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Detection of 90K/MAC-2BP in the Seminal Plasma of Infertile Males With Accessory Gland Infection and the Autoimmune Pathogenetic Hypothesis

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Abstract

The purpose of the study was to evaluate 90K/MAC-2BP, a glycoprotein member of the Scavenger Receptor Cystein Rich superfamily, in the seminal plasma of infertile male patients with male accessory gland infection in order to investigate a putative autoimmune pathogenesis. 90K seminal concentration and sperm parameters were evaluated in 50 patients with male accessory gland infection at baseline and after cycles of treatment with Levofluoxacin 500 mg daily for 15 days plus serratiopeptidase 10 mg daily for 30 days. Treatment was continued for up to 6 cycles in cases of persistant bacteriospermia and/or clinical and ejaculatory signs of the disease. Patients with persistant male accessory gland infection after 6 cycles were defined as nonresponders. The same parameters were evaluated at baseline and after a 2-month period in 30 healthy controls. Patients with male accessory gland infection showed impaired sperm parameters and had lower seminal 90K concentration compared to controls. After treatment, seminal 90K level significantly increased in patients compared to controls. Twenty-two patients responded to treatment (44%), while 28 were nonresponders (56%). No difference in pretreatment and posttreatment sperm parameters and seminal 90K was observed between the 2 subgroups. Thirteen patients (26%) had identifiable bacteriospermia: significantly less pretreatment seminal 90K was observed compared to patients without bacteriospermia. Seminal 90K is decreased in patients with male accessory gland infection, and may be

restored by a treatment with quinolones. However, the clinical utility of a 90K assay in these patients remains uncertain, as its level is not predictive of response to treatment.

Key words: Male infertility, autoimmunity, prostatitis, quinolones

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Male accessory gland infection commonly occurs in subfertile males (World Health Organization, 1987), and it is presumed to affect male fertility due to direct bacterial and immunologic effects on sperm function (Diemer et al, 2000).

As bacterial infection is identifiable only in a minority of cases (Weidner et al, 1991), the possible role of autoimmunity in the pathogenesis of male accessory gland infection has been postulated (John et al, 2003). As corroborative evidence, increased seminal interleukin (IL) 6 and IL-8 was observed in patients with chronic prostatitis compared to men with normal ejaculates (Matalliotakis et al, 2002; Paulis et al, 2003), and the injection of homogenates of mouse prostates in syngeneic mice induced differing degrees of prostatic inflammation in the injected mice (Keetch et al, 1994). However, the mechanisms involved in the pathogenesis of a putative local immune response remain unknown.

Several studies recently investigated the role of 90K/MAC-2BP (90K) in the immune response of patients suffering from a variety of diseases. 90K is a glycoprotein member of the Scavenger Receptor Cystein Rich superfamily originally described as a tumor-secreted antigen (Iacobelli et al, 1986) and subsequently observed to participate in the immune defense against cancer and other pathogens, such as viruses (Ulrich et al, 1994). In particular, 90K was observed to demonstrate stimulatory activity on NK and LAK cytotoxic effector cells, critical elements in the body's immune response, and to increase IL-2 secretion (Ulrich et al, 1994).

We thus sought to evaluate seminal 90K in infertile male patients with male accessory gland infection in order to investigate a putative autoimmune pathogenesis.

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Patients

Fifty male patients, ages 25–45 (mean 34.3 ± 5.2) years, referred to our center with a history of primary infertility (defined as the lack of conception after 2 or more years of unprotected intercourse), were enrolled in the study.

Patients were diagnosed to have male accessory gland infection when they fulfilled 2 or more of the following criteria: presence of 1 or 2 conventional ejaculatory signs (high viscosity, delayed or incomplete liquefaction, ejaculate volume <2 mL, leukocytospermia), 1 or 2 cultures with significant bacteriospermia (>10^5 CFU/mL) or detection of *Chlamydia trachomatis* by immunofluorescence, or signs of accessory gland inflammation at physical examination (Vicari, 1999).

Exclusion criteria were systemic autoimmune diseases, history of orchitis, testicular torsion, or trauma.

Thirty subjects with normal sperm parameters and no clinical or seminal signs of male accessory

gland infection served as the control group.

Study Design

Each subject underwent a careful physical examination to verify the presence of clinical signs of accessory gland inflammation. Clinical signs of epididymitis were painful palpation of the epididymis and swelling and/or microcysts or micronodularity in the caput or caudal region on one or both sides. Clinical signs of prostatitis were pelvic pain for a period of >3 months and voiding symptoms. Ultrasound was also performed to assess the presence of seminal vesicle inflammation.

