G. Manfredi[1], L. Dell'Aera[2], R. Liguori[2]

Overcoming recurrent spontaneous abortions in women suffering from IgG subclass deficiency: high efficiency of low dose intravenous immunoglobulins treatment

[1]Allergy and Clinical Immunology Unit, Clinical Medicine Dept., Religious Gen. Reg. Hospital "F. Miulli", Acquaviva (BA), Italy
[2]Obstaetric and Ginecology Unit, Mother-Child Dept., Religious Gen. Reg. Hospital "F. Miulli", Acquaviva (BA), Italy

Key Words

Idiotype-antiidiotype network restoration in pregnancy; IgG subclasses unbalance in habitual abortions; restoring maternal-foetus immunologic tolerance

Corresponding author
Giacomo Manfredi
Corso Italia 125, 70123 Bari, Italy
Phone/fax: +39 080 52 77 317
Phone: +39 333 40 74 527
E-mail: giacomo.manfredi@alice.it

Summary

Problem. *It's well known that iv. immunoglobulins may be useful to overcome habitual abortions, but the mechanisms at the base of a successful outcome and the likelihoods are still unknown.* ***Method of study****. In one hundred and sixty women with habitual abortions and one hundred and sixty healthy mothers, we evaluated blood IgG subclasses; among the patients, sixteen merely showed IgG subclass deficiency, after leaving out any autoimmunity and/or coagulation disorders. All the patients (100%) showed IgG3, twelve (75%) IgG1, eight (50%) IgG4 and six (37,5%) IgG2 deficiency; healthy control people's IgG subclasses fell in normal range in 156 women, but just four women showed IgG2 and IgG4 deficiency with neither immune deficiency's clinical marks nor increased vulnerability to infections. All the patients were treated with whole immunoglobulins iv. infusion (200 mg/kg/monthly) all over the pregnancy.* ***Results.*** *The successful pregnancy rate is very high (> 90%): 100% out of women showing IgG1 (12/12), 87,5% of IgG3 (14/16), 75% of IgG4 (6/8) and 66% of IgG2 deficiency (4/6) had successful pregnancies. The Odd's Ratio between IgG subclass deficiency and recurrent abortions is 4,33 with confidence interval of 95%; chi square value is 7.68 ($p < 0.025$).* ***Conclusions.*** *Low dose immunoglobulin infusion is the only effective way to reach successful pregnancy, despite previous habitual abortions in patients suffering from IgG subclass deficiency without autoimmunity and/or coagulation disorders, likely restoring idiotype-antiidiotype network; showing evidence of IgG subclasses deficiency (mostly IgG1 and IgG3) may help identify patients who can benefit from iv. immunoglobulin treatment.*

Introduction

Habitual pregnancy loss may be determined both by foetus and/or mother related causes; as to foetus-related, the main causes are genetic (aneuploidy); as to mother-related, the main causes are genetic, abnormal uterine structure, hormones, toxic exposure, malnutrition, metabolic, coagulation and/or immunologic disorders (1,2). Regarding immunologic disorders, both organ and non-organ specific autoimmune diseases, as well as immunodeficiencies, may lead to a pregnancy loss (3); the mechanisms leading to habitual abortions in case of immunodeficiencies may be very different. An anti-idiotypic antibodies unbalance may lead to overthrow both the maternal tolerance against semiallogeneic trophoblast cells (4,5,6) and the right myometrium tone (7). In fact, it has been shown (4) that the sera of women undergoing habitual abortions hold antibodies playing anti-idiotypic activity (8,9); moreover, a whole idiotypic network is essential to attain a correct blastocysts implantation and to overcome the rejection against trophoblast cells (10). On the other hand, the whole idiotypic network is fundamental to ensure a correct uterine contractility (11,12); the ability to bind idiotypes is related to higher molecule's flexibility (13) and this is a IgG1 and IgG3 peculiar property (14). This serendipitous study started in 2003, when the case of a 34 year-old woman who underwent five abortions without any apparent clear cause (the search for hyperhomocysteine, co-

agulation abnormalities, autoimmunity and/or LAC activity was negative) occurred to our observation. The immunologic characterization didn't show any cellular abnormality but only IgG1 and IgG3 deficiency, as referred to healthy controls. We decided to treat her with a whole immunoglobulins low dose iv., and the woman happily carried on her sixth pregnancy (15).

