Activation of Jun N-terminal Kinase/Stress-activated Protein Kinase Pathway by Tumor Necrosis Factor α Leads to Intercellular Adhesion Molecule-1 Expression*

Paola De Cesaris‡, Donatella Starace, Giuseppe Starace§, Antonio Filippini, Mario Stefanini, and Elio Ziparo¶

From the Istituto Pasteur-Fondazione Cenci Bolognetti, Department of Histology and Medical Embryology, University of Rome "La Sapienza," 00161 Rome, Italy, the ‡Department of Experimental Medicine, University of L'Aquila, and §Institute of Experimental Medicine, CNR, Rome, Italy

Tumor necrosis factor α (TNF-α) is a cytokine implicated in the pathogenesis of numerous chronic and acute inflammatory conditions. We have previously shown that mouse Sertoli cells respond to TNF-α by increasing interleukin-6 production and intercellular adhesion molecule-1 (ICAM-1) expression (1). In this cell type TNF-α activates the mitogen-activated protein kinase (MAPK) pathways p42/p44 MAPK, JNK/SAPK, and p38, the last of which is responsible for interleukin-6 production (2). To determine which MAPK signaling pathway is required for TNF-α induction of ICAM-1 expression, we have utilized the protein kinase inhibitor dimethylaminopurine, demonstrating that treatment of Sertoli cells with such compound significantly reduced ICAM-1 expression and JNK/SAPK activation. Moreover, dimethylaminopurine treatment increased the expression of MAPK phosphatase-2, providing a possible mechanism of action of this compound. By using agonist antibodies to p55 and to p75 TNF-α receptors and both human and mouse TNF-α, we demonstrate that both TNF receptors are expressed and that only the p55 receptor is involved in ICAM-1 expression. The p55 receptor activates all of the three pathways, whereas p75 failed to activate any of the MAPKs. Altogether our results demonstrate that TNF-α up-regulates ICAM-1 expression through the activation of the JNK/SAPK transduction pathway mediated by the p55 receptor.

Mammalian cells respond to a broad variety of extracellular stimuli by activating protein kinase "cascades" that are involved in the amplification of the signal allowing a diverse array of cellular responses to take place. Tumor necrosis factor α (TNF-α)[1] is a potent multifunctional cytokine produced predominantly by activated macrophages (3) that induces many physiological effects on a wide variety of cells. Its involvement in the induction of an inflammatory state is well established and includes the induction of the expression of adhesion molecules (4–6). In endothelial cells the induction of ICAM-1, VCAM-1, and E-selectin has been reported to be regulated at the level of gene transcription and to require binding of the nuclear transcription factor κB (NF-κB) to the regulatory regions within the promoters of each of these genes (7–10).

The acquisition of surface adhesive properties by cells is important for inflammatory processes dependent on cell migration. Moreover, TNF-α alters the barrier function of endothelium by increasing the permeability of endothelial cells to macromolecules (11, 12).

Much information is known about the TNF-α receptors and how TNF-α interacts with them (13–15), but there is limited knowledge about the signal transduction mechanisms involved. The biological activities of TNF-α are mediated by two structurally related but functional distinct receptors, p55 and p75, belonging to the TNFR gene family (16–19).

TNF-α initiates its pleiotropic action by binding to either of two receptors that do not contain an intrinsic protein kinase activity. The two receptors are coexpressed on the surface of most cell types. The receptors are activated by the clustering induced upon binding of their respective oligomeric ligands. Surface-associated p75 is postulated to enhance p55-dependent responses by recruiting TNF-α to the cell membrane and passing the ligand to p55 according to the ligand-passing model (20). However, it has recently been demonstrated that ligand-passing is not the only mechanism for the enhancement of specific p55 responses by p75 but that partially overlapping intracellular signaling events are also triggered by both p55 and p75 (21).

The lack of an intrinsic kinase activity implies the interaction of TNF-R with accessory proteins that couple the receptor to signaling pathways. In mammalian cells three distinct and parallel so called mitogen-activated protein kinase (MAPK) cascades have been discovered: p42/p44 MAPKs (22, 23), p38 kinase (24–27), and JNK/SAPK (28, 29). So far at least 10 different protein kinases have been identified as members of such cascades (30). The large number of these enzymes and their overlapping specificities *in vitro* has made it extremely difficult to identify the physiological roles and the substrates of individual members. This problem is particularly relevant in the case of MAPKs and JNK/SAPK because they are activated by the same extracellular agonists and because they all phosphorylate serine and threonine residues that are followed by proline (31–33).

Sertoli cells, also known as "nurse cells" are responsible for the maintenance of the microenvironment in which postmeiotic development takes place and are the target for the hormones regulating spermatogenesis. In the testis TNF-α is known to be produced by germ cells (round spermatids) (34) and to affect

¶ To whom correspondence should be addressed: Dept. of Histology and Medical Embryology, University of Rome "La Sapienza," Via A. Scarpa 16-00161 Rome Italy. Tel.: 39-06-4976-6582; Fax: 39-06-446-2854; E-mail: ziparo@uniroma1.it.

