Cytokine levels in seminal plasma

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Summary

We have studied IL-2, SIL-2R, and sCD4 concentrations in the seminal plasma of 20 healthy men (controls), 20 oligozoospermics and 20 azoospermics by the "sandwich" enzyme immunoassay technique. We have also measured IL-2, SIL-2R and sCD4 in the two fractions of the split ejaculate of 10 healthy men.

Our results show that the mean IL-2 and sCD4 concentrations in the seminal plasma were significantly higher compared to the levels in the serum of normal men; furthermore, there were no significant differences of SIL-2R and sCD4 between the group of normal men and the groups of men with oligozoospermia or azoospermia. On the contrary, significantly higher levels of IL-2 and sCD4 were found in the first compared to the second fraction of the split ejaculates.

Our results support the view that the estimation of IL-2 in seminal plasma has value concerning the quality of semen. On the contrary, the estimation of SIL-2R and sCD4 in seminal plasma are valueless concerning normal or abnormal semen.

Finally, the prostate seems to be the main site or origin of IL-2 and sCD4.

Key words: IL-2, SIL-2R, sCD4; Seminal plasma.

Introduction

The term cytokine was originally coined by Cohen [1] to describe soluble mediators produced by lymphoid and non-lymphoid cells that induce specific effects in target cells [1, 2]. More recently this definition has been expanded so that a cytokine is a soluble (glyco) protein released by cells which act non-enzymatically to regulate cellular function [3]. Whereas most cytokines function locally in a paracrine or autocrine nanner, some exert their effects distally in an endocrine manner. Although originally defined by their effect on, or derivation from one particular group of cells, it is now evident that many cytokines are derived from several cell types and can have actions on several tissues and organs as well [2, 4].

In endometriosis, commonly associated with infertility, several products of immunocompetent cells, including interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-a) [4-7] have been found in the peritoneal fluid. In male patients, chronic urogenital infections of humoral immune responses have been suggested to play a critical role in reduced sperm quality and fertility [8-10].

Recent studies report the presence of IL-2, SIL-2R, IL-6, IL-8 and IL-11 in human seminal plasma in concentrations much higher than those encountered in the serum of normal persons. The site of origin of the above cytokines in the seminal plasma has not yet been established, and very little is known at present regarding the diagnostic value of their measurement, in normal or abnormal semen [8-15].

The aim of our study was to further assess IL-2, SIL-2R, and sCD4 activities in the seminal plasma of men with normal spermiograms and men with oligo-asthenozoospermia and azoospermia, and to measure their levels in the two fractions of split ejaculates in order to study their origin in the genital tract.

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Materials and Methods

The studied population consisted of 60 males, classified into two groups on the basis of a normal or abnormal spermiogram (Table 1).

The semen was collected by masturbation into sterilized glass containers after three to five days of abstinence. After evaluation of the liquefaction and measurement of the volume and viscosity, an aliquot of the semen was used for the spermiogram, and the remaining was centrifuged and the seminal plasma separated and stored at -20°C until the time of assay.

In ten men with normal spermiograms IL-2, SIL-2R, and sCD4 were measured in the seminal plasma of the two fractions of a split ejaculate (Table 2). For comparison reasons IL-2, SIL-2R, and sCD4 were measured in the serum of ten normal men.

Statistical analysis of the results was performed by the paired t-test after long transformation of the values.

IL-2, SIL-2R, and sCD4 levels were determined by the "sandwich" enzyme immunoassay technique. The percentage of motile spermatozoa was analysed by the multiple-exposure photography (MFP) method [16]. Sperm concentration of undiluted semen specimens was measured using Makler's Counting Chamber [17], and sperm morphology was evaluated from Papanicolaou smears [18, 19].

