Journal of Andrology, Vol. 15, No. 3, May/June 1994

Increased Levels of Interleukin-6 in Seminal Plasma of Infertile Men

RAJESH K. NAZ* AND PAUL KAPLAN†

*From the *Reproductive Immunology and Molecular Biology Laboratory, Department of Obstetrics and Gynecology, The Albert Einstein College of Medicine, Bronx, New York and the †Department of Obstetrics, Gynecology and Reproductive Science, Mount Sinai School of Medicine, New York, New York.*

ABSTRACT: The presence of various cytokines, namely the tumor necrosis factor (TNF-α), interferon (IFN-γ), and interleukins (IL-1β and IL-6), was investigated in seminal plasma of fertile, infertile, and immunoinfertile men using specific immunoradiometric assays. TNF-α and IL-1β were not detected. IFN-γ was detected, but the differences between the levels of fertile and infertile/immunoinfertile were not significant ($P > 0.05$). IL-6 was detected in seminal plasma with significantly higher levels in infertile/immunoinfertile men compared to those of fertile men. IL-6 was also present in sera, and interestingly, the levels in sera were lower than those in seminal plasma. IL-6 levels in seminal plasma correlated significantly with some sperm parameters and penetration rates in the human sperm penetration assay (SPA). These findings suggest that IL-6 is associated with infertility and may be of importance in specific diagnosis and treatment of male infertility.

Key words: Cytokines, IL-6, human sperm, semen, infertility.
J Androl 1994;15:220–227

Antispermatozoal antibodies have been implicated as a cause of infertility in humans (Mathur, 1993; Bronson et al, 1984). However, the role of cell-mediated immunity (CMI) in infertility has remained underexplored. Recent advances in the fields of DNA recombinant and hybridoma technologies have made available the purified soluble mediators (cytokines) of CMI and their highly specific monoclonal antibodies (MAbs) to investigate their effects on reproductive cells *in vitro* and to explore their relevance *in vivo*. These cytokines have been shown to have distinct biological effects on growth and differentiation in a variety of somatic/cancer cell lines and have also recently been tested for their effects on reproductive cells *in vitro*. Many of these cytokines such as interferons (IFN-α and IFN-γ) and tumor necrosis factor (TNF-α) were found to have cytotoxic effects, and others such as transforming growth factors (TGF-α and TGF-β) were without any effect on sperm cells when tested *in vitro* (Anderson and Hill, 1988; Daya and Clark, 1993; Naz, 1993). However, their presence and relevance to infertility *in vivo* have not been investigated.

The present study was conducted to investigate the presence of various cytokines, namely TNF-α, IFN-γ, IL-1β, and IL-6, in the seminal plasma and/or sera of infertile men having infertility attributed to male/idiopathic/immunologic factors. If present, their concentrations were correlated with various seminal parameters to determine whether they had any relevance to infertility.

Supported by NIH grant HD 24425 (R.K.N.).

Correspondence to: Dr. Rajesh K. Naz, Reproductive Immunology and Molecular Biology Laboratory, Ullmann Research Building, Room 123, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461.

Received for publication June 23, 1993; accepted for publication September 22, 1993.

Materials and Methods

Patient Population

The seminal plasma and/or sera were collected from healthy fertile and infertile individuals for measurement of various cytokines, namely TNF-α, IFN-γ, IL-1β, and IL-6.

Seminal Plasma

Seminal plasmas were collected from the liquefied (37°C, 30 minutes) semen of fertile men ($n = 10$, patient nos. 1–10) and infertile men ($n = 15$, patient nos. 11–25) (both groups 30–37 years old) (Table 1) by centrifugation, 1 mM protease inhibitor phenylmethylsulfonyl fluoride (PMSF) was added, and the specimens were aliquoted and stored immediately at −20°C until used. The addition of 1 mM PMSF significantly inhibited the proteolytic activity of seminal plasma as studied by the BioRad substrate gel technique (cat. no. 500-0011; BioRad, Richmond, California); there was a complete block of proteolytic activity in majority of the samples tested. The infertile men had infertility attributed to male factor or idiopathic etiology and had abnormal semen analysis and/or defective sperm function as demonstrated by reduced (0–4%) sperm penetration rates in the assay of human sperm penetration of zona-free hamster oocytes (SPA) (Table 1). The infertile men did not reveal the presence of antisperm an-

Table 1. *Levels of various cytokines in seminal plasma of fertile and infertile men*

