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Original Article

Quantification of anti-sperm antibody and soluble MICA/MICB levels in the serum of infertile people of the Li ethnic group in China

Xiaobin Wei[1,2], Zhouxin Han[3], Biqiong Ren[1], Xi Xiao[2], Feng Li[2], Danqin Lin[3], Bin Luo[3], Xianxian Fu[2], Chunyun Li[2], Huan Xia[2], Ping Yu[1]

[1]Department of Immunology, College of Basic Medical Sciences, Central South University, 88 Xiangya Road, Changsha 410008, China; [2]Clinical laboratory, Affiliated Haikou Hospital of Xiangya Medical College in Central South University, Haikou 570208, China; [3]The Second People's Hospital of Hainan Province, 24 Aimin Road, Wuzhishan 572200, China

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Abstract: Objective: To investigate the presence of anti-sperm antibodies (AsAb) and the correlation between AsAb positivity and the expression of soluble major histocompatibility complex class I chain-related A and B (sMICA or sMICB) in the sera of infertile people of the Li nationality from Hainan, China. Method: A total of 136 people (68 couples) from five villages in the Wuzhishan region, Hainan province participated in this study. Among them, 31 couples were included in the fertile group and 37 couples in the infertile group. AsAb and sMICA/sMICB levels in serum were detected by ELISA. The median sMICA/sMICB levels between and among groups were compared by Mann-Whitney rank U testing and Kruskal-Wallis H testing, and the AsAb positivity rate was compared by Pearson Chi-Square testing. Correlation analysis was performed by calculating the Spearman's rho coefficient for nonparametric data. Results: The serum levels for the fertile group (AsAb: 15.5 [4.0~127.0] U/ml, sMICA: 18.33 [13.30~52.40] pg/ml, sMICB: 27.72 [18.63~47.43] pg/ml) were not statistically different from those for the infertile group (AsAb: 18.0 [9.8~95.0] U/ml, sMICA: 20.95 [15.78~23.81] pg/ml, sMICB: 26.26 [18.06~61.38] pg/ml). However, grouping based on AsAb positivity revealed a statistically significant difference for the sMICA/sMICB levels (AsAb positive group: sMICA: 5.56 [4.30~17.23] pg/ml, sMICB: 16.13 [7.54~25.43] pg/ml; AsAb negative group: sMICA: 22.00 [18.05~66.13] pg/ml, sMICB: 36.51 [20.53~67.22] pg/ml; $P < 0.01$). These results suggest that AsAb is negatively associated with both sMICA (Spearman's coefficient, -0.475, $P < 0.01$) and sMICB (Spearman's coefficient, -0.381; $P < 0.01$). The analysis also shows that sMICA levels are positively associated with sMICB levels (Spearman's coefficient, 0.635; $P < 0.01$). Conclusion: AsAb can be detected in the serum of fertile and infertile Li people. However, there appears to be limited clinical value in the conventional detection of AsAb, sMICA and sMICB in serum for diagnosing infertility. People with positive AsAb expression have lower levels of sMICA/sMICB expression in serum, which may be one mechanism by which people produce AsAb.

Keywords: The Li nationality, anti-sperm antibody, infertility, sMICA/sMICB

Introduction

As a boundary island between subtropical and tropical zones, Hainan province, China, has a unique climate and unique geographical conditions. The Li people are a minority, with characteristic genetic background. The Li people are centralized within Hainan province and are spread throughout the province, but mainly live in the central and southern regions, with an estimated population of 1.2 million [1]. Traditional Li villages are based on clanship and kinship relationships that embody the region's cultural style and have developed over many years. For a long time, infertility has been the main disease that has troubled the Li people. About 15% of the married couples of child-bearing age in this population are infertile, among which 20%~30% of the cases may be associated with immunologic factors [2]. However, the scientific basis for the infertility etiology of the Li people has not been elucidated.