Results

The mean IL-2 concentration in the seminal plasma (Table 3) was about 3.1 times higher compared to the IL-2 levels in the serum of normal men. Furthermore, the mean SIL-2R levels in the seminal plasma (543.9 U/ml) was nearly equal to the SIL-2R levels in the serum of normal men (295.4 U/ml) (Table 3). Finally, the mean sCD4 concentration in the seminal plasma was about 2.8 times hi-

Table 1. — *Parameters of the spermiograms in the groups classified according to normal or abnormal spermiograms.*

Spermiogram	Volume (ml)	Sperm concentration (x10⁶/ml)	Motility 1st hour (%)	Normal Forms (%)
1. Normal (N = 20)	3.5±1.4 (2.1-6.2)	72.3±30.4 (40-120.0)	52.3±8.0 (43-70.1)	46.7±6.8 (41-62)
2. Abnormal (N = 40)				
a. Oligoasthenozoospermia (N = 20)	3.1±1.7 (1.7-5.6)	7.8±4.1 (1.7-15.0)	21.6±10.2 (8.1-35.4)	11.1±8.0 1-24)
b. Azoospermia (N = 20)	2.1±1.2 (0.8-4.5)	0	0	0

Table 2. — *Parameters of the spermiograms of the two fractions of a split ejaculate in ten men with normal spermiograms.*

Fractions	Volume (ml)	Sperm concentration (x10⁶/ml)	Motility 1st hour (5)	Normal Forms (%)
First Fraction	1.1±0.5 (0.6-1.8)	140±60.9 (80.0-240.0)	75.0±18.2 (50.0-91.2)	48.1±12.3 (39-69)
Second Fraction	2.4±1.1 (0.6-3.7)	41.4±36.3 (12.1-99.0)	36.0±14.2 (20.0-55.1)	27.6±13.4 (15-46)

gher compared to the sCD4 level in the serum of normal men (Table 3).

A wide overlapping of IL-2, SIL-2R, and sCD4 levels was found between men with normal spermiograms and oligo-asthenozoospermics or azoospermics. There were no statistically significant differences for these three cytokines between men with normal spermiograms and those with oligo-asthenozoospermia or azoospermia. However, there was a statistically significant difference of IL-2 levels between men with normal spermiograms and those with oligo-asthenozoospermia and azoospermia ($p<0.05$) (Table 5).

Mean values of IL-2, SIL-2R, and sCD4 in the seminal plasma of the two fractions of a split ejaculate (Table 4) in the ten normal controls were significantly higher ($p<0.01$, $p<0.05$ and $p<0.05$, respectively) in the first compared to the second fraction.

Finally, there was no statistically significant correlation between IL-2, SIL-2R, and sCD4 and the number of spermatozoa/ml, the percentage of motile spermatozoa and the percentage of normal sperm forms.

Discussion

During the last decade, consistent data have been produced demonstrating the existence of functional relationships between immunity, hematopoiesis and inflammation. Conseguently, the list of polypeptides involved in the regulation of such systems has grown rapidly. Of these, interleukin-2 (IL-2), IL-6, tumor necrosis factor-alpha (TNF-a) and IL-8 have been shown to exert important additional influences [20].

Table 3. — *IL-2, SIL-2R, and sCD4 levels (mean + SD in the seminal plasma and serum in the various groups.*

	Number of cases	IL-2 (Pg/ml)	SIL-2R (U/ml)	sCD4 (U/ml)
1. *Seminal plasma* all cases	60	342.7±37.7 (28-510.2)	543.9±904.1 (36-5861)	41.6±33.9 (3.4-142.2)
2. *Seminal plasma* (control)	20	222.0±41.1 (28-301.1)	352.2±644.7 (36-2067)	55.7±42.1 (12.4-142.2)
3. *Seminal plasma* (men with oligo-asthenozoospermia)	20	411.1±35.5 (108-510.1)	262.9±258.0 (311-580)	43.2±32.5 (5.4-103.2)
4. *Seminal plasma* (men with azoospermia)	20	395.1±36.7 (102-501.2)	1016.7±1809.6 (39-5861)	26.8±27.2 (3.4-85.1)
5. *Serum* (normal men)	10	112±15.6 (38-950	295.4±75.9 (198-381)	14.7±8.4 (8-20.4)

In particular, some evidence suggests their involvement, together with other cytokines and colony stimulation factors (CSF), in the control of reproduction function [21].

