Histamine Antagonizes Tumor Necrosis Factor (TNF) Signaling by Stimulating TNF Receptor Shedding from the Cell Surface and Golgi Storage Pool*

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Tumor necrosis factor (TNF) activates pro-inflammatory functions of vascular endothelial cells (EC) through binding to receptor type 1 (TNFR1) molecules expressed on the cell surface. The majority of TNFR1 molecules are localized to the Golgi apparatus. Soluble forms of TNFR1 (as well as of TNFR2) can be shed from the EC surface and inhibit TNF actions. The relationships among cell surface, Golgi-associated, and shed forms of TNFR1 are unclear. Here we report that histamine causes transient loss of surface TNFR1, TNF shedding, and mobilization of TNFR1 molecules from the Golgi in cultured human EC. The Golgi pool of TNFR1 serves both to replenish surface receptors and as a source of shed receptor. Histamine-induced shedding is blocked by TNF-α protease inhibitor, an inhibitor of TNF-α-converting enzyme, and through the H1 receptor via a MEK-1/p42 and p44 mitogen-activated protein kinase pathway. Cultured EC with histamine-induced surface receptor loss become transiently refractory to TNF. Histamine injection into human skin engrafted on immunodeficient mice similarly caused shedding of TNFR1 and diminished TNF-mediated induction of endothelial adhesion molecules. These results both clarify relationships among TNFR1 populations and reveal a novel anti-inflammatory activity of histamine.

The immunological and inflammatory capacities of vascular endothelial cells (EC) are activated in response to binding of homotrimeric TNF with cell surface receptors of 55 (TNFR1 or TNFR2) and 75 kDa (TNFR2 or CD 120b) kDa (1). TNF-α is the predominant receptor involved in new EC gene expression, although TNFR2 may increase the sensitivity of EC to TNF (2). New gene transcription results from activation of parallel signaling pathways involving several protein kinases, notably IkB kinase (IKK), various MAP kinases (including c-Jun N-terminal kinase, p42/44 MAP kinase and p38 MAP kinase), and protein kinase B (also known as Akt) (1). IKK is central to the TNF activation response because this kinase uniquely phosphorylates IkB proteins, such as IkBα, thereby releasing their degradation and thereby releasing sequestered transcription factor, NFκB (3). NFκB is essential for the transcription of almost all of the pro-inflammatory gene products induced by TNF. IKK activation through TNFR1 is initiated by recruitment of the adaptor protein TNF receptor-associated death domain-containing protein to the cytoplasmic death domain of the ligand-occupied receptor molecule. Although the majority of TNFR1 molecules are located within the Golgi apparatus, TNF receptor-associated death domain associates with surface-expressed but not Golgi-associated receptors (4, 5). The significance of the Golgi pool of TNFR1 molecules is unclear. One hypothesis is that it may act as a reservoir to increase surface receptor expression density, thereby sensitizing EC to the actions of TNF. There is precedence for this idea in smooth muscle cells, in which the TNF receptor family member Fas localizes predominantly to the Golgi, from where it can be translocated to the cell surface, thereby sensitizing cells to Fas ligand-induced killing (6).

Both types of TNF receptors can be released from the cell surface by the actions of a metalloproteinase called TNF-α-converting enzyme (TACE) (7). The shed extracellular domains of the receptors are soluble in water and are referred to as sTNFR1 or sTNFR2 (8). Receptor shedding, which can reduce the surface expression of TNFR1 and TNFR2, may desensitize cells to TNF actions. Additionally, because TNFRs maintain their ability to bind ligand, they may serve as physiological neutralizing agents for TNF (9, 10), further dampening inflammatory responses. This idea is supported by the observation that patients with structural mutations in TNFR1 that prevent shedding by TACE are hypersensitive to TNF (11). Thus a second potential function of the Golgi pool of TNFR1 molecules is to serve as a reservoir for sTNFR1, reducing EC responses. TACE, which was initially identified as pro-inflammatory because of its role in TNF secretion (12, 13), may be either pro-

