Title

SARS-CoV-2-specific T cell responses and immune regulation in infected pregnant women

Authors

Hsieh, Li-En

Grifoni, Alba

Dave, Hiral

et al.

Publication Date

2022-02-01

DOI

10.1016/j.jri.2021.103464

Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Reproductive Immunology 149 (2022) 103464

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jri

SARS-CoV-2-specific T cell responses and immune regulation in infected pregnant women

Li-En Hsieh [a], Alba Grifoni [b], Hiral Dave [a], Jasmine Wang [a], Diana Johnson [a], Jennifer Zellner [a], John Sidney [b], Christina Chambers [a,1], Alessandra Franco [a,*,1]

[a] University of California San Diego, School of Medicine, Department of Pediatrics, United States
[b] La Jolla Institute for Immunology, Division of Vaccine Discovery, United States

ARTICLE INFO

Keywords:
SARS-CoV-2
Pregnancy
T cells
Regulatory T cells
Tolerogenic dendritic cells
Immune regulation

ABSTRACT

We studied the T cell response to SARS-CoV-2 spike and non-spike peptide epitopes in eight convalescent pregnant women together with the immune monitoring that included innate tolerogenic dendritic cell populations important to maintain the immunological mother/fetus interface to address a potential risk for the antiviral cellular response in the outcome of pregnancy. Four subjects had pre-existing chronic inflammatory conditions that could have potentially affected the SARS-CoV-2-specific T cell response. Seven of eight subjects responded to SARS-CoV-2 peptides with differences within CD4+ T helper (Th) and CD8+ cytotoxic T cells (CTL). SARS-CoV-2-specific inducible regulatory T cells (iTreg) were numerous in circulation. CD4+ T cell memory included central memory T cells (T_{CM}) and effector memory (T_{EM}). As far as the CD8+ memory repertoire, T_{CM} and T_{EM} were very low or absent in eight of eight subjects and only effector cells that revert to CD45RA+, defined as T_{EMRA} were measurable in circulation. T cells were in the normal range in all subjects regardless of pre-existing inflammatory conditions. The immune phenotype indicated the expansion and activation of tolerogenic myeloid dendritic cells including CD14+ cDC2 and CD4+ ILT-4+ tmDC. In summary, SARS-CoV-2 infection induced a physiological anti-viral T cell response in pregnant women that included SARS-CoV-2-specific iTreg with no negative effects on the tolerogenic innate dendritic cell repertoire relevant to the immune homeostasis of the maternal-fetal interface. All eight subjects studied delivered full-term, healthy infants.

1. Introduction

Early data suggest that the special population of pregnant women is more susceptible to severe disease when infected with SARS-CoV-2 (Delahoy, 2020; Du Fosse et al., 2020; Ellington, 2020; Zambrano, 2020), and is at increased risk for adverse outcomes such as preterm delivery (Woodworth, 2020). Immune function is uniquely modified during pregnancy to allow for tolerance of the fetal allograft. The immune cells that reside at the interface between the placenta and the uterus are in fact enriched or excluded from the decidua depending upon their regulatory or inflammatory function (Red-Horse et al., 2004; Moffett and Loke, 2006; Mold and Mccune, 2012; Erlebacher, 2013). Specifically, dendritic cells (DC), the antigen presenting cells (APC) that activate T cells *via* the presentation of peptides through the human leucocyte antigen (HLA), are very sparse in the decidua. The maternal adaptive immunity that responds to pathogens is "confined" to prevent bi-directional trafficking across the placenta. The immune cells that constitute the maternal-fetal interface are the immune cells that populate the decidua. Specific lineages of suppressive myeloid dendritic cells prevent miscarriage of the pregnancy *via* IL-10 secretion and by priming regulatory T cells (Treg): tolerogenic dendritic cells (tmDC) defined by the expression of CD4, CD14, the immunoglobulin-like transcript (ILT)-4 with the canonical markers $CD11^c$ and $CD11^b$ (Burns et al., 2013). HLA-G expression on DC leads to the polarization of T cell lineages

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; Th, T helper cells; CTL, cytotoxic T cells; T_{CM}, central memory T cells; T_{EM}, effector memory T cells; T_{EMRA}, terminally-differentiated effector T cells; Treg, regulatory T cells; iTreg, peripherally-induced Treg; DC, dendritic cells; APC, antigen-presenting cells; tmDC, tolerogenic myeloid DC; ILT-4, immunoglobulin-like transcript-4; cDC, classical DC; MP, megapool; AIM, activation-induced cell markers; SI, stimulation index.

* Corresponding author at: 9500 Gilman Drive, La Jolla, CA 92093-0641, United States.
E-mail address: alfranco@health.ucsd.edu (A. Franco).
[1] Drs. Chambers and Franco are senior authors on this paper.

https://doi.org/10.1016/j.jri.2021.103464
Received 10 September 2021; Received in revised form 17 November 2021; Accepted 10 December 2021
Available online 11 December 2021

toward a regulatory phenotype *via* the ILT-4/HLA-G pathway (Gregori et al., 2010; Amodio et al., 2015). At the maternal-fetal interface, ILT-4+, HLA-G+ DC, named DC-10, are very important in orchestrating the early interaction with the trophoblast (Amodio et al., 2013; Gregori et al., 2015). Within the adaptive immunity, maternal Treg play a critical role in the immune homeostasis at the maternal-fetal interface. Treg restore immune tolerance to *self* and allo-antigens in humans (Roncarolo and Battaglia, 2007; Von Boehmer and Melchers., 2010; Wing and Sakaguchi, 2010).

Immune response to infection with the novel SARS-CoV-2 virus in pregnancy has not been well characterized.

In a recent case report, it was suggested that an infected pregnant woman had a good antibody response and there was no evidence of fetal damage (Du Fosse et al., 2020). Here we sought to characterize SARS-CoV-2-specific CD4+ and CD8+ T cell responses and the innate and adaptive immune phenotype in a small sample of pregnant women convalescent from SARS-CoV-2 infection.

2. Materials and methods

2.1. Study population

The study sample included eight pregnant women ranging in age from 26 to 40 years who were documented to be infected with SARS-CoV-2 at varying times in gestation. Four women were healthy prior to SARS-CoV-2 infection, one was autoimmune diagnosed with polyarthritis rheumatism, one was allergic with atopic dermatitis, and two had ulcerative colitis (Table 1).

Eligible subjects were selected for this study from pregnant women residing in the U.S. who were enrolled in the MotherToBaby prospective cohort study conducted by the OTIS Research Center at UC San Diego (Chambers et al., 2001, 2010; Chambers et al., 2013; Bandoli and Chambers, 2017; Chambers et al., 2018, 2019). All enrolled pregnant women provide detailed information about their demographics, health history, and status of SARS-CoV-2 infection through maternal interviews and medical record review. None of the subjects had a severe SARS-CoV-2 infection and hospitalization was not required. The subjects with pre-existing inflammatory conditions were not on steroids medications at the time of the study. The two subjects with ulcerative colitis were on mercaptopurine and anti-α4β7 integrin receptor respectively during pregnancy. Each subject provided a blood sample through a Quest Laboratory closest to their residence, which was subsequently sent by overnight mail to UC San Diego for processing and analysis. The study was approved by the Human Research Protection Program at UC San Diego, and all women provided written informed consent to participate.

