

1, 2, 3, 4, 1, 1, 1

Antiendometrial antibodies in peritoneal fluid from patients with endometriosis

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=Abstract=

We have previously demonstrated that specific antigens involved in autoimmunity in endometriosis may be endometrial proteins with molecular weight(mw) of 71, 92, and 103 kilodalton(kDa). The purposes of this study were to determine the incidence of IgG antibodies against these endometrial antigens in peritoneal fluid of patients with endometriosis and to evaluate the antigenic differences between the endometria of patients with and without endometriosis. Forty peritoneal fluid(PF) from 24 patients with endometriosis and 16 patients without endometriosis(control patients) were tested against endometrial protein from patients(n=8) with endometriosis and from control patients (n=10) by western blot. Fifteen(62.5%) of 24 PF samples from patients with endometriosis had specific Immunoglobulin(Ig) G antibodies against one of three endometrial proteins with mw of 71, 92 and 103 kDa but none of PF samples from control patients had these antibodies. The electrophoretic pattern of endometrial proteins from patients with endometriosis was similar to that from control patients. Furthermore there was no significant difference in specific PF Immunoglobulin G binding to endometrial proteins regardless of origin of these proteins. Our data indicate that specific humoral immune response can be found in PF of patients with endometriosis and that specific antigens inducing this immune response are present in human endometrium and that there is no antigenic difference between the endometria of patients with and without endometriosis.

Key Words: Antiendometrial antibodies, Antigenic difference, Peritoneal fluid, Endometriosis

가

10- 15%

가

가

가

가

가

(immunogenic)

(natural killer cell)

(Garzetti et al.,1995; Ho et al.,1995),

가(Halme et al., 1984),

T

(Vigano et al., 1991)가

가

(Confino et al., 1990)

가

가

(Badawy et al., 1984;

Meek et al., 1988),

(Meek et al., 1988; Wild et al.,

1991,1992),

(Badawy et al., 1990; Garza et al., 1991), western blot

(Mathur et al.,1988,1990; Rajkumar et al.,1992; Gorai et al.,1993),

(Fernandez- Shaw et al.,1993; Odukoya et al.,1995)

(Kim et al.,1995)

western blot

48.1%, 55.1%

가

71, 92, 103

kDa

가

1.

, , ,
 24
 ,
 () 16
 (1985) 1 가 6 , 2 가 8 , 3 가 5
 , 4 가 5 가

2.

1)

,
 1000 x g 10 -20 가
 6 , 4
 -70 가
 4 , 4

2)

(protease) phenylmethylsulfonyl fluoride 1 M/L 가
 phosphate buffered saline(PBS) polytron
 (hemogenizer) Dounce

10 3000 x g 0.1% sodium azide
 50µl -70 ° C 가 .

3) Bicinchoninic acid(BCA)

BCA
 (bovine serum albumin) 100, 80, 60, 40, 20, 10, 5 µg/ml
 , 1:10, 1:20, 1:40 10 µl
 BCA 4% CuSO₄ · 5H₂O 50:1 200 µl
 (microplate) 37 ° C 30 562nm
 (optical absorbance) IBM computer curve fitter program

4) Western blot

(3 mg/ml) 62.5mM Tris, 10% glycerol , 2 % sodium dodecyl sulfate(SDS), 10% 2-mercaptoethanol, 0.05% bromphenol blue
 1:4 5 가 . 3% polyacrylamide stacking
 7.5% - 15% gradient polyacrylamide running
 . coomasie brilliant blue western blot
 . western blot transblot 0.3% Tris base , 1.44% glycine , 20% methanol
 . ponceau S 5 2
 . 10
 . 2% skim milk Tris
 buffered saline(TBS , pH 7.3) 2 가 .
 0.05% Tween-20 PBS(PBS-T) 10 3
 PBS-T , 2% skim milk PBS-T 100
 2 .
 (immunoglobulin; Ig) TBS
 . PBS-T 10 3 2% skim milk
 PBS-T 1:1000 goat antihuman Ig G F(ab)₂ peroxidase
 (conjugate) 1 PBS-T 10 1 , PBS
 2 . 0.025% diaminobenzidine, 0.075%

