Cytokines and Chemokines in Testicular Inflammation: A Brief Review

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ABSTRACT A wide spectrum of data in the literature shows the relevance of cytokines as paracrine regulators of spermatogenesis and steroidogenesis in the normal testis. In this brief review, we highlight the relevance of cytokines in the testis during inflammation. This phenomenon involves complex and multiple interactions among immune and germ cells generally resulting in the alteration of spermatogenesis. The complexity of these cell interactions is multiplied because Sertoli and Leydig cells are also producers of pro- and anti-inflammatory cytokines and chemokines. Also, cytokines are pleiotropic and they exert opposite and/or redundant effects in different conditions. However, in spite of this bidirectional immunoregulatory function of cytokines, the mass of the data, reported from experiments of acute testicular inflammation, shows upregulation of interleukin (IL)-1β, IL-1α, IL-6, and tumor necrosis factor alpha (TNF-α), which induce adverse effects on germ cells. In autoimmune orchitis, a chronic testicular inflammation, chemokines such as CCL2, CCL3, and CCL4 induce attraction and extravasation of immune cells within the testicular interstitium. These cells alter the normal immunosuppressor microenvironment principally through the secretion of proinflammatory cytokines, interferon-γ initially, and IL-6 and TNF-α thereafter. Germ cells expressing TNFR1, IL-6R, and Fas increase in number and undergo apoptosis, through the TNF-α/TNFR1, IL-6/IL-6R, and Fas/Fas L systems. The knowledge of immune-germ and somatic testicular cell interactions will contribute to the understanding of the mechanisms by which chronic inflammatory conditions of the testis can disrupt the process of spermatogenesis. *Microsc. Res. Tech. 00:000–000, 2009.*

INTRODUCTION

Spermatogenesis and steroidogenesis are the major functions of the testis. Although the importance of follicle-stimulating hormone, luteinizing hormone (LH), and androgens for the initiation and maintenance of spermatogenesis has been clearly demonstrated, the function of autocrine and paracrine regulatory factors is only partially understood. Among these factors, cytokines play a relevant role in the development and normal function of the testis. They also act as modulators of Leydig cell steroidogenesis (see reviews of Bornstein et al., 2004; Diemer et al., 2003; Hales, 2002; Hales et al., 1999).

The testis is considered an immune-privileged organ because it is able to tolerate those autoantigens expressed in germ cells that appear after puberty when immunocompetence is already established. The mechanisms of testis autoimmune disease prevention involve the existence of an incomplete immunological blood testis barrier (BTB), a structure that limits the access of germ cell antigens to the immune cells and antibodies, the presence of regulatory T cells (Treg) in the testicular interstitium, and the secretion of multiple immunosuppressor factors mainly by macrophages, Sertoli, and Leydig cells (Tung et al., 2002). Cytokines are especially involved in the maintenance of testicular immunoprivilege inducing an immunosuppressor microenvironment (Hedger and Meinhardt, 2003). The ability of certain cytokines to modulate the opening and closing of the inter-Sertoli tight junctions that constitute the BTB was also demonstrated (Lui and Cheng, 2007). Other recent data showed that activation of toll-like receptors present in Sertoli cells increases the production of inflammatory cytokines suggesting that these cells may play important roles protecting the seminiferous epithelium from invading pathogens and autoantigens (Girling and Hedger, 2007; Wu et al., 2008). Also, it has been recently reported that a malfunction of the clearance mechanisms for apoptotic cell debris following imbalance in phagocyte receptors or cytokines acting on Sertoli cells induces breakdown of self-tolerance during spontaneous orchitis (Pelletier et al., in press).

In this brief review, we will focus on the role of cytokines and chemokines in testicular inflammation.

Infection and inflammation of the male reproductive tract are widely accepted as important etiological factors of subfertility or infertility. Infections account for almost 15% of the cases of male infertility seen in fertility

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Abbreviations used: BTB, blood testis barrier; CMTM, conditioned medium of testicular macrophages; EAO, experimental autoimmune orchitis; hCG, human chorionic gonadotropin; IFN, interferon; IL, interleukin; KO, knockout; LH, luteinizing hormone; LPS, lipopolysaccharide; MoAb, monoclonal antibody; NO, nitric oxide; NOS, nitric oxide synthase; TGF, transforming growth factor; TNF-α, tumor necrosis factor alpha; Treg, regulatory T cells; VCAM, vascular adhesion molecule

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clinics. Inflammation of the testis (orchitis) or epididymo-orchitis occurs mainly by retrograde ascent of urethral bacterial pathogens or systemic viral infections (Schuppe and Meinhardt, 2005). Granulomatous orchitis may also occur as a primarily chronic disease of unknown etiology or as a manifestation of systemic infections.

