytokine and Chemokine

- Maternal and umbilical cord blood were collected and centrifuged
- Concentrations of 10 different cytokine receptors from the plasma were measured

Leukocytes

- Maternal and umbilical cord blood were collected, incubated, and lysed
- Immunophenotyping encompasses the process of identifying the leukocyte populations present in the solution and counting the absolute number of cells in each population

ROS Production

- Maternal and umbilical cord blood were stimulated with buffer and acetate, incubated, and lysed
- The resulting leukocytes were centrifuged and resuspended in buffer to measure ROS production by neutrophils and monocytes

Single-cell RNA Sequencing

Single-cell suspensions

Prepared by straining tissue suspensions, centrifuging them, and then resuspending them

Single-cell libraries

Cell suspensions were generated into gel beads and reverse mRNA transcripted to prepare the single-cell library construct. The libraries were then sequenced

Viral reads

Viral reference genomes and genomes from the DNA and cells collected were constructed and studied in Viral-Track

Genotyping

Purified DNA samples were obtained and genotyped using two different platforms

Gene Expression

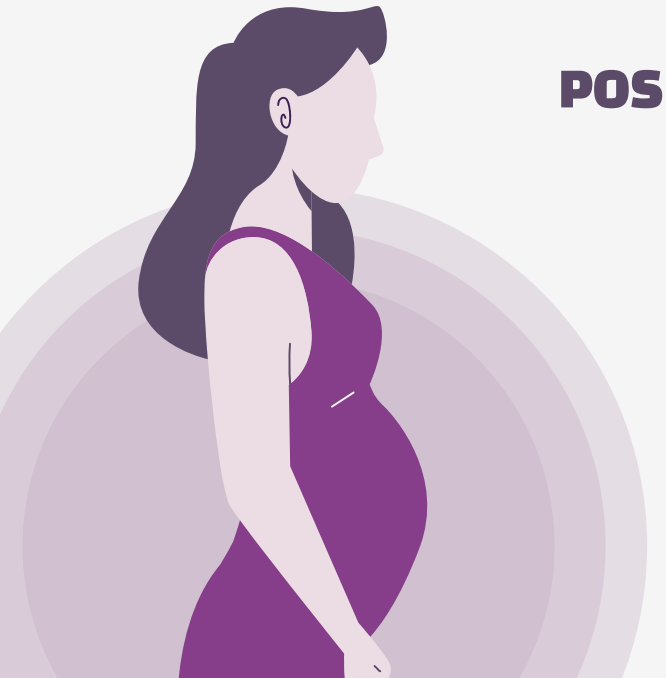
Differentially expressed genes were identified by creating collections of cells of similar origins/types

Comparison

The single-cell library showing the effects of SARS-CoV-2 was compared to data collected from another study

Results

Characteristics of Study Population



SIZE: 23 women

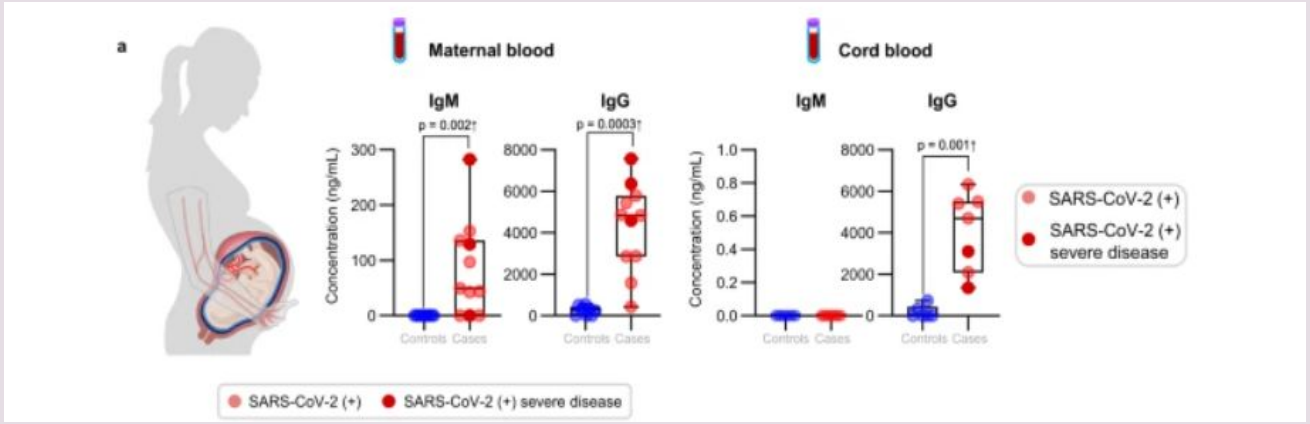
POSITIVE: 12 women (8 asymptomatic; 1 with mild symptoms; 3 had severe COVID-19 (requiring oxygen supplementation)