[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, interleukin; JNK/SAPK, c-Jun N-terminal protein kinase/stress-activated protein kinase; MAPK, mitogen activated protein kinase; DMAP, dimethylaminopurine.

This paper is available on line at http://www.jbc.org

Sertoli cell activity (35). We have previously demonstrated that cultured Sertoli cells treated with TNF-α increase surface expression of adhesion molecules (ICAM-1 and VCAM-1) and IL-6 production (1).

More recently we have analyzed the intracellular signaling pathways that bring about these responses. We demonstrated (2) that in mouse Sertoli cells TNF-α rapidly (within minutes) induces the phosphorylation and hence the activation of the p38 kinase and of activating transcription factor-2. Moreover, it activates the p42/p44 MAPK pathway, as demonstrated by the increased phosphorylation of its substrate Elk-1 and JNK/SAPK as revealed by the phosphorylation of c-Jun. These data indicate that TNF-α activates all of the three parallel MAPK cascades. By using small cell-permeant compounds SB203580 (24, 30, 36) and PD98059 (30, 37, 38), which are specific inhibitors of p38 and p42/p44 MAPKs, respectively, we identified the biological role of these enzymes. We could in fact ascertain that, in Sertoli cells, the biological responses to TNF-α are subject to a dual control; the activation of p38 leads to IL-6 production, whereas neither p38 nor p42/p44 MAPKs regulate the induction of ICAM-1 and VCAM-1 (2).

In this study, by using the protein kinase inhibitor dimethylaminopurine (DMAP) (39, 40), we present evidence that further dissects the signal transduction pathway of TNF-α leading to ICAM-1 induction on Sertoli cells. Recently DMAP was identified as an useful reagent for the characterization of TNF signaling in endothelial cells. In these cells, in fact, TNF activates JNK/SAPK and ceramide-activated protein kinases and augments Jun-b expression. DMAP abrogates or attenuates these events without affecting TNF binding, suggesting that its effects result from action at post receptor sites (41). The ability of DMAP to affect signaling induced by TNF, but not by histamine, demonstrated that the effects of DMAP on the responses to TNF are specific (41). Here, we report in mouse Sertoli cells that DMAP inhibits the TNF-α up-regulation of ICAM-1 and the activation of JNK/SAPK, thereby providing strong evidence for the essential role of the JNK/SAPK pathway in the induction of ICAM-1 by TNF-α in these cells.

The DMAP pretreatment, followed by TNF-α exposure, significantly increased the expression of MKP-2, which is a dual specificity phosphatase that recognizes the homologous tripeptide phosphorylation sites required for activation of JNK/SAPK (42, 43). Finally we demonstrate that the effect of DMAP is specific because IFN-γ up-regulation of ICAM-1 is not affected by DMAP treatment.

Furthermore, by using agonist antibodies to the p55 and to the p75 TNF-α receptors, we demonstrate the involvement of only the p55 receptor in ICAM-1 up-regulation. These results are in agreement with data previously described, indicating that p55 is the primary signaling receptor through which the majority of inflammatory responses classically ascribed to TNF-α occur (44–46).

<center>EXPERIMENTAL PROCEDURES</center>

Materials—DNase, collagenase, recombinant murine, and human TNF-α were purchased from Roche Molecular Biochemicals, trypsin was from DIFCO (Detroit, MI), and DMAP was from Sigma.

Sertoli Cell Cultures—Sertoli cells were prepared from CD1 mice as described previously (47). 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 essential medium (Life Technologies, Inc.). 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 (48). Sertoli cell cultures were routinely checked for possible contamination by macrophages and peri-

tubular myoid cells by indirect immunofluorescence with anti-macrophage monoclonal antibody (Mac-1 antigen CD11/b, Roche Molecular Biochemicals) and by histochemical detection of alkaline phosphatase activity (49).

At the 4th day of culture, Sertoli cell monolayers were treated with 20 ng/ml murine TNF-α or 500 units/ml IFN-γ for 18 h with or without pretreatment of 15 min with different concentrations of DMAP. Parallel experiments were performed using human TNF-α or agonist monoclonal antibodies specific for the two receptors p55 and p75 at 10, 20, and 40 μg/ml (the antibodies were kindly provided by Dr. W. A. Buurman, University of Maastricht) as described in Refs. 50 and 51. At the indicated time Sertoli cells were analyzed for ICAM-1 expression by flow cytometric analysis or utilized for measuring JNK/SAPK activity.

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 ICAM-1 expression on Sertoli cell surface we used the fluorescein isothiocyanate-conjugated hamster IgG anti-mouse CD54 (ICAM-1) monoclonal antibody (Pharmingen, San Diego, CA). Specific monoclonal antibody or the appropriate isotypic control monoclonal antibody was 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. 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.

