BRAVERMAN
IVF & Reproductive Immunology, P.C.
Answers for Infertility & Recurrent Pregnancy Loss

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Patient Consult Report

Clinical History

- Currently 36 years old
- History of 4-year old daughter conceived with 5th IUI cycle following 1 year of primary infertility
  - Followed by spontaneous loss at 14 weeks followed by 19-week loss with PPROM
    - 19-week loss was karyotypically normal
- Family history (mother) of positive ANA and ATA and lupus-like symptoms
- Family history of T1DM (father)
- Family history of AODM (uncle and grandmother)

KIR Analysis

- HLA-C
  - You
    - 02, 06
    - C2/C2
  - Partner
    - 04, 06
    - C2/C2
- Maternal KIR haplotype
  - A and B haplotype genes present so KIR AB haplotype
  - KIR2DS1 present
- Assessment
  - KIR AB haplotype with KIR2DS1 puts you in the protective effect category

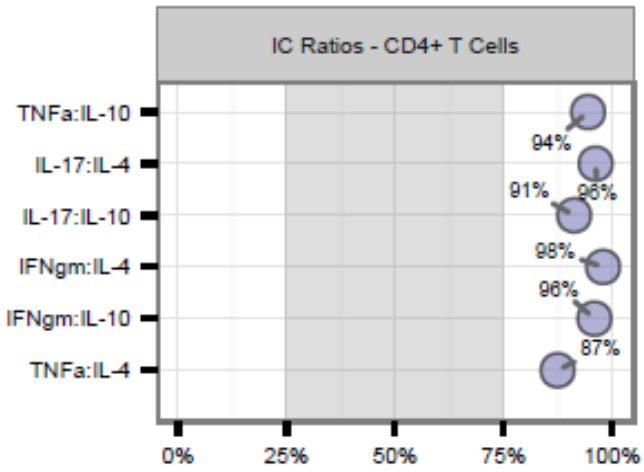
HLA Analysis

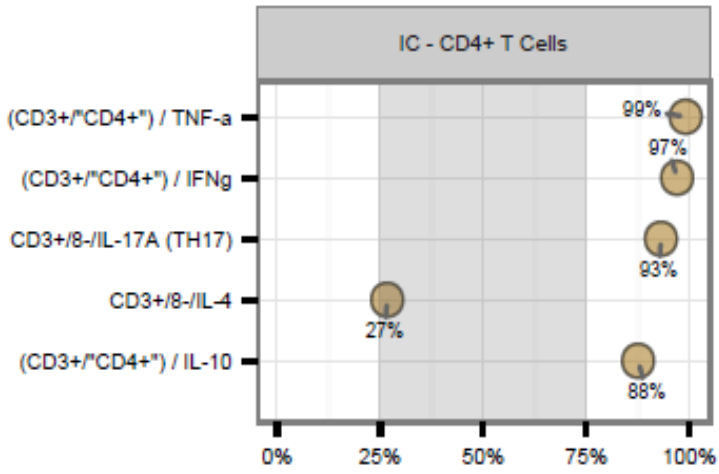
NOTE: The analysis below indicates HLA alleles and haplotypes that have been shown to predispose to certain autoimmune conditions. The presence of one or more predisposing HLA alleles/haplotypes is not diagnostic of the existence of an autoimmune condition. However, when combined with other analysis in this report, the presence of specific alleles/haplotypes can provide valuable insight into the state of the patient's immune system that could be contributing to failure to initiate or maintain pregnancy. HLA alleles and haplotypes that contribute to autoimmunity may also directly lead to an inability to appropriately establish maternal immune tolerance to an embryo or fetus.