Patient no.	Semen analysis						SPA	TNF-α		IFN-γ		IL-1β		IL-6	
	Total sperm ($\times 10^6$)	Motility (%)	Velocity	Lin	ALH	Best frequency	Ova penetrated (%)	pg/mg	pg/ml	IU/mg	IU/ml	pg/mg	pg/ml	pg/mg	pg/ml
Fertile men															
1.	167	47	36.4	4.4	1.8	13.1	70	0	0	0.02	0.2	0	0	2.2	23
2	94	50	32.2	4.5	1.5	11.6	58	0	0	0.02	0.2	0	0	2.1	19
3	155	61	37.4	4.1	2.2	13.0	100	0	0	0.04	0.4	0	0	4.3	34
4	132	64	40.5	4.5	2.3	12.0	61	0	0	0.02	0.2	0	0	2.6	27
5	166	80	33.2	1.8	1.2	8.4	57	0	0	0.03	0.3	0	0	4.1	41
6.	126	61	38.5	4.6	2.1	14.0	27	0	0	0.02	0.2	0	0	1.5	15
7.	310	78	36.5	4.2	2.2	14.1	68	0	0	0.02	0.2	0	0	2.3	21
8.	289	82	33.5	4.3	1.9	13.8	92	0	0	0.02	0.2	0	0	1.7	16
9.	234	59	32.8	4.3	1.9	13.0	82	0	0	0.02	0.3	0	0	1.7	16
10.	189	72	37.2	4.8	2.2	13.2	78	0	0	0.03	0.3	0	0	2.2	24
Infertile men															
11.	133	56	35.7	3.3	2.1	12.0	0	0	0	0.03	0.3	0	0	1.8	19
12.	20	20	47.6	3.8	2.8	11.6	0	0	0	0.03	0.3	0	0	5.6	60
13.	55	22	35.7	2.7	1.8	11.1	0	0	0	0.04	0.4	0	0	1.4	15
14.	76	27	31.8	4.2	1.5	13.0	4	0	0	0.04	0.4	0	0	4.1	45
15.	78	8	37.0	2.9	1.1	9.2	0	0	0	0.13	0.5	0	0	3.9	31
16.	55	22	41.0	4.1	2.2	13.0	0	0	0	0.02	0.2	0	0	4.6	57
17.	85	15	33.5	4.8	1.7	14.6	3	0	0	0.02	0.2	0	0	3.7	41
18.	113	9	36.0	2.3	2.1	5.7	0	0	0	0.03	0.3	0	0	2.8	35
19.	2	22	49.0	1.0	2.0	10.1	0	0	0	0.07	0.5	0	0	6.8	52
20.	138	16	39.7	4.2	2.3	12.0	0	0	0	0.02	0.2	0	0	3.4	62
21.	54	14	31.2	2.9	2.2	11.4	0	0	0	0.02	0.2	0	0	4.1	48
22.	48	10	35.6	3.7	1.7	12.1	0	0	0	0.03	0.3	0	0	5.1	59
23.	58	21	33.0	2.3	1.1	9.2	0	0	0	0.03	0.3	0	0	4.5	55
24.	20	20	37.2	2.1	2.7	14.6	2	0	0	0.04	0.4	0	0	5.2	58
25.	8	15	39.7	1.0	1.1	5.6	0	0	0	0.04	0.4	0	0	6.6	53

tibodies in their sera by the immunobead technique (IBT) (Bronson et al, 1985).

Seminal plasmas were also collected from another group of fertile men (n = 10, patient nos. 1–10) and infertile men (n = 10, patient nos. 11–20) (both groups 29–36 years old) (Table 2) and stored at −20°C as described above. The infertile men had infertility attributed to immunologic reasons as evidenced by the presence of antisperm antibodies in their sera by the IBT and will be referred to as "immunoinfertile."

Sperm Motion Analysis—Semen from these fertile and infertile individuals was analyzed for total sperm count, percent motility, and various sperm motility characteristics, namely velocity, linearity, amplitude of lateral head displacement (ALH) and beat frequency (Tables 1 and 2) using computer-assisted sperm analysis (CASA). For CASA, an aliquot (7 μl) of sperm suspension was placed into a Makler chamber (Sefi-Medical Instruments, Haifa, Israel), and the sperm motion characteristics were determined using a computerized semen analyzer (Cell Soft Cryo Resources Ltd., New York, New York), as described elsewhere (Naz et al, 1992a,b). The following parameter settings were used throughout the study: 30 frames analyzed at an image frequency of 30 Hz, 3 frames minimum sampling for motility, 15 frames minimum sampling for both velocity and ALH measurements, 10 μm/second threshold velocity, minimum linearity of 2.5 for ALH measurement, and cell size range of 4 to 40 pixels with a magnification calibration of 0.688 μm/pixel.