2.2. Characterization of SARS-CoV-2-specific T cells

All the SARS-CoV-2 peptides were derived by the bioinformatics analyses and synthesized as crude material (T.C. laboratory, San Diego, CA), resuspended in DMSO, and pooled according to megapool (MP) composition followed by sequential lyophilization steps. SARS-CoV-2 CD4 and CD8 peptides MP have been designed and validated in acute and convalescent samples, as previously reported (Grifoni et al., 2020a, 2020b, Mateus et al., 2020; Weiskopf et al., 2020). The MP design was carried out on the Wuhan-Hu-1 reference isolated (GenBank Accession Number: MN908947). To ensure a comprehensive assessment of CD4-spike specific reactivity, the main target of vaccine candidates, overlapping 15-mers by 10 spanning the entire protein have been synthetized and pooled separately (Spike; n = 253). The remainder of the SARS-CoV-2 proteome was filtered applying the "7-allele-method" CD4-T cell prediction with a cutoff of ≤ 20, aiming to predict promiscuous epitopes with the capability to bind across the most common HLA class II specificities (Non-spike; n = 221).

SARS-CoV-2 CD8 MP epitopes were predicted using the 12 most frequent HLA A & B alleles with broad worldwide coverage in human population (A*01:01, A*02:01, A*03:01, A*11:01, A*23:01, A*24:02, B*07:02, B*08:01, B*35:01, B*40:01, B*44:02, B*44:03) and applying the IEDB recommended NETMHCpan4.0 EL algorithm. The top 100 resulting predicted epitopes for each HLA allele ranked based on prediction were selected and clustered using the IEDB cluster 2.0 tool applying the cluster-break method with 70 % cut off for sequence identity Grifoni et al., 2020a, 2020b, Mateus et al., 2020; Weiskopf et al., 2020) for a total of 628 peptides divided in two CD8 MPs (A and B) composed by 314 peptides each.

Peripheral mononuclear cells (PBMC) were separated from heparinized whole blood from the subjects by Ficoll-Hypaque density gradient. 1×10^6 PBMC were stimulated in 96 wells U bottom plates with 1 μg/mL of peptide MPs. PBMC cultured with 0.1 % DMSO, the same concentration of DMSO (solvent) in the MP-stimulated cultures, served as un-stimulated controls. 24 h after cultures, T cell activation was determined by the activation-induced cell markers (AIM) assay by measuring the co-expression of 4-1BB and OX40 on CD4+ T cells, and 4-1BB and CD69 on CD8+ T cells. The memory phenotypes of AIM+ T cells defined effector memory T cells (T_{EM}, CD45RA− CCR7−), central memory T cells (T_{CM}, CD45RA− CCR7+), and terminally differentiated effector T cells (T_{EMRA}, CD45RA+ CCR7−). CD4+ $CD25^{high}$ regulatory T cells (Treg) that responded to SARS-CoV-2 MPs were also studied. The monoclonal antibodies used for these assays included anti-CD3-AF700 (clone OKT3, mouse IgG2aκ, Biolegend), anti-CD4-BV605 (clone RPA-T4, mouse IgG1κ, BD Bioscience), anti-CD8-BV650 (RPA-T8, mouse IgG1κ, Biolegend), anti-4-1BB-Allophycocyanin (clone 4B4-1, mouse IgG1κ, Biolegend), anti-OX40-PE/Cy7 (clone Ber-ACT35, mouse IgG1κ, Biolegned), anti-CD69-PE (clone FN50, mouse IgG1κ, BD Bioscience), anti-CD45RA-BV421 (clone HI100, mouse IgG1κ, Biolegend), anti-CCR7-FITC (clone G043H7, mouse IgG2aκ, Biolegend), anti-CD25-BUV395 (clone M-A251, mouse IgG1κ, BD Bioscience). Data were recorded on LSRFortessa X-20 (BD Biosceince) and analyzed with FlowJo software version 10 (Tree Star).

2.3. Immune phenotype

Innate myeloid, plasmacytoid and uterus resident immune cells were defined from the same PBMC preparation used to determine SARS-CoV-

Table 1
Characteristics of sample of pregnant women infected with SARS-CoV-2.

Subject #	Maternal Health Status Prior to Pregnancy	Age	Race	Weeks' Gestation Onset of SARS-CoV-2 Infection	Weeks' Gestation at Sample Collection	Weeks from Infection Onset to Sample Collection	Outcome	Weeks' Gestation at Delivery
1	Healthy	29	Caucasian	9	12.4	3	Livebirth	39.1
2	Healthy	40	Caucasian	18	25.0	7	Livebirth	39.0
3	Healthy	32	Caucasian	29	37.9	8	Livebirth	40.3
4	Healthy	26	Caucasian	6	34.7	28	Livebirth	38.7
5	Polyarthritis rheumatism	36	Caucasian	29	35	6	Livebirth	41.3
6	Atopic dermatitis	30	Caucasian	29	35	6	Livebirth	38.1
7	Ulcerative colitis	35	Caucasian	35	38.1	3	Livebirth	40.9
8	Ulcerative colitis	33	Caucasian	23	35.3	12	Livebirth	37.6

2-specific T cell responses by surface markers by staining with monoclonal antibodies and analyzed by flow cytometry gating on specific populations: anti-human CD11c-Allophycocyanin, clone B-ly6, mouse IgG1κ; anti-human CD11b-Allophycocyanin/Cy7, clone ICRF44, mouse IgG1κ; anti-human CD14-PE/Cy7, clone M5E2, mouse IgG2aκ (BD Biosciences); anti-human CD123-BV421, clone 6H6, mouse IgG1κ; anti-human BDCA-1-PE/Dazzle594, clone L161, mouse IgG1κ (Biolegend); anti-human ILT-4-PerCp/eF710, clone 42D1, rat IgG2aκ (eBioscience); anti-human CD4-AF700, clone RPA-T4, mouse IgG1κ (BD Biosciences); anti-human HLA-G-PE, clone 87 G, mouse IgG2aκ (Biolegend); anti-human CD56-PE/Cy7, clone CMSSB, mouse IgG1κ (eBioscience); anti-human-CD209-BV421, clone 9E9A8, mouse IgG2aκ (Biolegend). The activation/maturation of the innate immune cells that present antigen to T cells was defined by the expression of CD86 by using anti-human-CD86 FITC, clone FUN-1, mouse IgG1κ (BD Biosciences). Naïve and activated CD4+ and CD8+ T cells were defined with anti-human CD4-PerCp/Cy5.5, clone RPA-T4, mouse IgG1κ; anti-human CD45RA-Allophycocyanin, clone HI100, mouse IgG2bκ; anti-human IL-7Rα-FITC, clone eBioRDR5, mouse IgG1κ; and anti-human CD56-PE/Cy7, clone CMSSB, mouse IgG1κ to define NKT cells (eBioscience) and anti-human CD8-AF700, clone RPA-T8, mouse IgG1κ (BD Biosciences). Data were acquired on BD CANTO II and analyzed with FlowJo software version 10 (Tree Star).