Table 1. Comparison of the quantitative test results [M(Q1~Q3)] for AsAb, sMICA and sMICB in the serum of infertile and fertile Li people

Group	Cases	AsAb (U/ml)	sMICA (pg/ml)	sMICB (pg/ml)
Infertile	74	18.0 (9.8~ 95.0)	20.95 (15.78~23.81)	26.26 (18.06~61.38)
Fertile	62	15.5 (4.0~127.0)	18.33 (13.30~52.40)	27.72 (18.63~47.43)
P-values*		0.360	0.643	0.854

**P*-values are calculated using the Mann-Whitney U Test for continuous variables.

Immunity-associated infertility is an autoimmune disease [3], accounted for primarily by the presence of anti-sperm antibodies (AsAb). The highly polymorphic nonclassical major histocompatibility complex class I chain-related genes A and B (MICA and MICB) encode stress-inducible glycoproteins expressed on various epithelial cells, including intestinal epithelial cells. MICA and MICB gene polymorphisms and variations in expression levels are associated with autoimmune diseases [4]. Recent studies demonstrate that the dysregulation of NKG2D and its ligands, leading to the activation of autoreactive effector cells, can also trigger autoimmune diseases, but soluble forms of these ligands can down-modulate NKG2D expression on T effector cells [5]. Furthermore, MICA protein expression is increased through binding to activated NKG2D receptors that are located in NK cells and the surface of some T cells [6, 7]. Thus, the regulation of immunity-mediated infertility is complex and may be multifactorial. Whether the MICA and MICB proteins in sera play a role in the occurrence of AsAb or the development of infertily is unknown.

The purpose of this study was to compare the occurrence of AsAb in the serum of infertile and fertile Li people, to assess levels of sMICA/sMICB expression and to analyze the possible correlation between sMICA/sMICB expression levels and AsAb positivity. Determination of the possible mechanisms of infertility in the Li people may help to provide a scientific basis for the treatment and prevention of infertility.

Subjects and methods

Patients and samples

From September 2012 through March 2013, blood samples of participants were collected from 5 villages in the Wuzhishan region, Hainan province, China. This included 68 couples (136 people in total): 31 couples in the fertile group (1-2 children) were composed of men aged 32 ± 7 and women aged 28 ± 6; 37 couples in the infertile group (sterile duration 1~10 years) were composed of men aged 35 ± 6 and women aged 32 ± 5. Serum was prepared from 3 ml peripheral venous blood and was stored at -20°C until use. All subjects volunteered for the study, and written informed consent was obtained prior to the collection of peripheral blood samples. The Ethics Committee of the Affiliated Haikou Hospital of Xiangya Medical College in Central South University approved this study.

Enzyme-linked immunosorbent assay (ELISA)

The occurrence of AsAb was assessed by enzyme-linked immunosorbent assay (ELISA) (IBL, Germany; batch number: ESU142) performed according to standard protocol. The kits for sMICA/sMICB detection were from Yihan Biochemistry Technology Co., LTD (China), and the antibodies were from Santa Cruz Biotechnology, Inc (USA). Absorbance values were determined using a microplate reader from Thermo/Labsystems (model: MK3; Finland). The experimental operation and analysis of results were implemented strictly in accordance with the kit instructions.

Statistical analysis

The SPSS11.0 statistical software package was used for statistical analysis. The experimental data were expressed as the medians and interquartile range. The Mann-Whitney U rank sum test was adopted for comparing medians between two groups, and the Kruskal-Wallis H test was adopted for comparing the medians among multiple groups. The Pearson Chi-Square test was used for comparing the positive AsAb rate. Correlation analysis was performed by calculating the Spearman's rho coefficient for nonparametric data. Significance for all tests was set at Pc = 0.01.

Table 2. Comparison of the As Ab positivity rates in the serum of infertile and fertile Li men and women

Group	Sex	Cases	AsAb positive cases	AsAb negative cases	Positivity rate	Total positivity rate*
Infertile	Male	37	12	25	32.40%	24.30%
	Female	37	6	31	16.20%	
Fertile	Male	31	8	23	25.80%	27.40%
	Female	31	9	22	29.00%	

**P*-values were calculated using chi-square tests for categorical variables. The value of the Pearson Chi-Square is 2.799, P = 0.424 > 0.05, therefore, it cannot be assumed that there are differences in the positivity rate for serum AsAb between the four groups.