Sperm Penetration Assay (SPA)—The fertilizing capacity of sperm present in semen of fertile and infertile men was investigated using the method of Yanagimachi et al (1976) with slight modification as described in detail elsewhere (Naz et al, 1992a,b). Briefly, the swim-up sperm (5–10 × 10^6 motile sperm/ml) collected in Biggers, Whitten, and Whittingham (BWW) medium were incubated for 7–8 hours at 37°C (in 5% CO_2 and 95% air mixture) and then co-incubated with zona-denuded hamster oocytes (40–50 eggs/treatment in each assay) for 3–4 hours. The oocytes were removed, washed thoroughly, fixed with 3% glutaraldehyde, and stained with acetocarmine solution. Penetration was determined by the presence of a swollen sperm head with discernible tail in the cytoplasm of the ovum. Motility of sperm before and after incubation with ova was recorded.

Sera

Sera were also collected from different groups of fertile (n = 10, patient nos. 1–10) and immunoinfertile men (n = 10, patient nos. 11–20) (both groups 28–35 years old) for measurement of cytokines and stored at −20°C until used. The fertile men did not reveal any antisperm antibodies, but the immunoinfertile men demonstrated various titers of antisperm antibodies in their sera when analyzed by the tray agglutination technique (TAT) (Friberg, 1974) and sperm immobilization technique (SIT) (Isojima et al, 1968) (Table 3). These infertile men had infertility attributed to immunologic factors.

Immunoradiometric Assay

Various cytokines were measured using specific immunoradiometric assay kits (TNF-α, code no. 30-175-20; IFN-γ, code no. 30-123-20; IL-β, code no. 30-121-20; and IL-6, code no. 30-125-00) obtained from Medgenix Diagnostics (Fleurus, Belgium) through the USA distributor, the Ventrex Laboratories (Portland, Maine), as described elsewhere (Naz and Kaplan, 1993). This assay is based on coated-tube separation and on the oligoclonal system using several MAbs directed against distinct epitopes of various cytokines. The capture MAbs are attached to the lower and inner surface on the plastic tube. Standards or samples added to the tubes bind to these capture antibodies and the immunological binding is enhanced by adding the second signal MAb-labeled with ^{125}I. After washing, the remaining radioactivity bound to the tube reflects the antigen concentrations, which are calculated using the standard curve. Each serum was run in duplicate and the mean of two readings was recorded.

The levels of various cytokines were expressed as either pg or IU/ml of the seminal plasma/serum or as the specific activity expressed as pg or IU/mg protein in the seminal plasma/serum. The protein in the seminal plasma and serum was measured by the method described by Bradford (1976).

Statistical Analysis

Paired as well as unpaired Student's t-test was used for evaluation of differences between levels of various cytokines in seminal plasma or sera of fertile and infertile/immunoinfertile groups. The correlation coefficient (r) between various seminal parameters/SPA was determined by analyzing for linear regression. A correlation coefficient with $P > 0.05$ was considered nonsignificant. The inter-assay and intra-assay variabilities in various immunoradiometric assays were calculated by finding out the population coefficient of variation, which was defined as the population standard deviation divided by the population mean × 100.

Results

Tumor Necrosis Factor-α (TNF-α)

TNF-α was not detected in seminal plasma of fertile, infertile, or immunoinfertile men (Tables 1 and 2). Due to insufficient samples, the assay for TNF-α could not be performed in sera of immunoinfertile men (Table 3).

Interferon-γ (IFN-γ)

IFN-γ was detected in seminal plasma of fertile and infertile men (mean ± SD, fertile men: 0.025 ± 0.009 IU/mg, 0.250 ± 0.071 IU/ml; infertile men: 0.039 ± 0.028 IU/mg, 0.327 ± 0.103 IU/ml). The differences between the levels of fertile and infertile men were nonsignificant ($P > 0.05$) when expressed as IU/mg and approached significance ($P = 0.05$) when expressed as IU/mg (Table 1). The seminal plasma from all the fertile men had IFN-γ levels <0.05 IU/mg (<0.5 IU/ml), but the seminal plasma of two infertile men (nos. 15 and 19) showed levels >0.05 IU/mg (= 0.5 IU/ml).