JNK/SAPK Kinase Assay—This assay was performed by using a JNK/SAPK assay kit purchased from New England Biolabs and following the manufacturer's instructions. The kit employs an N-terminal c-Jun bound to Sepharose to selectively "pull down" JNK/SAPK from cell lysate, after which the kinase reaction is carried out in the presence of cold ATP (28, 29). c-Jun phosphorylation is selectively measured using phospho-specific c-Jun antibodies.

The cell lysates were prepared from Sertoli cells pretreated for 15 min with 1 mM DMAP before addition of TNF-α. After 25 min the kinase reaction was terminated by the addition of 3× SDS sample buffer, and the samples were run in a 12% SDS-polyacrylamide gel electrophoresis followed by transfer onto nitrocellulose (Hybond C, Amersham Pharmacia Biotech). The membrane was subsequently incubated with phospho-c-Jun antibodies and then with secondary horseradish peroxidase-conjugated anti-rabbit antibody, and finally the chemiluminescence detection was performed with LumiGLO provided with the kit.

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, leupeptin, aprotinin, and antipain (10 μg/ml each); 1 mM phenylmethylsulfonyl fluoride (Sigma), and the following phosphatase inhibitors: 10 mM sodium fluoride, 0.4 mM sodium orthovanadate, and 10 mM pyrophosphate.

The protein concentration of each sample was determined by using the micro BCA method (Pierce). Equal amounts of proteins (70 μg) were subjected to SDS-polyacrylamide gel electrophoresis and then transferred onto nitrocellulose. The filters were saturated with 5% nonfat dry milk in Tris-buffered saline. Phospho-specific anti-p38 and phospho-specific anti-p42/p44 MAPK and phospho-specific anti-JNK/SAPK were purchased from New England Biolabs. Rabbit polyclonal antibodies against MKP-2 and p55 TNF-α receptor were from Santa Cruzx, and the antibody against the p75 TNF-α receptor was from HyCult Biotechnology. The secondary antibodies were horseradish peroxidase-conjugates (Zymed Laboratories Inc.). After the first and second antibodies, the membranes were washed three times for 15 min with Tris-buffered saline containing 0.05% Tween, and the detection was performed by using the chemiluminescence system (ECL, Amersham Pharmacia Biotech).

<center>RESULTS</center>

Activation of JNK/SAPK Pathway by TNF-α Leads to ICAM-1 Expression—Our previous results showed the involvement of p38 in the production of IL-6 by Sertoli cells in response to TNF-α treatment. Moreover by using specific inhibitors of p38 and p42/p44 MAPKs, we further demonstrated that neither p38 nor p42/p44 MAPKs take part in the induction of ICAM-1 (2). To investigate the mechanisms involved in this biological response of Sertoli cells to TNF-α, we utilized the protein kinase inhibitor DMAP to evaluate the possible involvement of JNK/SAPK. By flow cytometric analysis we ob-
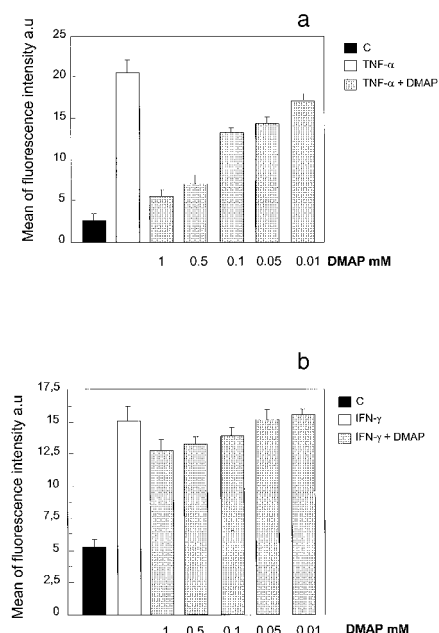
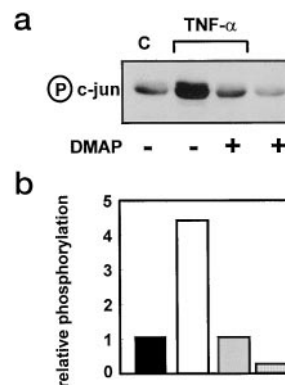FIG. 1. **Flow cytometric analysis of cell surface expression of ICAM-1 in mouse Sertoli cells treated with TNF-α or IFN-γ after pretreatment with DMAP.** Cells were preincubated for 15 min with different concentrations of DMAP and then 20 ng/ml TNF-α (*a*) or 500 units/ml IFN-γ (*b*) was added for 18 h. Immunofluorescence staining was performed with fluorescein isothiocyanate anti-ICAM-1 antibody. Control cells (*C*) are untreated Sertoli cells. Each point represents the mean of triplicate samples of at least three experiments. The *error bars* represent the S.E.