3. Results

3.1. Characterization of pro-inflammatory, regulatory and memory T cell responses to SARS-CoV-2

PBMC from the eight subjects were cultured 24 h with peptide pools to study T cell activation, antigen-specific Treg expansion and T cell memory in response to SARS-CoV-2.

The assay involved PBMC separation and 24 -h stimulation with SARS-CoV-2 peptides that included spike and non-spike epitopes for CD4+ T cell responses and spike and non-spike epitopes for CD8+ cytotoxic T cell (CTL) responses. The readout was an activation induced markers (AIM) assay based on flow cytometry to detect the early expression of molecules that define CD4+ and CD8+ T cell stimulation *via* T cell receptor signaling. Memory T cells on AIM + were enumerated by gating on CD45RA− and further defined as CCR7− effector memory T cells (T_{EM}) and CCR7+ central memory T cells (T_{CM}). T_{EMRA} memory cells are effector cells defined by reverting to a naïve CD45RA+

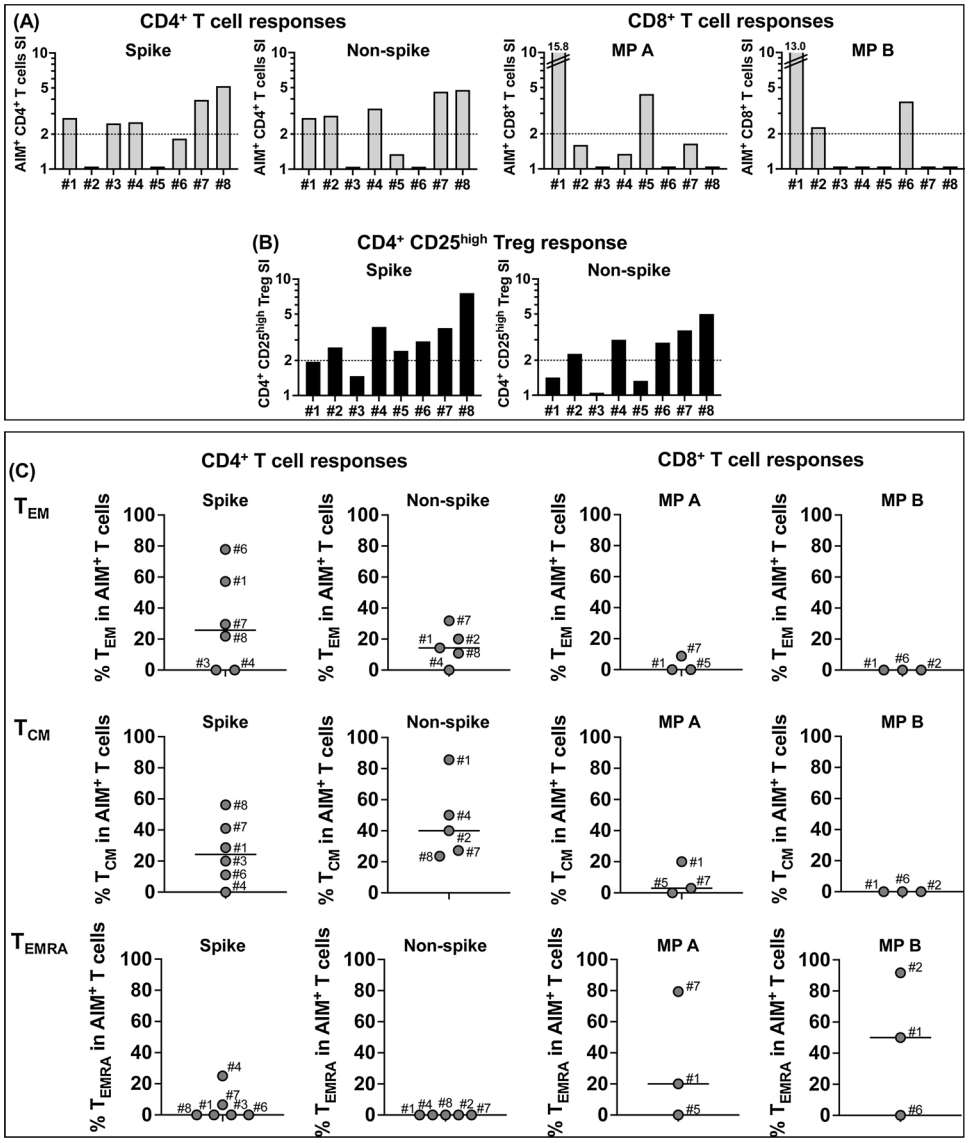


Fig. 1. CD4+ and CD8+ T and CD25^{high} regulatory T (Treg) cell responses to SARS-CoV-2 peptide epitopes and the memory phenotypes of the antigen-specific CD4+ and CD8+ T cells. PBMC from eight pregnant women infected by SARS-CoV-2 were separated from heparinized whole blood using Histopaque. PBMC were stimulated with SARS-CoV-2 CD4 spike (253 epitopes), CD4 non-spike (221 epitopes) peptide MPs, or CD8 (MP A and MP B, 314 epitopes each) for 24 h followed by the staining using a combination of monoclonal antibodies to study the T cell and iTreg activation in response to stimulation. The T cell memory phenotype of the antigen-specific AIM+ CD4+ and CD8+ T cells was also studied 24 h after stimulation. T cell and iTreg activation is shown as stimulation index (SI) by calculating the percentage of activated T cells in peptide -stimulated PBMC cultures divide by the percentage of activated T cells in the unstimulated control. A SI $\geq$ 2 was considered a T cell response to peptide epitopes. Memory phenotype of the AIM + T cells were identified as effector memory (T_{EM}; CD45RA− CCR7−), central memory (T_{CM}; CD45RA− CCR7+) T cells and terminally-differentiated effector that revert to a naïve phenotype (T_{EMRA}; CD45RA+ CCR7−). **(A)** CD4+ and CD8+ T cell responses to SARS-CoV-2 peptide epitopes. All the subjects showed a CD4+ T cell activation in response to SARS-CoV-2 CD4 peptides except for two subjects (#5 and #6). Four subjects (#1, #2, #5, and #6) of the eight subjects also showed CD8+ T cell responses to SARS-CoV-2 CD8 peptides. **(B)** CD4+ CD25^{high} iTreg expansion after SARS-CoV-2 CD4 peptides stimulation was found in six (#2, #4 - #8) of eight subjects. **(C)** Enumeration of SARS-CoV-2-specific memory T cells. CD4+ and CD8+ T_{EM}, T_{CM} and T_{EMRA} are shown. Each symbol represents the percentage of T_{EM} and T_{CM} and T_{EMRA} in the AIM+ CD4+ or CD8+ T cell populations. T_{EM} and T_{CM} were measurable in the AIM+ CD4+ T cell population but not CD8+ T cells. Conversely, T_{EMRA} were only AIM+ CD8+ T cells.

phenotype, although antigen experienced T cells. Representative flow images and flow gating strategies can be found in Supplementary Fig. 1. Treg were defined by the expression of $CD25^{high}$ measured on CD4+ populations stimulated with peptide MPs.