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‡ The abbreviations used are: EC, endothelial cells; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor; TACE, TNF-α-converting enzyme; MAP, mitogen-activated protein; MEK, MAP kinase/extracellular signal-regulated kinase; PRC, protein kinase C; FACS, fluorescence-activated cell sorter; HUVEC, human umbilical vein EC; PMA, phorbol 12-myristate 13-acetate; PBS, phosphate-buffered saline; FCS, fetal calf serum; TBS, Tris-buffered saline; BSA, bovine serum albumin; RT, reverse transcriptase; gfp, green fluorescent protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IKK, IkB kinase; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; PIPES, 1,4-piperazinediethanesulfonic acid; CT, threshold cycle; CD120a or 75 (TNFR2 or CD 120b) kDa (1). TNFR1 is the predominant receptor involved in new EC gene expression, although TNFR2 may increase the sensitivity of EC to TNF (2). New gene transcription results from activation of parallel signaling pathways involving several protein kinases, notably IkB kinase (IKK), various MAP kinases (including c-Jun N-terminal kinase, p42/44 MAP kinase and p38 MAP kinase), and protein kinase B (also known as Akt) (1). IKK is central to the TNF activation response because this kinase uniquely phosphorylates IkB proteins, such as IkBα, thereby triggering their degradation and thereby releasing sequestered transcription factor, NFκB (3). NFκB is essential for the transcription of almost all of the pro-inflammatory gene products induced by TNF. IKK activation through TNFR1 is initiated by recruitment of the adaptor protein TNF receptor-associated death domain-containing protein to the cytoplasmic death domain of the ligand-occupied receptor molecule. Although the majority of TNFR1 molecules are located within the Golgi apparatus, TNF receptor-associated death domain associates with surface-expressed but not Golgi-associated receptors (4, 5). The significance of the Golgi pool of TNFR1 molecules is unclear. One hypothesis is that it may act as a reservoir to increase surface receptor expression density, thereby sensitizing EC to the actions of TNF. There is precedence for this idea in smooth muscle cells, in which the TNF receptor family member Fas localizes predominantly to the Golgi, from where it can be translocated to the cell surface, thereby sensitizing cells to Fas ligand-induced killing (6).

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LPR, late phase reaction; TAPI, TNF-α protease inhibitor; t-NMMA, NG'-monomethyl-L-arginine.
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Measurement of Cell Surface TNF Receptor Expression by Flow Cytometry—HUVEC were seeded into 6-well tissue culture plates (1.5 × 10⁶ cells per well), and 24 h later the confluent cells were treated with histamine 100 μM for 0.5–16 h. For experiments using brefeldin A (10 μg/ml) or TAPI (25 μM), EC were pretreated with either agent for half an hour before treatment with histamine. After each treatment, cells were harvested using a nonenzymatic cell suspension solution (EDTA in Hank’s balanced salt solution), washed twice with 1% FCS in PBS, and then incubated with primary antibody on ice for 40 min. Cells were then washed twice and incubated with secondary antibody for another 40 min on ice. EC were then washed three times and resuspended in 500 μl of 2% paraformaldehyde in PBS. Fixed cells were analyzed by flow cytometry using FACSCalibor machine (BD Biosciences). Data were analyzed using WinMDI software.

Detection of Soluble Receptors by ELISA—HUVECs grown to confluence in T75 flasks (3 × 10⁵) were washed twice in ice-cold PBS, pH 8.0. The membrane-impermeable biotinylation reagent, NHS-SS-Biotin was added to a final concentration of 0.5 mg/ml in PBS, and the cells were incubated at 4 °C for 30 min. The cells were then washed twice with ice-cold PBS and incubated with complete media at 37 °C for 15 min. Cells were then treated with 100 μM histamine or 0.1 μM PMA for 30 min. After treatment, the supernatants were removed, and the cells were then lysed using 25 mM Tris base, 150 mM NaCl, 1 mM Pefabloc, 1% Nonidet P-40, protein inhibitor mixture, 0.5 mM phenylmethylsulfonyl fluoride, and 25 μM TAPI for 30 min. Lysates were centrifuged at 10,000 rpm for 5 min, and the clarified supernatant was transferred to tubes containing Neutrividin beads. After incubation for 1 h the beads were centrifuged down and washed. The supernatant (cytosolic fraction) or beads (containing the biotinylated membrane proteins) were boiled in sample buffer (125 mM Tris/HC1, 15% sucrose, 4% SDS, 10 mM EDTA, 0.1 mg/ml bromphenol blue, 2% mercaptoethanol) for 3 min and analyzed by immunoblotting as described below.

IκB-α Degradation Assay—HUVECs were grown to confluence in 6-well plates and then treated with or without 100 μM histamine for various time points. The membrane-impermeable biotinylation reagent, NHS-SS-Biotin was added to a final concentration of 0.5 mg/ml in PBS, and the cells were incubated at 4 °C for 30 min. The cells were then washed twice with ice-cold PBS and incubated with complete media at 37 °C for 15 min. Cells were then treated with 100 μM histamine or 0.1 μM PMA for 30 min. After treatment, the supernatants were removed, and the cells were then lysed using 25 mM Tris base, 150 mM NaCl, 1 mM Pefabloc, 1% Nonidet P-40, protein inhibitor mixture, 0.5 mM phenylmethylsulfonyl fluoride, and 25 μM TAPI for 30 min. Lysates were centrifuged at 10,000 rpm for 5 min, and the clarified supernatant was transferred to tubes containing Neutrividin beads. After incubation for 1 h the beads were centrifuged down and washed. The supernatant (cytosolic fraction) or beads (containing the biotinylated membrane proteins) were boiled in sample buffer (125 mM Tris/HC1, 15% sucrose, 4% SDS, 10 mM EDTA, 0.1 mg/ml bromphenol blue, 2% mercaptoethanol) for 3 min and analyzed by immunoblotting as described below.

