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Immunodominant Semen Proteins III: IgG_1 and IgG_4 Linkage in Female Immune Infertility

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Abstract

Active immune mechanism in the female reproductive tract may produce high levels of anti-seminal/sperm antibodies. Generated antibodies in the process of isoimmunization seem to be associated with female immune infertility. The aim of our study consists in the profiling of specific serum immunoglobulin classes and subclasses in infertile women. We focus on the distribution of serum seminal/sperm-specific antibodies in order to find those apparently related to female isoimmunization. Immunoglobulins G_{1-4}, $A_{1,2}$, M and E were measured by ELISA in serum from 30 infertile and 10 fertile females. Anti-seminal/sperm IgG_1 and IgG_4 fractions were predominantly detected. Anti-seminal IgG_1 and IgG_4 were observed approximately in the 2:1 ratio, anti-sperm fraction in the 1:2 ratio. Strikingly, the approximate ratio between IgG_1 and IgG_2 was 3:1 in seminal specific and 2:1 in sperm specific antibodies. Surprisingly, IgG_3 antibodies were nearly negative for both antigen fractions, seminal and sperm. Concerning our results, the proportionality does exist between seminal and sperm antibody fractions. Based on the poorly detectable levels of semen specific IgE, M, $A_{1,2}$, G_3, the markers of pathologic female isoimmunization appear to be the serum IgG_1 and IgG_4. These preliminary findings may contribute to a detailed patient diagnosis and an improved therapy.

Keywords: Antibodies, ELISA, Seminal fluid, Sperm

1. Introduction

Human semen, that is defined as a complex fluid containing sperm, cellular vesicles and other cells and components (e.g., leucocytes, environmental antigens of microbial origin), could provoke the immune reaction of the female genital tract (Moldoveanu *et al.*, 2005; Brazdova *et al.*, 2013). Semen immunoregulatory factors as well as immunogenic agents are, thus, the potential targets of activated inflammatory cytokines, initiate leukocyte infiltration and complement cascade in the female genital tract. The active local immunoregulatory mechanism of the female reproductive tract is related to the fertility potential (Wirth, 2007; Brazdova, 2014). Sperm is able to induce the production of sperm-reactive T-cells in men as well as in women. Anti-sperm antibodies interfere with the antibody-mediated infertility through various pre/post-fertilization processes (Kurpisz and Kamieniczna, 2009; Brazdova *et al.*, 2013). Seminal antibody-binding proteins contribute to sperm metabolism, passage in the female reproductive tract and block an interaction with immune effectors. Seminal Fluid (SF) induces pro-inflammatory cytokines and suppresses the cell-mediated cytotoxicity (Chiu and Chamley, 2002; Brazdova *et al.*, 2012a). In primary response to some allergens/antigens, IgE antibodies might be prevalent. The so-called switch into IgG and IgA antibodies is induced at the late phase of primary immune response and/or after the repeated exposure to the same antigen (Batard *et al.*, 1993). After the chronic antigen exposure, IgG_1 and IgG_4 become the predominantly produced subclasses of IgG isotype. In addition, IgG_4 is unable to activate the classical complement pathway and is then known as an anti-inflammatory immunoglobulin and a blocking antibody towards IgE antibodies. Still, it remains unclear whether IgG_4 is a protective or rather sensitizing antibody

List of abbreviations :AP: Alkaline Phosphatase; ELISA: Enzyme-Linked Immunosorbent Assay; F: control sera of Fertile women; IF: sera of Infertile Female patients; L: sperm Lysate; ND: Non Detectable levels; SD: Standard Deviation; SF: Seminal Fluid.

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(Aalberse and Schuurman, 2002; Guma and Firestein, 2012).

In the present paper, the profile of antibody-based immune response to seminal/sperm proteins in infertile and fertile women is studied to document which class or subclass of serum immunoglobulin might be mostly involved in the immune rejection of sperm associated with female immune infertility.

2. Materials and Methods

2.1. Sample preparation

Semen samples from 8 normozoospermic (WHO, 2010) donors (aged 25-30) were treated as previously described (Brazdova *et al.*, 2012a; Brazdova *et al.*, 2013). Sperm-free SF was processed untreated; the sperm disintegration was processed with Triton X-100. To increase the amount of potential antigens in the protein extracts and to eliminate individual variations, the obtained individual sperm Lysates (L) were pooled as well as individual SF. The samples were stored at -20 °C until assayed. All experiments were performed after obtaining informed written consent.