Semen analysis and sperm and urine culture were performed in patients and controls. Patients then underwent treatment with levofloxacin 500 mg (Tavanic 500 mg; Aventis Pharma S.p.A., Milan, Italy) once a day for 2 weeks, and serratiopeptidase (Danzen; Takeda Italia, Rome, Italy) 10 mg daily for 30 days. Use of condoms in sexual intercourse was strongly recommended during treatment.

At the end of every cycle patients underwent physical examination as well as semen analysis and semen and urine culture. Treatment was continued for up to 6 cycles in case of persistant bacteriospermia and/or clinical and ejaculate signs of male accessory gland infection; patients without clinical or laboratory signs of male accessory gland infection after 1–6 cycles were considered to be responders, while patients with persistant signs of male accessory gland infection after 6 cycles of treatment were considered nonresponders.

No treatment was prescribed to controls. Semen analysis, semen and urine culture, and seminal 90K concentration was re-evaluated in the control group after 2 months.

Semen Analysis and Culture

Semen samples were obtained from patients and controls after 3–4 days of sexual abstinence, allowed to liquefy at room temperature, and evaluated under a light microscope to assess sperm count, motility (expressed as percentage of motile sperm), and morphology according to WHO criteria. Sperm count was determined using a hemacytometer chamber, sperm motility was calculated by assigning individual motile sperm to categories on the basis of progression, and sperm morphology was evaluated in air-dried May-Grünwald/Giemsa stained smears. All tests began within 1 hour after sample collection and were performed by the same operator. Semen samples were also processed to detect presence of *Ureaplasma urealyticum*, Enterobacteriaceae, enterococci, and staphylococci. Urine samples were processed by immunofluorescence to identify *Chlamydia trachomatis* infection.

90K Seminal Plasma Assay

After sperm parameters were assessed, sperm samples were centrifuged at 1500 rpm for 10 minutes, and then the seminal plasma was separated from the pellet and stored in Eppendorf microtubes at −20°C until assayed.

After thawing, samples were diluted 1:101 in buffer solution, then seminal plasma 90K concentration was assayed using a commercial kit (Diesse; Siena, Italy) which utilizes an enzyme-linked immunosorbent assay (ELISA) sandwich method optimized for biological fluids other than serum. Standards were obtained by a recombinant preparation of 90K protein, and immunocomplexes were detected using the conjugate composed of the same monoclonal antibody labeled with peroxidase. Seminal 90K concentration was assayed in duplicate for the first 20 samples to demonstrate reliability, which was observed.

Statistical Analysis

Data analysis was performed using SPSS (Chicago, Ill). Seminal 90K and sperm parameters in patients were compared to those of controls by one-way analysis of variance (ANOVA). Pretreatment and posttreatment parameters were compared in patients and in controls by paired *t* test. Pearson's test was employed to investigate the correlation between 90K concentration and sperm parameters.

In all analyses, *P* less than .05 was considered to be statistically significant.

Ethical Guidelines

No intervention out of those routinely employed in the medical care and treatment of male accessory gland infection was made. The 90K seminal plasma assay was performed on sperm samples obtained during patients' routine evaluation.

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All patients completed the study. No adverse events related to quinolone therapy were noted.

At the time of the first evaluation, patients with male accessory gland infection demonstrated a wide range of sperm parameters, from severe oligoasthenoteratospermia to normal sperm parameters. In aggregate, patients' sperm count, motility, and morphology were significantly lower compared to controls (Table 1). Similarly, patients' seminal 90K was significantly lower compared to controls; however, no significant correlation was observed between seminal 90K and sperm parameters.

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Table 1. *Comparison of sperm parameters and seminal 90 K/MAC-2BP (90K) level in patients with infection of the accessory glands and in controls**

After treatment with Levofluoxacin plus serratiopeptidase, patients demonstrated a small, significant increase in sperm count compared to their pretreatment value, while sperm motility and morphology remained unchanged. Seminal 90K significantly increased after treatment compared to controls. Sperm parameters and seminal 90K were not significantly changed in the second, confirmatory evaluation performed in controls.

Table 2 displays the results obtained by dividing patients in 2 subgroups on the basis of their response to treatment. Twenty-two patients (44%) responded to treatment after a 2.5 ± 1.8-month period (range 1–6), while 28 men still manifested seminal and/or clinical signs of male accessory gland infection after treatment.

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Table 2. *Comparison of sperm parameters and seminal 90K/MAC-2BP (90K) level in patients with recovery of the infection (responders) or no recovery (nonresponders) after medical treatment**

Responders displayed comparable pretreatment and posttreatment sperm parameters and seminal 90K compared to nonresponders. Sperm parameters did not vary significantly between subgroups after treatment with quinolones, while seminal 90K increased after treatment in both.