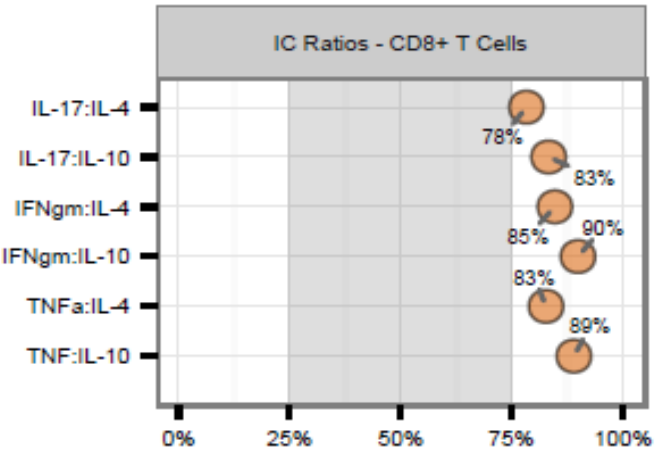
- Partial lack of class II allele mismatching
  - 1 total class II mismatch (out of 6)
  - 0 HLA-DRB1 mismatches (out of 2)
  - 0 HLA-DR supertype mismatches (out of 2)
- No homozygosity of class II alleles
- You carry DRB1*01:01 which is a "shared epitope" allele for rheumatoid arthritis (RA)
- You harbor DRB1*07 in combination with DQA1*02:01 and DQB1*02:02 which is associated with Graves' disease
- You harbor DRB1*07 and DRB4*01 which have been associated with primary antiphospholipid syndrome

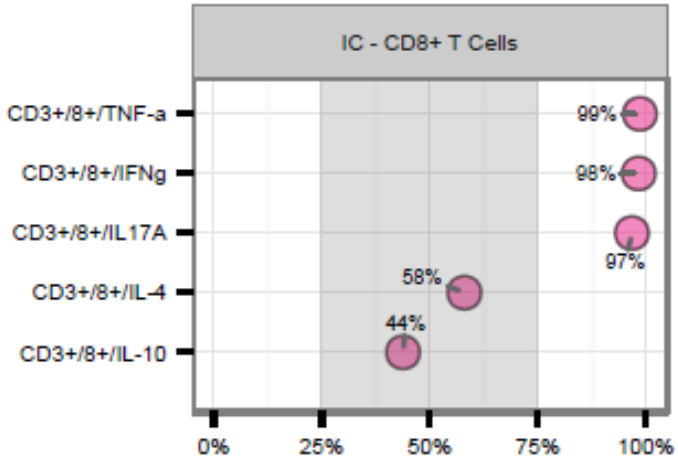
- You have 1 copy of HLA-B*27
  - HLA-B*27 is strongly associated with ankylosing spondylitis and other spondyloarthropathies, sarcoidosis, and primary biliary cirrhosis
- You harbor C*06:02 which is associated with psoriasis and psoriatic arthritis
  - You have 0 copies of the HLA-G 14bp ins allele
- HLA class II HY restricting alleles (class II HYrHLA)
  - DQB1*05:01 detected

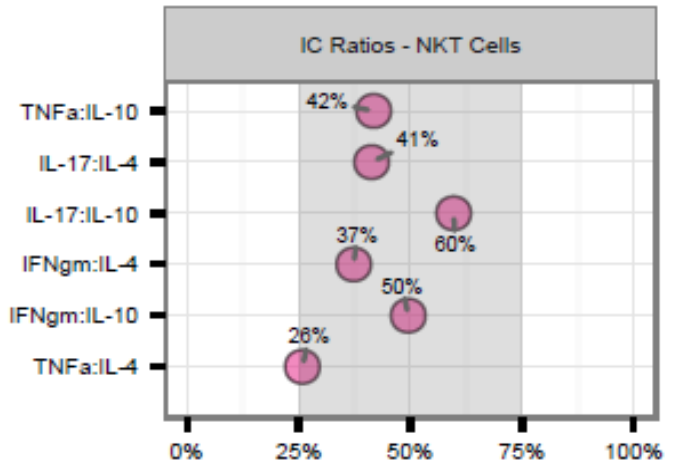
Intracellular Cytokine Analysis (IM-Xpress)

