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Tumor Necrosis Factor-α Induces Interleukin-6 Production and Integrin Ligand Expression by Distinct Transduction Pathways*

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Tumor necrosis factor-α (TNF-α) is a pleiotropic cytokine that elicits a large number of biological effects. However, the intracellular signaling mechanisms that are responsible for the TNF-α effects remain largely unknown. We have previously demonstrated that cultured mouse Sertoli cells, after TNF-α treatment, increase the surface expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) and interleukin-6 (IL-6) production (Riccioli, A., Filippini, A., De Cesaris, P., Barbacci, E., Stefanini, M., Starace, G., and Ziparo, E. (1995) *Proc. Natl. Acad. Sci. U.S.A.* 92, 5808–5812). Here, we show that, in cultured Sertoli cells, TNF-α activates the mitogen-activated protein kinase pathway (p38, c-Jun N-terminal protein kinase/stress-activated protein kinase, and the p42/p44 mitogen-activated protein kinases) as revealed by an increased phosphorylation of p38, activating transcription factor-2, c-Jun, and Elk-1. Furthermore, our data indicate that the biological effects induced by TNF-α in Sertoli cells (enhancement of ICAM-1, VCAM-1, and IL-6 expression) depend on the activation of different signaling pathways. SB203580, a highly specific p38 inhibitor, does not affect ICAM-1 and VCAM-1 expression, but strongly inhibits IL-6 production. Moreover, interferon-γ, which up-regulates adhesion molecule expression and reduces IL-6 production, does not induce phosphorylation of p38. Our data strongly support the hypothesis that, in response to TNF-α, activation of p38 leads to IL-6 production, whereas ICAM-1 and VCAM-1 expression could be induced by activation of the c-Jun N-terminal protein kinase/stress-activated protein kinase pathway.

TNF-α[1] has long been known to be a potent mediator of inflammation (1). Even though much information is known about the TNF-α receptors and how this cytokine interacts with them, there is limited knowledge regarding the signal transduction mechanisms involved (2).

We have previously demonstrated that cultured mouse Sertoli cells treated with proinflammatory cytokines, including TNF-α, undergo an increase in surface expression of adhesion molecules (ICAM-1 and VCAM-1) and IL-6 production (3). IL-6 is one of the most potent cytokines known to activate T and B lymphocytes (4, 5), and the deregulation of IL-6 gene expression may be involved in the pathogenesis of autoimmune diseases (6–8). The response of Sertoli cells to inflammatory factors could therefore contribute to the activation of lymphocytes and to their migration through the blood-tubular barrier to reach autoantigenic germ cells, resulting in the insurgence of autoimmune disorders (3, 9).

In the testis, TNF-α is known to be produced by germ cells (round spermatids) (10) and to affect Sertoli cell activity (11) by binding to the p55 receptor (12). However, little definitive data have come forth delineating the post-receptor molecular events. TNF receptors do not contain an intrinsic kinase domain, and recently, the involvement of serine/threonine protein kinases, including the so-called mitogen-activated protein kinases (MAPKs), has been proposed by a number of investigators (13–17). MAPKs form a large family of serine/threonine protein kinases activated by separate cascades and are important mediators of signal transduction from the cell surface to the nucleus. In mammalian cells, three distinct and parallel MAPK cascades have been identified: p42/p44 MAPKs (18, 19), p38 (20–23), and JNK/SAPK (24, 25). The activation of p42/p44 MAPKs constitutes a crucial step in the pathway mediating cell proliferation in response to growth factors (26–28), whereas p38 kinase and JNK/SAPK mediate signals in response to cytokines and environmental stress such as heat shock, osmotic stress, and UV light (20–22, 24, 25, 29). The fact that these biological responses are distinct implies that the specificity of external stimuli has to be maintained throughout each of the cascades. This specificity can result from selective phosphorylation (hence activation) operated by an upstream kinase (MEK1 (MAPK/extracellular signal-regulated kinase kinase) or MEK2 for p42/p44 MAPKs, MKK3/MKK6 (MAPK kinase) for p38, and MKK4/MKK7 for JNK/SAPK). To elucidate the biological function of the different MAPKs and/or p38, PD98059 and SB203580 have been used as specific inhibitors of MEK1 and of the activity of p38, respectively (recently reviewed in Ref. 30). In this report, we demonstrate that, in mouse Sertoli cells, TNF-α induces IL-6 production and ICAM-1/VCAM-1 expression by the simultaneous activation of two distinct signal transduction pathways, p38 and JNK/SAPK, respectively.