2.2. Patients

This study was approved by the institutional ethical committees and informed written consent was returned by patients. Sera were obtained from 30 women with a fertility disorder (patients with repeated in vitro fertilization failure, aged 29-38) and from 10 women (control group, aged 28-37) with proven fertility (1-2 healthy children). The serum samples were frozen at -20 °C until assayed.

2.3. Quantification of Serum Anti-Seminal/Sperm Immunoglobulins

Serum anti-seminal/sperm IgG_{1-4}, $IgA_{1,2}$, IgM, IgE and then the SF/L reactivity of patient and control sera were tested by ELISA developed in our laboratory.

In the first protocol, to obtain the standard calibration curves, the micro-plates (MaxiSorp^{TM}, Denmark) were coated with either mouse anti-human IgG_{1-4} (Calbiochem, United States), $IgA_{1,2}$ (Abcam, United Kingdom), goat anti-human IgM (Sigma-Aldrich, United States) or IgE (Sigma-Aldrich, United States) in a 50 mM carbonate-bicarbonate buffer (Friguet *et al.*, 1985) overnight at 4 °C. The plates were saturated with 0.5% gelatine (Sigma-Aldrich, United States) in PBS-Tw 0.1% (0.01 M Phosphate Buffered Saline with NaCl 0.15 M, pH 7.4 supplemented with Tween; Brazdova *et al.*, 2012a,b) for 2 h at room temperature. The wells were incubated with the individual patient or control sera or human IgG_{1-4}, $IgA_{1,2}$, IgM, IgE (Calbiochem, United States) as standards of known concentration in serial dilution for 2 h at 37 °C in PBS-Tw 0.1% and then with alkaline phosphatase (AP)-conjugated, either mouse anti-human IgG_{1-4} (Calbiochem, United States), $IgA_{1,2}$ (Beckman Coulter, United States), goat anti-human IgM or IgE (Sigma-Aldrich, United States) for 2 h at 37 °C. The AP activity was detected by p-nitrophenyl phosphate disodium (Sigma-Aldrich, United States). Optical density was measured at 405 nm versus 630 nm with a multichannel spectrophotometer (Tecan Group Ltd., Switzerland).

In the second protocol, to quantify the serum anti-seminal/sperm IgG_{1-4}, $IgA_{1,2}$, IgM, IgE, the micro-plates were coated with SF/L in a 50 mM carbonate-bicarbonate buffer overnight at 4 °C. The following procedure was identical to the first protocol. The anti-seminal/sperm immunoglobulin concentrations were obtained by linear regression in comparison with the standard calibration curve obtained in the first protocol.

3. Results

Figure 1 shows the concentration of seminal/sperm-specific IgG_{1-4} in the individual sera of infertile/fertile females. In the sera of infertile women, the predominant anti-SF IgG subclasses were IgG_1 and IgG_4, reaching maximum concentration of 45 µg/ml (IgG_1) and 38 µg/ml (IgG_4). In particular, anti-SF IgG_1 antibodies were detected in 100% of sera tested with the mean of 22 µg/ml (SD 9), IgG_2 in 97% with the mean of 8 µg/ml (SD 6), IgG_3 in 6% with the mean of 0.1 µg/ml (SD 0.05), IgG_4 in 90% with the mean of 12 µg/ml (SD 9). In the sera of infertile women, the prevalent anti-sperm IgG subclass was IgG_4 ranging from 9 to 45 µg/ml with the mean of 23 µg/ml (SD 10). Anti-sperm IgG_{1-4} antibodies were detected within all patient sera processed with the mean of 11 (SD 5, IgG_1), 7.5 (SD 5, IgG_2), 0.3 (SD 0.1, IgG_3), 23 µg/ml (SD 10, IgG_4). Twenty percent of control sera contained seminal-specific IgG_1 and 30% contained seminal-specific IgG_2, both of which ranged from 0.2 to 0.5 µg/ml. Sperm-specific $IgG_{1,2}$ were detected in 30% of control sera reaching the top value of 0.5 µg/ml. None of the control sera had the detectable levels of anti-seminal/sperm $IgG_{3,4}$.