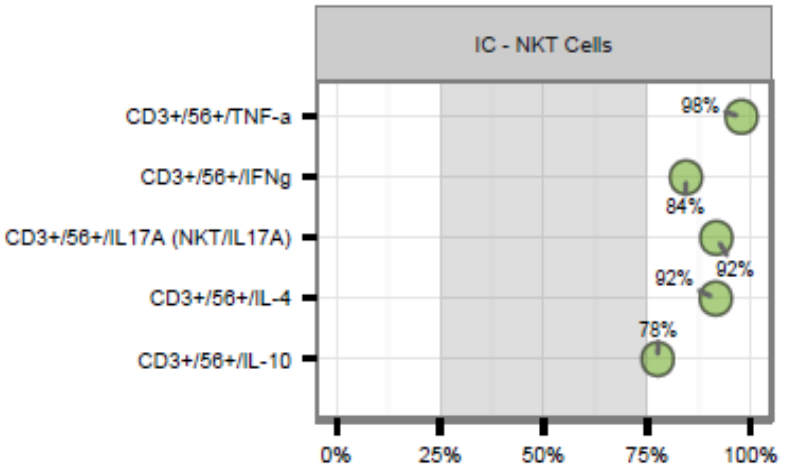


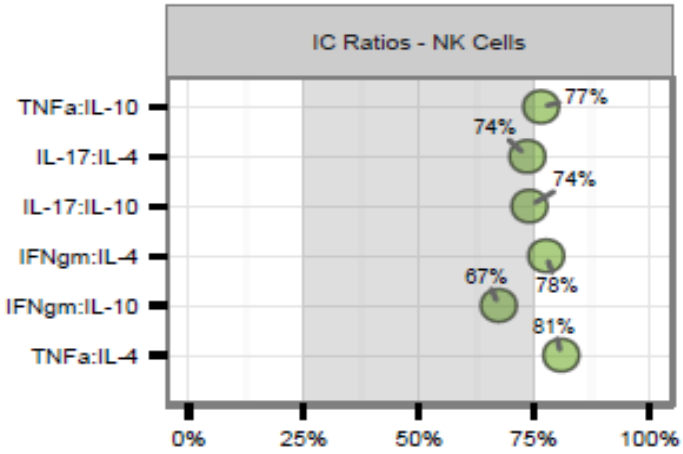


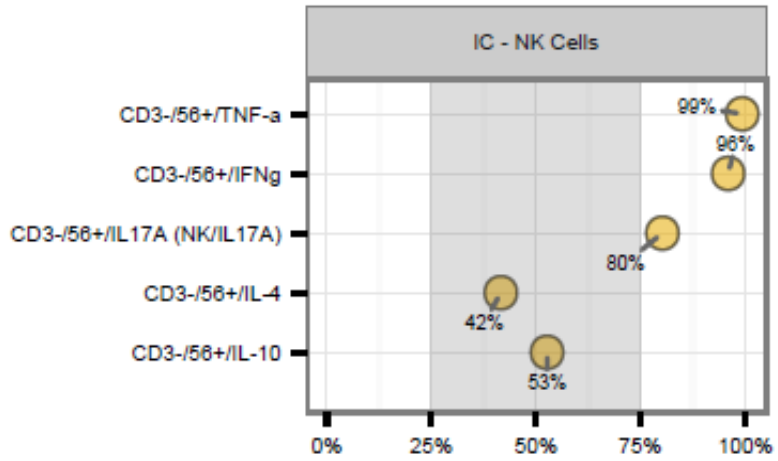






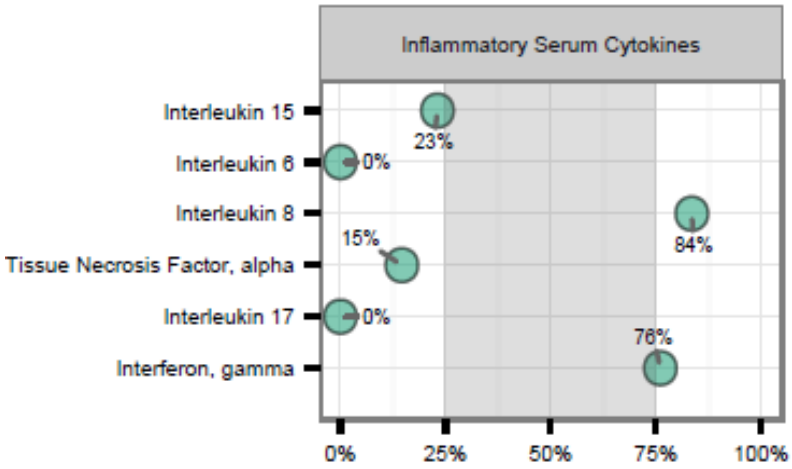


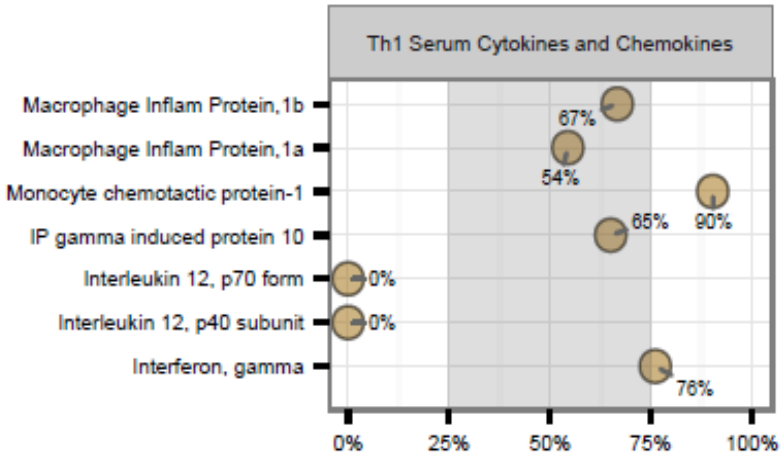




- Your intracellular cytokine (IC) ratios indicate a strong Th1 bias with a CD4+ T cell IFNγ:IL-4 ratio in the 98th percentile. Together with the strong Th1 bias, all CD4+ T cell and CD8+ T cell IC ratios are elevated. All NK cell IC ratios are also mildly elevated or borderline elevated. These elevated IC ratios are largely a function of elevated levels of TNFα positive, IFNγ positive, and IL-17 positive CD4+ T cells, CD8+ T cells, and NK cells. Almost all of these cells are at levels above the 90th percentile. Your TNFα positive, IFNγ positive, and IL-17 positive NKT cells are also all elevated, although all NKT cell IC ratios are within normal ranges due to elevated levels of IL-4 positive and IL-10 positive NKT cells. These data are indicative of significant activation of your immune system at the cellular level with a bias to Th1 (cellular) immunity, including significant CD8+ T cell involvement.