EXPERIMENTAL PROCEDURES

Materials—DNase, collagenase, recombinant murine TNF-α, and murine IFN-γ were purchased from Boehringer Mannheim (Mannheim, Germany). Trypsin was from Difco. The pyridinylimidazole SB203580

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[1] The abbreviations used are: TNF-α, tumor necrosis factor-α; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; IL-6, interleukin-6; MAPK, mitogen-activated protein kinase; JNK/SAPK, activated c-Jun N-terminal protein kinase/stress-activated protein kinase; IFN-γ, interferon-γ; ATF-2, activating transcription factor-2; HMA, herbimycin A.

was from SmithKline Beecham and was dissolved in Me_2SO. PD98059 was purchased from New England Biolabs Inc. and was dissolved in Me_2SO.

Sertoli Cell Cultures—Sertoli cells were prepared from CD1 mice as described previously (31). Briefly, testes from 15-day-old animals were sequentially digested for 20 min, first with Hanks' solution containing 0.25% trypsin + 10 μg/ml DNase and then with Hanks' solution supplemented with 0.1% collagenase + 10 μg/ml DNase, to remove interstitial tissue and peritubular cells. Fragments of seminiferous epithelium, mainly composed of Sertoli cells, were cultured at 32 °C in 95% air and 5% CO_2 in serum-free minimum Eagle's medium (Gibco-BRL, Paisley, Scotland). After 3 days, Sertoli cell monolayers were incubated at room temperature with 20 mM Tris-HCl buffer (pH 7.4) for 2 min to remove residual germ cells present in the culture (32). Sertoli cell cultures were routinely checked for possible contamination by macrophages and peritubular myoid cells by indirect immunofluorescence with anti-macrophage monoclonal antibody (Mac-1 antigen CD11/b, Boehringer Mannheim) and by histochemical detection of alkaline phosphatase activity (33).

On the fourth day of culture, Sertoli cell monolayers were treated with recombinant murine TNF-α or IFN-γ. At the indicated times, Sertoli cell-conditioned media were collected and frozen (−20 °C) before measurement of IL-6, while the cells were analyzed for ICAM-1 and VCAM-1 expression by flow cytometric analysis or were lysed for Western blotting and immunoprecipitation experiments.

Flow Cytometry—Control and treated Sertoli cells were detached with 0.02% EDTA and washed with cold phosphate-buffered saline + 1% bovine serum albumin. For detection of adhesion molecules on the cell surface of Sertoli cells, the following monoclonal antibodies were used: fluorescein isothiocyanate-conjugated hamster IgG anti-mouse CD54 (ICAM-1) or fluorescein isothiocyanate-conjugated rat IgG_{2a} anti-mouse VCAM-1 (INCAM-110) (Pharmingen, San Diego, CA). Specific monoclonal antibodies or the appropriate isotypic control monoclonal antibodies were used at 1 μg/10^6 cells for 30 min on ice. Cells were then washed twice with phosphate-buffered saline + 1% bovine serum albumin and analyzed with a FACSTAR flow cytometer (Becton Dickinson Labware). Cells were gated using forward *versus* side scatter to exclude dead cells and debris. The fluorescence of 10^4 cells/sample was acquired in logarithmic mode for visual inspection of the distributions and in linear mode for quantitating the expression of the relevant molecules by calculating the mean fluorescence intensity.

Assay for IL-6 Activity—Supernatants from mouse Sertoli cells untreated or treated with TNF-α or SB203580 ± TNF-α were assayed for IL-6 activity. IL-6 was measured using a B9 cell hybridoma growth factor assay (34) in which Sertoli cell-conditioned media were used to supplement the IL-6-dependent B9 cell line (kindly provided by Dr. Lucien Aarden, Central Laboratory of the Netherlands Red Cross, Amsterdam). The proliferative response of B9 cells to IL-6 is expressed relative to a standard curve with known amounts of IL-6. One unit of IL-6 is defined as the reciprocal of the dilution giving 50% maximal stimulation of proliferation.