The main histopathological characteristic of testicular inflammation is the presence of inflammatory cells in the interstitium, mainly lymphocytes, macrophages, and mast cells. Although massive cell infiltration, mainly of neutrophils, occurs in acute bacterial epididymo-orchitis in both interstitial and tubular compartments, focal or multifocal peritubular and interstitial lymphomononuclear cell infiltrates are present in chronic inflammatory reactions (Schuppe et al., 2008). In the normal human testis, mast cells are mainly found in the tunica albuginea and in the interstitium close to Leydig cells, whereas in the testis of infertile men, there is a shift of these cells from the interstitium to the tubular walls (Meineke et al., 2000). A relevant role in the induction of testicular fibrosis was ascribed to mast cell-secreted serine protease tryptase (Frungieri et al., 2002b). Recently, an increase in the transcription of inflammation-related genes, some of them encoding mast cell products, were detected by microarray analysis of testicular biopsy samples of infertile men (Spiess et al., 2007).

Fifty-six percent of subfertile or infertile patients present different degrees of testicular lymphocyte infiltrates, a characteristic not restricted to infectious agents but also found in different pathologies including neoplasia, trauma, toxic agents, and cryptorchidia. Lymphocyte and macrophage infiltrates are generally associated to seminiferous tubules with partial or complete loss of germ cells (Frungieri et al., 2002a; Schuppe and Meinhardt, 2005). The release of spermatic antigens from impaired seminiferous tubules presenting germ cell degeneration and sloughing induces inflammation and autoimmune responses.

Cytokines are polypeptide mediators that function as immune modulators and also have a wide range of other biological activities. As highlighted by Hales et al. (1999), their two main roles in the testis are as follows: (a) as growth and differentiation factors that help to orchestrate interactions during normal physiological functions and (b) as mediators of pathophysiological outcomes of immune-endocrine interactions during inflammatory disease.

Chemotactic cytokines or chemokines are a complex family of low-molecular-mass proteins that are regulators of the leukocyte recruitment process with an important role in inflammation. Depending on the chemokine family involved, they mainly attract neutrophils or monocytes and T lymphocytes to the target organ.

EXPERIMENTAL MODELS OF TESTICULAR INFLAMMATION

Acute Testicular Inflammation

The administration of lipopolysaccharide (LPS), an endotoxin derived from the walls of gram negative bacteria, to experimental animals induces an array of pathophysiological responses and characteristic symptoms, without the presence of living pathogens. LPS induces the production of proinflammatory cytokines, mainly interleukin (IL)-1, IL-6, and tumor necrosis factor alpha (TNF-α) by activated immune cells, as well as the inducible form of nitric oxide synthase (NOS) and increase of plasma and tissue levels of NO (nitric oxide) (Hales et al., 1999).

Injection of human chorionic gonadotropin (hCG) in adult male rats induces a testicular inflammation-like effect characterized by a transient surge of proinflammatory cytokines mainly produced by Leydig cells (Assmus et al., 2005).

Chronic Testicular Inflammation

Models of experimental autoimmune orchitis (EAO), useful to study chronic organ-specific inflammation, can be elicited by three experimental approaches: (a) immunization with testis antigens with or without adjuvants, (b) passive transfer of T lymphocytes previously sensitized with sperm antigens, or (c) manipulation of the normal immune system, such as thymectomy at day 3 after birth or the transfer of defined T cell populations from normal inbred mice to syngeneic athymic nu/nu mice recipients (Lustig and Tung, 2006). Autoimmune orchitis was also described in the contralateral testis of mice unilaterally injected into the testis with *Listeria monocytogenes* (Mukasa et al., 1995) or Sendai virus, closely related to mumps virus (Melaine et al., 2003). Despite the absence of detectable microorganisms, the contralateral testis showed an extensive inflammatory response and damage of the germinal epithelium providing evidence of an autoimmune response.

A great quantity of information on autoimmune orchitis induced in mice arises from the pioneering work of Tung et al. (1987, 2002). Our results described throughout this review were obtained in an experimental model of autoimmune orchitis induced in adult rats by active immunization with three doses of testis homogenate (or saline for controls rats) in complete Freund's adjuvant using *Bordetella pertussis*, as coadjuvant (Doncel et al., 1989). In the experimental rats (injected with testis homogenate), focal orchitis is induced 50 days after the first immunization and a severe and extended disease develops by 80 days. Testicular histopathology is characterized by interstitial lymphomononuclear cell infiltrates associated with damage of seminiferous tubules that exhibit germ cell apoptosis and severe cell sloughing followed by aspermatogenesis and tubular atrophy. We demonstrated that ED1+ED2+ macrophages, intermediate stage between the resident macrophage (ED2+ cell subset) and monocytes recently arrived to the testis from circulation (ED1+ cell subset), increased in number in rats with EAO (Rival et al., 2008). Also, an increase in the number of effector CD4+ and CD8+ as well as regulatory T lymphocyte subsets (Jacobo et al., 2007; Lustig et al., 1993) and semimature dendritic cells was detected in EAO (Rival et al., 2006a, 2007). An increased number of mast cells was also observed mainly distributed at perivascular and peritubular interstitial areas of the testis contrasting with the restricted subalbuginea localization detected in normal and control rats (Iosub et al., 2006; Lustig et al., 1995).

