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CASE REPORT

Coexistence of lymphocyte dysregulation, alloimmunity and autoimmunity in a patient with recurrent failed in vitro embryo transfer fertilization

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KEYWORDS
Infertility;
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Abstract
Objective: To describe a patient with primary infertility and recurrent implantation failure (RIF) and coexistence of peripheral blood immunophenotypic dysregulation of lymphocytes and alloimmune and autoimmune abnormalities. The hypothesis is that functionally distinct immunological abnormalities might better explain the immunological etiology of RIF than individual abnormalities in some patients.
Subjects and methods: We present clinical and immunological data.
Results: A patient with primary infertility and RIF had peripheral blood immunophenotypic abnormalities of T, B and NK-cells, unusually high shared HLA antigens with her partner, and antiphospholipid antibodies.
Conclusion: Functionally distinct immunological abnormalities may coexist in some women with RIF after in vitro fertilization.

PALABRAS CLAVE
Infertilidad;
Fallo de implantación;
Células NK;
Activación linfocitaria;
HLA

Coexistencia de alteracion inmunofenotipica linfocitaria, aloinmunidad y autoinmunidad en una paciente con fallo de implantacion recurrente tras fecundacion in vitro

Resumen
Objetivo: Describir un caso de fallo recurrente tras fecundación in vitro en el que coexisten varias alteraciones inmunológicas potencialmente relacionadas con este problema. La hipótesis es que esta coexistencia de factores podría explicar mejor la etiología inmunológica que alteraciones individuales.
Sujetos y métodos: Se presentan datos clínicos e inmunológicos.

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Resultados: Una paciente con infertilidad primaria y fallo recurrente tras 4 intentos de fecundacion in vitro tenía alteraciones inmunofenotípicas de células T, B y NK, antígenos compartidos por la pareja en una frecuencia inusualmente alta y anticuerpos antifosfolípidos.
Conclusiones: Distintas alteraciones inmunológicas pueden coexistir en casos aislados de fallo recurrente tras fecundación in vitro.

Introduction

The immunological mechanisms that are involved in the initial steps of pregnancy are quite complex. These include specific lymphocyte subsets, protective cytokine profiles (Th2 > Th1) and HLA antigens (HLA-G) and not well defined immunoregulatory mechanisms.[1] Recurrent implantation failure (RIF)[2] refers to a failure to achieve a clinical pregnancy after transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles in a woman under the age of 40 years. In this case report we describe a patient with primary unexplained infertility and RIF in whom peripheral blood immunophenotypic dysregulation of lymphocytes, alloimmune and autoimmune abnormalities coexisted.

Description of the case

A 33-year-old woman with a history of primary infertility and four attempts of in vitro fertilization embryo transfer without success. Previous history included one episode of retinal thrombosis. Physical examination was unremarkable. Blood testing was performed 3 months after the first implantation failure. Distinct immunological abnormalities were observed. Peripheral blood natural killer cell abnormalities: she was found to have increased percentages of peripheral blood NK-cells (CD3-CD16+CD56+: 24%). Mean ± 1SD NK-cell count of 38 healthy women was 12 ± 6% in our center. T and B-cell immunophenotypic dysregulation: peripheral blood immunophenotypic abnormalities included (as compared with mean ± 1SD values of 38 healthy women): higher CD4 central memory cells (CD4+CD45RA-CCR7+: 55 vs 38%); higher activated CD4 lymphocytes (CD4+CD25low: 59 vs 44%), higher CD19+CD5+ and CD19+CD5+CD40+ B-cells (44 vs 26% and 43 vs 26%, respectively). Alloimmune abnormalities: the patient shared 8 HLA antigens with her partner (A29, B44, Cw16, Bw4, DR7, DR11, DR53 and DQ2). The number of shared HLA antigens among 41 recurrent spontaneous miscarriage couples was 4 ± 1.9 in our center (mean ± 1 standard deviation of mean [SD]). Autoimmune abnormalities: low positive (above normal range) IgG anti-beta-2-glycoprotein-I antibodies were demonstrated in more than two determinations with an interval of 3 months between determinations. Anticardiolipin antibodies (IgG, IgM), lupus anticoagulant, antinuclear antibodies, anti-thyroid antibodies and celiac disease associated antibodies were negative.