As shown in Fig. 1A, eight of eight subjects showed a T cell response to SARS-CoV-2 with some differences within the Th and CTL repertoire as previously observed in convalescent adults (Grifoni et al., 2020b). Within the four healthy women before infection, only subject #1, had expanded CD4+ T cells specific for spike and non-spike proteins and CD8+ T cells. In this subject, the CD8+ T cell expansion was very pronounced. Coordinated CD4+ and CD8+ T cell responses were also detectable in subject #2: this subject showed only non-spike-specific CD4+ T cells. Subject #4 did not show CD8+ T cell expansion but CD4+ T cells recognized spike and non-spike proteins, as subject #3, that had only CD4+ spike-specific T cells in circulation. Subject #5 with polyarthritis rheumatism and subject #6 with allergy showed only CD8+ T cell responses but not Th responses. In contrast, subjects #7 and #8 with ulcerative colitis had a very pronounced CD4+ T cell response to spike and non-spike proteins but not CD8+ T cell responses.

In pregnancy, regulatory T cells are of the most importance in the maternal-fetal interface. To address the antigen- specific SARS-CoV-2 adaptive regulation, we enumerated CD4+ $CD25^{high}$ Treg within the AIM+ T cells (Fig. 1B). Eight of eight subjects had numerous spike-specific and non-spike-specific inducible Treg (iTreg) (Fig. 1C and Supplementary Fig. 1), indicating a role in controlling the anti-viral response.

The development of T cell memory plays an important role not only to insure protection for the mother from possible secondary SARS-CoV-2 infection, but also protection of the child during birth and possibly later through maternal immunological microchimera and milk transfer (Hsieh et al., 2019; Franco, 2020). When we examined T_{EM} and T_{CM} by gating in AIM+ T cells, we found differences depending upon timing of infection (Fig. 1C). The main differences were observed between spike-specific CD4+ T_{EM} (numerous in six of eight subjects) and non-spike CD4+ T_{EM} repertoire which occurred at a much lower rate (Fig. 1C). Of note, none of the eight subjects showed a T_{EM} or a T_{CM} CD8+ T cell response irrespective of the timing of infection (Fig. 1C). Within the CD8+ T cell memory populations, T_{EMRA} were inversely numerous within CD8+ T cells but not CD4+ T cells (Fig. 1C). T cell numbers were in the normal range and comparable between subjects with CD4+ T cells ranging from 29.6 to 58.7% (median 46.9 %) and CD8+ T cells ranging from 9.4 to 24.6 % (median 19.8 %). CD4+ and CD8+ CD45RA+ naïve T cells were in the normal range, 54.8–88.9 % (median 79.8 %). Antigen experienced IL-7R+ T cells were comparable between subjects, 42.1–82.6 % (median 67.8 %) (Fig. 2).

3.2. The immune phenotype suggested expansion of tolerogenic innate cells

Next, we explored the antigen presenting cell (APC) compartments for T cell presentation including myeloid cells important for the immune tolerance for the protection of the fetus from the same PBMC preparation tested for SARS-CoV-2 T cell responses. The most relevant regulatory innate cells in pregnancy include three myeloid lineages that abundantly secrete the suppressive lymphokine IL-10: CD14+ cDC2 and tmDC defined by the expression of ILT-4 and CD4 that can be found in circulation and tissue resident DC10, defined by the expression of ILT-4 and HLA-G. We included monocytes, macrophages, pro-inflammatory cDC1, CD14− cDC2 and plasmacytoid DC. The expression of CD86 served to define cell activation/maturation in these innate populations. The gating strategies for the analysis of the populations by flow cytometry can be found in Fig. 3A. We also enumerated in circulation tissue resident DC-10, myeloid DC with suppressive function defined by the expression of the HLA-G and ILT-4 in addition to HLA-G+ regulatory decidual macrophages, and CD209+ decidual NK cells. These cells are important for the physiological formation of the villi and the protection of the fetal compartment from pro-inflammatory T cell responses that should not cross the myometrium that could be tracked in the periphery heading to tissues.

Tolerogenic innate cells CD14+ cDC2 and tmDC were numerous in seven of the eight subjects with the exception of subject #8 who had ulcerative colitis, although not statistically significant (Fig. 3B). When we measured the CD86 expression, we found that CD14+ cDC2 and tmDC were fully activated and mature, suggesting that immune tolerance was the most relevant efferent arm of the antigen presentation irrespective of maternal health status before pregnancy and the timing in pregnancy of SARS-CoV-2 infection (Fig. 3B).

Classical APC enumeration within the myeloid compartment included: monocytes, macrophages, cDC1, CD14− cDC2. The results pointed to few differences between subjects and a much lower activation of these cells compared with the tolerogenic counterpart (Fig. 3B). cDC1 and CD14− cDC2, canonical APC for T cell responses, were found in the normal range. Levels of CD86 expression, a marker of activation/maturity, were high in the two subjects whose samples were collected three weeks after onset of the infection (#1 and #7, respectively), suggesting that the anti-viral T cell response was still ongoing. We also enumerated CD123+ plasmacytoid DC, relevant in the innate response to viruses. These were found to be very low (lower than 6% in total PBMC) and again, not activated in the subjects whose samples were collected close to onset of the SARS-CoV-2 infection (Fig. 3C).