Protein concentration was determined using BCA protein assay kits (Pierce Biotechnology). Human recombinant TNF-α and human recombinant IL-1α were purchased from R&D Systems Europe (Abingdon, UK). Goat anti-mouse fluorescein isothiocyanate-conjugated antibody was from Dako (Glostrup, Denmark). Goat anti-human TACE antibody and rabbit anti-human IκB-α antibody were from Santa Cruz Biotechnology. Horse anti-goat and goat anti-rabbit horseradish peroxidase-conjugated antibodies were from Vector Laboratories and Bio-Rad, respectively. TNF-α protease inhibitor (TAPI), a specific inhibitor of TACE, was purchased from Peptides International (Louisville, KY). Proteinase inhibitor mixture was from Roche Diagnostics. The ECL system was from Amersham Biosciences. Bisindolylmaleimide, PD98059, and SB202810 were from Calbiochem. Sulfo-NHS-biotin and Neutravidin were from Pierce. Unlabeled antibodies were from Santa Cruz Biotechnology. Horse anti-goat IgG and rabbit anti-goat IgG were purchased from Jackson ImmunoResearch Laboratories. Peroxidase-conjugated secondary antibodies were from Amersham Biosciences. Biotinylated Protein A Affinity Gel was from Amersham Pharmacia. Histamine, TAPI, trypsin, trypsin inhibitor, and paraformaldehyde in PBS were purchased from Sigma. HUVEC were seeded into 6-well tissue culture plate as described above 24 h before each experiment. Cells were then washed in media containing 10% heat-inactivated FCS and then treated with histamine or PMA for 1 h. In experiments using brefeldin A or TAPI, the agents were added half an hour before addition of histamine or PMA; other agents were added 15 min before treatment with histamine or PMA. After treatment the media from each well were collected and centrifuged at 1500 rpm (380 × g) for 5 min, and the clarified supernatants were collected and stored at −20 °C for 1–2 weeks until analyzed. ELISAs for sTNFR1 and sTNFR2 were performed following the manufacturer’s instructions. Developed absorbance values were read at 450 and 540 nm with Titertek Multiscan plate reader, and the results were calculated using a standard curve generated each time an assay was performed.

Cell Surface Labeling and Sample Preparation for TACE—HUVEC grown to confluence in 6-well flasks were centrifuged and washed twice with 1% BSA in 0.1 M ice-cold PBS, pH 8.0. The membrane-impermeable biotinylation reagent, NHS-SS-Biotin was added to a final concentration of 0.5 mg/ml in PBS, and the cells were incubated at 4 °C for 30 min. The cells were then washed twice with ice-cold PBS and incubated with complete media at 37 °C for 15 min. Cells were then treated with 100 μM histamine or 0.1 μM PMA for 30 min. After treatment, the supernatants were removed, and the cells were then lysed using 25 mM Tris base, 150 mM NaCl, 1% Nonidet P-40, protein inhibitor mixture, 0.5 mM phenylmethylsulfonyl fluoride, and 25 μM TAPI for 30 min. Lysates were centrifuged at 10,000 rpm for 5 min, and the clarified supernatant was transferred to tubes containing Neutrividin beads. After incubation for 1 h the beads were centrifuged down and washed. The supernatant (cytosolic fraction) or beads (containing the biotinylated membrane proteins) were boiled in sample buffer (125 mM Tris/HC1, 15% sucrose, 4% SDS, 10 mM EDTA, 0.1 mg/ml bromphenol blue, 2% mercaptoethanol) for 3 min and analyzed by immunoblotting as described below.

Cell Culture—Human umbilical vein EC (HUVEC) were isolated from human umbilical cords and serially cultured in modified M199 culture medium, containing 20% fetal calf serum (FCS), 100 μg/ml heparin sodium salt, 30 μg/ml endothelial cell growth supplement, 2 mM glutamine, 60 units/ml penicillin, and 0.5 μg/ml streptomycin at 37 °C, in 5% CO₂ on gelatin-coated tissue culture plastic (Appleton Woods, UK) as described previously (23). Cells were used at passages 2–4. Such cultures are free of detectable leukocytes by immunostaining for CD45.
then washed twice with PBS, 1% BSA. Cells were then incubated with mouse monoclonal anti-HERF1 in PBS, 1% BSA for 1 h. After washing three times with PBS, 1% BSA, EC were incubated with secondary fluorescein isothiocyanate-conjugated antibody for 45 min. EC were washed twice with PBS, 1% BSA and once in PBS, and coverslips were mounted in Citifluor (Agar Scientific Ltd., Essex, UK) before viewing in a Leica TCS-NT Confocal Microscope (Leica Microsystems Ltd., Milton Keynes, UK).