4-chloro-1-naphthol, 0.08% H₂O₂

10 가

(reactive antigen)

(lysozyme 18.5kDa, soybean trypsin inhibitor 27.5 kDa, carbonic anhydrase 32.5kDa,
Ova albumin 42kDa, BSA 80kDa, phosphoylease B 106kDa)
(relative mobility index)

5)

IBM computer SAS program

Fisher's exact test

p < 0.05

1)

Western blot

25, 26, 28, 30, 34, 36, 47,

54, 56, 59, 61, 71, 77, 81, 85, 92, 103, 107, 116, 127 kDa

가

(Fig. 1).

2)

Western blot

TBS

goat antihuman Ig G F(ab')₂ - peroxidase

26, 54, 85 kDa

3

가

28, 107, 116 kDa

가

가

IgG 34, 36, 56, 59, 77/81 kDa

34, 36kDa

IgG

가

IgG가

71, 92, 103 kDa

가 20.8 %, 37.5
 % (p<0,005), 62.5% (p<0.00005) 1
 IgG 62..5% (15/24) (Table. 1, Fig 2).

3) IgG

IgG 가

IgG

가 (Fig. 3).

가

(soluble) CD23가

B

Odukoya (1996)

- globulin

(Badawy et al., 1984).

western blot

가

가

Mathur (1988,1990)

western blot

가

26, 34kDa

26, 34, 42, 82, 94, 100, 120,140 kDa

Rajkumar

(1992)

34kDa

가

가

Gorai (1993)

western blot

72.2- 83.3%

26, 34, 42 kDa

33.3-44.4%

Switchenko (1991)

(re activity)

Wild (1992)

가 (Kim et al.,1995) 71, 92, 103kDa

가

IgG

(Badawy et al., 1984; Meek et al., 1988), (Meek et al., 1988;

Wild et al., 1991,1992), (Badawy et al., 1990; Garza et al., 1991),

western blot (Mathur et al.,1988,1990; Rajkumar et al.,1992; Gorai et al.,1993),

(Fernandez- Shaw et al.,1993; Odukoya et al.,1995)

가

Western blot

10- 100 %

(Mathur et al.,1988; Gorai et al.,1993).

IgG가

71, 92, 103 kDa 가

20.8 %, 37.5 %, 62.5% 1

IgG 62..5%

(Kim et al.,1995) wetem blot

55.1%

Ig

Meek (1988)

IgG , IgA. IgM 가

Liu (1987)

Ig 가

Confino

(1990)

Ig 가

IgG

IgG

(Wild et al,1992). Badawy (1990)

가가

western blot

가

B

IgG

가

(Badawy et al., 1989)

histone

(Confino et al,1990)

가

.

,

가

(immunogenic)

.

가

, Garza (1991)

가가

.

IgG 가

IgG

가

IgG

Rajkumar (1992)

Odukoya (1995)

. Ota Igarashi (1993)

(human leukocyte

antigen) DR

가

.

.

가 (Dmowski et al,1995)

가

가

(Sueldo et al,1987)

(Morocos et al,1985)

가

가

.

IgG 가

가

가

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Table 1. Results of sperm count, percentage motility, sperm kinetics and fertilizing capacity in relation to antisperm antibody negative donors proven to be fertile and to antisperm antibody positive patients

	Sperm Count ($\times 10^6/\text{Ml}$)	Percentage Motility	Sperm Kinetics	Fertilizing Capacity
ASA negative (n=96)	95.13 \pm 57.56	65.31 \pm 13.55	2.83 \pm 0.47	7.24 \pm 7.90
ASA positive (n=67)	58.36 \pm 50.52*	46.72 \pm 20.11*	2.44 \pm 0.73*	2.19 \pm 2.29*
Head (n=31)	56.10 \pm 47.31*	51.29 \pm 19.15*	2.55 \pm 0.51*	2.44 \pm 2.64*
Tail (n=24)	68.67 \pm 62.21*	42.50 \pm 16.48*	2.29 \pm 0.86*	2.02 \pm 1.95*
Head+Tail (n=12)	43.58 \pm 25.09*	46.67 \pm 23.77*	2.42 \pm 0.90*	1.88 \pm 2.06*