B9 proliferation was measured using the Biotrak cell proliferation enzyme-linked immunosorbent assay system (Amersham Pharmacia Biotech), which is based on the incorporation of 5-bromo-2′-deoxyuridine into DNA of proliferating cells, followed by the addition of peroxidase-labeled anti-5-bromo-2′-deoxyuridine antibodies. Briefly, 5×10^3 B9 cells in 100 μl of complete RPMI 1640 medium (Gibco-BRL) supplemented with 10% fetal calf serum were plated onto a 96-well microtiter plate and incubated at 37 °C in the presence of serial dilutions of Sertoli cell supernatants or recombinant mouse IL-6 (Genzyme Corp., Cambridge, MA). 48 h later, treatment with 5-bromo-2′-deoxyuridine was performed for 16 h. After fixation, peroxidase-labeled anti-5-bromo-2′-deoxyuridine antibodies were added. The immunocomplexes were detected by incubation with a chromogenic substrate (as indicated in the manufacturer's protocol), and the resultant color was read at 450 nm in a microtiter plate spectrophotometer. This assay was previously determined to be insensitive to TNF-α. All data points illustrated are the average of three wells and of three independent experiments.

Western Immunoblotting—Total Sertoli cells lysates were prepared by lysing and scraping the cells off the culture plate with 10 mM Tris-HCl (pH 6.8), 0.4 mM EDTA, 2% SDS, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml antipain, 1 mM phenylmethylsulfonyl fluoride (Sigma), and the following phosphatase inhibitors: 10 mM sodium fluoride, 0.4 mM sodium orthovanadate, and 10 mM pyrophosphate. The nuclear fraction was prepared by hypotonic lysis with 10 mM Tris-HCl (pH 7.5) and protease and phosphatase inhibitors. After 5 min on ice, cells were homogenized with 20 strokes of a Dounce homogenizer and centrifuged for 7 min at 900 × g. The pellet (nuclei) was resuspended in 2% SDS. The protein concentration of each sample was determined using the micro-BCA method (Pierce).

Equal amounts of proteins (70 μg) were subjected to SDS-polyacrylamide gel electrophoresis and then transferred onto a nitrocellulose (Hybond C, Amersham Pharmacia Biotech) or polyvinylidene difluoride (Immobilon-P, Millipore Corp.) membrane depending on the primary antibody used. When anti-phosphotyrosine monoclonal antibodies (Upstate Biotechnology, Inc.) were used, the nitrocellulose was saturated with 3% gelatin in Tris-buffered saline; otherwise, nonfat dry milk was used.

Phospho-specific anti-p38 and phospho-specific anti-ATF-2 antibodies were purchased from New England Biolabs Inc. After the first and second antibodies, the membranes were washed three times for 15 min with Tris-buffered saline + 0.05% Tween. The secondary antibodies were horseradish peroxidase conjugates (Zymed Laboratories, Inc.), and detection was performed using the chemiluminescence system (ECL, Amersham Pharmacia Biotech).

Immunoprecipitation—For the immunoprecipitation experiments, the lysates were prepared in 20 mM Tris-HCl (pH 7.5), 0.15 M NaCl, 0.5% Triton X-100, 1 mM $MgCl_2$, and 1 mM $CaCl_2$ supplemented with protease and phosphatase inhibitors. 4 μg of anti-phosphotyrosine monoclonal antibody were added to 1 mg of cell lysates and incubated overnight at +4 °C. The samples were challenged with 50 μl of protein G-Sepharose beads (Amersham Pharmacia Biotech) for 2 h at +4 °C and subsequently microcentrifuged. The beads were washed three times with ice-cold lysis buffer and then resuspended in Laemmli sample buffer. The samples were boiled for 5 min and run on a 12% SDS-polyacrylamide gel. The proteins were transferred onto nitrocellulose, and the membrane was incubated with anti-p38 antibody (C-20, Santa Cruz Biotechnology). Detection was performed using the chemiluminescence system.

JNK/SAPK Kinase Assay—This assay was performed using the JNK/SAPK assay kit purchased from New England Biolabs Inc. and following the manufacturer's instructions. The kit employs an N-terminal c-Jun bound to Sepharose to selectively "pull down" JNK/SAPK from the cell lysate, after which the kinase reaction is carried out in the presence of unlabeled ATP (24, 25). c-Jun phosphorylation is selectively measured using phospho-specific c-Jun antibodies.