TNFR1 fusion constructs containing enhanced green fluorescent protein (eGFP-TNFFR1) were introduced into HUVECs by transient transfection. In brief, HUVECs grown to 70% confluence on 100-mm diameter plastic culture plates were transfected 18 h after passage with gfp-TNFFR1 (24) using a modified DEAE-dextran protocol as described previously (49). Cadaveric human skin was obtained from discarded specimens harvested by the skin bank at Yale University, School of Medicine, and skin was engrafted under a protocol approved by the Yale Animal Care and Use Committee and by the Yale Human Investigation Committee.

To examine the effects of histamine on TNFR1 expression, grafts were injected with 10 µl of histamine (Histatol, composed of 0.1 mg/ml histamine base and 0.275 mg/ml histamine phosphate, Center Laboratories, Port Washington, NY) or 10 µl of saline or untreated and harvested 30 min later. The tissue was then prepared for immunoelectron microscopy (see below).

To examine the effects of TNF responses, one skin graft on each mouse was injected with 10 µl of histamine, and the other graft was injected with physiological saline, 30 min prior to TNF (R&D Systems, Minneapolis, Minn.) administration. Two weeks after each dose, 1-100, 300, and 1000 ng of TNF were injected subcutaneously into the scapular region, well separated from the graft site. Animals were euthanized, and skin grafts were harvested 6 h after TNF injection. Harvested grafts were snap-frozen in liquid nitrogen and stored at −80°C until assay for mRNA content.

Electron Microscopy of Skin Grafts—Human skin grafts tissue was dissected into pieces of less than 1 mm in thickness and fixed by immersioin in 2% formaldehyde (J. T. Baker Inc.) in 0.1 M PIPES buffer, pH 7.6, for 1.5 h at 4°C. The tissue was processed for freeze substitution and low temperature embedding for immunogold electron microscopy as described previously (26). In brief tissue was cryo-protected in 30% ethylene glycol for 1 h at 4°C for 24 h, at −70°C for 24 h, and at −50°C for 24 h. The tissue was then impregnated with Lowicryl HM 20 over a period of 3 days, and the resin was polymerized by ultraviolet irradiation at a temperature of −50°C. Ultrathin sections 70 nm in thickness were cut on a Leica Ultracut-S (Leica Vienna) ultramicrotome and mounted on Formvar-coated grids.

Immunogold Labeling for Electron Microscopy—The grids were incubated section down, for 0.5 h at room temperature in blocking buffer containing 10% FCS in TBS to suppress nonspecific antibody binding. Excess blocking buffer was removed, and they were incubated over night at 4°C with either mouse anti-TNFFR1 or mouse anti-hCytokeratin (MN116, Dako, UK) at 0.5 dilution in blocking buffer. Omission of primary antibody and use of isotype-specific primary antibody or nonimmune serum were used as negative controls.

After rinsing extensively with TBS, the grids were incubated with goat anti-mouse conjugated with either 1- or 20-nm colloidal gold particles (British Biocell International Ltd., Cardiff, UK) at 1:100 dilution in a solution of 2% BSA in 0.1 M PIPES buffer, pH 7.6, for 60 min at 4°C. After rinsing in TBS, grids were labeled with 1-nm colloidal gold wound with silver enhancement solution (British Biocell International Ltd., Cardiff, UK) for 4 min and washed in deionized water. All grids were then contrast-stained with uranyl acetate and lead citrate for 15 min each. They were then rinsed in a Phillips TEM 410 electron microscope (Cambridge, UK) at an accelerating voltage of 80 kV. To quantify the labeling of membrane/extracellular versus intracellular TNFR1, gold particles were counted in 10 fields containing on average 8 keratinocytes at a magnification of x<3000 using a small screen attached to the microscope. Counting was repeated using 3 different grids for each experiment.

Quantitative RT-PCR—Total RNA was isolated from skin grafts as follows. Frozen skin was placed into 1 ml of Trizol (Invitrogen) and homogenized using a Polytron tissue grinder until smooth. Samples were further processed according to the manufacturer’s instructions and modified by centrifugation of the homogenate at 12,000 × g at 4°C for 10 min to remove insoluble materials. Following Trizol extraction, RNA was further purified using a Qiagen RNeasy (Valencia, CA) clean-up protocol with a DNase digestion step.