Pre-treatment work-up consisted of pelvic ultrasound scan and hysterosalpingography. No hydrosalpinx or other anatomic factors were detected. There was no evidence of endometritis. Parental karyotype, endocrine, hormonal (serum progesterone in the mid-luteal phase, follicle-stimulating hormone, luteinising hormone and ovarian response to gonadotrophin stimulation) were normal. Semen analysis was normal. HIV, hepatitis B and hepatitis C serology was negative. Rubella serological status was positive. Thrombophilia studies (Factor V Leiden, prothrombin G20210A and MTHFR genes, protein C and S deficiency, antithrombin III deficiency) were normal.

Oocyte retrieval was carried out using transvaginal ovarian puncture guided by ultrasound scan. In the failed cycles a total of eight good-quality fresh ($n = 7$) or frozen ($n = 1$) embryos were transferred. After discussion with the patient, and due to the multiple immunological abnormalities observed, several of which may be modulated by intravenous immunoglobulin (IVIG), in the second and third in vitro fertilization attempts IVIG (compassionate use) of a 5% preparation at a single dose of 400 mg/kg was administered 72 h before embryo transfers without success. Pre-conceptional low-dose acetyl salicylic acid 100 mg oral was also administered in the three latest attempts to conceive using IVF.

Discussion

Immunological abnormalities are observed in some patients with unexplained RIF although none of these are included in guidelines for the evaluation of this reproductive problem. Simultaneous evaluation of distinct immunological alterations including autoimmune, NK-cell counts, lymphocyte subsets and HLA testing has not been performed in previous RIF studies. Among 26 women with RIF evaluated in our center, two women including the presented case, had increased percentages of peripheral blood NK cells (>15%) at the same time with positive antiphospholipid antibodies (7.6%). Four of 85 women with unexplained recurrent abortion disclosed this simultaneous association (4.7%). On the other side, none of 35 women with recurrent abortion and two among 72 healthy women (2.7%) were found to have simultaneously increased NK cell count, higher percentages of central memory CD4 T-cells and of CD19+CD5+ B-cells. In addition, our patient was found to share even more HLA antigens with her partner than women with recurrent abortion studied in our center. These data suggest that the number of simultaneous immunological alterations that we observed in our patient was unusually high.

All these immunological abnormalities have been previously described in association with RIF. Uterine natural killer (uNK) cells are the most abundant leukocytes in preimplantation endometrium and early pregnancy decidua. Maternal uNK cells are adjacent to, and have the ability to interact directly with, fetal trophoblasts.[3] Increased numbers of peripheral blood NK cells have been correlated with increased numbers of decidual NK cells.[4] On the other hand, increased levels of NK progenitors in the decidua of women with early spontaneous abortion have been demonstrated.[5]

Higher percentages of peripheral blood NK cells have been described in women with RIF.[6,7] Our patient was found to have a hundred percent increase in the peripheral blood NK cell percentages. During implantation, maternal immunoactivation and tolerance not only are limited to the decidua but are also observed in the periphery.[8,9] Our patient was found to have an immunophenotypic profile that included increased percentages of central memory and activated CD4+ T-cells coexpressing the alpha chain of the IL-2 receptor which might be surrogates of increased T-helper 1 (Th1) immunity. CD4+ central memory cells contain a higher number of Th1 (IFN-γ producing) cells.[10] It has been suggested that infertile women who fail alloimmune and autoimmune therapy have been described to have significant alterations in cellular immunity involving CD19+/CD5+ B cells.[6] Our patient was found to have higher percentages of CD19+/CD5+ B-cells and failed to become pregnant despite IVIG therapy. Antiphospholipid antibodies have been described in patients with RIF.[11] We found persistently positive low-titer antiphospholipid antibodies in the presented case. Couples with RIF are not believed to share more HLA antigens with their spouses than expected by chance. An increased incidence of shared HLA antigens among couples experiencing recurrent spontaneous abortion or infertility has been described.[12] Creus et al.[13] suggested that some cases of implantation failure after IVF and embryo transfer might be caused by underlying close histocompatibility between partners.

Dysregulation of all these pathways might have contributed to RIF in this case. This complexity makes unlikely that any single immunological marker could explain by itself immune mediated recurrent reproductive failure when it occurs in a women. We suggest the hypothesis that functionally distinct immunological abnormalities (i.e. immunophenotypic abnormalities of T and B-cells, autoimmune and alloimmune factors) might explain better the potential immunological etiology of RIF in some cases. Whether a profile of immune abnormalities might be more accurate way to evaluate immune mediated RIF than single testing of immunological biomarkers warrants evaluation in future studies.[14]

Ethical responsibilities

Protection of people and animals. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Data confidentiality. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

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Conflict of interest

The authors declare no conflict of interest.

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