The cell lysates were prepared from Sertoli cells treated for 10, 16, and 25 min with TNF-α. The kinase reaction was terminated by the addition of 3× SDS sample buffer, and the samples were run on a 12% SDS-polyacrylamide gel, followed by transfer onto a polyvinylidene difluoride membrane. The membrane was subsequently incubated with phospho-c-Jun antibodies and then with secondary horseradish peroxidase-conjugated anti-rabbit antibody, and finally, chemiluminescence detection was performed with LumiGLO provided with the kit.

MAPK Assay—This assay was performed using the MAPK assay kit purchased from New England Biolabs Inc. Briefly, phospho-specific antibodies to MAPKs were utilized to selectively immunoprecipitate active MAPKs from Sertoli cell lysates under control conditions after treatment for 25 min with TNF-α or with TNF-α + PD98059. PD98059 at 75 and 25 μM was added 1 h before TNF-α treatment. The resulting immunoprecipitate was then incubated with an Elk-1 fusion protein in the presence of ATP, which allows immunoprecipitated active MAPK to phosphorylate Elk-1 (35, 36). The kinase reaction was terminated by the addition of 3× SDS sample buffer, and the samples were run on a 12% SDS-polyacrylamide gel, followed by transfer onto a polyvinylidene difluoride membrane. The membrane was subsequently incubated with phospho-Elk-1 antibodies and then with secondary horseradish peroxidase-conjugated anti-rabbit antibody, and finally, chemiluminescence detection was performed with LumiGLO provided with the kit.

RESULTS

TNF-α Induces Phosphorylation of p38 and ATF-2—To investigate the possible involvement of tyrosine phosphorylation in the signal transduction mechanisms utilized by TNF-α in Sertoli cells, we assayed the effect produced by the protein-tyrosine kinase inhibitors herbimycin A (HMA) and genistein on TNF-α-induced ICAM-1 and VCAM-1 expression.

As shown in Fig. 1*A*, HMA reduced, in a dose-dependent manner, the expression of both adhesion molecules, whereas genistein was ineffective on VCAM-1 expression and positively up-regulated ICAM-1 (Fig. 1*B*). The latter result is in agreement with the data reported by Tiisala *et al.* (37) that show that pretreatment of endothelial cells with genistein, followed by TNF-α stimulation, induces ICAM-1 expression.

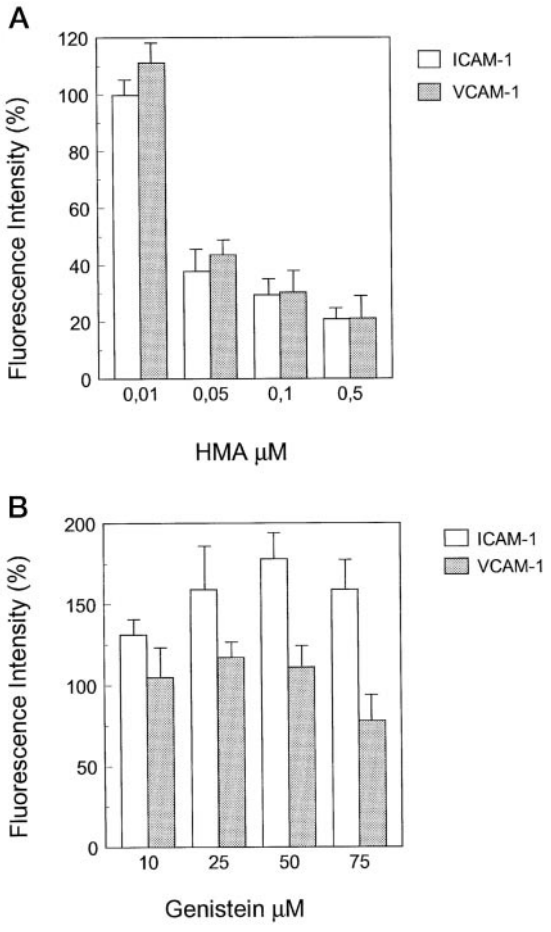

FIG. 1. **Flow cytometric analysis of the expression of ICAM-1 and VCAM-1 in mouse Sertoli cells treated with TNF-α after pretreatment with tyrosine kinase inhibitors.** Cells were preincubated for 24 h with the indicated concentrations of HMA (*A*) and genistein (*B*), and then TNF-α (20 ng/ml) was added for 16 h. The results are expressed as percent of mean fluorescence intensity of TNF-α-treated cells.