Materials and methods

Blood IgG subclasses of one hundred and sixty women having clinical history of more than 2 spontaneous abortions and one hundred and sixty healthy mothers without any history of abortions as control people were evaluated by nephelometry (Beckman). In the patients suffering from IgG subclass deficiency (16/160), the abortions occurred both very early, starting from the fourth week, and later, till the twentieth week; the symptoms were bleeding and/or myometrium contraction resistent to all tocolytic drugs. The search for organ and non organ-specific autoimmune diseases, including Hughes Syndrome (antids-DNA, antiENA, P-and C-ANCA, antinuclear autoantibodies, IgG and IgM antibeta2glycoprotein 1, IgM and IgG antiphospholipid and anticardiolipin autoantibodies, Lupus anticoagulant), hyperhomocysteinemie and coagulation diseases (C and S Prot., P.T., aP.T.T., D-Dimer) was performed. Since the early b-HCG increase all the patients showing sole IgG subclass deficiency were treated with whole immunoglobulins iv. infusion (200 mg/kg/monthly) all over the pregnancy in order to restore humoral immunity, hoping to gain a successful pregnancy; this low therapeutic dose has been shown to be safe and effective in treating unexplained recurrent spontaneous abortions by increasing the blood s-HLAG and IL-10 tolerogenic cytokine in a prospective clinical trial (16), while higher doses (0,4-1 g/kg) are indicated in habitual autoimmune disease-driven abortions (17,18,19). The study was approved by local ethical committee and informed consent was asked and attained by the patients. No placebo control therapeutic intervention was planned because of ethical reasons; on the other hand, the patients hadn't had any benefit from previous treatments with conventional therapies (hormones, tocolytic drugs). Statistical analysis was performed using chi square test and Odd's ratio.

Results

Among one hundred and sixty patients just sixteen 24-42 year-old women (10%) showed IgG subclass deficiency: all of these (100%) showed IgG3, twelve (75%) IgG1, eight (50%) IgG4 and six (37.5%) IgG2 deficiency. As to one hundred and sixty healthy control people, just four (2.5%) showed IgG subclasses deficit, involving IgG2 and IgG4 only, without any clinical symptom, while one hundred and fifty-six women had IgG subclasses falling under normal range as referred to more than eight year-old people (**table 1**). The statistical association between IgG subclass deficiency and habitual abortions significantly reaches an Odd's ratio of 4.33 with confidence interval 95%, while chi square test is 7.68 ($p < 0.025$) (**table 2**). Among the patients' group, symptoms resistant to every other therapy, symptoms consisting both in early bleeding, and later incoercible uterus contractions, were fully bridled thanks to iv. immunoglobulins treatment. A successful pregnancy was reached by all (100%) patients showing IgG1 deficiency (12/12), 87.5% (14/16) out of those with IgG3 deficit, 75% (6/8) with IgG4 deficit, and 66% (4/6) out of those showing IgG2 deficiency. No detrimental effect was registered either on mothers or on children.

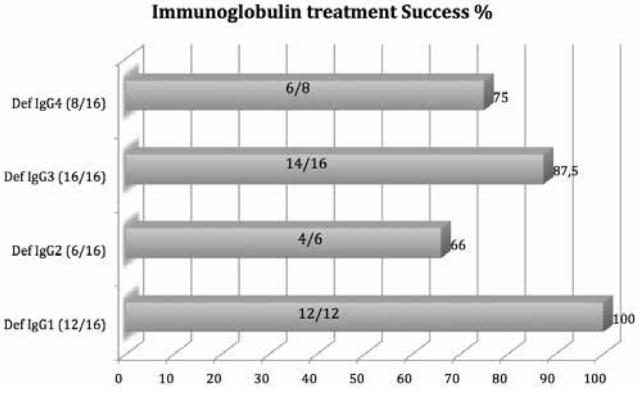
Table 1 *- IgG subclass normal values (women more than 8 years-old).*

	Mg/dl	%
IgG1	580-700	60
IgG2	290-350	30
IgG3	68-82	7
IgG4	29-35	3

Table 2 *- Contingency Table between IgG subclass deficiency and habitual abortions: chi square = 7.68 (p < 0.025); Odd's ratio = 4.33 (confidence interval 95%).*

		Habitual abortions		Total
		+	-	
IgG subclass deficiency	+	16	4	20
	-	144	156	300
	Total	160	160	

Figure 1 *- Relationship between immunoglobulin treatment and successful pregnancy % in sixteen women suffering from IgG subclass deficiency with habitual abortions.*