FIG. 2. **Activation of JNK/SAPK by TNF-α.** JNK/SAPK activity was evaluated by a specific immunoprecipitation/kinase assay as described under "Experimental Procedures." Phosphorylation of c-Jun was visualized by Western blot using phospho-c-Jun antibody. Sertoli cells were pretreated for 15 min with 1 mM DMAP, and TNF-α was added at 250 ng/ml for 25 min (*a*). The *bar graph* indicates the relative intensities of the signals (*b*). *Lane C*, control.
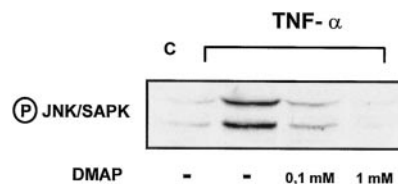
FIG. 3. **Effect of DMAP on JNK/SAPK phosphorylation.** Sertoli cells were pretreated for 15 min with 0.1 or 1 mM DMAP, and TNF-α was then added at 250 ng/ml for 30 min. Sertoli cell whole extracts were immunoblotted with antibodies specific for the phosphorylated form of JNK/SAPK. *Lane C*, control.

FIG. 4. **Effect of DMAP on MKP-2 expression.** Sertoli cells were pretreated for 15 min with 0.1 or 1 mM DMAP, and TNF-α was added at 250 ng/ml for the indicated times. Sertoli cell whole extracts were immunoblotted with antibodies against the phosphatase MKP-2. *Lane C*, control.

served that DMAP significantly inhibits ICAM-1 induction by TNF-α (Fig. 1*a*). A relevant reduction of ICAM-1 expression is obtained at 0.05 mM DMAP, and 1 mM DMAP completely abolishes the effect of TNF-α. In contrast all the concentrations of DMAP tested failed to inhibit ICAM-1 up-regulation induced by IFN-γ (Fig. 1*b*), indicating that the effect of DMAP on the biological response to TNF-α is specific. Cell viability after exposure to DMAP was checked by the trypan blue dye exclusion test and was found to be not affected even at 1 mM DMAP.

Parallel experiments were performed to analyze the possible involvement of the JNK/SAPK cascade. By using an *in vitro* kinase assay, we evaluated the activation of JNK/SAPK by measuring the phosphorylation of c-Jun, which is a substrate that is phosphorylated by activated JNK/SAPK. Treatment of Sertoli cells for 25 min with 250 ng/ml of TNF-α significantly increases the degree of phosphorylation of c-Jun. Pretreatment for 15 min with 1 mM DMAP completely inhibits the described effect (Fig. 2*a*). Densitometric analysis revealed that TNF-α induces a 4-fold enhancement of the phosphorylation of c-Jun, whereas DMAP pretreatment reduced c-Jun phosphorylation down to the control level. DMAP alone reduces the basal phosphorylation of control condition (Fig. 2*b*). Moreover, DMAP (1 and 0.1 mM) completely abolishes the phosphorylation of JNK/SAPK as demonstrated by Western blot by using antibodies against the phosphorylated forms of JNK/SAPK (Fig. 3, *third* and *fourth lanes*), thus indicating that DMAP inhibits the upstream kinase that phosphorylates JNK/SAPK.
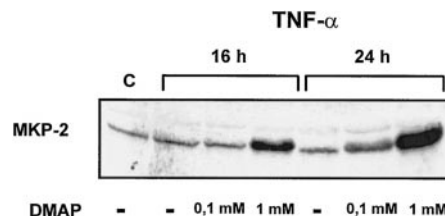
To investigate the possible involvement of the dual specificity phosphatases MKP in the modulation of JNK/SAPK activity by DMAP, we performed Western blotting experiments by using antibodies against the phosphatase MKP-1 and MKP-2. The results obtained are reported in Fig. 4, showing that DMAP significantly increases the expression of the phosphatase MKP-2, whereas the MKP-1 levels were not modified (not shown).

The p55 TNF-α Receptor Is Involved in ICAM-1 Up-regulation—The presence of the p55 TNF-α receptor on Sertoli cells has been described (34). As shown in Fig. 5 we confirm by Western blotting the expression of the p55 TNF-α receptor (Fig. 5*A*), and we demonstrate that Sertoli cells express the p75 TNF-α receptor (Fig. 5*B*).