Table 3. Comparison of the quantitative test results [M(Q1~Q3)] for AsAb, sMICA and sMICB levels in the serum of males and females from infertile and fertile groups

Group	Sex	Cases	AsAb (U/ml)	sMICA (pg/ml)	sMICB (pg/ml)
Infertile	Male	37	24.0 (10.5~176.5)	19.58 (14.58~23.77)	28.72 (16.39~62.07)
	Female	37	16.0 (7.5~36.5)	21.19 (17.50~23.88)	23.79 (18.86~57.18)
Fertile	Male	31	14.0 (8.0~100.0)	20.17 (13.41~139.43)	28.25 (21.31~47.22)
	Female	31	19.0 (4.0~140.0)	17.67 (10.55~25.45)	26.37 (17.19~48.05)
P-values*			0.334	0.625	0.899

**P*-values were calculated using Kruskal-Wallis H testing for continuous variables. *P*-values are greater than 0.01, therefore, it cannot be assumed that there are differences in quantitative test results for AsAb between any two groups.

Results

Quantification of serum AsAb, sMICA and sMICB levels from infertile and fertile couples

To assess differences in the levels of AsAb, sMICA and sMICB in serum from infertile and fertile Li couples, we collected blood samples from 136 people (31 infertile couples and 37 fertile couples. Serum levels were calculated by ELISA (**Table 1**). The quantitative test results show that there is no statistical significance of differences in AsAb, mMICA or sMICB levels between the two groups (P > 0.01).

Quantification of test results for AsAb, sMICA and sMICB in the males and females from the infertile and fertile groups

Theoretically, the levels and effects of AsAb expression could be expected to vary for females or males. To determine whether grouping by gender for the infertile and fertile couples might reveal differences in AsAb expression, we calculated average values for each subgroup (**Table 2**). The AsAb positive rate for infertile males was 32.4%, and the AsAb positive rate for infertile females was 16.2%; while the AsAb positive rate for fertile males was 25.8%, and the AsAb positive rate for fertile females was 29.0%. *P*-values, calculated using the Kruskal-Wallis H test for continuous variables, were greater than 0.01; therefore, it cannot be assumed that there are differences in the quantitative test results for AsAb between fertile and infertile males and females.

Comparison of sMICA and sMICB levels in AsAb-positive and AsAb-negative groups

To further assess whether differences might be observed by grouping AsAb-positive and AsAb-negative individuals, we chose a cutoff for forming groups, with ≥ 90 U/ml representing the AsAb-positive group and < 90 U/ml representing the AsAb-negative group. No statistical significance could be observed for the positive rate from serum AsAb according to male-female statistics (Pearson Chi-Square 2.799; P = 0.424; **Table 3**). Interestingly, the sMICA/sMICB levels in the AsAb positive group were lower than in the AsAb negative group (P < 0.01; **Table 4**). These results suggest that AsAb levels may be negatively correlated with sMICA/sMICB expression.

Correlation analysis for AsAb, sMICA and sMICB levels

To verify that AsAb levels are negatively correlated with sMICA and sMICB expression, we performed correlation analysis. Our results

Table 4. Comparison of the test results [M(Q1~Q3)] for sMICA and sMICB levels in the serum of AsAb-positive and AsAb-negative people

Group	Cases	sMICA (pg/ml)	sMICB (pg/ml)
AsAb-positive	35	5.56 (4.30~17.23)	16.13 (7.54~25.43)
AsAb-negative	101	22.00 (18.05~66.13)	36.51 (20.53~67.22)
P-values*		0.000	0.000

*P-values were calculated using the Mann-Whitney U test for continuous variables.