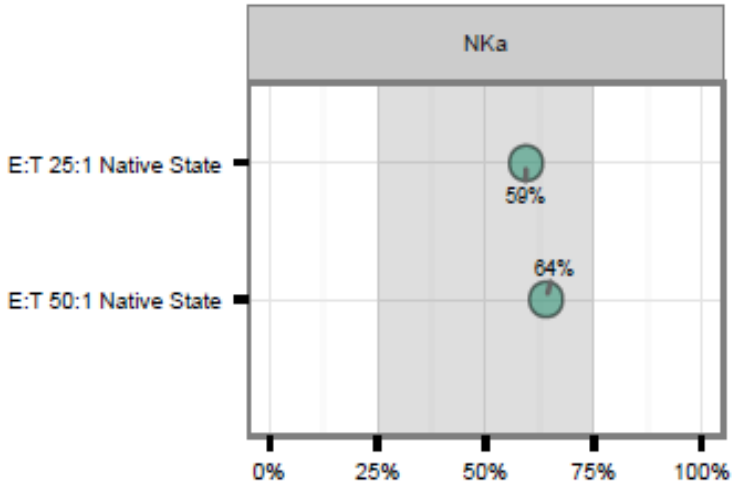
Serum Cytokines Analysis





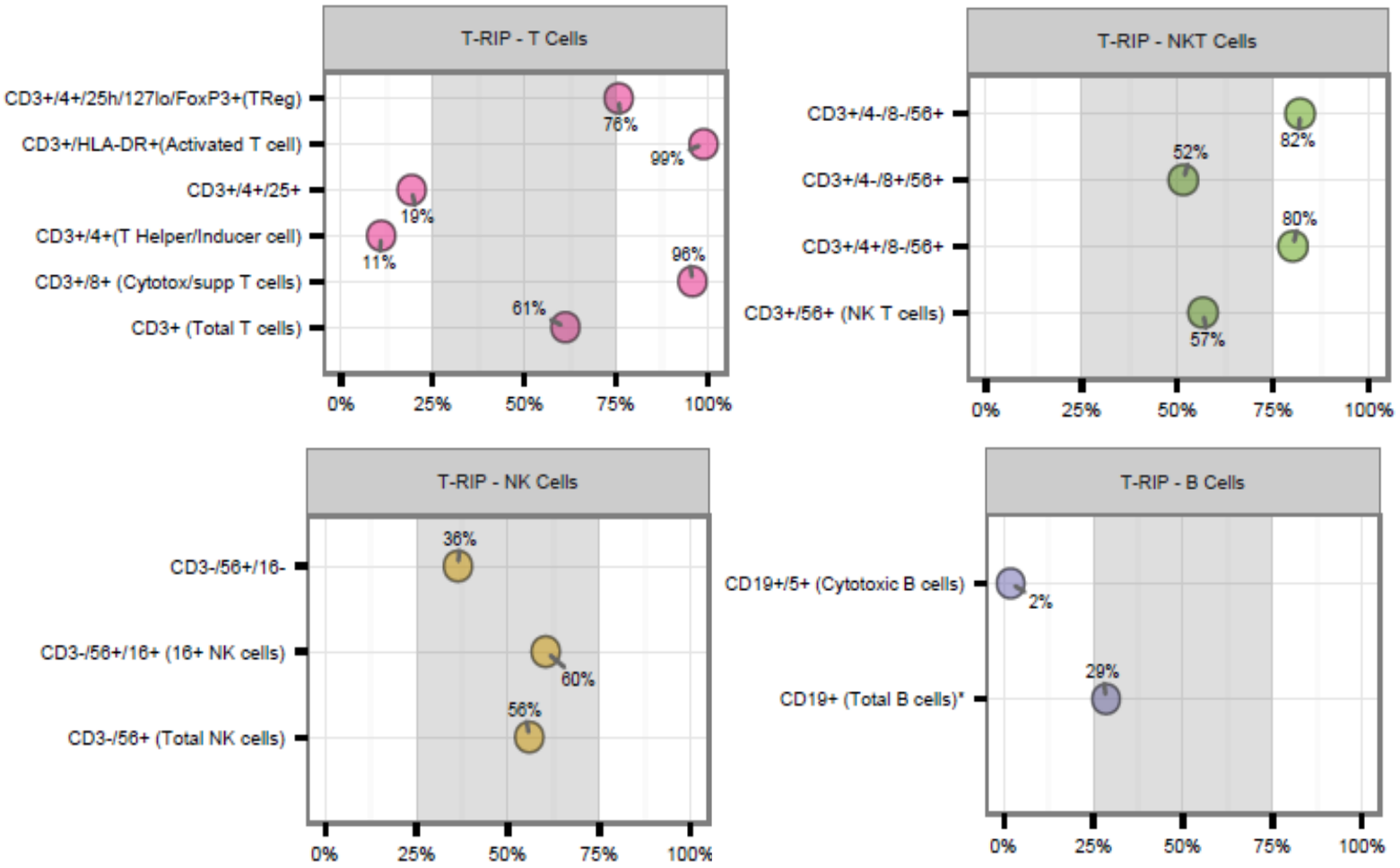
- You have elevated serum levels of several cytokines and chemokines, including IFNγ, IL-8, MCP-1, IL-13, IL-1Rα, and GRO. Eotaxin is also borderline elevated.

NK Cell Activity Analysis



- Your NK cell cytotoxic activity (NKa) is within normal ranges.

Reproductive Immunophenotype and CBC Analysis



- T-RIP
  - You have highly elevated levels of CD8+ and HLA-DR+ T cells. Your CD4+ and CD4-CD8- NKT cells are also elevated, while your CD5+ B cells are low and your total B cells are borderline low.
- CBC
  - WBCs = 6.1
  - Low MCHC (31.0)

Anti-HLA Antibodies Analysis

- Class I antibodies
  - 4K-10K MFI:
    - A11, B35, B56, B71, A43, B53, B49, B50, B75, B62, B51, B72, B58, B57, B78, B63, B77, B52, A25, A3, A*66:01, A1
  - 1.5K-4K MFI:
    - B46, B54, A26, B42, Cw9, B67, B8, B18, Cw10, A29, A34, B55, B73, B39, B7, A69, A68, B76, B41, B45, B82, B61, B48, B*27:08, B60, Cw1, A33, B81, Cw8, A36, B64
  - Partner-specific
    - A1 (4,000), A11 (9,000), B35 (9,000), B57 (6,500)
  - Partner-specific and complement-fixing
    - A11 (16,000), B35 (12,000)
- No class II antibodies detected

Other

- Positive for THAB (74.0)
- Heterozygous for the MTHFR Al298C polymorphism
- Negative for RF (11)
- Negative for anti-TPO (2.0)
- Normal TSH (1.01)
- Negative for TSH receptor antibody (<6.00)
- Negative for anti-CCP antibodies (<16)
- Negative for ANAs
- Negative for APAs
- Normal total IgG (1300)
- Normal total IgM (175)
- Normal total IgA (293)
- Normal total IgE (9)
- Total 25 Hydroxy Vit D sufficiency (37.7)
    - 25 Hydroxy Vit D2 = <5.0
    - 25 Hydroxy Vit D3 = 37.7
- Normal C3 complement activity (125)
- Normal C4 complement activity (24)
- Normal homocysteine (6.0)
- Negative (normal) for the MTHFR C677T polymorphism