In an effort to investigate which receptor is involved for the stimulation of ICAM-1 expression, we used the known differential binding characteristics of human and mouse TNF-α to the mouse TNF receptors. In fact, whereas mouse TNF-α binds both receptors, human TNF-α only binds to mouse p55 (52). Mouse Sertoli cells were therefore stimulated with human TNF-α or with mouse TNF-α, and then ICAM-1 expression was evaluated by flow cytometric analysis. The results obtained revealed that the activation of the p55 receptor by human TNF-α produces ICAM-1 induction to an extent similar to that obtained by the simultaneous triggering of both receptors by mouse TNF-α (Fig. 6). Furthermore we confirmed these results by using agonist antibodies directed respectively to p55 and to p75 TNF-α receptors. Flow cytometric analysis demonstrated that the antibody agonist for p55 receptor significantly up-
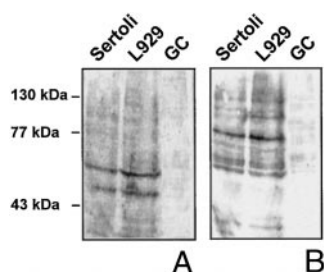
FIG. 5. **Expression of p55 and p75 TNF-α receptors on Sertoli cells.** Western analysis was performed by using specific antibodies against p55 (*a*) and p75 (*b*) TNF-α receptors. L929 (58) and germ cells (GC) (34) were used respectively as positive and negative control cells.
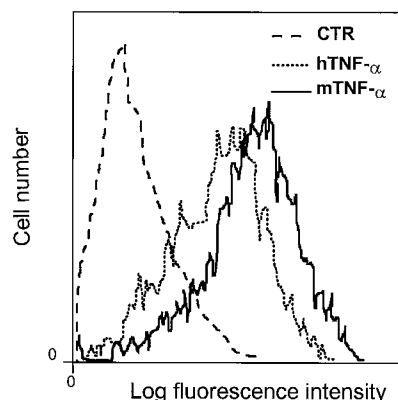

FIG. 6. **Flow cytometric analysis of cell surface ICAM-1 expression following stimulation through one or both TNF-α receptors.** Sertoli cells were treated with 50 ng/ml mouse TNF-α (*mTNF-α*) or human TNF-α (*hTNF-α*) for 18 h, and then immunofluorescence staining was performed with fluorescein isothiocyanate anti-ICAM-1 antibody. Control cells (*CTR*) are untreated Sertoli cells. The diagram is representative of four independent experiments.

regulates ICAM-1 expression, whereas the anti-p75 antibodies induced a barely detectable response. The combination of both antibodies did not produce a synergistic effect on ICAM-1 expression (Fig. 7). The concentration of agonist antibodies has been derived from previous dose-response experiments (data not shown). The effective concentrations, at the lower doses, was 20 μg/ml for p55. For the p75 receptor none of the concentrations tested (up to 40 μg/ml) was effective.

Signal Transduction Pathways of p55 and p75 TNF-α Receptors—To analyze the transduction pathways used by either TNF-α receptor and to establish which pathway is involved in ICAM-1 up-regulation, we stimulated Sertoli cells with agonist antibodies against the p55 or the p75 receptor. After treatment, total cell lysates were blotted with anti-phospho p42/p44 MAPK (Fig. 8*a*), anti-phospho p38 (Fig. 8*b*), and anti-phospho JNK/SAPK (Fig. 8*c*) antibodies. The results obtained show that the p55 receptor is able to activate all the MAPK signal transduction pathways, the optimal effect was observed at 20 μg/ml of agonist antibody. The p75 receptor is not involved in the activation of the kinase pathways analyzed (up to 40 μg/ml of agonist antibody). DMAP pretreatment inhibits the phosphorylation of p38 and of JNK/SAPK, whereas p42/p44 MAPK phosphorylation is enhanced. Taken together our data demonstrate the involvement of the JNK/SAPK pathway, through the p55 TNF-α receptor, in the induction of ICAM-1 in Sertoli cells by TNF-α.

DISCUSSION

Delineating the molecular events underlying the signal transduction pathways that link TNF-α receptors to down stream gene activation has become a major focus for research
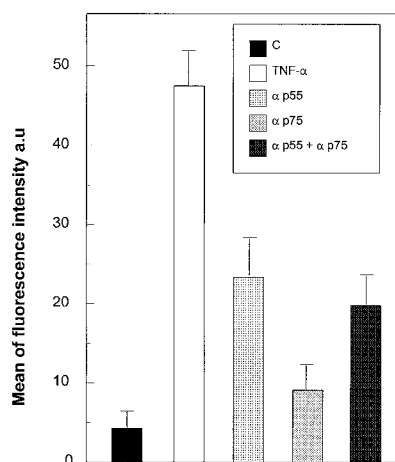

FIG. 7. **Flow cytometric analysis of cell surface ICAM-1 expression after stimulation with agonist antibody specific for either p55 and p75 TNF-α receptors.** Sertoli cells were treated with either or both the agonist and selective monoclonal antibodies for the p55 and p75 TNF receptors (20 μg/ml) for 18 h. Cells were reacted with fluorescein isothiocyanate anti-ICAM-1 antibody, and fluorescence intensity was measured with linear amplification.
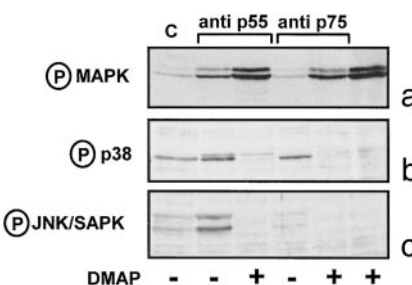