Human semen contains spermatozoa and a population of round cells comprised primarily of immature germ cells and leukocytes. Large numbers of leulkocytes have been detected in some semen samples associated with male genital tract infection and infertility [8].

Table 4. — *IL-2, SIL-2R, and sCD4 levels (mean + SD) in the seminal plasma in the two fractions of split ejaculate of ten normal men.*

Fractions	IL-2 (Pg/ml)	SIL-2R (U/ml)	sCD4 (U/ml)
First Fraction	612.1±75.1 (70.1-801)	17.2±16.5 (5-30)	55.4±41.2 (5.8-106.4)
Second Fraction	44.1±8.7 (17.1-65)	335.7±275.2 (47-797)	8.4±11.9 (3.7-10.1)

Table 5. — *Statistical test differences in seminal plasma levels of IL-2, SIL-2R and sCD4 in the various groups.*

Comparative Groups	IL-2 (Pg/ml)	SIL-2R (U/ml)	sCD4 (U/ml)
Normal versus oligo-asthenozoospermia	$p<0.05$	N.S.	N.S.
Normal versus azoospermia	$p<0.05$	N.S.	N.S.
Oligo-asthenozoospermia versus azoospermia	N.S.	N.S.	N.S.
First fraction versus second fraction	$p<0.01$	–$p<0.05$	$p<0.05$

*N.S. = Not significant

Little is known about IL-2 and SIL-2R levels in seminal plasma [8-10]. Paradisis *et al.* (1995) [22] confirm the precence of IL-2 secretion in an infertile group with respect to a fertile group, show a negative correlation between IL-2 levels and the main spermiogram parameters, and do not show a correlation between IL-2 levels and leukocyte count suggesting an IL-2 role in infertility independent from a leukocytospermia condition.

No data are available concerning IL-2R levels in the seminal plasma of men classified into two groups on the basis of normal or abnormal spermiograms (Tables 1, 5). Our results show that there was a wide overlapping of the values of this interleukin between men with normal and those with abnormal spermiograms.

Statistical analysis of IL-2 levels between men with normal spermiograms and those with oligo-asthenozoospermia and azoospermia showed significant differences (Table 5), thus rendering determination of seminal plasma IL-2 with value concerning the quality of semen.

The prostate appears to be the main site of origin of IL-2 in seminal plasma, as indicated by the significantly higher levels ($p<0.01$) in the first fraction compared with the second of the split ejaculate of our ten normal controls (Table 5).

Regarding SIL-2R and sCD4 we found a wide overlapping of levels between men with normal spermiograms and oligo-asthenozoospermics or azoospermics. Furthermore, there were no significant differences in SIL-2R and sCD4 levels in those groups.

The spermatocyst seems to be the main site of origin of SIL-2R in seminal plasma as indicated by the significantly higher levels in the second, compared to the first fraction of the split ejaculates (Table 5).

On the contrary, the prostate seems to be the main site of origin of sCD4 in seminal plasma, as indicated by the significantly higher levels in the first, compared to the second fraction of the split ejaculates.

Finally, the higher levels of IL-2, SIL-2R and sCD4 in the seminal plasma to those in the serum suggest local production of cytokines in the male genital system.

References

[1] Cohen S., Pick E., Oppenheim J.: "In biology of the Lymphokines". Pick E. & Oppenheim J. (Eds), New York: Academic Press, pp. 1.

[2] Mitchell M. D., Trautman M. S., Dudey D. J.: "Cytokine networking in the placenta". *Placenta*, 1993, *14*, 249.

[3] Nathan C., Sporn M.: "Cytokines in contest". *Journal of Cell Biology*, 1991, *113*, 981.