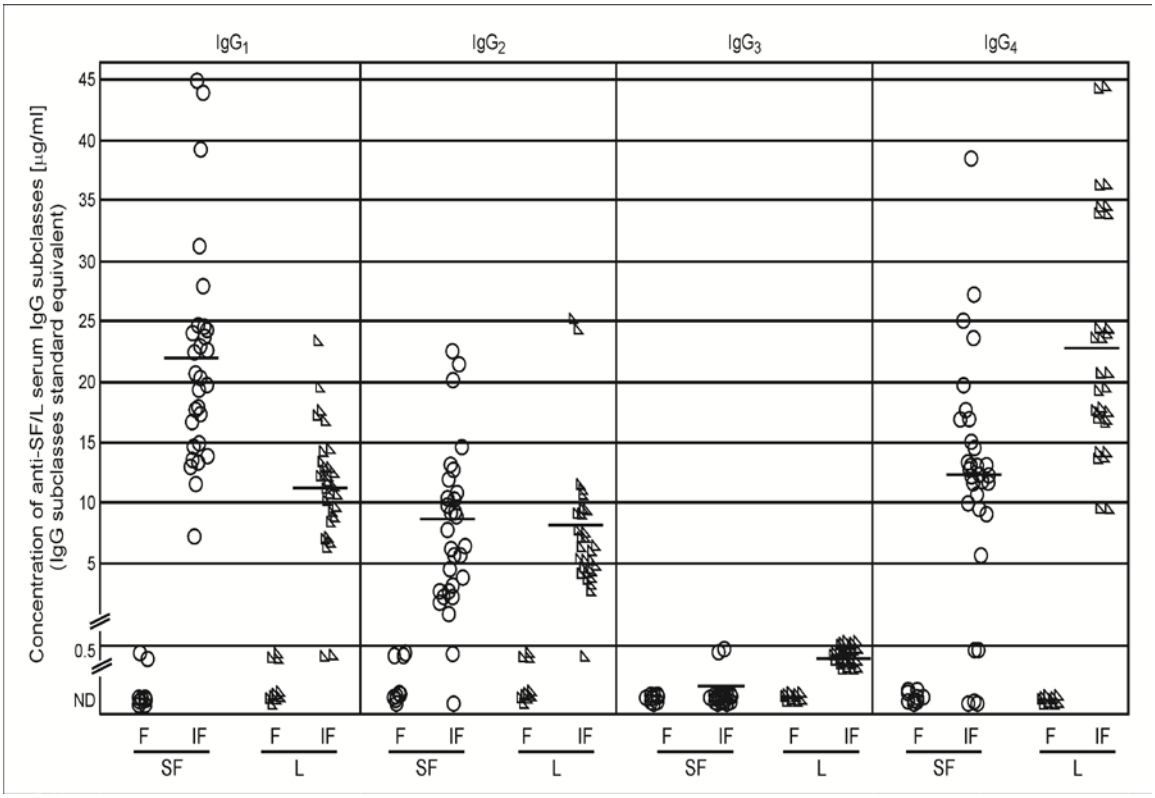

Figure 1. Quantified female serum anti-seminal/sperm $IgG_{1,2,3,4}$. IF: sera of infertile patients (30), F: control sera of fertile women (10), ND: nondetectable level, ○: anti-seminal antibodies, ◺ : anti-sperm antibodies, bars: arithmetic means.

Figure 2 shows seminal/sperm-specific $IgA_{1,2}$, IgM, IgE concentrations in the individual sera of infertile/fertile women. Anti-seminal/sperm IgE, IgM and IgA_1 were not detected in the patient sera. Only 1 patient serum out of 30 contained anti-sperm IgA_2 (0.18 µg/ml). Then, 1 control serum out of 10 contained anti-seminal IgA_1 (1.2 µg/ml), IgA_2 (1.8 µg/ml) and anti-sperm IgA_1 (1.9 µg/ml), IgA_2 (2.1µg/ ml).