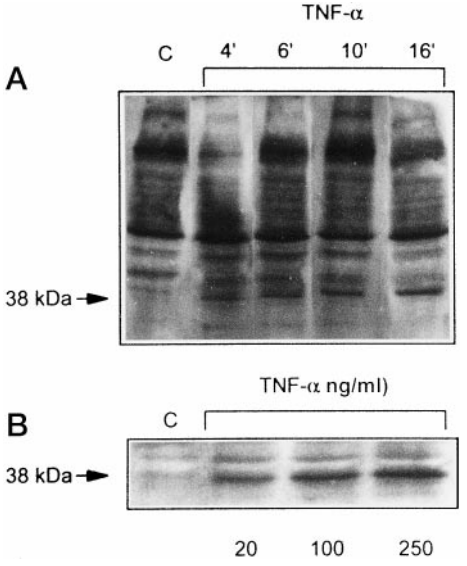

FIG. 2. **Anti-phosphotyrosine Western blot of Sertoli cell whole extracts.** Cells were treated with TNF-α for the indicated times (minutes) (*A*) and at the indicated doses (*B*). *C*, control.

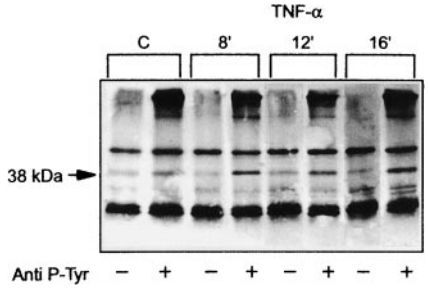

FIG. 3. **TNF-α up-regulates tyrosine-phosphorylated p38.** Sertoli cells lysates (1 mg) were immunoprecipitated with anti-Tyr(P) monoclonal antibodies. After SDS-polyacrylamide gel electrophoresis and Western blotting, the filter was incubated with specific antibodies against p38.

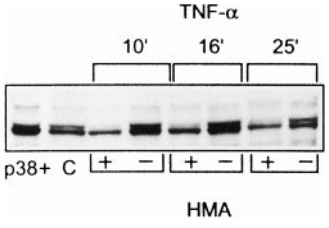

FIG. 4. **Effect of HMA on the phosphorylation of p38.** Shown are Western blots of Sertoli cell whole extracts treated for the indicated times (minutes) with 250 ng/ml TNF-α ± 0.5 μM HMA and immunoblotted with specific antibodies to the phosphorylated form of p38. Densitometric analysis revealed that, considering control (*C*) = 1, the relative phosphorylation was as follows: 10 min + HMA = 0.56, 10 min = 1.86, 16 min + HMA = 1.09, 16 min = 2.26, 25 min + HMA = 0.49, and 25 min = 1.14. *p38+* represents the phosphorylated protein from C6 glioma cells treated with anisomycin.

Such results prompted us to investigate the pattern of tyrosine phosphorylation in Sertoli cells stimulated for different times with TNF-α with or without HMA and genistein pretreatment. Using anti-phosphotyrosine antibodies in Western blotting, we demonstrated that TNF-α treatment was responsible for a rapid and dose-dependent tyrosine phosphorylation of a protein of ~38–40 kDa (Fig. 2, *A* and *B*). Immunoprecipitation with anti-Tyr(P) antibodies and subsequent blotting with anti-p38 antibodies demonstrated that TNF-α indeed induced the phosphorylation of p38 (Fig. 3). The definitive confirmation of this result was obtained using antibodies that specifically recognize p38 in the phosphorylated form. Fig. 4 shows that, under control conditions, Sertoli cells contained the phosphorylated form of p38 and that, after 10 min of TNF-α stimulation, the phosphorylation (hence the activation) of p38 significantly increased. Moreover, as shown in Fig. 4, HMA treatment greatly reduced the phosphorylation of p38.

Since the transcription factor ATF-2 is the substrate that is phosphorylated with great avidity by activated p38 and by JNK/SAPK (38), we analyzed the effect of TNF-α on ATF-2. Using specific anti-phospho-ATF-2 antibodies, we demonstrated that TNF-α induces the rapid phosphorylation of ATF-2 from Sertoli cell nuclear fractions and that the maximal effect is reached after 16 min (Fig. 5).