Neonates were not RTC-PCR tested for SARS-CoV-2

No differences in demographic and clinical characteristics found between study groups

Preg Women with SARS-CoV-2 infection and their neonates exhibit distinct IgM responses

- Detectable levels of IgM suggest that the fetus was infected with SARS-CoV-2, given that this immunoglobulin can not cross the placenta due to its large molecular weight
- IgG was increased in the cord blood of neonates born to women infected with SARS-CoV-2 infection but IgM was undetected
- Serological data implies that none of the neonates were infected with SARS-CoV-2

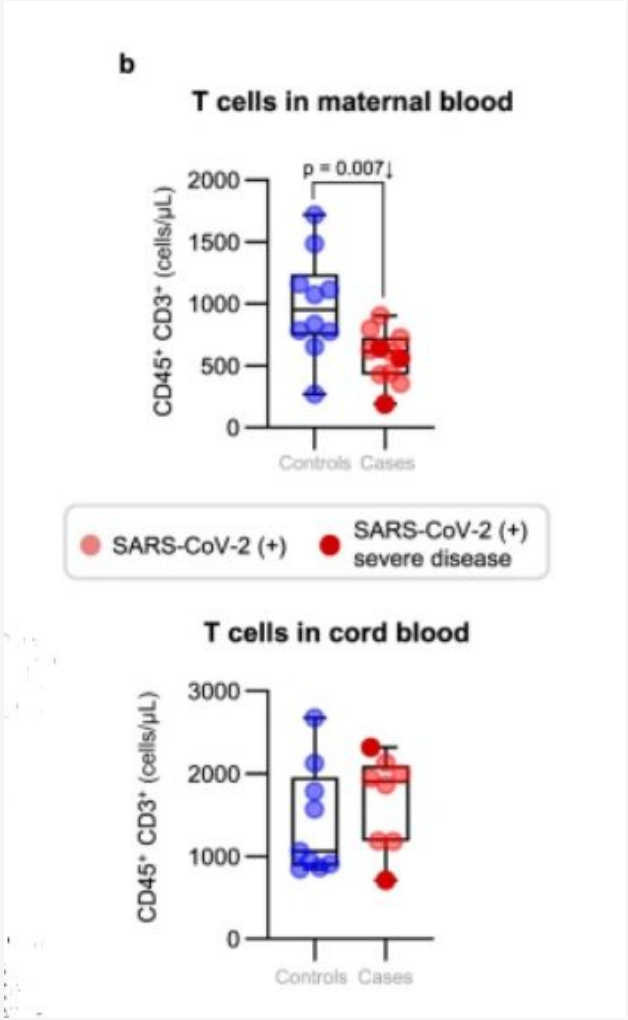

a
Maternal blood
Cord blood
IgM
IgG
IgM
IgG
p = 0.002†
p = 0.0003†
p = 0.001†
Concentration (ng/mL)
Concentration (ng/mL)
300
200
100
0
8000
6000
4000
2000
0
1.0
0.8
0.6
0.4
0.2
0.0
Controls Cases
Controls Cases
Controls Cases
Controls Cases
SARS-CoV-2 (+)
SARS-CoV-2 (+) severe disease
SARS-CoV-2 (+)
SARS-CoV-2 (+) severe disease

Pro-inflammatory cytokine responses are displayed in the circulation of pregnant women with SARS-VoV-2 infection and their neonates

- Neonates born to women infected with SARS-CoV-2 had increased concentrations of IL-8 (2-FC) compared to those born to control mothers
- Cytokine response is observed in both the maternal and neonatal circulation upon maternal infection with SARS-CoV-2

Pregnant women with SARS-CoV-2 infection, but not their neonates, undergo a T-cell reduction in the circulation

- Used immunophenotyping to investigate whether changes in cellular immune repertoire occur or not
- No statistical differences observed in the mother or the neonate in the total number of general leukocyte subpopulations or in the monocyte, neutrophil, activated T-cell, and B-cell subsets


b
T cells in maternal blood
p = 0.007↓
CD45⁺ CD3⁺ (cells/µL)
2000
1500
1000
500
0
Controls
Cases
SARS-CoV-2 (+)
SARS-CoV-2 (+) severe disease
T cells in cord blood
CD45⁺ CD3⁺ (cells/µL)
3000
2000
1000
0
Controls
Cases

- Differences in abundance among cell type clusters were observed between placental compartments as well as between tissues from women with SARS-CoV-2 infection and those from controls
- The majority of the differentially expressed genes (DEGs, Supplementary Data 1) between SARS-CoV-2-positive cases and controls belong to immune cells from the CAM, namely maternal T cells (89 DEGs) and macrophages (12 DEGs)
- Other fetal cell types (e.g., trophoblasts and T cells) in the CAM and PVBP were minimally altered by the presence of SARS-CoV-2 infection in the mother

SARS-CoV-2 infection during pregnancy does not compromise the sterility of the placenta.