CYTOKINES

Interleukin-1

IL-1 has long been known to be an important mediator of inflammatory process through chemotactic, pyro-

genic, and immunoregulatory actions (recently reviewed by Amjad et al., 2006). IL-1 occurs as two isoforms: IL-1α and IL-1β, two single glycosylated chains sharing only 22% amino acid homology that bind to the same receptors and have similar biological activities. The other components of the IL-1 family are the receptors type I and II and the high affinity IL-1 receptor antagonist (IL-1ra) that binds to both receptors but is unable to transmit a biological signal.

In adult rat and human testis, IL-1α, predominantly secreted by Sertoli cells, plays a physiologic function as a mitogen regulating spermatogonial and preleptotene spermatocyte DNA replication. IL-1α also acts as an autocrine and paracrine factor stimulating the production of lactate and transferrin and in vitro Sertoli cell mitosis and also modulating Leydig cell steroidogenesis. Data suggest that IL-1α inhibits LH-stimulated testosterone production but can stimulate basal steroidogenesis under appropriate conditions (Svechnikov et al., 2001). Although the involvement of IL-1 in normal spermatogenesis and steroidogenesis was clearly demonstrated, interestingly, no major alterations in the testicular physiology were detected in IL-1 knockout (KO) mice, suggesting that the effects of most of the multiple cytokines present in the testicular microenvironment are redundant. Recent data highlighted the regulatory role of IL-1α (Sarkar et al., 2008) transforming growth factor (TGF)-β3 and TNF-α on Sertoli tight junction dynamics (Lui and Cheng, 2007; Yan et al., 2008). The intratesticular administration of acute doses of these cytokines in rats has been shown to "open" the BTB, suggesting that IL-1α facilitates the tight junction opening by affecting the actin cytoskeleton while TGF-β3 and TNF-α, do so by lowering occludin levels.

Unlike IL-1α, IL-1β does not appear to be produced in significant amounts in the normal testis. However, a different situation occurs in infection and inflammation where an upregulation of inflammatory IL-1β might contribute to the injury of testicular tissue. LPS was shown to stimulate IL-1β secretion by monocytes–macrophages in the testis as well as the IL-1α secreted by Sertoli cells (Stéphan et al., 1997). Intratesticular IL-1β injection in adult rats was demonstrated to induce vascular inflammation and a potent inflammatory response (Bergh and Söder, 1990). Also, the high intratesticular IL-1 and NO levels detected in rats with varicocele have been proposed as the reason for the severe alterations of spermatogenesis (Amjad et al., 2006). In recent years, a number of potentially serious side effects of hCG used clinically to treat cryptorchidia have been reported. The adverse effects on germ cells were ascribed to the transient testicular inflammation with high local levels of IL-1α, IL-1β, TNF-α, and other cytokines induced by hCG (Thorsson et al., 2007).

Interleukin-6

IL-6 is one of the most potent cytokines that promote inflammatory events through expansion and activation of T cells, differentiation of B cells, and induction of the acute phase response. IL-6 actions are mediated by IL-6R, a glycoprotein of 80 kDa (gp80), considering to be the α-subunit of IL-6R. Binding of IL-6 to IL-6R leads to the homodimerization of gp130, considered to be the β-subunit of IL-6R, and activation of the signal transduction pathways, which generates opposite responses such as cell growth and differentiation or growth arrest and apoptosis (Kamimura et al., 2003; Oritani et al., 1999).

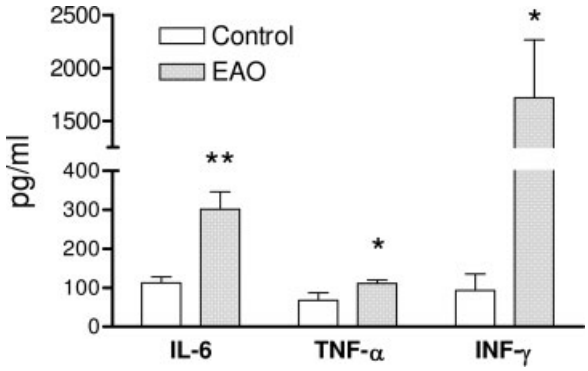

Fig. 1. IL-6, TNF-α, and IFN-γ production by testicular macrophages. Cytokines were measured by ELISA in the CMTM obtained from rats with severe orchitis. The conditioned medium was obtained by culturing the macrophages obtained from one testis per rat. Each column represents the mean ± SEM of four to seven rats/group. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus respective control.

In normal rats, IL-6 is produced by most of testicular somatic cells: interstitial macrophages (Bryniarski et al., 2005; Kern et al., 1995), Leydig cells (Boockfor et al., 1994), Sertoli cells (Cudicini et al., 1997; Rival et al., 2006b), peritubular, and germ cells (Potashnik et al., 2005). The last authors demonstrated the involvement of gonadotropins and testosterone in the regulation of IL-6 expression (Potashnik et al., 2005).