* Significantly different from ASA negative group; P<0.01, Duncan multiple range test

Table 2. Results of sperm count, percentage motility, sperm kinetics and fertilizing capacity in relation to the distribution of antibodies and antibody isotypes on spermatozoa

	Sperm Count ($\times 10^6/M\emptyset$)	Percentage Motility	Sperm Kinetics	Fertilizing Capacity
ASA negative (n=96)	95.13 \pm 47.56	65.31 \pm 13.55	2.83 \pm 0.47	7.24 \pm 4.90
HG+ (n=35) [#]	54.57 \pm 39.32**	52.57 \pm 20.63**	2.60 \pm 0.60*	2.36 \pm 2.56**
HA+ (n=19) [#]	41.63 \pm 43.81**	47.90 \pm 18.36**	2.47 \pm 0.70*	1.13 \pm 0.84**
HM+ (n=7) [#]	50.43 \pm 29.77*	37.14 \pm 14.68**	2.14 \pm 0.69**	2.81 \pm 2.77*
TG+ (n=30) [#]	58.37 \pm 57.69**	46.50 \pm 19.26**	2.47 \pm 0.86*	2.20 \pm 2.07**
TA+ (n=23) [#]	65.30 \pm 62.03*	45.43 \pm 18.64**	2.39 \pm 0.89*	1.48 \pm 1.46**
TM+ (n=3) [#]	70.00 \pm 27.84*	30.00 \pm 17.32**	2.00 \pm 0.00*	1.17 \pm 0.75**

* Significantly different from ASA negative group, P<0.05, Student's t-test

** Significantly different from ASA negative group, P<0.01, Student's t-test

HG+: ASA IgG positive on sperm head; HA+: ASA IgA positive on sperm head; HM+: ASA IgM positive on sperm head;

TG+: ASA IgG positive on sperm tail; TA+: ASA IgA positive on sperm tail; TM+: ASA IgM positive on sperm tail

Table 3. Correlation coefficient between selected variables

	Sperm Count	Percentage Motility	Sperm Kinetics	Fertilizing Capacity	HG+	HA+
Sperm Count	1.0000					
Percentage Motility	0.4248**	1.0000				
Sperm Kinetics	0.2928**	0.4948**	1.0000			
Fertilizing Capacity	0.3234**	0.3445**	0.3089**	1.0000		
HG [#]	0.2570**	0.1524	0.0582	0.3103**	1.0000	
HA [#]	0.2694**	0.1986*	0.1148	0.3100**	0.4153**	1.0000
HM [#]	0.1211	0.2401*	0.1805	0.1053	0.2577**	0.3003**
TG [#]	0.1986*	0.2958**	0.1555	0.2979**	0.1372	0.1235
TA [#]	0.1152	0.2760**	0.1822*	0.3162**	0.0455	0.0724
TM [#]	0.0265	0.2086**	0.1483	0.1159	0.0396	0.0925

* Significantly different from ASA negative group, P<0.05, Student's t-test

** Significantly different from ASA negative group, P<0.01, Student's t-test

HG+: ASA IgG positive on sperm head; HA+: ASA IgA positive on sperm head; HM+: ASA IgM positive on sperm head;

TG+: ASA IgG positive on sperm tail; TA+: ASA IgA positive on sperm tail; TM+: ASA IgM positive on sperm tail

Table 4. Slective variables affecting fertilizing capacity (multiple regression analysis: stepwise)*

Variables		B	P value
Percentage Motility	0.134253	0.020846	0.0990
HG+**	-0.201613	0.856855	0.0076
TA+**	-0.227031	0.954751	0.0015
Sperm Kinetics	0.170581	0.595816	0.0300
HA+**	0.163615	1.105802	0.0309

* $R^2=0.028402$, P,0.0001

** HG+: ASA IgG positive on sperm head; HA+: ASA IgA positive on sperm head; TG+: ASA IgG positive on sperm tail