Blood RNA sequencing reveals shared and distinct immune responses to SARS-CoV-2 infection

- SARS-CoV-2 infection was associated with the dysregulation of 425 transcripts in maternal blood: 165 upregulated and 260 downregulated
- Biological processes enriched in the upregulated DEGs in maternal blood included humoral responses such as complement activation, adaptive immune responses, and immunoglobulin-mediated immune response, whereas those enriched in downregulated DEGs included phagocytosis and extracellular matrix organization
- SARS-CoV-2 infection induced significantly different responses in the maternal blood compared to the cord blood for 34 genes
- These findings also suggest that, although SARS-CoV-2 infection does not trigger fetal hematopoietic immune responses in the placenta as evidenced by our scRNA-seq data, it affects the neonatal immune system.

SARS-CoV-2 RNA and proteins are not detected in the placentas of infected women.

- First, the researchers investigated whether viral sequences were found in the scRNA-seq data of CAM and PVBP from women with SARS-CoV-2 infection using a method called Viral-Track.
- Positive controls (bronchoalveolar lavage from SARS-CoV-238-infected patients) contained SARS-CoV-2 virus sequences, but placental tissues did not.

Discussion

Immune Responses

- SARS-CoV-2 infection in pregnancy is primarily related with maternal inflammatory responses in the circulation and at the maternal-fetal interface
  - Only IgG was found in the cord blood of newborns of pregnant women with SARS-CoV-2 infection, although both IgM and IgG levels were raised in the peripheral circulation
  - SARS-CoV-2 causes modest systemic inflammation in pregnant women
    - Infected moms with SARS-CoV-2 and their newborns both reported elevated IL-8 levels in their blood
  - SARS-CoV-2-infected neonates showed dysregulated immunological and non-immune processes

Existence of SARS-CoV-2 In Placenta

BACTERIAL MICROBIOME

The placentas of women who gave birth by cesarean section did not consistently contain a microbiome of bacterial DNA, which is consistent with earlier investigations.

PLACENTAL INFECTION

SARS-CoV-2 was not detected in the placentas of infected women, nor was the sterility of the placenta compromised by this virus.

RNA Sequencing of Maternal and Cord Blood

- RNA was isolated from maternal and cord blood and raw data was inputted into a RNA-seq library
- Differences between SARS-CoV-2 maternal/cord blood samples and immune cell types found in placenta were analyzed

Molecular Microbiology

1

Sample Collection

Swabs from different areas of placenta

2

DNA Extraction

Swab samples were centrifuged, washed, and incubated

3

Quantitative Real-time PCR

Bacterial DNA abundance was measured via amplification

4

Sequencing and Processing

Gene libraries were prepared and analyzed for possible contaminants

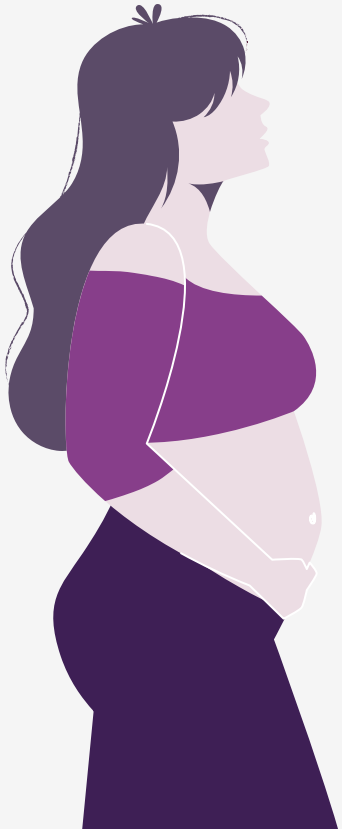
5

Statistical Analyses

5426 sequences were randomly sampled for structural differences

Statistical Analysis

- Linear mixed effects models were used to compare concentrations
- Significance of coefficient was based on the likelihood ratio test based on infection status
- P-values and Z-scores were visualized to compare SARS-CoV-2 positive and Control groups



Discussion Questions

- What is the timing of the mother-child transmission?
- How does SARS-CoV-2 infect placental cells?
- Would the rate or timing of placental infection change among differing demographic characteristics, such as ethnicity/age?
- Do you think the data would look any different if there was a larger symptomatic population?

REFERENCE

Garcia-Flores, V., Romero, R., et al (2022). Maternal-fetal immune responses in pregnant women infected with SARS-CoV-2. *Nature Communications*, *13*(1), 320. https://doi.org/10.1038/s41467-021-27745-z


Thanks for
listening