Table 5. Correlation analysis on quantitative test results of AsAb, sMICA and sMICB Correlations

			ASAB	MICA	MICB
Spearman's rho	ASAB	Correlation Coefficient	1.000	-.475**	-.381**
		Sig. (2-tailed)	-	.000	.000
		N	136	136	136
	MICA	Correlation Coefficient	-.475**	1.000	.635**
		Sig. (2-tailed)	.000	-	.000
		N	136	136	136
	MICB	Correlation Coefficient	-.381**	.635**	1.000
		Sig. (2-tailed)	.000	.000	-
		N	136	136	136

**Correlation is significant at the 0.01 level (2-tailed).

show that AsAb levels are negatively related to both sMICA (Spearman's coefficient -0.475, P < 0.01) and sMICB levels (Spearman' scoefficient -0.381, P < 0.01) (**Table 5**). Furthermore, sMICA expression is positively related to sMICB expression with statistical significance (Spearman's coefficient is 0.635, P < 0.01). These results suggest that AsAb, sMICA and sMICB levels are associated with each other, though the levels do not appear to be associated with the fertility status of the Li males or females.

Discussion

The antigenic properties of human sperm were reported as early as the end of 19th century [8]. Since then, AsAb has been considered as a possible causative factor in infertility, with significant levels of AsAb detected in the semen of 5-15% of infertile men but only 1-2% of fertile men [9-12]. In China, the positive rate of serum AsAb from infertile males is reported to be as high as 20%-30% [13, 14], with the total positive rate of AsAb for fertile males of 5-7.5% [14, 15]. Another report suggests that AsAb is found in the serum of 60% of healthy men. Moreover, AsAb has been found in cervical mucus for 2% of women [16]. Leushuis et al. [17] showed that AsAb may reduce the chance of pregnancy in a cohort of infertile couples, although the effect did not reach statistical significance. Other reports suggest that AsAb is not associated with infertility [16]. Therefore, the prominence of AsAb and whether AsAb is associated with infertility remains controversial.

A number of mechanisms have been proposed to explain the potential detrimental effects of AsAb on sperm function and fertility potential. AsAb in semen has been associated with reduced sperm motility, likely resulting from antibody binding to the sperm tail and subsequent agglutination [18, 19]. AsAb has also been shown to interfere with sperm-oocyte binding and penetration into hamster oocytes, although the antigenic specificity of the AsAb may be important in this respect [20]. AsAb may impede cervical mucus penetration and inhibit the transport of the sperm through the female reproductive tract [21-24]. In addition to these direct mechanisms, AsAb may adversely impact male fertility indirectly by mediating the release of cytokines that can impair sperm function [21-24]. Despite these suggested mechanisms, other studies have suggested that AsAb has no influence on fertilization rates and that the sperm concentration, percentage of progressive motility, and percentage of strict morphology are not significantly different in AsAb-positive and AsAb negative samples. [25-29]. Still other studies show an inverse relationship between AsAb levels and sperm motility [19, 20]. These contradictory results suggest that whether or not AsAb deters fertility and its mechanism of action may depend on other factors for specific individuals.

To further explore the possible relationship between AsAb levels and infertility, we exam-

ined AsAb levels in the Li people, a geographically-isolated minority population for which about 15% of the married couples of child-bearing age are infertile [2]. Our results show that there is no statistical difference for the positive AsAb rate between infertile and fertile groups. Furthermore, the average rate of AsAb positivity (16%-32.4%) is higher than for other populations [16]. The high AsAb positivity rate from the Li people may be related to the living habits, status and cultural practices, including the low usage rate of condoms in this population and the extended time-to-treat for genital tract infections due to limited medical availability. The lack of correlation of AsAb levels with fertility suggests that additional factors may regulate the high infertility in the Li population, either independently or synergistically with the high overall AsAb positivity rate.