Activation of p38 Mediates IL-6 Production, but Not ICAM-1 and VCAM-1 Induction—Both TNF-α and IFN-γ induce ICAM-1 and VCAM-1 expression in Sertoli cells, whereas only TNF-α treatment increases the production of IL-6 (3). These data suggest that differential signal transduction pathways are involved in the different response of Sertoli cells to TNF-α. To clarify the role of p38 in the biological activity of TNF-α on Sertoli cells, we treated the cells with SB203580, a specific inhibitor of p38 kinase activity (20, 30, 39).

Fluorescence-activated cell sorting analysis demonstrated that neither ICAM-1 (Fig. 6) nor VCAM-1 (not shown) expression, induced by TNF-α, was affected by increasing doses of SB203580 (up to 20 μM). Conversely, under the same experi-

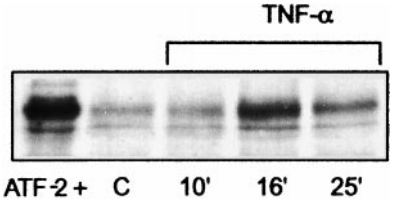

FIG. 5. **Time course of ATF-2 phosphorylation induced by TNF-α.** Sertoli cells were treated for the indicated times (minutes) with 250 ng/ml TNF-α. The antibody used is specific for the phosphorylated form of ATF-2. *ATF-2+* is the phosphorylated protein from NIH 3T3 cells treated with anisomycin.

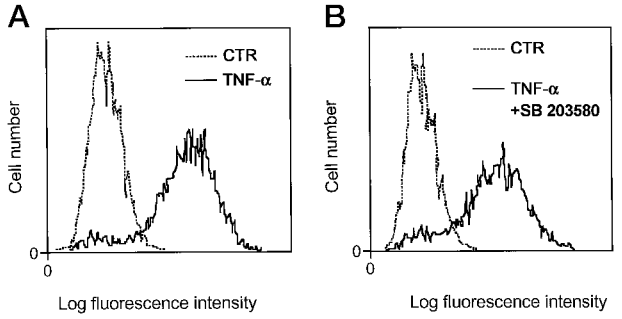

FIG. 6. **Flow cytometric analysis of the expression of ICAM-1.** Sertoli cells were treated with 50 ng/ml TNF-α for 18 h (*A*). 20 μM SB203580 was added 2 h before TNF-α treatment (*B*). *CTR*, control.

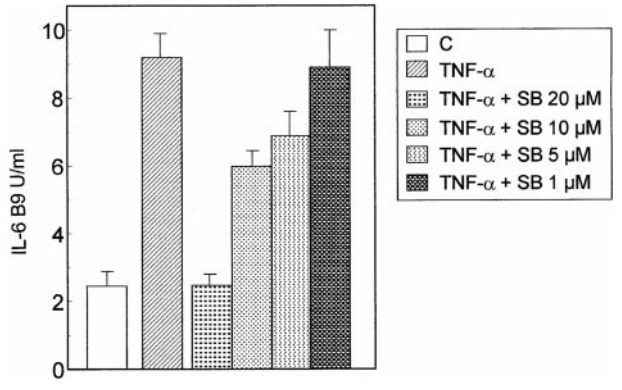

FIG. 7. **Effect of SB203580 on IL-6 production induced by TNF-α.** Sertoli cells were preincubated for 2 h with the indicated concentrations of SB203580 (*SB*) before the addition of 50 ng/ml TNF-α for 18 h. Conditioned media were assayed for IL-6 activity using the proliferative response of B9 hybridoma cells. Each point represents the mean of triplicate samples of at least three experiments.