FIG. 8. **Activation of p42/p44 MAPK, p38, and JNK/SAPK by p55 and p75 TNF-α receptors.** Sertoli cells were treated for 25 min with agonist antibodies against the p55 or p75 TNF-α receptors at the concentration of 20 μg/ml. Where indicated 1 mM DMAP was added 15 min prior to stimulation. All the antibodies used were against the phosphorylated form of p42/p44 MAPK (*a*), p38 (*b*), and JNK/SAPK (*c*). *Lane C*, control.

efforts in the biology of TNF-α. Recent studies using molecular cloning with a yeast two-hybrid system have identified "adapter proteins" that associate with TNF receptors and are directly or indirectly involved in coupling receptors to different responses (16, 53–56). Specifically p55 receptor interacts with a protein called TNF receptor-associated death domain and with the receptor interacting protein, both receptors interacting with the TNF receptor-associated factor 1 and 2. The dissection of the different signal transduction pathways triggered by TNF-α is crucial to understand the regulation of the pleiotropic effects induced by this cytokine.

We have previously demonstrated that TNF-α induces IL-6 production and ICAM-1 expression by distinct transduction pathways. We have in fact shown that brief exposure (10–30 min) of Sertoli cells to TNF-α activates the three MAPK families: p42/p44 MAPKs, JNK/SAPK, and p38. Moreover, the biological responses of Sertoli cells to TNF-α are under a dual control; the activation of p38 leads to IL-6 production, whereas neither p38 nor p42/p44 MAPKs regulate the induction of ICAM-1 and VCAM-1 (2).

In the present study we have further characterized the signaling of TNF-α in Sertoli cells by demonstrating the involvement of the JNK/SAPK cascade in the up-regulation of ICAM-1. Recently the protein kinase inhibitor DMAP has been identified as an useful reagent for the characterization of TNF-α signaling in endothelial cells where it has been demon-

JNK/SAPK Activation by TNF-α Leads to ICAM-1 Expression

strated that the effects of DMAP on responses to TNF-α are specific (41). By using DMAP we have significantly reduced ICAM-1 induction by TNF-α but not ICAM-1 induction by IFN-γ. DMAP pretreatment completely abolished the TNF-α-induced phosphorylation of JNK/SAPK and of c-Jun, which is one of the targets of activated JNK/SAPK. DMAP treatment also negatively affected the phosphorylation of p38. However, the involvement of p38 in ICAM-1 induction can be ruled out because our previous results demonstrated that treatment with the p38-specific inhibitor SB203580 does not affect ICAM-1 expression (2). Moreover, DMAP pretreatment increased the expression of the dual specificity phosphatase MKP-2. These data could indicate a mechanism by which DMAP inhibits TNF-α -induced JNK/SAPK phosphorylation. Because MKP-2 is constitutively expressed in unstimulated Sertoli cells, it could be hypothesized that DMAP interferes with the degrading pathway of MKP-2, thereby allowing the accumulation of the phosphatase that we in fact observe starting from 4 h with a peak at 24 h from the beginning of the treatment.

TNF-α induces ICAM-1 up-regulation by activation of the p55 receptor. In fact by using the differential binding characteristics of human and mouse TNF-α to the mouse TNF receptors and by using agonist antibodies to the p55 and to the p75 receptors, we demonstrated the involvement of the p55 receptor in ICAM-1 induction. These data are in agreement with other groups (10, 52, 57) showing that the majority of TNF-α effects are mediated by the p55 receptor.

In conclusion we have defined a TNF-α signaling pathway involving the p55 receptor and JNK/SAPK leading to ICAM-1 induction. This is the first evidence of the involvement of the JNK/SAPK cascade in the modulation of the adhesion properties of a cell type under the control of the inflammatory cytokine TNF-α. An understanding of the signal transduction pathway triggered by inflammatory mediators may provide new targets for the modulation of the inflammatory process. In fact new antinflammatory therapies, based on the selective inhibition of a specific step among the multiple signaling transduction pathways activated by TNF-α could be designed to target the function of adhesion molecules in the extravasation process.