First strand synthesis was performed using TaqMan Gold RT-PCR kit (Applied Biosystems of PerkinElmer Life Sciences) following the manufacturer’s instructions. Random hexamers were used as primers to reverse transcribe 700 ng of total RNA per 35-µl reaction, and RT reactions were performed in a PTC-150 Minicycler (MJ Research; Watertown, MA). Real time quantitative RT-PCR was performed using the TaqMan assay and PCR amplifications in Bio-Rad iCycler IQ Multicolor Real Time Detection System (Bio-Rad) as described previously (50). Briefly, a solution of 2× TaqMan Universal PCR Master Mix (Applied Biosystems, PerkinElmer Life Sciences) containing primers and probes were prepared and aliquoted into individual wells of iCycler iQ PCR Plates (Bio-Rad) and cDNA as added to give a final volume of 25 µl. Conditions for PCRs included 2 min at 50°C, 10 min at 95°C, and 50 cycles of denaturation at 95°C for 15 s, and annealing/extension at 60°C for 1 min. Threshold cycle (CT) during the exponential phase of amplification was determined by real time monitoring of fluorescent emission after cleavage of sequence-specific probes by nuclease activity of Taq polymerase. An increase in fluorescence is proportional to the amount of PCR product, and the amplification cycle at which the reporter dye fluorescence passes a selected base line is the CT. Low CT values reflect a high copy number and vice versa. CT values were exported to Excel for calculations.

ICAM-1, ICAM-2, E-selectin, and GAPDH RNA levels were quantified. ICAM-2 is not regulated by TNF and was used as an internal control gene to normalize values for ICAM-1. E-selectin was normalized to GAPDH. Primers for ICAM-1 were purchased from Applied Biosystems of PerkinElmer Life Sciences. Primers for E-selectin, ICAM-2, and GAPDH were designed using Primer 3 software and synthesized by the Keck Foundation Bioresource Laboratory at Yale University. Sequences are as follows: E-selectin forward, CTGGGACCAGCCGAGTT-GTA; E-selectin reverse, GAGTGTGATCAGGATCACA; ICAM-2 forward, CTGACTGTTGGCCCTCTTCCTC; ICAM-2 reverse, CACGGTG-TAAGATACTTCTC; GAPDH forward, GAGAAGTGGAGAGAGG-AGT; and GAPDH reverse, GAAGAAGTGGATTGATGCT.

Probes were purchased from Applied Biosystems of PerkinElmer Life Sciences with 6-carboxyfluorescein as the emitter at the 5′ end and 6-carboxytetramethylrhodamine as the quencher at the 3′ end.

Statistics—The significance of differences between experimental values was assessed by means of the paired Student’s t test.

RESULTS

Effects of Histamine on Endothelial Cell Surface TNF Receptor Expression and Shedding—As reported previously, cultured HUVEC express TNFR2 and to a lesser extent TNFR1 on their cell surface (2). Treatment of confluent EC monolayers with histamine (100 µM) for 30 min reduced cell surface expression levels of both receptors as detected by FACS analysis. The level of TNFR1 on the cell surface had recovered to basal level by 1 h, whereas the recovery of TNFR2 was slower (Table I). Concomitant with its effects of surface receptor expression, histamine treatment induced an increase in soluble TNFR1 and TNFR2 shed into the culture media. Receptor shedding was maximal during the 1st h of histamine treatment (Fig. 1). Over this time period histamine-induced shedding of TNFR1 was agonist concentration-dependent and inhibited by the histamine H1 receptor antagonist diphenhydramine but not by the H2 antagonist cimetidine (Table II). Similar results were found for shedding of TNFR2, although the total amount shed was less. Cumulatively, these data suggest that histamine-stimulated TNFR reduction on the surface was caused by histamine-stimulated receptor shedding.

Role of TACE in Histamine-induced Shedding—TACE has been reported to cleave both TNF receptors from the cell surface, and it was previously noted that receptor shedding-
Histamine transiently reduces endothelial cell surface TNF receptor expression

HUVEC were treated with 100 μM histamine for the time indicated, and TNF receptor expression was measured by FACS analysis as described under "Experimental Procedures." Values are means ± S.E. from three separate experiments, corrected for background staining in each experiment.

| Histamine treatment | Corrected values of mean fluorescence intensity |
|---------------------|-----------------------------------------------|
|                     | TNFR1 | TNFR2 |
| h                   |       |       |
| 0                   | 1.79 ± 0.4 | 2.82 ± 0.2 |
| 0.5                 | 1.16 ± 0.5\(^a\) | 1.75 ± 0.1\(^b\) |
| 1                   | 1.58 ± 0.1 | 2.11 ± 0.3\(^b\) |
| 2                   | 1.48 ± 0.6 | 2.08 ± 0.2\(^b\) |
| 4                   | 1.56 ± 0.5 | 2.15 ± 0.4\(^b\) |
| 16                  | 1.63 ± 0.6 | 2.72 ± 0.3 |

\(^a\) Values are \(p < 0.01\) compared to zero time.
\(^b\) Values are \(p < 0.05\).

