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LIPOPOLYSACCHARIDE, LATEX BEADS AND RESIDUAL BODIES ARE POTENT ACTIVATORS OF SERTOLI CELL INTERLEUKIN-1α PRODUCTION

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SUMMARY: Several recent studies indicate that interleukin-1 (IL-1) may be one of the major regulators of spermatogenesis. In the present work, the effects of various agents on rat Sertoli cell IL-1 production were investigated *in vitro* at different ages. In control cultures the IL-1 production by Sertoli cells from immature rats (20 days) was barely detectable; it markedly increased with the advancing age of the donors (35 and 45 days). Lipopolysaccharide and latex beads, two inducers of monocyte-macrophage IL-1 production, were able to stimulate the release of IL-1 by Sertoli cells at all ages studied; a decrease in the relative response to these inducers was noted as the age of the Sertoli cell donors increased. Under all the experimental conditions tested it appeared that the IL-1 produced was IL-1α, not IL-1ß. Whereas pachytene spermatocytes and early spermatids had no effect on IL-1α production, residual bodies/cytoplasts from elongated spermatids dramatically stimulated Sertoli cell IL-1 production. In addition, FSH had no effect on IL-1α levels. It is concluded that Sertoli cell IL-1 secretion can be stimulated *in vitro* by well known monocyte-macrophage activators. Furthermore, Sertoli cell IL-1 production is most probably crucially dependent on the phagocytosis of residual bodies *in vivo*.

Sertoli cells are testicular somatic cells which synthetize and secrete factors that are essential for germ cell proliferation and differentiation such as energy metabolites, transport proteins and growth factors (1,2). Interleukin-1 (IL-1) has initially been described as a macrophage-derived protein and is now known to be synthetized by a large variety of cells (3-6). IL-1 is involved in a wide range of biological functions including cell proliferation and differentiation (3-6). Recent studies have identified an IL-1α-like factor in the rat and human testis (7-10) which originates from Sertoli cells (11). Of particular interest are the recent observations showing that: 1) the onset of Sertoli cell IL-1α production coincides with the onset of the meiotic process (9); 2) IL-1 receptors are localized on germ cells in the mouse

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Abbreviations used: IL-1, interleukin-1; IL-2, interleukin-2; LPS: lipopolysaccharide; SPC, pachytene spermatocytes; SPT, early spermatids; RB/CES, residual bodies/cytoplasts from elongated spermatids.

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testis (12) and 3) IL-1α has been shown to stimulate germ cell DNA synthesis (13-15). Therefore, Sertoli cell IL-1 may be a major regulator of spermatogenesis.

The production and the release of IL-1 by monocytes-macrophages is induced by agents which cause membrane perturbation and phagocytosis such as latex beads and by the bacterial wall component named lipopolysaccharide (LPS)(3-5,16,17). Similar to monocytes-macrophages, Sertoli cells are able to phagocytose latex beads (18); under physiological conditions they phagocytose degenerating germ cells, sperm and germ cell materials including the residual bodies which are the cytoplasmic fragments of late spermatids that are shed at the time of sperm release (2,19,20). Furthermore, in the rat testis, IL-1 production is very low at stage VII of the cycle of the seminiferous epithelium but rises markedly at stage VIII-IX (14), the time of residual bodies formation and phagocytosis (20,21). The aim of the present study was therefore, to investigate the involvement of phagocytosis and more specifically of phagocytosis of residual bodies, in the regulation of Sertoli cell IL-1 production *in vitro*. In addition to latex beads and elutriated residual bodies, the effects of FSH, LPS as well as of enriched populations of pachytene spermatocytes and early spermatids on Sertoli cell IL-1 release were also investigated.