REFERENCES

1. 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
2. De Cesaris, P., Starace, D., Riccioli, A., Padula, F., Filippini, A., and Ziparo, E. (1998) *J. Biol. Chem.* **273,** 7566–7571
3. Tracey, K. J., and Cerami, A. (1993) *Annu. Rev. Cell Biol.* **9,** 317–343
4. Bevilacqua, M. P. (1993) *Annu. Rev. Immunol.* **11,** 767–804
5. Pober, J. S., Gimbrone, M. A. J., Lapierre, L. A., Mendrick, D. L., Fiers, W., Rothlein, R., and Springer, T. A. (1986) *J. Immunol.* **137,** 1893–1896
6. Pai, R., Bassa, B., Kirschenbaum, M. A., and Kamanna, V. S. (1996) *J. Immunol.* **156,** 2571–2579
7. Lewis, H., Kaszubska, W., DeLamarter, J. F., and Whelan, J. (1994) *Mol. Cell. Biol.* **14,** 5701–5709
8. Read, M. A., Whitley, M. Z., Gupta, S., Pierce, J. W., Best, J., Davis, R. J., and Collins, T. (1997) *J. Biol. Chem.* **272,** 2753–2761
9. Iademarco, M. F., McQuillan, J. J., Rosen, G. D., and Dean, D. C. (1992) *J. Biol. Chem.* **267,** 16323–16329
10. Hou, J., Baichwal, V., and Cao, Z. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 11641–11645
11. Worrall, N. K., Chang, K., LeJeune, W. S., Misko, T. P., Sullivan, P. M., Ferguson, T. B. J., and Williamson, J. R. (1997) *Am. J. Physiol.* **273,** 2565–2574
12. Curtis, T. M., Rotundo, R. F., Vincent, P. A., McKeown-Longo, P. J., and Saba, T. M. (1998) *Am. J. Physiol.* **275,** 126–138
13. Rothe, J., Gehr, G., Loetscher, H., and Lesslauer, W. (1992) *Immunol. Res.* **11,** 81–90
14. Tartaglia, L. A., and Goeddel, D. V. (1992) *Immunol. Today* **13,** 151–153
15. Bazzoni, F., and Beutler, B. (1996) *N. Engl. J. Med.* **334,** 1717–1725
16. Vandenabeele, P., Declercq, W., Beyaert, R., and Fiers, W. (1995) *Trends Cell Biol.* **5,** 392–399
17. Smith, C. A., Davis, T., Anderson, D., Solam, L., Beckmann, M. P., Jerzy, R., Dower, S. K., Cosman, D., and Goodwin, R. G. (1990) *Science* **248,** 1019–1023
18. Loetscher, H., Pan, Y. C., Lahm, H. W., Gentz, R., Brockhaus, M., Tabuchi, H., and Lesslauer, W. (1990) *Cell* **61,** 351–359
19. Schall, T. J., Lewis, M., Koller, K. J., Lee, A., Rice, G. C., Wong, G. H., Gatanaga, T., Granger, G. A., Lentz, R., and Raab, H. (1990) *Cell* **61,** 361–370
20. Tartaglia, L. A., Pennica, D., and Goeddel, D. V. (1993) *J. Biol. Chem.* **268,** 18542–18548
21. Weiss, T., Grell, M., Hessabi, B., Bourteele, S., Muller, G., Scheurich, P., and Wajant, H. (1997) *J. Immunol.* **158,** 2398–2404
22. Sturgill, T. W., and Wu, J. (1991) *Biochim. Biophys. Acta* **1092,** 350–357
23. Boulton, T. G., Nye, S. H., Robbins, D. J., Ip, N. Y., Radziejewska, E., Morgenbesser, S. D., DePinho, R. A., Panayotatos, N., Cobb, M. H., and Yancopoulos, G. D. (1991) *Cell* **65,** 663–675
24. Lee, J. C., Laydon, J. T., McDonnell, P. C., Gallagher, T. F., Kumar, S., Green, D., McNulty, D., Blumenthal, M. J., Heys, J. R., Landvatter, S. W., *et al.* (1994) *Nature* **372,** 739–746
25. Han, J., Lee, J. D., Bibbs, L., and Ulevitch, R. J. (1994) *Science* **265,** 808–811
26. Freshney, N. W., Rawlinson, L., Guesdon, F., Jones, E., Cowley, S., Hsuan, J., and Saklatvala, J. (1994) *Cell* **78,** 1039–1049
27. Rouse, J., Cohen, P., Trigon, S., Morange, M., Alonso Llamazares, A., Zamanillo, D., Hunt, T., and Nebreda, A. R. (1994) *Cell* **78,** 1027–1037
28. Derijard, B., Hibi, M., Wu, I. H., Barrett, T., Su, B., Deng, T., Karin, M., and Davis, R. J. (1994) *Cell* **76,** 1025–1037
29. Kyriakis, J. M., Banerjee, P., Nikolakaki, E., Dai, T., Rubie, E. A., Ahmad, M. F., Avruch, J., and Woodgett, J. R. (1994) *Nature* **369,** 156–160
30. Cohen, P. (1997) *Trends Cell Biol.* **7,** 353–361
31. Clark-Lewis, I., Sanghera, J. S., and Pelech, S. L. (1991) *J. Biol. Chem.* **266,** 15180–15184
32. Songyang, Z., Blechner, S., Hoagland, N., Hoekstra, M. F., Piwnica-Worms, H., and Cantley, L. C. (1994) *Curr. Biol.* **4,** 973–982