Tolerogenic tissue resident DC-10 and decidual macrophages were

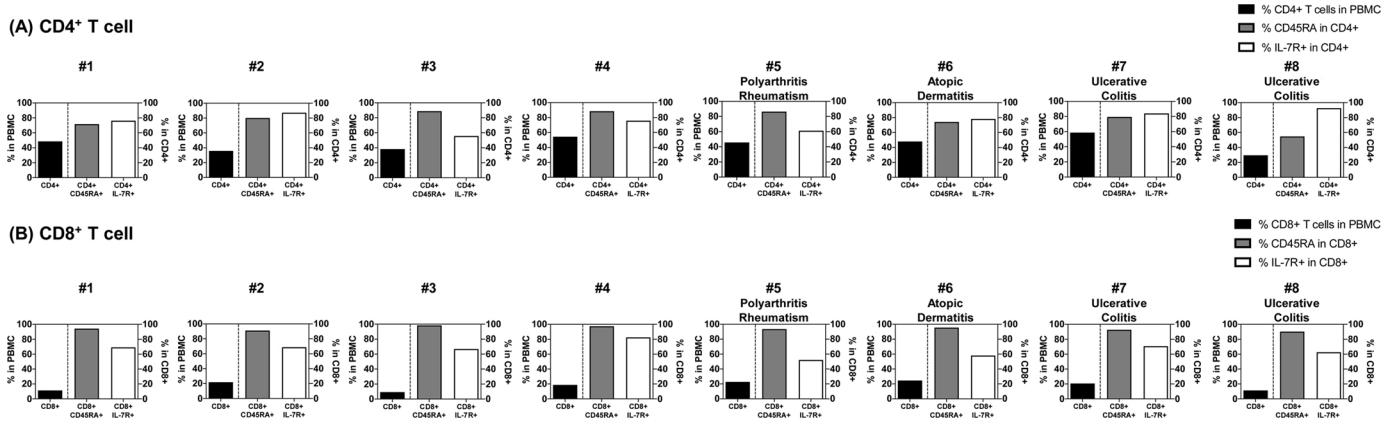


Fig. 2. Characterization of CD4+ and CD8+ T cells. CD4+ **(A)** and CD8+ **(B)** T cells in PBMC were enumerated and further characterized as CD45RA+ naïve T cells and antigen-experienced IL-7R+ T cells. CD4+ and CD8+ T cells in PBMC are shown with black bars, naïve CD45RA+ CD4+ and CD8+ T cells are shown with grey bars and antigen-experienced IL-7R+ CD4+ and CD8+ T cells are shown with white bars. No differences could be appreciated between SARS-CoV-2 convalescent healthy pregnant women and SARS-CoV-2-convaleacent pregnant women with pre-existing inflammatory conditions.

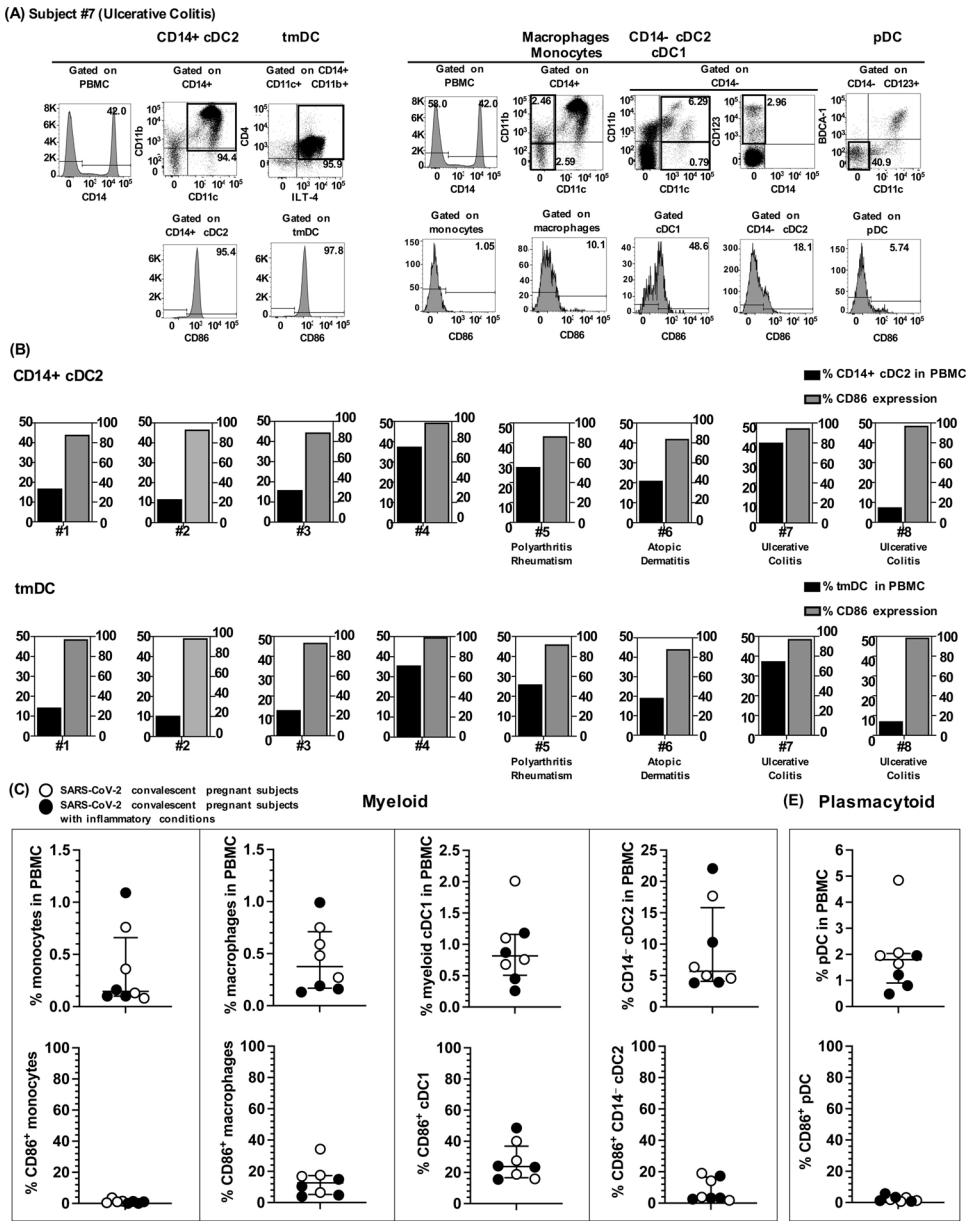


Fig. 3. Enumeration of circulating innate antigen presenting cell (APC) populations. PBMC were incubated with a combination of monoclonal antibodies to study different innate APC populations relevant to the antigen presentation to T cells and the immune regulation. **(A)** Representative FACS plots showing the gating strategies to identify cell populations: Classical CD14+ type 2 dendritic cells (CD14+ cDC2) were defined as CD14+ CD11c+ CD11b+ and tolerogenic myeloid dendritic cells (tmDC) were defined by the co-expression of ILT-4 and CD4 by CD14+ CD11c+ CD11b+ cells. **(B)** Circulating regulatory innate cells, CD14+ cDC2 and tmDC, and their expression of maturation/activation marker CD86. No significance of the percentages of CD14+ cDC2 or tmDC in the PBMC from the two cohorts. CD86 expressions were high in both CD14+ cDC2 and tmDC from both cohorts. Black bars: percentage of different regulatory innate cell populations. Grey bars: percentage of CD86+ in different regulatory innate cell populations. **(C)** Enumeration of CD14+ CD11c− CD11b− monocytes, CD14+ CD11c− CD11b+ macrophages, classical type 1 CD14− CD11c+ CD11b− myeloid dendritic cells (cDC1), and CD14− CD11c+ CD11b+ cDC2 and plasmacytoid dendritic cells defined by the expression of CD123, and lack of the expression of CD14, CD11c, and BDCA-1. The lower panel shows the expression of CD86, activation/maturation marker. SARS-CoV-2 convalescent pregnant subjects healthy before infection are shown with empty symbols, SARS-CoV-2 convalescent pregnant subjects with inflammatory conditions are shown with filled symbols.