MATERIALS AND METHODS

Sertoli cell isolation, culture and coculture. Sertoli cells were prepared from 20, 35 and 45-day-old Sprague-Dawley rats (Elevage Janvier, Le Genest Saint Isle, France) according to methods described elsewhere (22,23) and cultured at 32°C in a humidified atmosphere of 5% CO_2-95% air in Ham's F12/DMEM (v/v) containing gentamicin (4 μg/ml; products from Gibco Europe, Cergy Pontoise, France) supplemented with insulin (10 μg/ml; Sigma Chemical Co, St Louis, MO, USA) and transferrin (5 μg/ml; Sigma). After 2-3 days of culture, Sertoli cell monolayers from 20, 35 and 45-day-old donors were exposed to a Tris-HCl buffer solution (20 mM, pH 7.4) for 2.5 min and 15 min respectively, to remove contaminating germ cells (24). The day after, the cells were incubated for additional 24 h in medium where transferrin was omitted, with or without either lipopolysaccharide (LPS, *E.Coli* 0.26:B6; 10 and 50 μg/ml; Sigma), latex beads (0.4-7x10^8 latex beads/ml; Sigma), FSH (NIADDK-oFSH-16: 1-1000 ng/ml; gift from the NIH, Bethesda, MD, USA) or elutriated populations of germ cells (see below). The media were recovered, centrifuged and kept at -80°C until measurement of IL-1. The cells were scraped and frozen at -80°C until DNA content assay (25).

Germ cell isolation and coculture procedures. Germ cells were prepared from adult Sprague-Dawley rat testes by trypsinisation and submitted to a centrifugal elutriation as previously described (26). The three enriched fractions obtained (purity $\approx$ 80%), namely pachytene spermatocytes (SPC), early spermatids (SPT) and residual bodies/cytoplasts from elongated spermatids (RB/CES) were added on Sertoli cell monolayers at the density of 4x10^6/ml to assess the cellular specificity of IL-1 activation. The number of cells used in this study is in accordance with a previous study (27) demonstrating the paracrine effect of germ cells to Sertoli cells. In another set of experiment, in order to study the dose-response effect of RB/CES on Sertoli cell IL-1 release, 0.25, 0.5, 1, 2, 4 and 8x10^6/ml of RB/CES were added to 20-day-old rat Sertoli cells. Cocultures were run for 24 hours. Media were then collected, centrifuged to remove floating cells and kept at -80°C until IL-1 bioassay.

Bioassay of IL-1. IL-1 was measured in the culture media using the murine thymocyte proliferation assay (28), with minor modifications. Briefly, thymocytes (15x10^6 cells/ml) from 4-6 week old CH_3/He female mice (Elevage Janvier) were incubated in the presence of phytohemagglutinin (PHA, 20 μg/ml; Difco, Detroit, MI, USA), with various doses of human recombinant IL-1α (Boehringer Mannheim, Indianapolis, IN, USA) or test material, for 48 h at 37°C. During the last 2 h of incubation, 1μCi of [^{3}H]thymidine (Amersham,

Arlington Heights, IL, USA) was added. Incorporated radioactivity was determined in counts per minute by liquid scintillation counting. In these assay conditions, one unit (U) of IL-1 activity was defined as the ability of test material to double [^{3}H]thymidine incorporation into thymocytes, when compared with PHA stimulated cultures (8,9). This assay is known to detect IL-2 as well as IL-1α and IL-1ß (4). However, previous studies have shown that no IL-2 activity could be detected in testicular extracts nor in Sertoli cell culture media using a specific IL-2 bioassay (7,11). In order to determine the nature of the IL-1 produced by Sertoli cells, culture media were incubated at room temperature with either sheep anti-murine IL-1α or sheep anti-murine IL-1ß antibodies kindly provided by Dr S. Poole (NBSB, Hertfordshire, UK), for 1 hour, before to be assayed for IL-1 bioactivity.

Statistical analysis. Results were expressed as means ± S.E.M. of IL-1 units per μg of Sertoli cell DNA (U/μg DNA). Significance of difference was determined by the use of the analysis of variance and the Student's t test.

RESULTS

Effect of age on Sertoli cell IL-1α production

In confirmation to our previous study (11), results here show that IL-1 was undetectable or barely detectable in the media of Sertoli cells prepared from rats of 20 days of age and cultured under basal conditions, whereas an increase in the levels of this cytokine was observed as testicular maturation progressed on (Fig. 1A,B,D,F). At all ages studied the anti-IL-1α antibody blocked the IL-1 bioactivity, whereas the anti-IL-1ß antibody had no effect (Fig. 1B,D,F).