Levels of soluble TNFR1 in the culture media following treatment of HUVEC for 1 h with histamine across the concentration range from 0 to 1000μM are as follows: 8.5 pg/ml (0 μM), 8.0 pg/ml (1 μM), 23.3 pg/ml (10 μM), 32.4 pg/ml (100 μM), 35.1 pg/ml (1000 μM). (Data are average values from two separate experiments with similar results.) Pretreatment with the H1 antagonist diphenhydramine (100 μM) or H2 antagonist cimetidine (100 μM) was performed for 15 min prior to treatment with histamine 100 μM for 1 h. The H1 antagonist blocked the histamine-induced shedding, whereas the H2 antagonist had no effect. Data are expressed as average values ± S.E. from three experiments.

| Treatment                      | sTNFR1 concentration (pg/ml) |
|--------------------------------|------------------------------|
| No treatment                   | 7.04 ± 2.0                   |
| Diphenhydramine                | 7.48 ± 2.5                   |
| Cimetidine                     | 7.77 ± 1.6                   |
| Histamine                      | 27.1 ± 6.0                   |
| Histamine + diphenhydramine    | 12.2 ± 2.4                   |
| Histamine + cimetidine         | 31.8 ± 7.0                   |

\(\text{Histamine Antagonizes TNF Signaling}\)

Histamine induces shedding of TNF receptors. HUVEC were treated with 100 μM histamine for the times indicated, and concentrations of sTNFR2 were detected. Most of the increased shedding caused by histamine occurred within the first half hour of treatment. After this time soluble receptors accumulated in the media of treated and untreated cells at a similar rate. Data are average values from three experiments.

Effect of Brefeldin A on Cell Surface TNF Receptor Expression

Effect of Brefeldin A on Cell Surface TNF Receptor Expression—The observation that endothelial cells express more TNFR2 than TNFR1 on their surface (4), yet release higher concentrations of TNFR1 into the medium in response to histamine, raises the possibility that intracellular TNFR1 molecules may contribute to the shed receptor pool. We therefore directly examined whether intracellular TNFR1 contributed to the amount of shed receptor. Brefeldin A is a fungal extract that can disrupt cellular protein transportation from the Golgi apparatus to the plasmic membrane (29). In 1 h, brefeldin A did not affect the amount of TNFR1 spontaneously shed into the media, but the cell surface level was reduced significantly (Table IV). This indicated that mobilization of TNFR1 from an intracellular compartment was required to maintain the constant cell surface level of the receptor. Brefeldin A also reduced TNFR2 levels indicating that maintenance of this receptor on the cell surface also depends on mobilization from an intracellular pool. Furthermore, brefeldin A reduced the amount of soluble receptors shed into the media in response to histamine (Table IV). This suggests that histamine-induced shedding also involves mobilization of intracellular receptors. Brefeldin A also reduced receptor shedding caused by PMA; sTNFR1 following treatment with PMA was 58.9 ± 1.3 pg/ml and was partially inhibited by pretreatment with brefeldin A (15.6 ± 2.0 pg/ml).

The Golgi pool constitutes the majority of TNFR1 molecules in EC. To determine whether histamine mobilizes TNFR1 from...
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Table III
Effect of TAPI on the histamine-induced reduction of TNF receptor cell surface expression and histamine-induced shedding of TNF receptors.

HUVEC were treated with histamine in the presence or absence of TAPI (25 μM) as described under “Experimental Procedures.” Cell surface TNF receptor levels were measured by FACS analysis, and shed receptors were measured by ELISA. TAPI blocked the reduction in the level of both cell surface receptors, which was induced by histamine. Data are mean ± S.E. in arbitrary units from three experiments.

| Treatment            | TNFR on the cell surface | sTNFR1 in media |
|----------------------|--------------------------|-----------------|
|                      | TNFR1 | TNFR2 | pg/ml |
| No treatment         | 1.13 ± 0.4 | 1.42 ± 0.3  | 8.9 ± 1.5 |
| TAPI                 | 1.4 ± 0.3  | 1.83 ± 0.1  | 4.8 ± 0.2 |
| Histamine            | 0.56 ± 0.5\(^{\ast}\) | 0.83 ± 0.1\(^{\ast}\) | 33.4 ± 6.4 |
| Histamine + TAPI     | 1.35 ± 0.3 | 1.73 ± 0.5  | 6.9 ± 0.91 |

\(^{\ast}\) p < 0.05 compared to untreated. TAPI also blocked the shedding of sTNFR1 into culture media induced by histamine. sTNFR1 following treatment with PMA was (68.4 ± 7.2 pg/ml), and this increase was also completely inhibited if cells were treated with TAPI and PMA (7.6 ± 1.5 pg/ml).

Table IV
Effect of brefeldin A on the histamine-induced reduction of TNF receptor cell surface expression and histamine-induced shedding of TNF receptors.