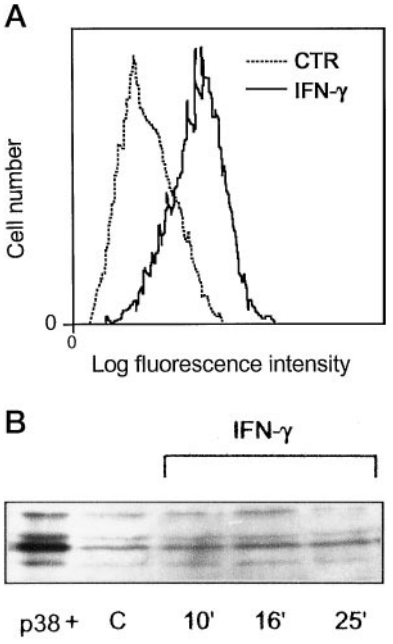

FIG. 8. **IFN-γ up-regulates ICAM-1 expression, but does not activate p38.** *A*, flow cytometric analysis of the expression of ICAM-1 in mouse Sertoli cells treated for 24 h with 500 units/ml IFN-γ; *B*, Western blot of Sertoli cell whole extracts treated for the indicated times (minutes) with 500 units/ml IFN-γ. The antibody used is specific for the phosphorylated form of p38. *p38+* is the phosphorylated protein from C6 glioma cells treated with anisomycin. *CTR* and *C*, control.

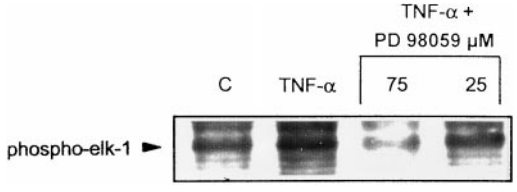

FIG. 9. **PD98059 inhibits p42/p44 MAPKs activated by TNF-α.** Sertoli cells were preincubated for 1 h with PD98059 at the indicated doses and then stimulated with TNF-α for 25 min. The activation of p42/p44 MAPKs was analyzed by a specific immunoprecipitation/kinase assay of its substrate (Elk-1) as described under "Experimental Procedures." Phosphorylation of Elk-1 was visualized by Western blotting using phospho-Elk-1 antibody. *C*, control.

mental conditions, the secretion of biologically active IL-6 was completely inhibited by SB203580 at 20 μM (Fig. 7). Moreover, IFN-γ treatment, which increased ICAM-1 expression (Fig. 8*A*), failed to activate the p38 pathway (Fig. 8*B*). We can therefore conclude that activation of the p38 pathway is involved in IL-6 production, but not in ICAM-1 and VCAM-1 expression.

TNF-α Activates p42/p44 MAPK and JNK/SAPK Signal Transduction Pathways—We analyzed whether, in Sertoli cells, TNF-α treatment induces the activation of the p42/p44 MAPK pathway using a MAPK assay. As shown in Fig. 9, TNF-α activated p42/p44 MAPKs, as demonstrated by the increased phosphorylation of its substrate, Elk-1. Pretreatment of Sertoli cells with 75 μM PD98059, a specific inhibitor of MEK1 (30, 40, 41), the upstream kinase that activates p42/p44 MAPKs, completely abolished the phosphorylation of Elk-1.

We therefore tested the effects of PD98059 on ICAM-1 and VCAM-1 expression and on IL-6 production. This treatment was without effect and failed to modify either adhesion molecule expression, as revealed by fluorescence-activated cell sorting analysis, or basal and stimulated IL-6 production. These data indicate that, although p42/p44 MAPKs are fully activated, they are not involved in the biological response of Sertoli cells to TNF-α.

We further investigated if, in Sertoli cells, TNF-α was able to activate the JNK/SAPK MAPK subtype, the activation of which leads to c-Jun phosphorylation. Using a JNK/SAPK assay, we measured the phosphorylation of c-Jun. Fig. 10 shows that SAPK-induced phosphorylation of c-Jun appeared after 16 min and increased strongly after 25 min of treatment with TNF-α.

We can therefore conclude that the p38 cascade is specifically involved in IL-6 production by Sertoli cells, as demonstrated by the fact that, following TNF-α treatment, stimulation of IL-6 secretion, but not of ICAM-1 and VCAM-1 expression, is prevented by the p38 kinase inhibitor SB203580. Moreover, treatment with IFN-γ, which induces ICAM-1 and VCAM-1 but not IL-6, does not affect p38 phosphorylation. In addition, the phosphorylation of c-Jun by TNF-α suggests the involvement of the JNK/SAPK pathway in the regulation of ICAM-1 and VCAM-1 in Sertoli cells.

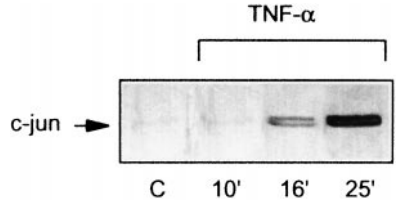

FIG. 10. **Activation of JNK/SAPK activity by TNF-α.** JNK/SAPK activity was evaluated by Western blot analysis using phospho-c-Jun antibody. TNF-α was added to Sertoli cells at 250 ng/ml for the indicated times (minutes).