Effect of LPS on Sertoli cell IL-1α production

Lipopolysaccharide is a constituent of the wall of gram negative bacteria and well known inducer of IL-1 production by macrophages and other phagocytic cells (4,5). The control media containing LPS displayed no significant proliferative effect on thymocytes (data not shown). Addition of LPS to Sertoli cell monolayers resulted in a dose-dependent increase of IL-1 release at all ages studied (Fig. 1A,B). Furthermore, this study shows that the LPS-induced IL-1 bioactivity observed at all ages was significantly inhibited by the anti-IL-1α antibody but not by the anti-IL-1ß antibody (Fig. 1B).

Effect of latex beads on Sertoli cell IL-1α production

The phagocytosis of latex beads by macrophages is known to activate IL-1 transcription, translation and processing (5). When Sertoli cells from immature rats were cultured with increasing concentrations of latex beads, a dose-dependent stimulation of IL-1 release was observed (Fig. 1C). The plateau level was attained at a concentration of 1.75×10^8 latex beads per ml. Figure 1D indicates that latex beads also stimulated Sertoli cell IL-1 release at the three ages studied although, as observed with LPS, the factor of stimulation progressively declined as the age of the Sertoli cell donors increased. That the IL-1 produced after activation by latex beads was IL-1α, not IL-1ß, is evidenced by the fact that only the IL-1α antiserum blocked the bioactivity measured after latex beads phagocytosis (Fig. 1D).

Effect of RB/CES on Sertoli cell IL-1α production

In order to indentify a possible physiological activator of Sertoli cell IL-1 production, Sertoli