HUVEC were treated with histamine (100 μM) in the presence or absence of brefeldin A (10 μg/ml); cell surface TNF receptor level was measured by FACS analysis, and shed receptors were measured by ELISA. Brefeldin A itself reduced cell surface TNF receptor level without any effect on shedding. Brefeldin A appeared to potentiate the reduction in the level of TNF cell surface receptors induced by histamine, and decreased the shedding of sTNFR1 induced by histamine. Data are mean ± S.E from three experiments.

| Treatment            | TNFR on the cell surface | sTNFR1 in media |
|----------------------|--------------------------|-----------------|
|                      | TNFR1 | TNFR2 | pg/ml |
| No treatment         | 1.51 ± 0.4 | 1.84 ± 0.8  | 7.5 ± 1.9 |
| Brefeldin A          | 0.78 ± 0.3\(^{\ast}\) | 1.01 ± 0.5\(^{\ast}\) | 7.7 ± 1.2 |
| Histamine            | 0.78 ± 0.3\(^{\ast}\) | 1.1 ± 0.5\(^{\ast}\) | 30.2 ± 0.7 |
| Histamine + brefeldin A | 0.68 ± 0.5\(^{\ast}\) | 0.95 ± 0.5\(^{\ast}\) | 13.7 ± 3.3 |

\(^{\ast}\) p < 0.05 compared to untreated.

Fig. 2. HUVEC expression of TACE. Cell surface and intracellular forms of TACE were prepared as described under “Experimental Procedures” and analyzed by Western blotting. The intracellular fraction shows two bands with molecular masses of 100 and 120 kDa, representing the mature and pro-enzyme forms of TACE, respectively. The membrane fraction contains only the mature 100-kDa form. Treatment with histamine for 30 min did not alter the relative expression of the pro-enzyme and mature forms in different cell fractions.

Fig. 3. Histamine and PMA cause mobilization of TNFR1 from the Golgi in HUVEC. TNFR1 in untreated HUVEC co-localizes with the Golgi marker BODIPY TR (a). The staining for TNFR1 disperses to a punctate pattern throughout the cytoplasm after treatment with histamine (b) or PMA (c), whereas the distribution of the Golgi marker is unchanged. HUVEC transfected with gfp-TNFFR1 show a Golgi pattern of fluorescence (d, A). Histamine caused mobilization of gfp-TNFFR1 (d, B).

The Golgi apparatus, we examined the distribution of TNFR1 in untreated and histamine-treated cells by confocal immunofluorescence microscopy. As expected TNFR1 co-localizes with a Golgi probe in untreated cells (Fig. 3a). Treatment with histamine disperses TNFR1 from the Golgi to give a punctate staining pattern throughout the cytoplasm (Fig. 3b). PMA has a similar but more pronounced effect (Fig. 3c).

To extend the results seen in fixed and permeabilized cells, we generated HUVEC transfected with EGFP-TNFR1 fusion protein (gfp-TNFR1), and we used these cells to observe translocation of the fluorescent-labeled receptor in real time. As shown in Fig. 3d, A, mock-treated cells show fluorescence localized to the perinuclear region consistent with receptor in the perinuclear region throughout the cytoplasm and, eventually, to loss of fluorescence from the cell. These results are consistent with observations in fixed and permeabilized cells, as well as with the results of ELISA and FACS studies, and demonstrate that histamine causes a redistribution of the receptor from the Golgi to the surface and into the medium.

Signaling Pathway of Shedding Caused by Histamine—To investigate the signaling pathway by which histamine activates TACE and causes shedding of TNFRs, the effects of several pharmacological agents were tested. Histamine activates nitric-oxide synthase in EC and, via NO, can activate soluble guanylyl cyclase and protein kinase G. The NO synthase inhibitor L-NMMA (1 mM) did not affect shedding caused by histamine or PMA. Histamine can activate protein kinase C in EC. Bisindolylmaleimide (30), a protein kinase C inhibitor,
inhibited shedding caused by PMA in a concentration-dependent manner but had no effect on the shedding caused by histamine (Fig. 4). Because the shedding of amyloid precursor protein, which is cleaved by TACE, involves MEK (16), we examined this pathway as well. The specific MEK-1 inhibitor PD98059 (25 μM) significantly inhibited shedding caused by histamine as well as PMA. In contrast, SB202810, the inhibitor of the p38 mitogen-activated protein kinase (p38 MAP kinase), did not affect either PMA or histamine-induced shedding (Fig. 4). These results are consistent with the hypothesis that MEK/p42/44 MAP kinase pathway is involved in TACE activation in EC but that the activation of this pathway by histamine is independent of PKC.