In the acute testicular inflammation induced by LPS, a stimulation of IL-6 synthesis occurs (Elhija et al., 2005). Similarly, IL-6 is upregulated in Sertoli cell in vitro in response to LPS and TNF-α (Riccioli et al., 1995; Stéphan et al., 1997). In EAO, we observed a significant increase of IL-6 content in the conditioned medium of testicular macrophages (CMTM) (Fig. 1) (Rival et al., 2006b). Only monocytes recently arrived to the testis from circulation (ED1+) expressed high IL-6, whereas resident testicular macrophages (ED2+) did not, highlighting the proinflammatory profile of ED1+ macrophages. Although activated testicular macrophages and peritubular cells exhibit an increased IL-6 reactivity during the development of EAO, Sertoli cells showed a downregulation of IL-6 expression associated to the morphological alteration present in these cells during the chronic stage of the disease. A significant increase in the number of IL6R+ germ cells (Fig. 2) occurred simultaneously with the increasing degree of testicular damage and increased number of apoptotic germ cells. Also, in vitro experiments showed that IL-6 induced germ cell apoptosis when added to the cultures of rat seminiferous tubules (Fig. 3) (Rival et al., 2006b; Theas et al., 2003). Overall results suggested the involvement of IL-6/IL-6R system in the pathogenesis of EAO through the stimulation of inflammation and the induction of germ cell apoptosis. In addition, the apoptotic effect of IL-6 on germ cells may be mediated through the modulated expression of pro- or antiapoptotic factors or through the inhibition of meiotic DNA synthesis as it was demonstrated in

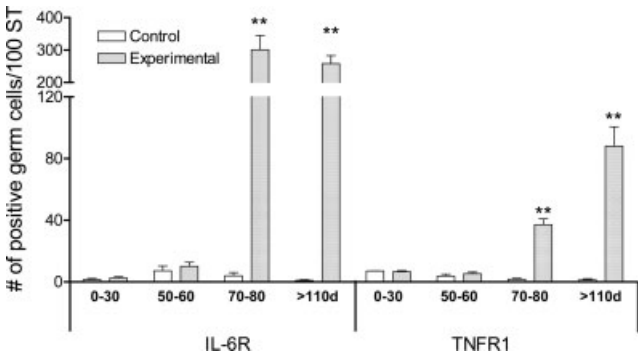

Fig. 2. Quantification of IL-6 receptor (IL-6R) and TNF-α receptor type I (TNFR1) by immunohistochemistry. The number of IL-6R+ and TNFR1+ germ cells was quantified in 100 seminiferous tubules (ST)/testis from control and experimental rats at different days (d) after the first immunization. Values represent mean ± SEM of four to five rats/group. $^{**}P < 0.01$ versus respective control.

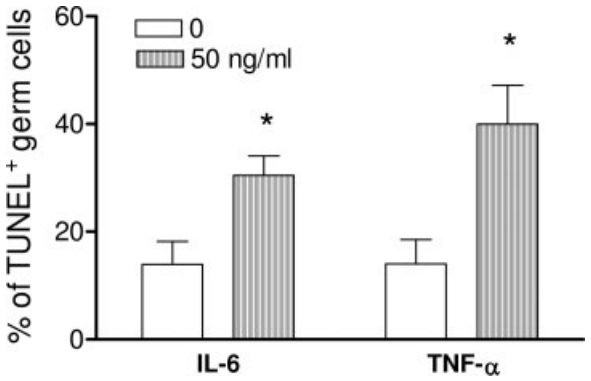

Fig. 3. In vitro effect of IL-6 and TNF-α on germ cell apoptosis evaluated by the TUNEL technique. Seminiferous tubules segments obtained from normal rats were incubated with or without the cytokines in DMEM/F12 supplemented medium for 18 h. Data are expressed as mean ± SEM of four independent experiments. $^{*}P < 0.05$ versus medium.

preleptotene spermatocytes and, to a lesser extent, in advanced spermatogonia in the normal testis (Hakorvita et al., 1995).

Interferon

Interferons (IFNs) are a family of secreted regulatory proteins with three main categories: IFN-α and IFN-β (type I IFNs) initially described as the product of virus-infected leukocytes and fibroblasts, and IFN-γ (type II IFN) produced by lymphocytes and natural killer cells stimulated by antigen or mitogenic substances (Balkwill, 1989). IFN-γ receptor (IFN-γR) has at least two subunits, α-chain (IFN-γRα) and β-chain (IFN-γRβ). Masanori and Morris (1998) detected the expression of both mRNAs IFN-γRα and IFN-γRb in immature and mature adult Leydig and Sertoli cells. In the normal testis, early spermatids expressed low levels of IFN-α/β and IFN-γ, whereas pachytene spermatocytes only express IFN-α/β (Dejucq et al., 1995). Dejucq et al. (1995) demonstrated the in vitro production of IFN-α/β by murine Sertoli and peritubular cells in response to infection with Sendai virus. Virus-exposed Leydig cells and macrophages exhibit high antiviral activity producing IFN-α/β/γ and IFN-α/β, respectively. It is possible that testicular macrophages and Leydig cells, both important IFN producers, can act as a first line of defense against viruses coming from the circulation. It seems likely that antiviral protection of spermatogonia that do not express IFN-γ and is localized outside of the BTB is essentially performed by the somatic peritubular and Sertoli cells. In addition to their immunomodulatory and antiviral action, IFNs might play an important role in the testis by controlling spermatogenesis because transgenic mice overexpressing either the IFN-α or IFN-β gene show disruption of the germinal epithelium and sterility (Hekman et al., 1988; Iwakura et al., 1988).