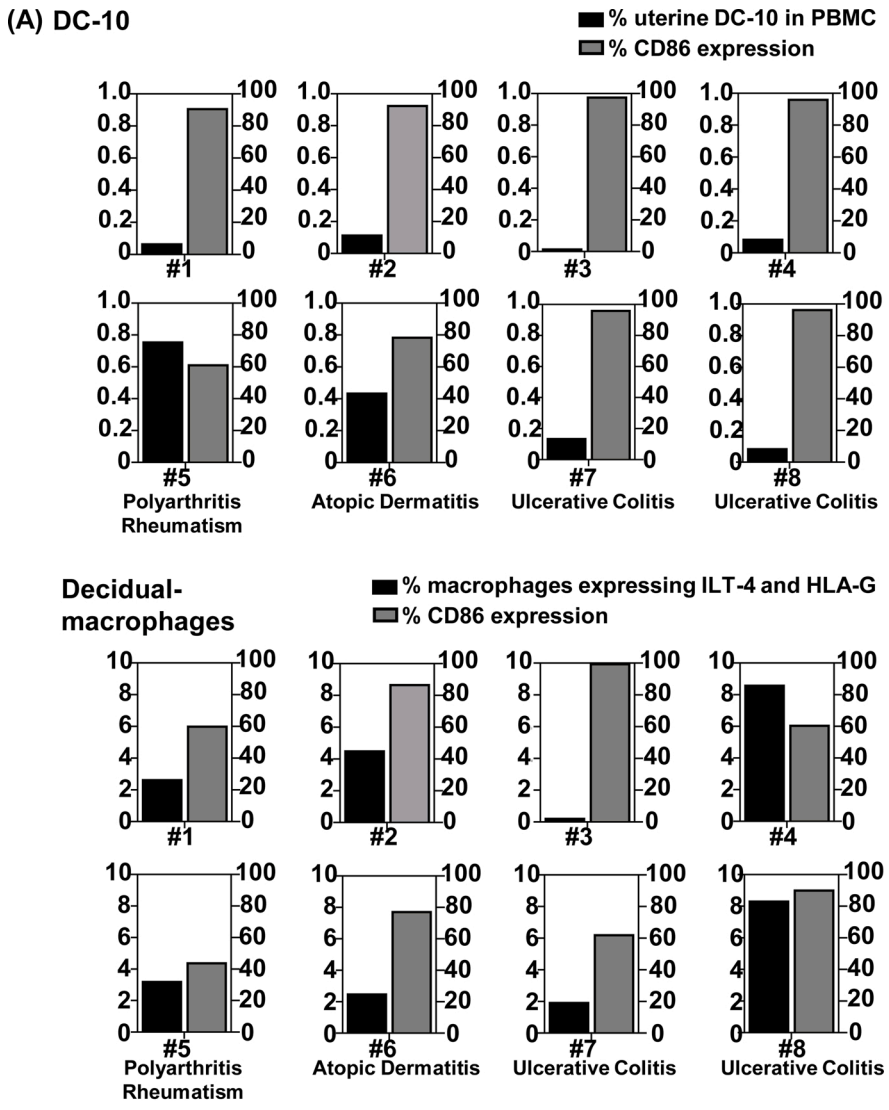
measurable in circulation in all the subjects studied with no significant differences in women with inflammatory conditions (Fig. 4A) as CD209+ decidual NK cells (Fig. 4B).

4. Discussion

Here we report the SARS-CoV-2-specific T cell response and immune phenotype in eight SARS-CoV-2 infected pregnant women. T cells are very important in controlling SARS-CoV-2 infection. It has been an open question about patterns of T cell recognition in infected pregnant women and the integrity of the mechanisms involved in protecting the fetus from pro-inflammatory T cell responses. The fetal HLA differs from maternal HLA and their physiology would suggest an immune response to “allogeneic” antigens. The delicate balance between the necessary pro-inflammatory systemic immune response and strong immune regulation within the placenta occurs through a very complex cross-talk between maternal immune cells and the placental trophoblast. Low numbers and/or functional defects within innate and adaptive cell lineages that regulate the immunity in the maternal-fetal interface contribute to several clinical complications including premature delivery. In physiological conditions, during a viral infection the maternal immune response clears the pathogen, avoiding fetal damage due to pro-inflammatory T cells in the placental interface. A mechanism that prevents T cell activation is the very low number of DC in the decidua that limits the ability to initiate adaptive immune responses in the draining lymph nodes not only to fetal antigens but also to antigens derived from viruses or pathogens (Erlebacher, 2013).

This work reports the T cell anti-viral response in eight SARS-CoV-2 infected pregnant women highlighting the CD4+ and CD8+ recognition of spike and non-spike peptides peptide epitopes. The SARS-CoV-2 proteome was probed using 1102 peptides (9–15 amino acids in length) spanning the whole genome, ensuring the detection of HLA class II-restricted CD4+ T cell responses and HLA class I-restricted CD8+ T cell responses (Grifoni et al., 2020a, 2020b, Lipsitch et al., 2020; Mateus et al., 2020; Rydyznski Moderbacher and Dan, 2020; Tarke et al., 2020).

The results indicated that coordinated CD4+ Th cell and CD8+ CTL responses to SARS-CoV-2 were found in subjects closer to the onset of infection (three weeks), and showed greater activation of classical myeloid APC for T cell presentation as cDC1 and CD14− cDC2. This suggested that the T cell response to the virus is still ongoing in subjects