Discussion

The prevalence of recurrent abortions (R.A.) in our hospital is about 1.5% (20/1420 gestations/year). The prevalence of IgG subclass deficiency in people suffering from R.A. is on average 10% (16/160 in the last eight years). Unexplained recurrent abortions have already been recorded as IgG subclass deficiency's clinical expression, as well as tooth decay, relapsing pharyngotonsillitis, H.P. infestation, mucocutaneous herpes, urticaria, asthma, heart's valves diseases, urinary tract infections (20). Abortions may occur both very early during pregnancy, from second to eighth gestation week, and also later, till the twentieth; in the former case, the first more common symptom is bleeding, in the latter, uncontrolled myometrium contractions; both these symptoms may be related to idiotypic network's unbalance. In a normal immunoglobulin structure, the idiotypic specificity inhabits the Fab portion of the molecule's frame; the idiotype's function and target binding ability may be modulated by a second anti-idiotype antibody able to bind idiotype's Fab; but also the anti-idiotypic antibody's function may be under- or up-regulated by a third anti-antiidiotype antibody. This idiotypic network may regulate the function of virtually every biologic receptor, including the myometrium muscarinic acethylcholine receptors; so, an immunologic unbalance of the idiotypic network may lead to uncontrolled myometrium contraction (21,22). The idiotypic network's unbalance may also overthrow both the immunologic recognition of antigens (auto-, allo- and iso-antigens) and the tolerance induction mechanisms, including the mother's immunologic tolerance against the foetus semiallogeneic hystocompatibility antigens. Moreover, during pregnancy the sole mother's immunoglobulin subclass entering foetus circulation is IgG1, thanks to the molecule's active carriage from mother to foetus' blood (23), so ensuring the right immunologic defence against endouterine infections (viruses, bacteria, parasites). The iv. Immunoglobulin treatment is known for bringing benefits among the patients undergoing habitual abortions (16,17); the invoked mechanisms go from natural killer cells down-regulation (24,25,26) to idiotypic network's restoration (18,19). Some reviews failing to find a benefit from iv. Immunoglobulins for treating unexplained recurrent miscarriage (27), don't consider the etiology of R.A., so that the heterogeneity of inclusion criteria may endanger conclusion's congruity.

Conclusions

The present study shows for the first time that: 1) IgG subclass deficiency (mostly IgG1 and IgG3) may lead to habitual abortions in about 10% of women having R.A.; 2) habitual abortions are included among the clinical features of IgG subclass deficiency; 3) the slow iv. infusion of low dose of whole immunoglobulins (200 mg/kg/monthly) all over the pregnancy since the first increase of β-HCG is the only effective treatment allowing to happily carry on the pregnancy in women having a clinical history of habitual abortion and isolated IgG subclass deficiency; 4) the successful pregnancy rate after this therapy in selected patients is very high (on the average > 90%). The IgG1 and IgG3 subclasses likely include most idiotype and antiidiotype antibodies, so that their deficiency may lead to idiotypic network's unbalance, allowing to overthrow maternal immunologic tolerance mechanisms against foetus semiallogeneic antigens and the right myometrium tone regulation during the pregnancy; the low dose of whole immunoglobulin treatment is the only effective way to overcome habitual abortions in women suffering from IgG1 and IgG3 subclass deficiency without autoimmunity and/or coagulation disorders.

References

1. Jarošová R, Mašata J, Stejskal D, Brandejská M. Recurrent pregnancy loss. Review Ceska Gynekol. 2013;78(2):200-5.
2. R Fraser, G StJ Whitley, AP Johnstone, AJ Host, NJ Sebire, B T, JE Cartwright. Impaired decidual natural killer cell regulation of vascular remodelling in early human pregnancies with high uterine artery resistance. J Pathol. 2012;228:322-32.
3. Girardi G, Bulla R, Salmon JE, Tedesco F. The complement system in the pathophysiology of pregnancy. Mol Immunol. 2006;43(1-2):68-77.
4. Saunders RD, Nakajima ST, Rai SN, Pan J, Gercel-Taylor C, Taylor DD. Alterations in antibody subclass immune reactivity to trophoblast-derived fetal fibronectin and α2-macroglobulin in women with recurrent pregnancy loss. Am J Reprod Immunol. 2012;68(5):438-49.
5. CJ Davies. Why is the fetal allograft not rejected? J Anim Sci. 2007;85(13)supplE32-E35.
6. Riley JK. Trophoblast immune receptors in maternal-fetal tolerance. Immunol Invest. 2008;37(5):395-426.
7. Garfield RE, Bytautiene E, Vedernikov YP, Marshall JS, Romero R. Modulation of rat uterine contractility by mast cells and their mediators. Am J Obstet Gynecol. 2000;183(1):118-25.
8. Hart E, Bersini H, Santos FC. How affinity influences tolerance in an idiotypic network. J Theor Biol. 2007;7;249(3):422-36.
9. Behar E, Carp H, Livneh A, Gazit E. Anti-idiotypic IgM antibodies to anti-HLA class I antibodies in habitual abortion. Am J Reprod Immunol. 1991;26(4):143-6.
10. L Ortiz-Ortiz, WO Weigle, DE Parks: Deregulation of idiotype expression. Induction of Tolerance in an Anti-Idiotypic Response. J Exp Med. 1982;156 (1):898-911.
11. Leiber D, Harbon S, Guillet JG, Andre C, Strosberg AD: Monoclonal antibodies to purified muscarinic receptor display agonist-like activity. Proc Natl Acad Sci. USA 1984;81(14):4331-4.
12. Papka RE, Traurig HH, Schemann M, Collins J, Copelin T, Wilson K. Cholinergic neurons of the pelvic autonomic ganglia and uterus of the female rat: distribution of axons and presence of muscarinic receptors. Cell Tissue Res. 1999;296(2):293-305
13. Roux KH, Tankersley DL. A view of the human idiotypic repertoire. Electron microscopic and immunologic analyses of spontaneous idiotype-anti-idiotype dimers in pooled human IgG. J Immunol. 1990;15;144(4):1387-95.