In EAO induced in mice by immunization with testicular germ cells, Itoh et al. (1998) reported that blockage of endogenous IFN-γ by one single injection of anti-IFN-γ monoclonal antibody (MoAb) prevented disease development only when the antibody was administered at onset of the EAO. In contrast, in a different EAO model induced by adoptive transfer of CD4+ T clones derived from mice actively immunized with testicular homogenate plus adyuvants (Yule and Tung, 1993), the incidence and severity of the disease was unaffected by the IFN-γ MoAb treatment.

In EAO induced in rats, we detected a high production of IFN-γ by interstitial testicular macrophages in both phases, focal and severe orchitis (Rival et al., 2008). High levels of IFN-γ in the CMTM were found early in development of the disease, in contrast with IL-6 and TNF-α, which increased during the chronic phase of EAO (Fig. 1).

Dal Secco et al. (2008) reported that IFN-γ can strongly upregulate the negative costimulatory ligand B7-H1 and induce MHC class II expression in prepubertal mice Sertoli cells, in vitro. Therefore, Sertoli cells could function as nonprofessional tolerogenic antigen-presenting cells capable of downregulating the local immune response.

Tumor Necrosis Factor Alpha

There are two biologically active forms of TNF-α: the full-length type II transmembrane protein and the soluble form resulting from proteolytic cleavage of transmembrane TNF-α by TNF-α-converting enzyme (Black, 2002). Transmembrane and soluble TNF-α exert their effects by binding, as a trimmer, to either cell membrane TNF-α receptor (TNFR) TNFR1 or TNFR2. TNFR1 contains a cytoplasmatic death domain (DD) and belongs to the family of death receptors responsible for the transduction of TNF-α-induced death signal through caspase activation; survival and proinflammatory signals may also be initiated by stimulation of TNFR1. Which type of signal predominates depends on the balance of intracellular adaptor proteins interacting with TNFR1. Because the intracellular domain of TNFR2 lacks DD, it efficiently activates NF-kB pathway and JNK and is generally unable to elicit apoptosis (Dempsey et al., 2003).

In the seminiferous tubules, TNF-α is synthesized by pachytene spermatocytes round and elongating spermatids (De et al., 1993; Siu et al., 2003). Very faint immunoreactivity of this cytokine was observed in the basal and adluminal compartment of rat adult seminiferous tubules suggesting that Sertoli cells may express TNF-α at a very low level. However, preceding sper-

miation, a surge in TNF-α immunoreactivity was observed surrounding elongated spermatids in the ectoplasmic specialization, at stages VI–VII (Siu et al., 2003).

Different interstitial cells such as mast cells (Rodriguez et al., 2006), lymphocytes (Jacobo, personal communication), and macrophages synthesize TNF-α (Bryniarski et al., 2005). Testicular macrophages released more TNF-α when stimulated by LPS highlighting the ability of these cells to modify the normal immunosuppressor microenvironment of the testis (Suescun et al., 2003; Xiong and Hales, 1993).

TNFR1 is the main receptor expressed in the murine and human testis by Leydig cells, macrophages, lymphocytes, and germ cells. TNFR2 expression was detected in human peritubular cell cultures and both receptors were observed in prepuberal rat Sertoli cells (De Cesaris et al., 1999; Pentikäinen et al., 2001; Schell et al., 2008; Suescun et al., 2003).

TNF-α is a multifunctional cytokine involved in spermatogenesis through the modulation of androgen receptor expression in Sertoli cells, transport of iron to germ cells, and lactate supply to postmeiotic germ cell and spermiation (Lysiak, 2004; Sui et al., 2003). TNF-α modulates testicular germ cell apoptosis and survival through the regulation of Fas/Fas L system (Pentikainen et al., 2001; Riccioli et al., 2000). A direct effect of TNF-α on germ cell survival mediated by the upregulation of Bcl-xL, a prosurvival protein of the Bcl-2 family protein, was also demonstrated (Suominen et al., 2004). On the other hand, TNF-α is able to induce the expression of proinflammatory mediators in the testis. In combination with other cytokines, TNF-α stimulates in vitro rat Sertoli cells to express inducible NOS and the release of NO (Bauché et al., 1998). TNF-α induces the expression of intracellular adhesion molecule-1, vascular adhesion molecule (VCAM-1), and IL-6 on cultured Sertoli cells and VCAM-1 and IL-6 on peritubular cells, suggesting that this cytokine may induce the migration of lymphocytes from vasculature to interstitium (Riccioli et al., 1995; Schell et al., 2008).