DISCUSSION

Sertoli cells play an important role in the regulation of spermatogenesis, including its hormonal control (42, 43). Moreover, this cell type is implicated in the immune tolerance of testicular autoantigens, both by establishing the blood-testis barrier, which segregates autoantigenic germ cells, and by secreting immunosuppressive factor(s) into the interstitial environment (44, 45).

The insurgence of autoimmune disorders in the testis has been reported (46, 47). In these pathologic conditions, a massive intratubular leukocyte infiltration has been described that allows leukocytes to reach immunogenic germ cells after crossing the blood-testis barrier and thus overwhelming the immune tolerance system (48).

The inflammatory process implies a series of different steps: "rolling" of leukocytes on the endothelial wall, adhesion, and finally migration to the adjacent tissue. Leukocyte extravasation requires the progressive expression of various adhesion molecules on the surface of endothelial cells (49). Among these molecules, ICAM-1 and VCAM-1 are inducible by inflammatory stimuli also in a set of non-vascular cells (50, 51).

TNF-α is a proinflammatory cytokine, and we previously demonstrated that it is a strong inducer of surface expression of ICAM-1 and VCAM-1 and of IL-6 production by Sertoli cells (3). These responses lead to increased adhesion between lymphocytes and Sertoli cells. Our data suggest that the biological effects of TNF-α on Sertoli cells may play a pathogenic role in the outbreak of autoimmune disorders in the testis. However, the intracellular signaling pathways that bring about these responses are largely unknown.

In this work, we have reported the early biochemical events triggered by TNF-α in mouse cultured Sertoli cell. We have shown that TNF-α induces a rapid and time- and dose-dependent tyrosine phosphorylation of p38 kinase. Moreover, pretreatment with HMA, a powerful inhibitor of tyrosine kinases, significantly decreases both the expression of ICAM-1 and VCAM-1 and the phosphorylation of p38. However, treatment of Sertoli cells with SB203580, a specific inhibitor of p38 kinase, does not affect the expression of ICAM-1 and VCAM-1 induced by TNF-α, indicating that p38 is not involved in the signal transduction pathway that leads to this response. On the other hand, the same treatment completely inhibited IL-6 production induced by TNF-α, providing strong evidence that TNF-α regulates IL-6 production through the activation of p38, as already demonstrated in the L929 cell line (52).

Using the p38-specific inhibitor SB203580, we further demonstrated that, in Sertoli cells, TNF-α regulates the expression of surface adhesion molecules and the secretion of IL-6 through two different transduction pathways. Such conclusions are further confirmed by the fact that IFN-γ, an inducer of ICAM-1 and VCAM-1 but not of IL-6, fails to activate p38 in these cells.

We then performed experiments to investigate the involvement of p42/p44 MAPKs in the biological responses of Sertoli cells to TNF-α. TNF-α activates this pathway as demonstrated by the phosphorylation of Elk-1, a substrate of p42/p44 MAPKs. However, treatment with PD98059, a specific inhibitor of MEK1 (the upstream kinase that activates p42/p44 MAPKs), failed to interfere with adhesion molecule expression and with IL-6 production.

In conclusion, we have demonstrated that the biological responses of Sertoli cells to TNF-α are under dual control: the activation of p38 leads to IL-6 production, but neither p38 nor p42/p44 MAPKs regulate the induction of ICAM-1 and VCAM-1. In Sertoli cells, TNF-α also activates JNK/SAPK as demonstrated by the increased c-Jun phosphorylation. The activation of this pathway could be responsible for the up-regulation of ICAM-1 and VCAM-1 in Sertoli cells. The different regulation of the biological responses induced by TNF-α could be due to the differential recruitment of distinct signal transducer proteins such as TRADD (TNF receptor-1-associated death domain protein), TRAF2 (TNF receptor-associated factor), and RIP (receptor-interacting protein) (53). In this regard, it might be of interest to analyze the role of the kinase RIP, a proximal intermediate of TNF-α signaling, and its involvement in JNK/SAPK activation (54). The dual control of the effects induced by TNF-α in Sertoli cells could represent a useful experimental model to further dissect the different signal transduction pathways mediated by TNF receptors.

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