33. Songyang, Z., Lu, K. P., Kwon, Y. T., Tsai, L. H., Filhol, O., Cochet, C., Brickey, D. A., Soderling, T. R., Bartleson, C., Graves, D. J., DeMaggio, A. J., Hoekstra, M. F., Blenis, J., Hunter, T., and Cantley, L. C. (1996) *Mol. Cell. Biol.* **16,** 6486–6493
34. De, S. K., Chen, H. L., Pace, J. L., Hunt, J. S., Terranova, P. F., and Enders, G. C. (1993) *Endocrinology* **133,** 389–396
35. Mauduit, C., Jaspar, J. M., Poncelet, E., Charlet, C., Revol, A., Franchimont, P., and Benahmed, M. (1993) *Endocrinology* **133,** 69–76
36. Lee, J. C., and Young, P. R. (1996) *J. Leukocyte Biol.* **59,** 152–157
37. Pang, L., Sawada, T., Decker, S. J., and Saltiel, A. R. (1995) *J. Biol. Chem.* **270,** 13585–13588
38. Dudley, D. T., Pang, L., Decker, S. J., Bridges, A. J., and Saltiel, A. R. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 7686–7689
39. Schlegel, R., Belinky, J. S., and Harris, M. O. (1998) *Cell Growth Differ.* **1,** 171–178
40. Neant, I., and Guerrier, P. (1988) *Exp. Cell. Res* **176,** 68–79
41. Marino, M. W., Dunbar, J. D., Wu, L., Ngaiza, J. R., Han, H., Guo, D., Matsushita, M., Nairn, A., Zhang, Y., Kolesnick, R., Jaffe, E. A., and Donner, D. B. (1996) *J. Biol. Chem.* **271,** 28624–28629
42. Denu, J. M., Zhou, G., Wu, L., Zhao, R., Yuvaniyama, J., Saper, M. A., and Dixon, J. E. (1995) *J. Biol. Chem.* **270,** 3796–3803
43. Hirsch, D. D., and Stork, P. J. (1997) *J. Biol. Chem.* **272,** 4568–4575
44. Barbara, J. A., Smith, W. B., Gamble, J. R., Van Ostade, X., Vandenabeele, P., Tavernier, J., Fiers, W., Vadas, M. A., and Lopez, A. F. (1994) *EMBO J.* **13,** 843–850
45. Rothe, J., Lesslauer, W., Lotscher, H., Lang, Y., Koebel, P., Kontgen, F., Althage, A., Zinkernagel, R., Steinmetz, M., and Bluethmann, H. (1993) *Nature* **364,** 798–802
46. Erickson, S. L., de Sauvage, F. J., Kikly, K., Carver-Moore, K., Pitts-Meek, S., Gillett, N., Sheehan, K. C., Schreiber, R. D., Goeddel, D. V., and Moore, M. W. (1994) *Nature* **372,** 560–563
47. Kohno, S., Ziparo, E., Marek, L. F., and Tung, K. S. (1983) *J. Reprod. Immunol.* **5,** 339–350
48. Galdieri, M., Ziparo, E., Palombi, F., Russo, M. A., and Stefanini, M. (1981) *J. Androl.* **5,** 249–254
49. Palombi, F., and Di Carlo, C. (1988) *Biol. Reprod.* **39,** 1101–1108
50. Benigni, F., Faggioni, R., Sironi, M., Fantuzzi, G., Vandenabeele, P., Takahashi, N., Sacco, S., Fiers, W., Buurman, W. A., and Ghezzi, P. (1996) *J. Immunol.* **157,** 5563–5568
51. Leeuwenberg, J. F., van Tits, L. J., Jeunhomme, T. M., and Buurman, W. A. (1995) *Cytokine* **7,** 457–462
52. Tartaglia, L. A., Weber, R. F., Figari, I. S., Reynolds, C., Palladino, M. A., Jr., and Goeddel, D. V. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 9292–9296
53. Hsu, H., Xiong, J., and Goeddel, D. V. (1995) *Cell* **81,** 495–504
54. Rothe, M., Sarma, V., Dixit, V. M., and Goeddel, D. V. (1995) *Science* **269,** 1424–1427
55. Hsu, H., Huang, J., Shu, H. B., Baichwal, V., and Goeddel, D. V. (1996) *Immunity* **4,** 387–396
56. Hsu, H., Shu, H. B., Pan, M. G., and Goeddel, D. V. (1996) *Cell* **84,** 299–308
57. Slowik, M. R., De Luca, L. G., Fiers, W., and Pober, J. S. (1993) *Am. J. Pathol.* **143,** 1724–1730
58. Sheehan, K. C., Pinckard, J. K., Arthur, C. D., Dehner, L. P., Goeddel, D. V., and Schreiber, R. D. (1995) *J. Exp. Med.* **181,** 607–617

**Activation of Jun N-terminal Kinase/Stress-activated Protein Kinase Pathway by
Tumor Necrosis Factor α Leads to Intercellular Adhesion Molecule-1 Expression**

Paola De Cesaris, Donatella Starace, Giuseppe Starace, Antonio Filippini, Mario Stefanini
and Elio Ziparo

Access the most updated version of this article at http://www.jbc.org/content/274/41/28978

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 58 references, 24 of which can be accessed free at
http://www.jbc.org/content/274/41/28978.full.html#ref-list-1