In the in vivo LPS model of inflammation, germ cell apoptosis occurs and the spermatogenic damage observed was proposed to be most likely because of the direct effect of the local inflammatory mediators rather than to the androgen withdrawal, because damage was confined to stages I–V which are not acutely affected by androgen withdrawal (O'Bryan et al., 2000). In fact, LPS induces in vivo expression of the TNF-α mRNA as well as release of IL-6 and IL-1β (O'Bryan et al., 2005). Moreover, we demonstrated that in EAO, interstitial macrophages release TNF-α and that lymphocytes express the membrane-bound form of this cytokine suggesting that these cells may also be a source of TNF-α (Jacobo, personal communication; Suescun et al., 2003; Theas et al., 2008). In EAO, we observed that TNFR1 is expressed by interstitial cells and by spermatocytes in the seminiferous tubules. The number of TNFR1+ spermatocytes increases during severe orchitis (Fig. 2) and most of these TNFR1+ germ cells are apoptotic. TNF-α induces in vitro apoptosis of germ cells, evidence that TNFR1 is functionally active (Fig. 3) (Theas et al., 2008). Apoptosis may be triggered by TNFR1-activated caspase 8 and/or by induced proteins that activate the mitochondrial pathway such as Bid and Bax (Dempsey et al., 2003; Pei et al., 2007). In EAO, we demonstrated caspase 8 activation and also Bax translocation to mitochondria, suggesting that TNF-α may activate these apoptotic pathways when it binds to TNFR1 in germ cells (Theas et al., 2006). We observed that CMTM from rats with severe orchitis induces apoptosis of germ cells in vitro and that contains high levels of TNF-α (Fig. 1). Neutralization of TNF-α employing Etanercept, a protein that mimics the inhibitory effects of soluble TNF receptors (Scallon et al., 2002), reverted CMTM apoptotic effect. The fact that we were unable to detect TNF-α in the serum of EAO rats supports the concept that this inflammatory cytokine secreted by testicular macrophages acts locally to induce testicular damage during the chronic phase of orchitis (Suescun et al., 2003; Theas et al., 2008). Other soluble factors such as IFN-γ, NO, and Fas L may be responsible for germ cell apoptosis during focal orchitis. We observed high content of IFN-γ and NO in the CMTM from rats with EAO and an increased number of Fas+ spermatocytes, being the majority of them apoptotic (Jarazo Dietrich, personal communication; Rival et al., 2008; Theas et al., 2003). Previous data on the efficiency of anti-TNF-α treatment in EAO were controversial. Initially, Teuscher et al. (1990) demonstrated that i.v. administration of anti-TNF-α IgG in mice actively immunized with spermatic antigens did not influence the pathogenesis of orchitis. However, these authors were unable to maintain a continued presence of anti-TNF-α IgG to cover the full period between the challenge and sacrifice of the animals. On the other hand, Yule and Tung (1993) defined TNF-α as an important cytokine in the pathogenesis of autoimmune orchitis developed in mice by adoptive transfer with T cells because i.p. neutralization of the cytokine reduced the incidence and severity of the disease.

A potential role for TNF-α in controlling testicular function in health and disease is suggested. TNF-α behaves as a survival factor at the physiologically low concentrations present in the normal testis. On the other hand, this cytokine may act as a proapoptotic factor during inflammatory conditions of the testis and also in human testicular pathologies such as Sertoli cell-only syndrome and germ cell arrest (Frungieri et al., 2002a; Li et al., 2006; Riccioli et al., 2000; Theas et al., 2008).

Transforming Growth Factor

The immunosuppressive and anti-inflammatory TGF superfamily includes several peptides such as TGF-βs, activins, inhibins, anti-Müllerian hormone, and other growth factors (see reviews of de Kretzer et al., 2004; Ingraham et al., 2000; Josso et al., 2001). In particular, TGF-βs belong to a family of at least five distinct dimeric proteins, of which, three are expressed in mammals (β1, β2, and β3) and are widely distributed in embryonic and adult tissues. In the testis, cytokines of the TGF-β family are expressed constitutively at high levels being produced mainly by Sertoli, peritubular, and Leydig cells. In postpubertal testis, TGF-βs are also expressed by early spermatids and pachythene spermatocytes, in a stage-specific manner. Most TGF-β is