Again, the differences between the levels of IFN-γ in seminal plasma of fertile and immunoinfertile men were statistically nonsignificant ($P > 0.05$), though the seminal plasma of immunoinfertile men had a tendency towards

Table 2. *Levels of various cytokines in seminal plasma of fertile and immunoinfertile men*

Patient no.	Semen analysis: Total sperm (×10⁶)	Motility (%)	Velocity	Lin	ALH	Beat frequency	Immunobead technique* IgG	IgA	IgM	TNF-α pg/mg	TNF-α pg/ml	IFN-γ IU/mg	IFN-γ IU/ml	IL-1β pg/mg	IL-1β pg/ml	IL-6 pg/mg	IL-6 pg/ml
Fertile men																	
1.	125	52	37	4.4	2.9	13.1	0	0	0	0	0	0.02	0.2	0	0	3.6	36
2.	312	40	33	4.7	1.7	11.8	0	0	0	0	0	0.04	0.3	0	0	5.9	47
3.	295	54	36	4.9	2.0	12.1	0	0	0	0	0	0.04	0.4	0	0	1.6	18
4.	390	55	49	4.6	2.9	12.8	0	0	0	0	0	0.04	0.4	0	0	2.7	34
5.	307	73	35	5.3	1.7	13.6	0	0	0	0	0	0.02	0.3	0	0	2.2	34
6.	252	62	39	5.1	1.6	13.3	0	0	0	0	0	0.02	0.3	0	0	2.6	32
7.	368	58	33	5.1	1.9	11.8	0	0	0	0	0	0.04	0.3	0	0	1.1	17
8.	208	72	37	4.8	2.7	12.1	0	0	0	0	0	0.03	0.2	0	0	1.9	21
9.	307	69	48	4.5	2.6	12.8	0	0	0	0	0	0.02	0.3	0	0	1.8	22
10.	196	76	45	4.4	2.9	12.8	0	0	0	0	0	0.04	0.4	0	0	1.1	16
Immunoinfertile men																	
11.	150	65	41	5.0	2.0	12.1	65 (TT)	0	0	0	0	0.03	0.2	0	0	7.1	51
12.	95	39	46	4.0	2.1	12.9	0	71 (H, TT)	0	0	0	0.02	0.2	0	0	2.9	28
13.	225	42	37	4.7	2.9	13.0	96 (H, TT)	0	0	0	0	0.05	0.5	0	0	3.2	35
14.	160	64	32	4.4	2.7	11.9	0	60 (H, TT)	0	0	0	0.07	0.5	0	0	4.6	33
15.	170	57	42	4.6	2.6	12.0	52 (H, TT)	0	0	0	0	0.04	0.4	0	0	2.4	25
16.	68	38	37	4.4	2.8	11.9	75 (TT)	0	0	0	0	0.05	0.6	0	0	4.6	55
17.	112	42	45	4.0	2.1	12.1	12 (H)	86 (H, T)	0	0	0	0.02	0.2	0	0	4.8	48
18.	132	58	42	4.0	2.1	12.1	50 (H)	40 (H)	0	0	0	0.05	0.7	0	0	2.7	36
19.	182	39	41	5.0	2.0	12.9	0	78 (H)	0	0	0	0.05	0.6	0	0	4.0	48
20.	216	62	32	4.6	2.6	11.9	10 (H)	40 (H, T)	0	0	0	0.03	0.4	0	0	3.6	46

* Expressed as percent sperm bound with the class of antibody; H means head bound; T means tail bound; TT means tail tip bound.

Table 3. *Levels of various cytokines in sera of fertile and immunoinfertile men**

Patient no.	Antisperm antibody (titer)		IFN-γ		IL-1β		IL-6	
	TAT†	SIT†	IU/mg	IU/ml	pg/mg	pg/ml	pg/mg	pg/ml
Fertile men								
1.	0	0	0.12	2.2	2.3	42	0	0
2.	0	0	0.08	1.8	1.5	32	0.20	5
3.	0	0	0.06	1.2	0.80	17	0.40	8
4.	0	0	0.07	1.3	0.63	12	0.30	6
5.	0	0	0.08	1.5	0.60	11	0.90	18
6.	0	0	0.11	2.0	0.60	11	0	0
7.	0	0	0.10	1.8	1.7	24	0.14	3
8.	0	0	0.08	1.6	0.50	10	0.60	13
9.	0	0	0.07	1.3	0.90	17	0.50	10
10.	0	0	0.09	1.7	1.0	19	0.30	6
Immunoinfertile men								
11.	512	0	0.07	1.5	5.2	111	4.9	105
12.	512	0	0.04	0.90	0.60	13	0	0
13.	2,048	32	0.04	0.70	0	0	0.63	12
14.	2,048	128	0.05	1.2	1.2	26	1.1	23
15.	2,048	128	0.05	1.0	0	0	0.63	12
16.	256	128	0.05	0.8	0	0	0.60	10
17.	132	132	0.06	1.4	1.8	41	1.3	31
18.	572	32	0.06	1.4	2.4	56	0.60	13
19.	256	0	0.25	3.7	6.1	90	0	0
20.	2,048	0	0.20	4.2	2.2	46	3.9	82