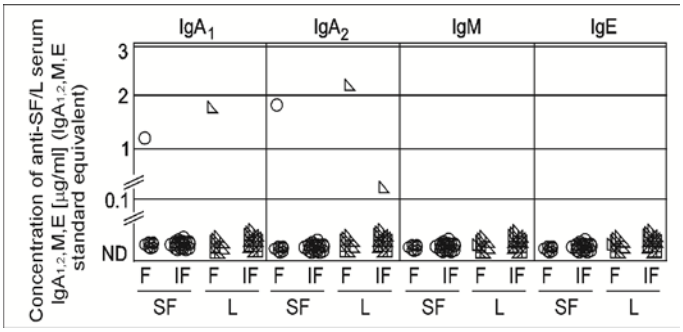

Figure 2. Quantified female serum anti-seminal/sperm $IgA_{1,2}$, IgM, IgE. IF: sera of infertile patients (30), F: control sera of fertile women (10), ND: nondetectable level, ○: anti-seminal antibodies, ◺ : anti-sperm antibodies.

4. Discussion

We present our essential preliminary findings concerning the distribution of serum anti-SF/L $IgG_{1,2,3,4}$, $IgA_{1,2}$, IgM and IgE as a consequence of pathophysiological female isoimmunization. We show IgG_1/IgG_4 predominance depending on the values themselves, 2:1 in anti-seminal specific antibodies, also valid in a reverse statement between anti-sperm IgG_1 and IgG_4 levels. The IgG_1:IgG_4 ratio in anti-sperm antibodies is in agreement with the working hypothesis of Batard *et al.* (1993) who proved that the prolonged exposure to immunogenic agents, such as grass pollen allergens, generates IgG_1 further shifting into IgG_4. Then, we speculate that the detected antigens might be the same since the distribution of tested antibodies follows the similar trend of IgG_1>IgG_2>IgG_3<<IgG_4. Based on barely detectable IgG_3 within the patient group, we assume that IgG_3 does not have any protective or inflammatory role in female immune infertility. Among the four IgG subclasses, $IgG_{1,3}$ activate complement cascade (Jefferis, 2012). Since patients do not suffer from the inflammatory symptoms, we suggest that complement cascade may not be activated by IgG_3 in the semen rejection. It was specified (Tamayo *et al.*, 2012) that the response to protein antigens is primarily mediated by IgG_1 and IgG_3, whereas IgG_2 especially and IgG_4 are induced in response to polysaccharide antigens. Since seminal/sperm-specific IgG_1 and IgG_4 were inversely correlated, IgG_1 could theoretically bind to seminal antigens of a protein character and IgG_4 to sperm structures of an oligosaccharide nature. Whether or not serum IgG_4 is a blocking or sensitizing antibody in infertile females remains unexplained, unlike in other studies dealing with other pathologies (Aalberse and Schuurman, 2002; Guma and Firestein, 2012). The absence of data following patients over time prevented us to better understand the role of IgG_4. Concerning male autoimmunity, IgA antibodies were proved to be associated with a systematic factor in male immune infertility (Kutteh *et al.*, 1994). The nondetectable levels of $IgA_{1,2}$ in 97% of female patient sera tested imply that either IgA_1 or IgA_2 do not correlate with systemic isoimmunization. However, we are able to affirm that only IgA_1 is not involved in the disease as it predominates in the sera (Woof and Kerr, 2006) examined in our study. Immunoglobulin M contributes to opsonization and is involved in the primary response to antigen exposure (Schoeder and Cavacini, 2010). Since the patient sera are neither collected nor tested immediately after the sensitization, the nondetectable level reflects the potential secondary immune response where IgM does not play a significant role. However, we cannot refute that IgM is not involved either in primary or secondary antibody response to isoimmunization. Not a single patient was diagnosed with semen hypersensitivity, which is in agreement with an absence of anti-seminal/sperm IgE, usually related to the pathophysiology of allergy (Brazdova *et al.*, 2012a,b).

5. Conclusion

The described distribution of seminal/sperm-specific $IgG_{1,2,3,4}$, $IgA_{1,2}$, IgM, IgE indicates that female iso-immunization seems to be IgG-dependent as immunoglobulins E, M, $A_{1,2}$ are not involved in this pathophysiological process. Specific IgG_1 predominates in anti-seminal serum antibody fraction; on a contrary, specific IgG_4 in anti-sperm serum antibody fraction. We believe that the determination of serum seminal/sperm-specific immunoglobulin G subclasses might make patient profiling more precise and complete the information for diagnosis. Anti-seminal/sperm IgG_1 and IgG_4 could be of interest for further therapy targets.

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