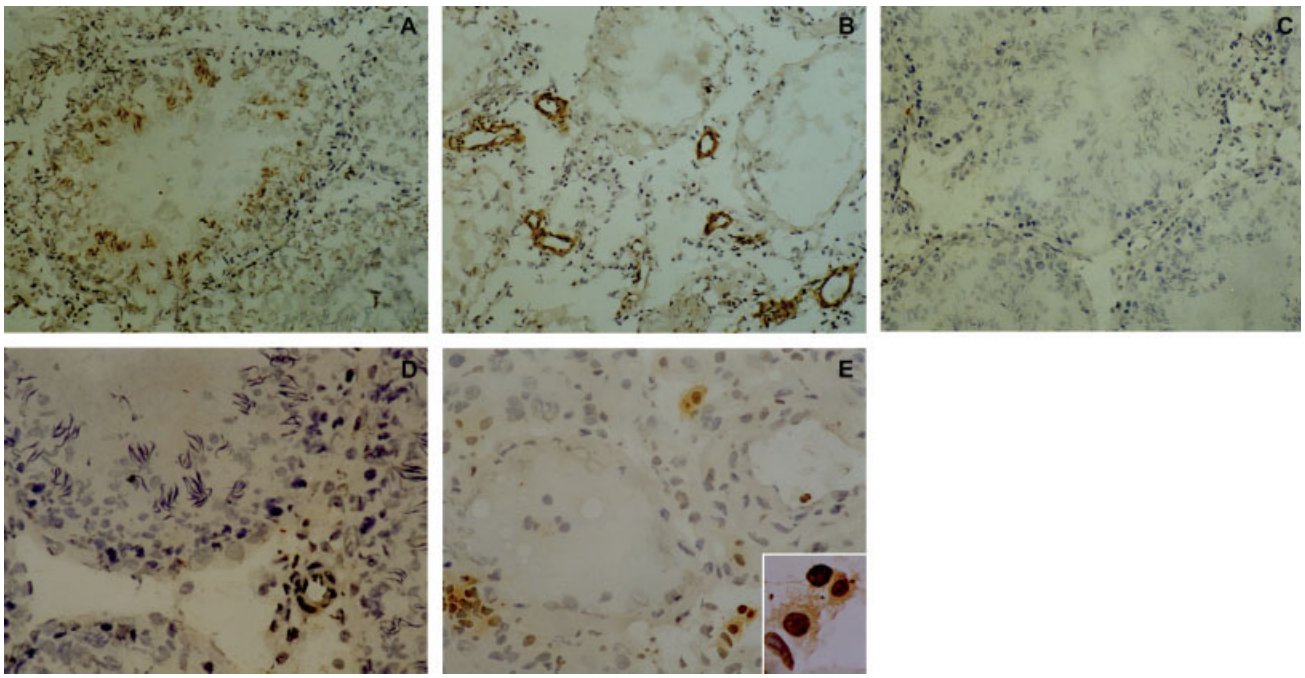

Fig. 4. Immunoperoxidase Technique. CCL4 (A, B, C) and CCR5 (D, E) immunoreactivity in cryostat testis sections. CCL4 expression in germ cells in the testis of a control rat (**A**) and in testicular blood vessel walls of a rat with severe orchitis (**B**) is observed. No staining is detected when antiserum to CCL4 is absorbed with recombinant blocking peptide CCL4 (**C**). A high number of lymphomononuclear cells CCR5+ ($4.87 \pm 0.24 \times 10^6$) is observed in a rat with orchitis (**E**) compared with control rat ($0.88 \pm 0.38 \times 10^6$). (**D**) The insert shows magnification of CCR5+ cells (×1500). Damaged seminiferous tubules of rats with orchitis show aspermatogenesis and smaller diameter compared with controls. A, B, C: ×300, D, E: ×500. Data are expressed as mean ± SEM of four rats/group.

present in the testis as the latent inactive precursor form and is activated at its site of action by local proteases (O'Bryan et al., 2005). TGF-βs are known to modulate multiple physiological functions in the testis including regulation of Leydig and germ cell proliferation, Leydig cell steroidogenesis, extracellular matrix synthesis, and testis development. TGF-βs contribute to the immunoprivileged status of the testis through their strong immunosuppressive ability (Pöllänen et al., 1993).

Activins have considerable structural homology to the TGF-β and possess predominantly paracrine functions exerting stimulatory and inhibitory effects on germ and Sertoli cell proliferation, respectively. Okuma et al. (2005) showed that IL-1α and β isoforms stimulate activin A secretion by Sertoli and peritubular cells in vitro. O'Bryan et al. (2005) showed that the upregulation of TGFβ1 and activin A after in vivo LPS treatment was not evident because expression of these cytokines in the interstitial testicular fluid was constitutively elevated even in normal conditions.

In the testis of rats with severe EAO, we were unable to detect TGF-β in the CMTM by ELISA. In these animals, we detected a fivefold decrease in serum inhibin B levels and a decrease in the inhibin-α subunit expression in Sertoli cells compared with control rats in correlation with the degree of damage of the germinal epithelium (Suescun et al., 2001). The dramatic decrease seen in the Sertoli cell capacity to either produce inhibin B or express the inhibin-α subunit demonstrates the functional impairment of this cell in rats with severe orchitis. In contrast, Leydig cells of EAO rats that do not show morphological and functional alterations and are able to express inhibin-α subunit and secrete testosterone (Suescun et al., 1997).

Chemokines

Chemokines are a large family of small chemoattractive cytokines grouped into the two major subfamilies on the basis of the arrangement of the two N-terminal cysteine residues, CXC and CC, depending on whether the first two cysteine residues have an amino acid between them (CXC) or are adjacent (CC). Two other classes of chemokines have been described: lymphotactin (C) and fractalkine (CX3C). Chemokines bind to seven transmembrane spanning G-protein-coupled receptors. Analogously to their ligands CCL (CC chemokine ligand) and CXCL (CXC chemokine ligand), chemokine receptors are divided into CCRs (CC chemokine receptors) and CXCRs (CXC chemokine receptors) (Zlotnik and Yoshie, 2000). About 50 chemokines and 20 chemokine receptors have been identified. Recently, Thelen and Stein (2008) summarized findings on the initial steps in chemokine receptor-induced signal transduction in leukocytes. Chemokines are involved in leukocyte extravasation, which includes the sequential use of selectins, chemokine receptors, and integrins to induce tethering and rolling, firm adhesion, and diapedesis.