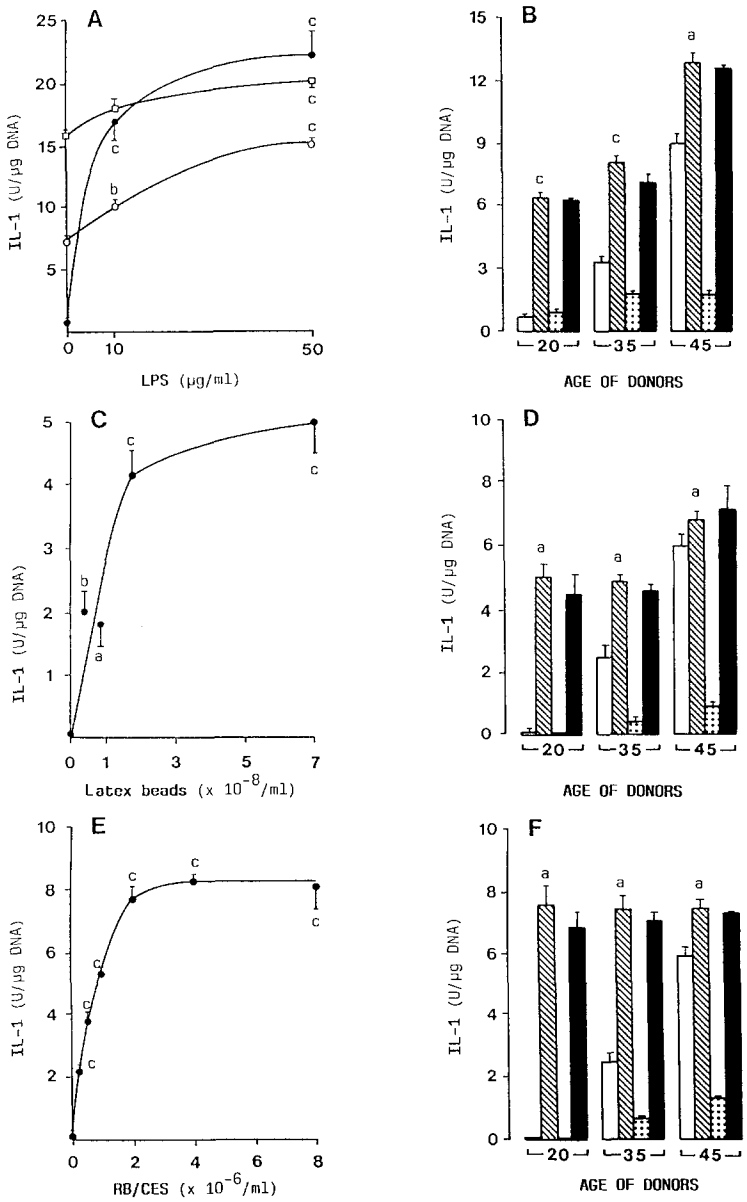

Figure 1. A-F: Effects of the addition of lipopolysaccharide (LPS), latex beads and residual bodies/cytoplasts from elongated spermatids (RB/CES) on IL-1 production by Sertoli cells isolated from 20 (●—●), 35 (○—○) and 45 (□—□)-day-old rats. In B, D, F are shown the effects of sheep anti-murine IL-1α (⚄) and of sheep anti-murine IL-1ß (■) antisera on the bioactivity of IL-1 secreted by Sertoli cells isolated from rats of 20, 35 and 45 days of age, after activation by LPS (10 μg/ml at 20 days, 50 μg/ml at 35 and 45 days of age), latex beads ($7x10^8$ per ml) and RB/CES ($4x10^6$ per ml), respectively (▨). □ : IL-1 secretion by Sertoli cells cultured under basal conditions. The data presented are representative of 2 to 4 experiments; values are means ± S.E.M (A: n = 6; B-F: n = 3); a: $p < 0.05$; b: $p < 0.005$; c: $p < 0.001$, compared to the basal IL-1 levels.

cells were cocultured with increasing concentrations of RB/CES. Figure 1E indicates that like LPS and latex beads, RB/CES activated in a dose-dependent fashion the IL-1 production by Sertoli cells collected from the younger donors. As little as $0.25x10^6$ RB/CES per ml were able to elicit a significant activation of Sertoli cells and the plateau was attained at the

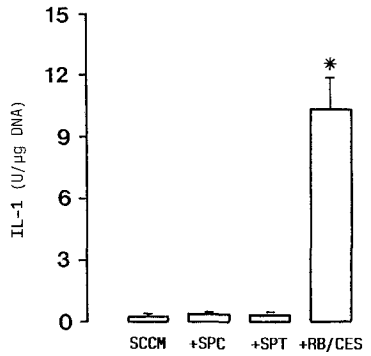

Figure 2. Effects of the addition of pachytene spermatocytes (+SPC), early spermatids (+SPT) and residual bodies/cytoplasts from elongated spermatids (+RB/CES) ($4x10^6$/ml for the 3 populations), on IL-1 production by Sertoli cells isolated from 20-day-old rats. SCCM: basal secretion of IL-1. Values are means ± S.E.M of 3 experiments (n=12); *: $p < 0.001$ when compared to the levels produced by Sertoli cells alone.

concentration of $2x10^6$ RB/CES per ml. A highly significant stimulation of IL-1 release in response to RB/CES was seen when Sertoli cells were prepared from 35 day-old donors (Fig. 1F). Though markedly reduced, the response of the older Sertoli cells to the presence of RB/CES was still significant (Fig. 1F). The RB/CES-stimulated IL-1 bioactivity was only inhibited by the IL-1α antiserum (Fig. 1F).

Cellular specifity of Sertoli cell RB/CES activation

In order to study the cellular specificity of the activation of IL-1 production, RB/CES, pachytene spermatocytes (SPC) and early spermatids (SPT) were cocultured with Sertoli cells from 20-day-old rats. Figure 2 indicates that contrarily to RB/CES which were very potent activators of IL-1, spermatocytes and spermatids did not significantly influence the release of this cytokine.

Effect of FSH on Sertoli cell IL-1 production

The addition of FSH (1-1000 ng/ml) to Sertoli cell cultures prepared from 20-day-old rats had no effect on IL-1 production (data not shown).

DISCUSSION

Testes of rats and humans produce large amounts of an interleukin-1α (IL-1α)-like factor (7-10). This study confirms our previous results showing that Sertoli cells are the source of the testicular IL-1 and that IL-1 production is a function of the age of the Sertoli cell donors (11). Previously it has been shown that Sertoli cells do not produce IL-2 and that the Sertoli cell IL-1 produced in culture was completely neutralized by an anti-IL-1α antiserum (11). The results of the present study also confirm the latter finding and indicate that Sertoli cells stimulated by LPS, latex beads or RB/CES only produce IL-1α.