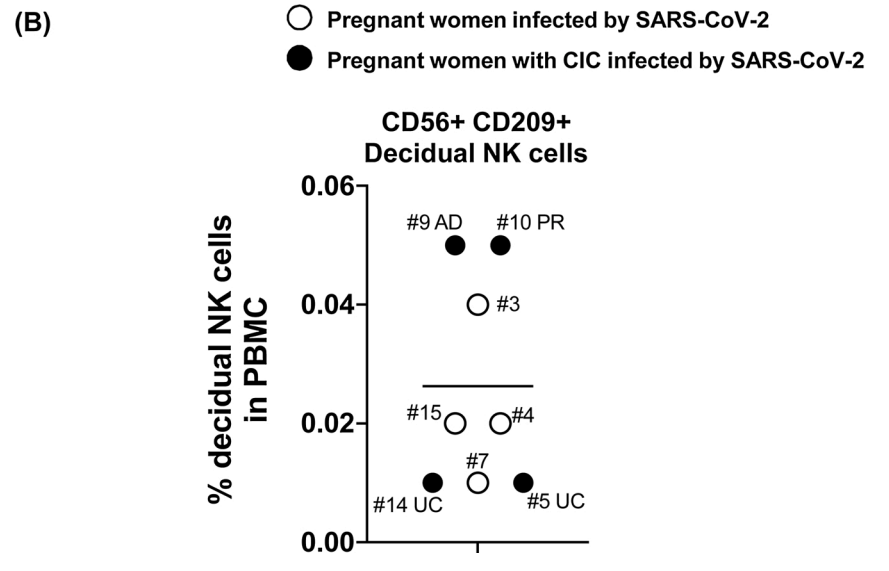


Fig. 4. Enumeration of pregnancy-related tissue resident regulatory innate cell populations in circulation. **(A)** Percentage of Uterine DC-10 (CD14+ CD11c+ CD11b+ co-expressing ILT-4 and HLA-G) and decidual macrophages (CD14+ CD11c− CD11b+ co-expressing ILT-4 and HLA-G), and their expression of maturation/activation marker CD86. Uterine DC-10 and decidual macrophages can be found in the PBMC from all the subjects. Subject #5 (polyarthritis rheumatism) and #6 (atopic dermatitis) showed higher percentages of uterine DC-10 then the other subjects with lower percentage of CD86+ cells. Decidual macrophages were found in 0.27 – 8.64 % of CD14+ CD11c− CD11b+ macrophage population with variable CD86 expressions among the subjects. Black bars: percentage of different regulatory innate cell populations. Grey bars: percentage of CD86+ in different regulatory innate cell populations. **(B)** Enumeration of CD209+ decidual NK cells. CD209+ decidual NK cells can be detected in the peripheral blood with a range between 0.01 – 0.05 %. No significant difference of the percentage of CD209+ decidual NK cells between the two cohorts was found.

infected months prior to the blood sample collection. Of interest, only the subject with allergy (#6, atopic dermatitis) did not respond to any of the SARS-CoV-2 peptide pools, although the T cell enumeration has not been informative in capturing differences with the other subjects as the number of CD4+ T cells were comparable.

Memory T cells, T_{EM} and T_{CM}, have been found confined to the CD4+ compartment and less numerous in healthy women close to delivery, raising the question of a possible specific homing to the uterus and T cell transfer to the fetus. The absence of T_{EM} and T_{CM} memory CD8+ T cells in circulation but numerous T_{EMRA} (these antigen-experienced effector T cells revert to a naïve CD45RA+ phenotype and are ready to expand upon encounter with the antigen), raises a question on the different timing within the T cell memory development between Th and CTL. This is an important point to better understand the risk of possible re-infection in convalescent subjects.

iTreg that recognized viral epitopes were numerous regardless of the timing of infection, timing of pregnancy or pre-existing conditions. We did not detect Treg in COVID-19 convalescent women (Hsieh et al., 2021) but further investigation is required to drive final conclusions about the unique presence of SARS-CoV-2-specific iTreg in pregnancy. In pregnancy, Treg play a critical role in preventing allogeneic T cell responses and our data indicate that SARS-CoV-2 infection elicits not only a pro-inflammatory T cell response in pregnant women but also a regulatory T cell response. Of interest, two subjects with ulcerative colitis showed the most pronounced response to the virus and the larger number of Treg in circulation. The gut and reproductive system share several lymphoid stations suggesting that women with gut inflammation require further control of pro-inflammatory T cells that may affect the maternal-fetal interface.

The predominant innate populations in circulation were tolerogenic in all the subjects studied: CD14+ cDC2 and tmDC were numerous and highly activated, indirectly suggesting that the immune homeostasis of the maternal-fetal interface is not compromised by SARS-CoV-2 infection. cDC1 and CD14− cDC2, classical APC for T cell presentation, were found in the normal range and activated in women studied close to the time of infection with no significant differences within the subjects.

In summary, SARS-CoV-2 infection does not jeopardize the physiology of the immunity at the maternal-fetal interface. Further evidence is the enumeration of tolerogenic tissue resident innate cells as DC-10 and decidual macrofages that we found in circulation in comparable numbers between healthy women and women with pre-existing conditions before SARS-CoV-2 infection.

This is supportive of the current recommendations regarding the safety of vaccination to SARS-CoV-2 any time during pregnancy. Furthermore, in support of this interpretation, all the subjects studied delivered full-term, healthy infants.

Little is known about the placental infection in SARS-CoV-2-infected pregnant women. In two case reports viral load has been found in the placenta associated either with pre-eclampsia at the third trimester (Hosier et al., 2020) or neurological pathology in the baby (Vivanti, 2020) suggesting that healthy deliveries of healthy babies in SARS-CoV-2-infected women does not involve placental infection. More studies are needed to validate this statement: we do not have information in our cohort but the low numbers of pDC, critical innate cells in the anti-viral response and CD14− cDC2 would suggest lack of placental infection.

We acknowledge the strengths and limitations of this work. The sample size of women with inflammatory disorders was small to generalize on all autoimmune and allergic conditions. We first explored the virus-specific T cell repertoire and immune phenotype in SARS-CoV-2 infected pregnant women. However, we did not examine cord blood tissues and blood samples from infants born to the study subjects. This is a limitation in addressing the homing of the T cells, in particular memory T cells, their relation to fetal protection and possible T cell transfer to the baby as a mechanism of protection.

Data availability

No data was used for the research described in the article.
Data will be made available on request.
All data is within the manuscript and figure
The authors do not have permission to share data.

Author contributions

Li-En Hsieh executed the experiments with the help of Jamine Wang under the supervision of Alessandra Franco, Alba Grifoni and John Sidney designed and selected SARS-CoV-2 peptide MPs for this study, Hiral Dave, Diana Johnson, and Jennifer Zellner helped with the recruitment of the subjects, Christina Chambers is the prinicipal investigator of the parent MotherToBaby Pregnancy Study, directed the recruitment of the subjects and edited the manuscript, Alessandra Franco designed, directed the study and wrote the manuscript.

Declaration of Competing Interest

The authors declare no competing interests. LJI has filed for patent protection for various aspects of vaccine design and identification of specific epitopes.

Acknowledgements

This work was partially supported by a grant from the National Institutes of Health (RO1AI43586) to AF, by a grant Resource-based Center for the study of the joint microenvironment in rheumatology; University of California San Diego, NIH Grant P30AR073761 to AF, a grant from the National Institutes of Health (75N9301900065) to AS, and a supplement from the National Institutes of Health (R34DA050341) to CC. The authors thank Alessandro Sette for rendering available SARS-CoV-2 peptide MPs. The authors also thank the women who contributed their time and information to participate in this study and Quest Laboratories for the blood drawing and shipment of the samples.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jri.2021.103464.

References

Amodio, G., Mugione, A., Sanchez, A.M., Vigano', P., Candiani, M., Somigliana, E., Roncarolo, M.G., Panina-Bordignon, P., Gregori, S., 2013. HLA-G expressing DC-10 and CD4+ T cells accumulate in human decidua during pregnancy. Hum. Immunol. 74, 406–411.

Amodio, G., Comi, M., Tomasoni, D., Gianolini, M.E., Rizzo, R., Lemaoult, J.L., Roncarolo, M.G., Gregori, S., 2015. HLA-G expression levels influence the tolerogenic activity of human DC-10. Cell Ther. Immunother. https://doi.org/10.3324/haematol.2014.113803.