* Due to insufficient samples, assay for TNF-α was not performed.
† TAT means tray agglutination technique; SIT means sperm immobilization technique.

higher levels (mean ± SD, fertile men: 0.031 ± 0.009 IU/mg, 0.31 ± 0.074 IU/ml; immunoinfertile men: 0.041 ± 0.016 IU/mg, 0.43 ± 0.183 IU/ml) (Table 2). The seminal plasma from all the fertile men showed levels <0.05 IU/mg (<0.5 IU/ml), but the seminal plasma from one immunoinfertile man (no. 14) showed an IFN-γ level >0.05 IU/mg, and three (nos. 16, 18, and 19) showed an IFN-γ level >0.5 IU/ml (Table 2).

IFN-γ was also detected in sera of fertile and immunoinfertile men, though the levels in sera were higher than those in the seminal plasma (Table 3). However, the differences between the levels of IFN-γ in sera of fertile and immunoinfertile men were statistically nonsignificant ($P > 0.05$) (mean ± SD, fertile men: 0.086 ± 0.019 IU/mg, 1.64 ± 0.32 IU/ml; immunoinfertile men: 0.088 ± 0.07 IU/mg 1.68 ± 1.23 IU/ml). The levels of IFN-γ in sera of all the fertile men were ≤0.12 IU/mg (≤2.2 IU/ml), and sera from two infertile men (nos. 19 and 20) showed >0.12 IU/mg (>2.2 IU/ml) levels of IFN-γ.

In Table 1, the levels of IFN-γ did not show any significant correlation ($r = -0.232$ to -0.410, $P > 0.05$) with any of the seminal parameters or SPA when expressed as IU/mg. However, when expressed as IU/ml, the levels of IFN-γ showed a significant correlation with total sperm number ($r = -0.436$, $P = 0.025$), linearity ($r = -0.626$, $P < 0.001$) and beat frequency ($r = -0.384$, $P = 0.05$). In Table 2, there was no significant correlation with any of the seminal parameters/SPA, except with beat frequency ($r = -0.433$, $P = 0.04$).

Interleukin-1β (IL-1β)

IL-1β was not detected in the seminal plasma of fertile, infertile, or immunoinfertile men (Tables 1 and 2). IL-1β was detected in the sera of fertile and immunoinfertile men, and the levels were slightly higher in the sera of immunoinfertile men; however, the differences were statistically nonsignificant ($P > 0.05$) (mean ± SD, fertile: 1.05 ± 0.59 pg/mg, 20.10 ± 10.91 pg/ml; immunoinfertile: 1.95 ± 2.15 pg/mg, 38.30 ± 38.77 pg/ml) (Table 3). The levels of IL-1β in the sera of fertile men were ≤2.3 pg/mg (≤42 pg/ml), and the three immunoinfertile men (nos. 11, 18, and 19) had >2.3 pg/mg; four (nos. 11, 18, 19, and 20) had >42 pg/ml levels of IL-6 (Table 3).

Interleukin-6 (IL-6)

IL-6 was detected in the seminal plasma of fertile as well as infertile men, with the statistically significant higher levels in infertile men (mean ± SD, fertile men: 2.47 ± 0.97 pg/mg, 23.60 ± 8.43 pg/ml; infertile: 4.24 ± 1.54 pg/mg, 46.00 ± 14.92 pg/ml; $P = 0.001$ to 0.003) (Table 1). The levels of IL-6 in seminal plasma of fertile men were ≤4.3 pg/mg (≤34 pg/ml), and in contrast, 7 infertile men (nos. 12, 16, 19, and 22–25) had >4.3 pg/mg and

12 infertile men (nos. 12, 14, and 16–25) had >34 pg/ml levels of IL-6 (Table 1).

Again IL-6 levels were significantly higher in seminal plasma of immunoinfertile men compared to those of fertile men (mean ± SD, fertile: 2.45 ± 1.43 pg/mg, 27.70 ± 10.34 pg/ml; immunoinfertile: 3.99 ± 1.38 pg/mg, 40.50 ± 10.36 pg/ml; $P = 0.01$ to 0.02) (Table 2). The levels of IL-6 in seminal plasma of fertile men were ≤5.9 pg/mg (≤47 pg/ml), and one immunoinfertile man (no. 11) had >5.9 pg/mg and four (nos. 11, 16, 17, and 19) had >47 pg/ml levels of IL-6 (Table 2).