Monocytes-macrophages are major IL-1 producers (3-6). Sertoli cell exhibits several characteristics typical of these cells, such as production of IL-1, enhancement of phagocytic

activity by tufsin and phagocytosis of various particles like latex beads (18). Keeping in view these observations, we investigated the effects of monocyte-macrophage inducers like LPS and latex beads and of phagocytosis of RB/CES, on Sertoli cell IL-1 production. Our results demonstrate for the first time that, like the monocyte-macrophage IL-1, Sertoli cell IL-1 can be activated by LPS and phagocytosis. The effects of LPS, latex beads and RB/CES were most striking when the cells were prepared from the younger animals (20 days). We consistently observed a progressive decrease in the responsiveness of Sertoli cells from older donors (35 and 45 days) to these agents. This is probably not due to an intrinsic reduction in the capability of Sertoli cells to respond to the activators. Sertoli cells prepared from 35 and 45-day-old donors may be activated *in situ* and therefore cannot be activated to a much greater extend *in vitro*. Furthermore, since germ cell contamination of Sertoli cells in culture markedly increase with the age of the donors (24), a further activation of the Sertoli cells may also results *in vitro* from the increase of the phagocytosis of degenerating germ cells. Of note is that the concentrations of LPS and of latex beads able to activate IL-1 production by Sertoli cells here are comparable to the ones that are active on murine and human monocytes-macrophages (3,16,17). Furthermore, in contrast to human monocytes-macrophages, Sertoli cells, at all ages and under the different conditions studied here, secrete IL-1α, not IL-1ß (4,5,17).

Binding and phagocytosis of residual bodies by Sertoli cells constitute one of the most striking aspect of Sertoli cell-late spermatid interrelationships (2). The regulation of Sertoli cell-residual bodies adhesive interaction is very complex and most probably involves specific spermatid membrane receptors concentrated on the residual body by selective partitioning of the spermatid plasma membrane and unique adhesion molecules expressed at the Sertoli-residual body interface (29,30). Pure residual body populations cannot be prepared *in vitro* (26,31). However, in a previous study we have demonstrated that the mixture of residual bodies and of cytoplasts from elongated spermatids at steps 10-19 of spermiogenesis obtained by centrifugal elutriation (the RB/CES fraction) bind very well to Sertoli cells *in vitro* (27,31) and that the different steps of their disposal by Sertoli cells *in vitro* are identical to the ones of residual bodies *in vivo* (31). Therefore, they can be used to study Sertoli cell-residual body interactions *in vitro*. Our previous experiments have shown that these structures had no effect *in vitro*, on Sertoli cell transferrin, inhibin and estradiol productions and only a marginal effect on ABP levels whereas pachytene spermatocytes and early spermatids either markedly stimulated (ABP, transferrin and inhibin) or inhibited (estradiol) these Sertoli cell parameters (27,32-34). This constrasts to the marked and specific stimulatory effect of RB/CES on IL-1α production observed in this study. IL-1 activity was found to be undetectable in tubular segments at stage VII of the seminiferous epithelium cycle in the rat, whereas maximum levels were reached at stages VIII and IX-X (14), which coincides with the release of spermatozoa and the phagocytosis of residual bodies *in vivo* (20,21). Our results showing that, *in vitro*, RB/CES activate or stimulate IL-1 release, strongly suggest that phagocytosis of residual bodies is a very potent regulatory mechanism by which IL-1α production is controlled in the seminiferous epithelium.

At present, the role of the testicular IL-1 is unknown. There are however indications that it may be mitogenic for germ cells: 1) administration of IL-1α to hypophysectomized rats may stimulate both mitotic and meiotic DNA synthesis (13); 2) levels of testicular IL-1 correlate with DNA synthetic activity during the cycle of the rat seminiferous epithelium (14,15); 3) *in vitro*, IL-1α also stimulates DNA synthesis in spermatogonia (stage I) and stimulates meiotic DNA synthesis at stages VII and stages VIII-IX respectively (15). These data are compatible with the localization of IL-1 receptors at the level of germ cells in the seminiferous epithelium (12). Therefore, an exciting hypothesis is that phagocytosis of residual bodies would trigger IL-1α production; IL-1α would then stimulates meiotic DNA synthesis in preleptotene spermatocytes. Further experiments are underway to explore this possible system of coordination of the spermatogenetic process.

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