Using an immunohistochemical technique with an antihuman CCL3 (MIP-1 α) antibody, Hakovirta et al. (1994) detected CCL3 in both, germ and Leydig cells in

contrast with Aubry et al. (2000a) who observed no CCL3 or CCL4 (MIP-1β) expression in primary cultures of Leydig, Sertoli, peritubular, or germ cells. Our results in an in vivo model of testicular inflammation show that spermatids of control rats and also the walls of testicular blood vessels of EAO rats express CCL4 (Figs. 4A and 4B). This is consistent with the high concentrations of CCL4 detected by ELISA in the testicular fluid of rats with orchitis (Guazzone et al., 2002). We also observed an increased number of CCR5+ lymphomononuclear cells infiltrating the testicular interstitium during the development of EAO (Fig. 4E). CCR5 has been described as a receptor of CCL3, CCL4, and CCL5 (Zlotnik and Yoshie, 2000). In relation to CCL3, the production of this chemokine by macrophages of experimental rats was higher during the immunization period, preceding testicular lesion (unpublished data).

Of the CC subfamily, CCL2 (MCP-1) the chemokine mostly studied in the testis, is present in this organ at low, but physiologically relevant levels (Gerdprasert et al., 2002; Guazzone et al., 2003). CCL2 is produced in vitro by human and rat peritubular and Leydig cells. CCL2 transcripts were detected by RT-PCR in Sertoli cells, but Northern blot analysis and ELISA did not confirm this finding. No reaction was detected in germ cells (Aubry et al., 2000a; Schell et al., 2008). These authors showed that CCL2 is regulated in cultured peritubular cells by IL-1α, IL-1β, TNF-α, IFN-γ, and LPS in a concentration and time-dependent manner. Exposure of rat testicular macrophages, Sertoli, Leydig, and peritubular cells to the Sendai virus led to the production of mRNA and protein for CCL2 and CCL5 (RANTES). Also, Sertoli cells respond to TLR agonists with a strong upregulation of CCL2 (Riccioli et al., 2006; Starace et al., 2008). In vivo injection of LPS induces a CCL2 increase in the testicular interstitial fluid of rats (Gerdprasert et al., 2002). Accordingly, our results in the EAO model show an increase of this chemokine in the testicular fluid and in the CMTM. This high CCL2 concentration is associated with the highest number of mononuclear cells expressing CCR2 (CCL2 receptor) observed within the testicular interstitium of rats with EAO (Guazzone et al., 2003).

Of the CXC chemokine subfamily, CXCL10 (IP-10) is expressed in rat Leydig cells, its function is inhibited by hCG and is induced by IFN-γ, IL-1α, and TNF-α (Hu et al., 1998). In addition, CXCL1 (GRO) and CXCL10 were produced by rat testicular macrophages and Sertoli, Leydig, and peritubular cells after exposure to the Sendai virus or after stimulation with proinflammatory cytokines (Aubry et al., 2000b; Le Goffic et al., 2002). In contrast, rat germ cells did not produce these chemokines. Le Goffic et al. (2002, 2003) detected CXCL10 synthesis in human Leydig cells exposed to the Sendai or to mumps virus suggesting that rats and humans display similar responses in term of chemokine production.

The sole member of the CX3CL chemokine subfamily, CX3CL1 (fractalkine), is constitutively expressed in Leydig, Sertoli cells, and pachytene spermatocytes. IL-1β, IL-6, and TNF-α stimulated the expression of CX3CL1 mRNA in Sertoli cells (Habasque et al., 2003).

Inflammation is associated with leukocyte infiltration in the target organ. Thus, the mechanisms of leukocyte attraction and extravasation in the testis and the role played by chemokines in these events are proving to be central.

Other Cytokines

Other cytokines such as macrophage migration inhibitory factor (MIF), Fas L, and IL-10 are also relevant in the immunoregulation of the testis in inflammatory conditions. The limitations of this brief review rule out their detailed description.

Future Perspectives

Infection and inflammation of the male reproductive tract are accepted as important etiological factors of infertility. Also, there is clinical and pathological evidence that chronic inflammatory conditions of the testes can disrupt spermatogenesis altering sperm number and quality. EAO is a good model to study chronic inflammatory testicular pathology. The data described here highlight the relevant role played by cytokines produced locally in the testis during inflammation. New experiments on autoimmune orchitis using KO mice or studies blocking endogenous cytokines will confirm the pathogenic role of these factors. Also, new approaches in the study of other cytokines like IL-12, IL-17, IL-18, and IL-23, in the analysis of Treg function and in the modulation of BTB permeability will contribute to the understanding of immune and testicular germ and somatic cell interactions in physiological and pathological conditions. Although it is not possible to directly extrapolate the data obtained from rodent to human testes mainly because the different composition and distribution of immune cells, data from experimental models of testicular inflammation allow a better understanding of the complex disturbances of the normal testicular immunoregulation. These studies together with a molecular approach to identify inflammation-related genes may provide the rationale to generate appropriate therapeutic tools to control testicular inflammation and spermatogenic failure.

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