IL-6 was also detected in sera of fertile and immunoinfertile men (Table 3). Interestingly, the levels of IL-6 in sera were lower than those in seminal plasma. Though the levels of IL-6 were higher in sera of immunoinfertile men than those in sera of fertile men, the differences were statistically nonsignificant ($P = 0.06$) (mean ± SD, fertile men: 0.33 ± 0.28 pg/mg, 6.90 ± 5.65 pg/ml; infertile men: 1.37 ± 1.67 pg/mg, 28.80 ± 35.75 pg/ml). The levels of IL-6 in sera of fertile men were ≤0.90 pg/mg (≤18 pg/ml), and four immunoinfertile men (nos. 11, 14, 17, and 20) had >0.90 pg/ml (>18 pg/ml) levels; the levels in two of these immunoinfertile men (nos. 11 and 20) were 4.3- to 5.8-fold higher than those of fertile men.

In Table 1, the levels of IL-6 correlated significantly ($P = 0.03$ to <0.001) with sperm number ($r = -0.663$), motility ($r = -0.663$), velocity ($r = -0.354$), linearity ($r = -0.407$), beat frequency ($r = -0.23$), and penetration rates ($r = -0.610$). The levels did not correlate significantly ($P > 0.05$) with ALH ($r = -0.357$). In Table 2, IL-6 levels correlated ($r = -0.102$ to -0.51) significantly ($P = 0.01$ to 0.06) with total sperm number and motility, but not with any other seminal parameter.

For various immunoradiometric assays of TNF-α, IFN-γ, IL-1β, and IL-6, the coefficients of variation were 3.4–5.2% for the intra-assay variabilities and 4.2–6.1% for the inter-assay variabilities.

Discussion

TNF-α was not detected in seminal plasma of fertile, infertile, or immunoinfertile men, indicating that TNF-α is absent in seminal plasma having normal or abnormal seminal parameters. Alternatively, it is also possible that the specific/nonspecific proteolytic activity present in seminal plasma may be inactivating/degrading the already low levels of TNF-α, thus making it nondetectable by our assay. It is equally possible that TNF-α is absent in seminal plasma of fertile men and is present in seminal plasma of infertile/immunoinfertile men, where it is bound to the sperm cells. In the present study, we did not investigate the presence of TNF-α in sperm of fertile, infertile, or immunoinfertile men. T-lymphocytes produce TNF-β, whereas the major source of TNF-α is the macrophage; TNF-α is considered an important mediator of inflammation (Anderson and Hill, 1988; Daya and Clark, 1993; Naz, 1993). TNF-α has been shown to cause a dose-dependent reduction of sperm motility, and it reduces the fertilizing capacity of human sperm in SPA (Anderson and Hill, 1988).

Similarly, IL-1β was not detected in the seminal plasma of fertile, infertile, or immunoinfertile men. It was detected in the sera of fertile and immunoinfertile men, with immunoinfertile men having higher but nonsignificant levels; three immunoinfertile men showed 1.1- to 2.5-fold higher values than those observed in sera of fertile groups. The nondetection of IL-1β in seminal plasma may indicate that it is absent in seminal plasma having normal or abnormal seminal parameters, or it may be due to similar reasons as those described above for TNF-α. IL-1 is a factor that could potentiate the response of T cells to antigens or mitogens; following immune recognition an antigen is processed by an antigen-presenting cell (mainly of the monocyte/macrophage lineage), which secretes IL-1 (Dinarello, 1988). IL-1-like factor has been detected in the testis and may regulate growth or immune suppression in the seminiferous tubule (Kahn et al, 1987). However, IL-1 has no adverse effect on sperm motility and fertilization except in very high concentrations (Anderson and Hill, 1988; Daya and Clark, 1993; Naz, 1993).