Summary

You and your partner have a partial lack of HLA class II allele mismatching with only 1 total mismatched class II allele. Your mismatched class II allele also is not at the DRB1 locus and therefore also does not include a DRB supertype mismatch. Therefore, your single HLA mismatch is not likely sufficiently antigenically distinct to be immunogenic. A lack of HLA class II allele mismatching can lead to a deficiency in the ability of the maternal immune system to generate tolerance for paternal antigens present on the embryo.

You also have a family history of autoimmune disease (T1DM and positive ANA and ATA with lupus-like symptoms), and you harbor several HLA alleles/haplotypes known to predispose to the development of various autoimmune conditions. These include DRB1*01:01 which is a "shared epitope" allele that predisposes to the development of rheumatoid arthritis, DRB1*07/DQA1*02:01/DQB1*02:02 which predisposes to the development of Graves' disease, DRB1*07/DRB4*01 which predisposes to the development of primary antiphospholipid syndrome, B*27 which predisposes to the development of ankylosing spondylitis and other spondyloarthropathies, sarcoidosis, and primary biliary cirrhosis, and C*06:02 which predisposes to the development of psoriasis and psoriatic arthritis. As stated above, these and other HLA predispositions are not diagnostic for the presence of one or more autoimmune conditions. However, HLA alleles and haplotypes are known to predispose to autoimmunity by inducing failure in mechanisms that promote tolerance for self-antigens. A failure in these same mechanisms can also cause a deficiency in the ability of the immune system to generate tolerance for non-self-antigens, such as when the maternal immune system is exposed to antigens present on an embryo of paternal origin.

You have elevated levels of TNFα positive, IFNγ positive, and IL-17 positive cells for all tested cell types (CD4+ T cells, CD8+ T cells, NKT cells, and NK cells) with almost all of these cells at levels above the 90th percentile. Largely as a result of these elevated levels of individual intracellular cytokine (IC) positive cells, your immune system has a strong Th1 bias (CD4+ T cell IFNγ:IL-4 ratio in the 98th percentile) together with elevation of all CD4+ T cell and CD8+ T cell IC ratios and mild elevation or borderline elevation of all NK cell IC ratios. You also have highly elevated levels of CD8+ and HLA-DR+ T cells, and elevated levels of CD4+ and CD4-CD8- NKT cells. These data are all consistent with significant activation of your immune system at

the cellular level with a bias to Th1 (cellular) immunity, including significant CD8+ T cell involvement. You also have elevated serum levels of IFNγ, IL-8, MCP-1, IL-13, IL-1Rα, and GRO, and a borderline elevated serum level of eotaxin, indicative of an elevated level of systemic inflammation.

You are positive for anti-thyroglobulin antibodies and your TSH is borderline low, indicative of thyroid autoimmunity which may either be Graves' disease or a hyperthyroid phase of Hashimoto's thyroiditis. Further investigation of your thyroid function is recommended. You are negative for all tested ANAs and APAs.

You are positive for several partner-specific class I anti-HLA antibodies (A1, A11, B35, B57) of which the A11 and B35 antibodies were found to fix complement (C1q). These HLA-A and HLA-B antibodies cover all of your partner's HLA-A and -B alleles and therefore any fetus will possess 2 HLA alleles for which you harbor antibodies. Although HLA-A and -B alleles are not expressed at early stages of embryonic/fetal development, anti-HLA antibodies are associated with later complications of pregnancy including PPROM and preterm labor. This is consistent with your history of losses at 14 and 19 weeks.

You also harbor a copy of DRB1*07 which is the second most frequent HLA allele found in our patients with endometriosis, as well as a copy of DQB1*03:01 which has been independently associated with a predisposition to the development of endometriosis. Your elevated levels of TNFα positive, IFNγ positive, and IL-17 positive NK cells, elevated/borderline elevated NK cell IC ratios, elevated serum levels of IL-8, MCP-1, and GRO, and borderline elevated serum level of eotaxin are also all consistent with the possible presence of endometriosis. Positive levels of anti-thyroid antibodies are also frequently observed in our patients with endometriosis.

You are heterozygous for the MTHFR Al298C polymorphism and use of a methylated form of folate during pregnancy could be considered.