In 13 patients (26%), identifiable bacteriospermia was observed in cultures. These patients, although revealing comparable sperm parameters (data not shown), had significantly lower pretreatment seminal 90K compared to patients without bacteriospermia: their seminal 90K significantly increased after treatment, and was significantly higher compared to the level observed after treatment in patients without bacteriospermia (see Table 3).

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Table 3. *Comparison of posttreatment seminal 90K/MAC-2BP (90K) level in patients with bacteriospermia (B) with those of responder (R) and nonresponder (NR) patients without identifiable bacteriospermia*†

All patients with bacteriospermia had negative cultures after treatment. However, 11 (84.6%) of these patients were defined to be nonresponders, as they persistantly exhibited clinical and ejaculate signs of male accessory gland infection.

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This prospective, controlled, clinical study investigated the role of 90K/MAC-2BP in the local immune response of infertile male patients with accessory gland infection. We assayed seminal plasma 90K/MAC-2BP in 50 infertile male patients in whom diagnosis of male accessory gland infection was made according to standardized criteria and in 30 controls without male accessory gland infection and with normal sperm parameters. Assays were also performed in patients after 1–6 cycles of treatment with levofloxacin 500 mg, a fluoroquinolone whose efficacy on microbiologic eradication rates is known (Bundrick et al, 2003), plus serratiopeptidase, a 50.6-kd metalloprotease that demonstrates strong proteolytic activity enhancing the penetration of antibiotics in infected sites (Yamazaki et al, 1967) and enhancing the activity of quinolones against the development of bacterial biofilms (Selan et al, 1993), while in controls seminal 90K was reevaluated after 2 months.

At baseline, seminal 90K was significantly lower in patients than in controls. This finding is unexpected, considering first the known immune stimulator activity of 90K in other tissues. Investigators previously demonstrated that 90K enhances generation of cytotoxic effector cells (Ulrich et al, 1994) as well as the secretion of IL-1, IL6, and tumor necrosis factor alpha (Powell et al, 1995). As those and other interleukins (IL-8 and IL-11) involved in the inflammatory reaction to tissue damage caused by infection (Comhaire et al, 1999) were previously observed to be increased in the ejaculate of patients with male accessory gland infection compared to controls (Alexander et al, 1998; Matalliotakis et al, 2002), a comparable increase of seminal 90K compared to controls could be expected in our patients. However, our finding of comparatively lower seminal 90K in patients is not surprising if we hypothesize that patients with male accessory gland infection harbor a disregulated local immune response.

Further evidence confirms this latter hypothesis of a dysregulated local immune response. Treatment with quinolones restored normal seminal 90K concentrations in patients, which reached and even exceeded the value observed in controls. This effect was probably due to the previously demonstrated immunomodulatory effect of quinolones, which were observed to alter the release of proinflammatory bacterial products, to modulate phagocytic capacity and intraleucocytic killing, and finally to stimulate IL-2 production by monocytes in a concentration-dependent manner and to decrease IL-1 beta production (Dalhoff et al, 2003). Thus quinolones could modulate local 90K production in patients with male accessory gland infection, possibly leading to recovery from the disease.

Twenty-two out of 50 patients (44%) responded to treatment; however, the remainder did not respond, although they had seminal 90K concentration comparable to responders. At this point, we are unable to explain this finding. A possible hypothesis could be that in nonresponders a failure of self-limiting inflammation occurs, as observed in other chronic diseases (Lawrence et al, 2002).

We suspect that the impaired seminal 90K response observed in our patients with bacteriospermia could be further evidence for the hypothesis of disregulation of the local immune response in patients suffering from male accessory gland infection. In support of this hypothesis, investigators previously reported that 90K concentration in human milk was inversely correlated to episodes of acute respiratory infections in breast-fed infants, suggesting a protective role of 90K against infections (Fornarini et al, 1998). Based on these results, one might reasonably infer that patients having lower seminal 90K are more susceptible to bacterial infection due to an altered local immune response. Supporting this inference, 84.6% of patients, although having negative cultures after treatment, still demonstrated clinical and seminal signs of male accessory gland infection.

In conclusion, this study demonstrates that 90K is decreased in the seminal plasma of patients with male accessory gland infection and that its seminal level can be restored by a treatment with quinolones. However, recovery of the disease clinical entity does not universally follow. Evidence obtained from the current study supports the hypothesis of a disregulated local immune response. Our study presents more of an investigative effort than a clinical tool, as seminal 90K was not found to predict response to treatment in patients suffering from male accessory gland infection. We cannot recommend the use of 90K analysis as a clinical tool in patients with accessory gland infection unless further studies comparing its level with those of other factors involved in the local immune response (interleukins, etc) would demonstrate its appropriateness.

Footnotes

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