IFN-γ was detected in seminal plasma as well as sera of fertile, infertile, and immunoinfertile men, and the serum levels were higher than the seminal plasma levels. The infertile and immunoinfertile men showed a tendency towards higher levels (both in sera and seminal plasma) than the fertile men, though the differences were statistically nonsignificant. IFN-γ levels correlated significantly with sperm number in the semen and some of the sperm motility characteristics, namely the linearity and beat frequency. IFN-γ is a product of T cells and natural killer (NK) cells, and it has been extensively demonstrated to have distinct biological effects on cell growth and differentiation in a variety of cell systems through enhancing the expression of IFN-γ-inducible regulatory genes, especially the 2'-5' (A) oligoadenylate (2'-5'[A]) synthetase system (Pestka et al, 1987). IFN-γ has been shown to have adverse effects on human sperm motility by a mechanism not involving the 2'-5' (A) synthetase system (Naz and Kumar, 1991). In another study, IFN-γ was identified in sera of 38% of women with antisperm antibodies, but only in 7% of women who lacked antibodies, and in 70% of these women with antibody and 0% of fertile controls, *in vitro* incubation of lymphocytes with sperm led to production of IFN-γ *in vitro* (Witkin and Chaudhry, 1989). Our present data are in agreement with these findings, indicating a tendency towards higher levels of IFN-γ in seminal plasma and sera of infertile and immunoinfertile

men compared to those of fertile men. In another study in mice, we found that when *in vivo* sensitized lymphocytes were incubated *in vitro* with specific sperm antigens, they were activated and secreted IFN-γ as the major cytokine in the conditioned medium (Naz and Mehta, 1989). All these cumulative findings indicate that IFN-γ may be one of the major cytokines involved in infertility.

The most significant finding of the present investigation was the levels of IL-6. Though IL-6 was detected in seminal plasma of fertile, infertile, and immunoinfertile men, its levels were significantly lower in the seminal plasma of fertile men as compared to those of infertile and immunoinfertile men. Similar trends were also noted in sera, with some immunoinfertile men having 4.3- to 5.8-fold higher values than those observed in sera of fertile men. Interestingly, the levels in seminal plasma were higher than those in the sera, indicating a local production of IL-6. The higher levels of IL-6 in the seminal plasma of infertile and immunoinfertile men seem to have clinical significance because they correlated significantly with total sperm number, penetration rates, and some sperm motion parameters. A recent report demonstrated significantly higher levels of another interleukin, IL-8, in the seminal plasma of infertile men having leukospermia compared to those in the seminal plasma of fertile men (Shimoya et al, 1993). IL-8 is a chemotactic factor for neutrophils; thus the higher IL-8 levels in the seminal plasma of infertile men than these of fertile men might explain the reason for the increased cell number of granulocytes in infertile semen.

IL-6 is produced by a variety of cell types, including macrophages, endothelial cells, fibroblasts, and trophoblasts and has been demonstrated to influence the growth and differentiation of B cells (Kishimoto et al, 1992). Because the male genital tract is an immunologically dynamic system and a number of studies have indicated an increased number of leukocytes in the ejaculate of infertile men as compared to fertile men (Menge and Edwards, 1993; Naz, 1993), the higher levels of IL-6 in seminal plasma of infertile and immunoinfertile men may be due to an increased number of leukocytes (secreting IL-6) in the semen of these patients. A recent study carried out in rats demonstrated that Sertoli cells, but not the spermatocytes, spermatids, and peritubular cells, secrete bioactive IL-6 when cultured *in vitro* (Syed et al, 1993). Interestingly, it was further found that FSH augments Sertoli cell IL-6 secretion in a dose-dependent manner, indicating that IL-6 secretion may be regulated by a complex interplay of various hormonal factors. Thus hormonal (especially FSH) imbalance may also be contributing to defective levels of IL-6 in some of these infertile men, because increased IL-6 levels correlated inversely with the sperm number in the ejaculate, and FSH has been shown to have a predominant role in regulating spermatogenesis. In our present study, IL-6 was assayed by the immunoradiometric method that measures immunoreactive IL-6. The bioactivity of the immunoreactive IL-6 needs to be evaluated. The inverse correlation between the sperm number/sperm motion parameters and the IL-6 levels is very intriguing. It remains to be determined whether it is due to the direct effect of IL-6 per se on sperm function or is due to the immune/hormonal phenomenon that also causes an enhanced IL-6 production. Presently we are investigating the effect of recombinant IL-6 on the fertilizing capacity of human sperm to test these hypotheses (Naz and Kaplan, 1994).

In conclusion, our data indicate for the first time that the seminal plasma of infertile and immunoinfertile men have significantly higher levels of IL-6 that are correlated significantly with sperm number in the ejaculate, penetration rates in SPA, and some sperm motion parameters. This novel finding may have implications in the specific diagnosis and treatment of male infertility, especially due to male factor/idiopathic/immunologic reasons. In the present study, we used a selective population of infertile men that had severe impairment of the sperm function in SPA. A multicenter trial including a larger random population of fertile and infertile men should be conducted to confirm the present findings.