Bandoli, G., Chambers, C.D., 2017. Autoimmune conditions and comorbid depression in pregnancy: examining the risk of preterm birth and preeclampsia. J. Perinatol. 37, 1082–1087.

Burns, J.C., Song, Y., Bujold, M., Shimizu, C., Kanegaye, J.T., Tremoulet, A.T., Franco, A., 2013. Immune-monitoring in Kawasaki disease patients treated with infliximab and intravenous immunoglobulin. Clin. Exp. Immunol. 174, 337–344.

Chambers, C.D., Braddock, S.R., Briggs, G.G., et al., 2001. Postmarketing surveillance for human teratogenicity: a model approach. Teratology 64, 252–261.

Chambers, C.D., et al., 2010. Birth outcomes in women who have taken leflunomide during pregnancy. Arthritis Rheum. 62, 1494–1503.

Chambers, C.D., et al., 2013. Risks and safety of pandemic h1n1 influenza vaccine in pregnancy: birth defects, spontaneous abortion, preterm delivery, and small for gestational age infants. Vaccine 31, 5026–5032.

Chambers, C., Johnson, D.L., Kiernan, E., 2018. Approach to evaluating pregnancy safety of anti-rheumatic medications in the otis mothertobaby pregnancy studies: What have we learned? Rheumatology 5 (supplement 5), v34–v39.

Chambers, C.D., Johnson, D.L., Xu, R., et al., 2019. Birth outcomes in women who have taken adalimumab in pregnancy: a prospective cohort study. PLoS One 14, e0223603.

Delahoy, M.J., et al., 2020. Characteristics and maternal and birth outcomes of hospitalized pregnant women with laboratory-confirmed COVID-19 - COVID-NET, 13 states, March 1-August 22, 2020. MMWR Morb. Mortal. Wkly. Rep. 69, 1347–1354.

Du Fosse, N.A., Bronsgeest, K., Arbous, M.S., et al., 2020. Deatailed immune monitoring of a pregnant women with critical COVID-19. J. Reprod. Immunol. 143, 103243.

Ellington, S., et al., 2020. Characteristics of women of reproductive age with laboratory-confirmed SARS-CoV-2 infection by pregnancy status - United States, January 22-June 7, 2020. MMWR Morb. Mortal. Wkly. Rep. 69, 769–775.

Erlebacher, A., 2013. Immunology of the maternal-fetal interface. Annu. Rev. Immunol. 31, 387–411.

Franco, A., 2020. Immune regulation is the most relevant arm of the immune response in infants and young children. Crit. Rev. Immunol. 40, 413–415.

Gregori, S., Tomasoni, D., Pacciani, V., Scirpoli, M., Battaglia, M., Magnani, C.F., Hauben, E., Roncarolo, M.G., 2010. Differentiation of type 1 T regulatory cells (Tr1) by tolerogenic DC-10 requires the IL-10-dependent ILT4/HLA-G pathway. Blood 116, 935–945.

Gregori, S., Amodio, G., Quattrone, F., Panina-Bordignon, P., 2015. HLA-G orchestrate the early interaction of human trophoblasts with maternal niche. Front. Immunol. 6, 281.

Grifoni, A., Sidney, J., Zhang, Y., et al., 2020a. A sequence homologyand bioinformatic approach can predict candidate targets for immune responses to SARS-CoV-2. Cell Host Microbe 27, 671–680.

Grifoni, A., Weiskopf, D., Ramirez, S.I., et al., 2020b. Targets of T cell responses to sars-cov-2 coronavirus in humans with covid-19 disease and unexposed individuals. Cell 181, 1489–1501.

Hosier, H., Farhandian, S.F., Morotti, R.A., et al., 2020. SARS-CoV-2 infection of the placenta. J. Clin. Invest. 130, 4947–4953.

Hsieh, L.-E., Benhamfar, N., Bode, L., Franco, A., 2019. The immunological chimera mother/baby persists and functionally reverses after birth: immune monitoring of the milk and its role in active immunotherapy. Curr. Trends Immunol. 20, 71–78.

Hsieh, L.-E., Grifoni, A., Sidney, J., Shimizu, C., Shike, H., Ramchandar, N., Moreno, E., Tremoulet, A.H., Burns, J.C., Franco, A., 2021. Characterization of SARS-CoV-2 and common cold coronavirus-specific T-cell responses in MIS-C and Kawasaki disease children. Eur. J. Immunol. https://doi.org/10.1002/eji.202149556. Online ahead of print.

Lipsitch, M., Grad, Y.H., Sette, A., Crotty, S., 2020. Cross-reactive memory T cells and herd immunity to SARS-CoV-2. Nat. Immunol. 20, 709–713.

Mateus, J., Grifoni, A., Tarke, A., et al., 2020. Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. Science 370, 89–94.

Moffett, A., Loke, C., 2006. Immunology of placentation in ehtherian mammals. Nat. Rev. Immunol. 6, 584–594.

Mold, J.E., Mccune, G.M., 2012. Immunological tolerance during fetal development: from mouse to man. Adv. Immunol. 115, 73–111.

Red-Horse, K., Zhou, Y., Genbacev, O., et al., 2004. Trophoblast differentiation during embryo implantation and formation of the maternal fetal interface. J. Clin. Invest. 114, 744–754.

Roncarolo, M.G., Battaglia, M., 2007. Regulatory T cell immunotherapy for tolerance to self antigens and to alloantigens in humans. Nat. Rev. 7, 585–598.

Rydyznski Moderbacher, R.I., Dan, J., et al., 2020. Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and association with age and disease severity. Cell 183, 996–1012.

Tarke, A., Sidney, J., Kidd, C.K., et al., 2020. Comprehensive analysis of T cell immunodominance and immunoprevalence of SARS-CoV-2 epitopes in COVID-19 cases. bioRxiv.

Vivanti, A.J., et al., 2020. Transplacental transmission of SARS-CoV-2 infection. Nat. Commun. 11, 3572.

Von Boehmer, H., Melchers, F., 2010. Checkpoints in lymphocytes development and autoimmune disease. Nat. Immunol. 11, 14–20.

Weiskopf, D., Schmitz, K.S., Raadsen, M.P., et al., 2020. Phenotype and kinetics of SARS-CoV-2-specific T cells in COVID-19 patients with acute respiratory distress syndrome. Sci. Immunol. 5 eabd2071.

Wing, K., Sakaguchi, S., 2010. Regulatory T cells exert checks and balances on self tolerance and autoimmunity. Nat. Immunol. 11, 7–13.

Woodworth, K.R., et al., 2020. Birth and infant outcomes following laboratory-confirmed SARS-CoV-2 infection in pregnancy - SET-NET, 16 jurisdictions, March 29-October 14, 2020. MMWR Morb. Mortal. Wkly. Rep. 69, 1635–1640.

Zambrano, L.D., et al., 2020. Update: Characteristics of symptomatic women of reproductive age with laboratory-confirmed SARS-CoV-2 infection by pregnancy status - United States, January 22-October 3, 2020. MMWR Morb. Mortal. Wkly. Rep. 69, 1641–1647.