14. Roux KH, Strelets L, Michaelsen TE: Flexibility of human IgG subclasses. J Immunol. 1997;1; 159(7):3372-82.
15. Manfredi G, Dell'Aera L, Derosa C, Ventura E, Caso R. Successful immunoglobulin treatment of recurrent abortions in a woman suffering from IgG1 and IgG3 deficiency. XXIII EAACI Congress, Amsterdam 2004, Abstract Book, 249, poster 840.
16. Sun XG1, Liu XY, Zhu R, Fan GS, Zhang Y, Chen FL. Effectiveness of intravenous immunoglobulin therapy in treating unexplained recurrent spontaneous abortion and its effect on the level of serum soluble human leucocyte antigen G. Zhongguo Yi Xue Ke Xue Yuan Xue Bao. 2010;32(5):483-7. doi:10.3881/j.issn.1000-503X.2010.05.002.
17. H Yamada, M Takeda, Y Maezawa, Y Ebina, R Hazama, K Tanimura, Y Wakui, and S Shimada. A High Dose Intravenous Immunoglobulin Therapy for Women with Four or More Recurrent Spontaneous Abortions. ISRN Obstet Gynecol. 2012;2012:512732. Published online 2012Sept11.
18. Bayary J, Dasgupta S, Misra N, Ephrem A, Duong Van Huyen JP, Delignat S, Hassan G, Caligiuri G, Nicoletti A, Lacroix-Desmazes S, Kazatchkine MD, Kaveri S. Intravenousimmunoglobulin in autoimmune disorders: an insight into the immunoregulatory mechanisms. Int Immunopharmacol. 2006;6(4):528-34.
19. Emmi L, Chiarini F. The role of intravenous immunoglobulin therapy in autoimmune and inflammatory disorders. Neurol Sci. 2002;23Suppl1:S1-8.
20. Manfredi G, Derosa C, Ventura E, Caso R. IgG subclass deficiency's clinical expression JACI 2007;119(1): S252, 988.
21. Cavill D, Waterman SA, Gordon TP. Antidiotypic antibodies neutralize autoantibodies that inhibit cholinergic neurotransmission. Arthritis Rheum. 2003;48(12):3597-602.
22. Ruzycky AL, DeLoia JA. Expression of beta adrenergic receptor kinase subtypes in the pregnant rat myometrium. Am J Obstet Gynecol. 1997;176(5):1077-83.
23. Malek A. Role of IgG Antibodies in Association With Placental Function and Immunologic Diseases in Human Pregnancy. Expert Rev Clin Immunol. 2013;9(3):235-49.
24. JYH Kwak, FMY Kwak, SW Ainbinder, AM Ruiz, AE Beer: Elevated Peripheral Blood Natural Killer Cells Are Effectively Downregulated by Immunoglobulin G Infusion in Women With Recurrent Spontaneous Abortions. Am J Reprod Immunol. 1996;35(4):363-9.
25. Moraru M, Carbone J, Alecsandru D, Castillo-Rama M, García-Segovia A, Gil J, Alonso B, Aguarón A, Ramos-Medina R, Martínez de María J, Oliver-Miñarro D, Rodríguez-Mahou M, Ortega V, Caballero P, Meliá E, Vidal J, Cianchetta-Sivori M, Esteban C, Vargas-HennyL, Dale J, Ortiz-Quintana L, Fernández-Cruz E, Sánchez-Ramón S. Intravenous immunoglobulin treatment increased live birth rate in a Spanish cohort of women with recurrent reproductive failure and expanded CD56(+) cells. Am J Reprod Immunol. 2012;68(1):75-84.
26. Shigeki Shimada, Masamitsu Takeda, Jun Nishihira, Masanori Kaneuchi, Noriaki Sakuragi, Hisanori Minakami, Hideto Yamada: A High Dose of Intravenous Immunoglobulin Increases CD94 Expression on Natural Killer Cells in Women with Recurrent Spontaneous Abortion. Am J Reprod Immunol. 2009;62(5):301-7.
27. Ata B1, Tan SL, Shehata F, Holzer H, Buckett W. A systematic review of intravenous immunoglobulin for treatment of unexplained recurrent miscarriage. Fertil Steril. 2011;1;95(3):1080-5.e1-2. doi:10.1016/j.fertnstert.2010.12.021. Epub 2011Jan12