Acknowledgments

We thank Ms. Daniela Pulitano for her excellent typing assistance and Carles Morte for his help in the statistical analysis. Some of these seminal plasma and sera were kindly provided by Dr. Alan Menge, University of Michigan Medical School, Ann Arbor, Michigan.

References

Anderson DJ, Hill JA. Cell-mediated immunity in infertility. *Am J Reprod Immunol* 1988;17:22–30.

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal Biochem* 1976;72:248–254.

Bronson RA, Cooper G, Hjort T, Ing R, Jones WR, Wang SX, Mathur S, Williamson HO, Rust PF, Mettler L. Antisperm antibodies detected by agglutination, immobilization, cytoxocity and immunobead binding assays. *J Reprod Immunol* 1985;8:279–287.

Bronson RA, Cooper GW, Rosenfeld DL. Sperm antibodies: their role in infertility. *Fertil Steril* 1984;42:171–182.

Daya S, Clark DA. Cytokines and reproduction. In: Naz RK, ed. *Immunology of Reproduction.* Boca Raton, Florida: CRC Press; 1993: 209–232.

Dinarello CA. Interleukin 1. *Ann NY Acad Sci* 1988;546:122–126.

Friberg J. A simple and sensitive micromethod of demonstration of sperm-agglutinating activity in serum from infertile men and women. *Acta Obstet Gynecol Scand* 1974;74:21–29.

Isojima S, Li TS, Ashitaka Y. Immunological analysis of sperm-immobilizing factor found in sera of women with unexplained infertility. *Am J Obstet Gynecol* 1968;101:677–683.

Kahn SA, Soder O, Seyd V, Gustafsson K, Lindh M, Ritzen EM. The rat testis produces large amounts of an interleukin-1 like factor. *Int J Androl* 1987;10:495–501.

Kishimoto T, Akira S, Taga T. Interleukin-6 and its receptor: a paradigm for cytokines. *Science* 1992;258:593–597.

Mathur S. Clinical significance of sperm antibodies. In: Naz RK, ed. *Immunology of Reproduction.* Boca Raton, Florida: CRC Press; 1993: 3–18.

Menge AC, Edwards RP. Mucosal immunity of the reproductive tract and infertility. In: Naz RK, ed. *Immunology of Reproduction.* Boca Raton, Florida: CRC Press; 1993:19–36.

Naz RK. Role of cell-mediated immunity in infertility. In: Naz RK, ed. *Immunology of Reproduction.* Boca Raton, Florida: CRC Press, 1993: 61–80.

Naz RK, Ahmad K, Kaplan P. Expression and function of ras proto-oncogene proteins in human sperm cells. *J Cell Sci* 1992a;102:487–494.

Naz RK, Kaplan P. Effects of epidermal growth factor on human sperm cell function. *J Androl* 1993;14:240–247.

Naz RK, Kaplan P. Interleukin-6 enhances the fertilizing capacity of human sperm by increasing capacitation and acrosome reaction. *J Androl* 1994;15(3):228–233.

Naz RK, Kaplan P, Goldstein AL. Thymosin alpha-1 enhances the fertilizing capacity of human sperm cell: implication in diagnosis and treatment of male infertility. *Biol Reprod* 1992b;47:1064–1072.

Naz RK, Kumar R. Transforming growth factor β_1 enhances expression of 50 kDa protein related to 2^1-5^1 oligoadenylate synthetase in human sperm cells. *J Cell Physiol* 1991;146:156–163.

Naz RK, Mehta K. Cell-mediated immune responses to sperm antigens: effects on mouse sperm and embryos. 1989;41:533–542.

Pestka S, Langer JA, Zoon KC, Samuel CE. Interferons and their actions. *Annu Rev Biochem* 1987;56:727–777.

Shimoya K, Matsuzaki N, Tsutsui T, Taniguchi T, Saji F, Tanizawa O. Detection of interleukin-8 (IL-8) in seminal plasma and elevated IL-8 in seminal plasma of infertile patients with leukospermia. *Fertil Steril* 1993;59:885–888.

Syed V, Gerard N, Kaipia A, Bardin CW, Parvinen M, Jegou B. Identification, ontogeny, and regulation of an interleukin-6-like factor in the rat seminiferous tubule. *Endocrinology* 1993;132:293–299.

Witkin SS, Chaudhry A. Circulating γ-interferon in women sensitized to sperm: a new mechanism of infertility. *Fertil Steril* 1989;52:867–869.

Yanagimachi R, Yanagimachi H, Rogers BJ. The use of zona-free animal ova as a test system for the assessment of the fertilizing capacity of human spermatozoa. *Biol Reprod* 1976;45:471–476.