[4] Matalliotakis I., Neonaki M., Zolindaki A., Hassan E., Georgoulias V., Koumantakis E.: "Changes in immunological variables (TNF-a, sCD8, and sCD4) during danazol treatment in patients with endometriosis". *Int. J. Fertil.*, 1997, *42*, 211.

[5] Koumantakis E., Matalliotakis I., Neonaki M., Froudarakis G., Georgoulias V.: "Soluble serum interleukin-2 receptor, interleukin-6 and interleukin 1a in patients with endometriosis and in controls". *Arch. Gynecol. Obstet.*, 1994, *255*, 107.

[6] Matalliotakis I., Panidis D., Vlassis G., Vavilis D., Neonaki M., Koumantakis E.: "PRL, TSH and their response to the TRH test in patients with endometriosis before, during and after treatment with danazol". *Gynecologic and obstetric investigation*, 1996, *42*, 183.

[7] Hill J. A.: "Immunology and endometriosis". *Fertil. Steril.*, 1992, *58*, 262.

[8] Shimoya K., Matsuzaki N., Tsutsui K., 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.

[9] Bronson R. A., Cooper G., Hjort T., Ing R., Rone W. R., Wang S. X., Mathur S. *et al.*: "Antisperm antibodies detected by aggutination, immobilization, cytoxicity and immunobead binding assay". *J. Reprod. Immunol.*, 1985, *8*, 279.

[10] Naz R., Kaplan P.: "Increased levels of interleukin-6 in seminal plasma of infertile men". *J. of Andrology*, 1994, *15*, 220.

[11] Gruschwitz M., Breznschek R., Brezinscek H. P.: "Cytokine levels in the seminal plasma of infertile men". *J. of Andrology*, 1996, *17*, 158.

[12] Matalliotakis I., Kyriakoyu D., Fragouli, Y., Sifakis S., Eliopoulos F. G., Koumantakis E.: "Interleukin-6 (IL-6) in seminal plasma of fertile and infertile men". *Arch. of Androl.*, 1998, *41*, 49.

[13] Paradisi R., Capelli M., Mandini M., Bellavia F., Focaggi M., Flamigni C.: "Interleukin-2 in seminal plasma of fertile and infertile men". *Arch. of Androl.*, 1995, *35*, 35.

[14] Miska W., Mahmoud M.: "Determination of soluble interleukin-2 receptor in human seminal plasma". *Arch. of Androl.*, 1993, *30*, 23.

[15] Hussenet F., Dousset B., Cordonnier J. L., Flognet B., Grignon G., Nabet P.: "Interleukin-2 et liquide seminal humain". *Contraception, fertlilete, sexualite*, 1993, *21*, 376.

[16] Makler A.: "A new multiple exposure photography method for objective spermatozoal motility determination". *Fertil. Steril.*, 1978, *30*, 192.

[17] Makler A.: "The improved ten-micrometer chamber of rapid sperm count and motility evaluation". *Fertil. Steril.*, 1980, *33*, 337.

[18] Matalliotakis I., Panidis D., Vlassis G., Kalogeropoulos A., Zolindakis A., Koumantakis E.: "The evaluation of morphological abnormalities of human spermatozoa in fertile men". *Eur. J. Obst. Gynec. and Repr. Biolo.*, 1996, *66*, 65.

[19] Eliasson R.: "Diagnosis in male infertility". *Int. J. Androl.*, 1978, *1*, 53.

[20] Matthias G., Ruth Bresinschek G., Brezinscheck H. P.: "Cytokine levels in seminal plasma of infertile males". *J. Androl.*, 1996, *17*, 158.

[21] Wegmann T.: "The cytokine basis for cross-talk between the maternal immune and reproductive systems". *Curr. Opin. Immunol.*, 1990, *2*, 765.

[22] Paradisi R., Capelli M., Mandini M., Bellavia E., Focaggi M., Flamigni C.: "Interleukin-2 in seminal plasma of fertile and infertile men". *Arch. of Andrology*, 1995, *35*, 35.

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