Sperm has antigens that stimulate T cells and can activate cross reactivity, which suppresses the T cell response; conversely, release of immune suppression can lead to the generation of AsAb. To assess a potential immune-mediated pathway of AsAb function, we assessed levels of MICA and MICB. Levels of soluble MICA are very low in the healthy population including healthy blood donors [30]. However, MICA can be activated by the stress response to tumorigenesis or virus infection. MICA protein expression is increased through binding to activated NKG2D receptors that are located on natural killer (NK) cells and the surface of some T cells [31, 32]. Both the membrane-bound and soluble forms of MICA molecules regulate lymphocyte function, but the effect is different according to the form of the protein. The membrane MICA protein is expressed on the cell surface. After binding of MICA to NKG2D, NKG2D binds DAP10, which in turn binds through its intracytoplasmic SH2 structural domain to the P85 subunit of phosphatidyl inositol 3-kinase (PI-3). PI-3 then transmits a signal to activate lymphocytes [33]. The soluble form of MICA protein (sMICA) is released from the cell surface by metalloproteinases [34]. When sMICA binds to NKG2D, internalization and degradation of NKG2D is induced, and consequently, immune surveillance against tumors is inhibited. Most tumor cells express MICA on their surface [35], and the content of sMICA in the serum is increased for some cancers [36], which suggest that MICA may take part in inhibiting immune suppression. Proteolytic shedding of sMICA and sMICB from cancer cells therefore constitutes a novel immune escape strategy that diminishes anti-tumor reactivity by NKG2D-bearing cytotoxic T lymphocytes.

Recent evidence also suggests that MICA and MICB may also be associated with autoimmune diseases. Polymorphisms and variable expression are associated with autoimmune diseases. sMICA is highly expressed in ulcerative colitis [37]. Furthermore, elevated sMICB levels are found in the serum of multiple sclerosis patients and related to immune-associated disease activity during relapses [38]. A profound dysregulation of NKG2D and its MIC ligands may cause autoreactive T cell stimulation, thus promoting the self-perpetuating pathology in rheumatoid arthritis and possibly other autoimmune diseases [39]. While hepatic autoimmune diseases have no general impact on the amount of circulating sMICA and sMICB, acute bacterial infections, renal insufficiency and cholestasis can lead to notably elevated serum levels of the NKG2D ligands [40]. MICA-mimicking peptides are useful for identifying the specific functional sites for the NKG2D-MICA interaction, but also are promising for explaining NKG2D-related autoimmunity [41]. Because AsAb-associated infertility can classified as an autoimmune disorder, we hypothesized that MICA/MICB might be correlated with AsAb levels.

The results of our study showed that the soluble MICA/B levels in the serum from patients with positive AsAb is lower than that from patients with negative AsAb ($P < 0.01$). Given that MICA/B expression is increased upon binding with NKG2D to tumors, we hypothesize that sMICA/B can reduce the expression of NKG2D receptor on T effector cells, which leads to autoimmune disease. The decrease of sMICA/B in patients with positive AsAb rates may be due to excessive depletion, leading to a decline in the body's immune suppression and in the production of AsAb. Therefore, sMICA/B is suggested to have a dual function: overexpression can restrain the body's immune system and cannot kill tumor cells, whereas excessive depletion or decreased expression can lead to autoimmune disease.

Conclusion

AsAb exists in the serum of the normal fertile group from Li People in China. There is limited

clinical value for conventionally detecting serum AsAb, sMICA and sMICB for infertility diagnosis for Li People. Low expression of serum sMICA/sMICB from the patients with positive AsAb may be one of the mechanisms by which the body produces AsAb. Therefore, adjusting sMICA/B expression may be an effective therapeutic regimen for inhibiting the occurrence and development of AsAb.

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Disclosure of conflict of interest

None.

Abbreviations

sMICA, soluble major histocompatibility complex class I chain-related A; sMICB, soluble major histocompatibility complex class I chain-related B; AsAb, anti-sperm antibody; MHC, major histocompatibility complex; NKG2D, natural killer group 2, member D; ELISA, Enzyme-linked immunosorbent assay; NK, natural killer; DAP10, Death Associated Protein 10; BD, Behçet's disease; GDCD8+, Gammadelta T lymphocytes CD8+; HC, healthy controls; UC, ulcerative colitis.

Address correspondence to: Dr. Ping Yu, Department of Immunology, College of Basic Medical Sciences, Central South University, 88 Xiangya Road, Changsha 410008, China. Tel: +86 731 82355010; Fax: +86 731 82650401; E-mail: yuping195311@163.com

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