Effect of Histamine-induced Alterations in TNF Receptors on TNF Responses—To investigate if the shedding of TNFR1 caused by histamine has any effect on TNF responses in HUVEC, TNF-induced degradation of Ixβα was analyzed by immunoblotting. On its own histamine had no direct effect on the level of cellular Ixβα at any time point from 0.5 to 12 h, whereas TNF, as reported previously (1), induced rapid Ixβα degradation (Fig. 5a). Pretreatment with histamine for half an hour prior to addition of TNF diminished the extent of TNF-induced Ixβα degradation; the effects of histamine pretreatment were lost at later time points. The time of maximal effect corresponds to the time of the greatest reduction in the cell surface level of TNFR1 (Table 1). Furthermore, blocking the shedding of TNFR1 induced by histamine with TAPI abolished the inhibitory effect of histamine on TNF-induced Ixβα degradation (Fig. 5b). IL-1 also induces Ixβα degradation in HUVEC, but IL-1Rs are not subject to TACE-mediated shedding. To examine the specificity of the histamine effect, histamine-pretreated cells were tested for IL-1 responsiveness. At no time point did histamine pretreatment show interference with IL-1-induced Ixβα degradation. These data cumulatively demonstrate that the effect of histamine on TNF responses in HUVEC can be attributed to the shedding of cell surface receptors. It is likely that cell surface receptor loss rather than neutralization of cytokine by sTNFR is responsible for the effect because the medium containing shed receptors was replaced before TNF treatment, and the effect of histamine was similar if TNF was added without replacing the media (data not shown). The absence of neutralizing properties in the medium can be explained by the concentrations of sTNFR receptors that are required to neutralize biological responses to TNF, which are ~5 ng/ml for sTNFR1 and 500 ng/ml for sTNFR2 (9). The concentration of sTNFRs in media after histamine treatment typically reached only 30–40 pg/ml.

Effect of Histamine on TNF Receptors and TNF Responses in Vivo—To determine whether the results observed with cultured HUVEC occur in vivo, we used a model involving transplantation of human skin grafts onto immunodeficient (SCID/beige) mice. First we injected replicate grafts with either saline or histamine and examined the tissue 30 min later by immunoelectron microscopy. TNFR1 molecules were most evident in the epidermis, associated with keratinocytes. Compared with saline-injected skin, histamine injection caused a marked accumulation of human TNFR1 in intercellular space and near cell junctions of keratinocytes in epidermis, whereas the distribution of cytokeratin was not altered (Fig. 6a). Quantification of TNFR1 labeling by counting immunogold particles revealed significantly more membrane/extracellular gold particles in histamine-treated tissue (Fig. 6b). A similar redistribution of TNFR1 within EC lining dermal microvessels was also noted (Fig. 6c), but the lesser frequency of these structures did not permit quantification.

To test the effect of histamine pretreatment on TNF responses in vivo, we used quantitative RT-PCR to evaluate ICAM-1 and E-selectin mRNA induction. E-selectin is re-
Histamine Antagonizes TNF Signaling

Histamine is a principal mediator of the immediate hypersensitivity reaction that follows interaction of antigen with specific IgE molecules on the surface of mast cells and/or basophils, and vascular endothelial cells are major targets for the biological actions of histamine. Vascular responses occur within minutes of antigenic challenge and are often followed several hours later by a late phase reaction (LPR) characterized by persistent edema and leukocyte infiltration. TNF is likely to be an important mediator of the LPR. In skin organ culture TNF derived from resident cells in the skin contributes to expression of E-selectin in elicited LPR (31), and in a murine model of IgE-dependent cutaneous LPR TNF contributes to mast cell-dependent recruitment of leukocytes (32). In addition mast cell-derived TNF is at least one of the mediators involved in the recruitment of neutrophils during IgE-dependent gastric inflammation in the mouse (33).

Mast cells contain preformed stores of biologically active TNF that can be released into the extracellular space on degranulation (34, 35). Mast cells thus provide a source for the early release of both histamine and TNF at sites of evolving allergic inflammation, and the biological actions of mast cell-derived TNF are likely to be important for the development of an LPR. Our results indicate that in this setting rapid actions of histamine may limit subsequent TNF actions through effects on TNF receptor shedding.

Histamine down-regulates both TNFR1 and TNFR2 on the cell surface of endothelial cells by enhancing receptor shedding. TACE can cleave both TNF receptors from the cell surface, and we have demonstrated that TACE is expressed at high levels in EC. Histamine appears to cause shedding through activation of TACE, as its effects could be completely blocked by the TACE inhibitor TAPI but not other metalloproteinase inhibitors, which is the characteristic profile for TACE (36). Although TNFR2 is the predominant endothelial cell surface TNF receptor (4), higher concentrations of TNFR1 were released into the media in response to histamine, raising the possibility that TNFR1 was mobilized from the Golgi pool. This idea is supported by the observation that brefeldin A disrupts the Golgi and reduces histamine-induced receptor shedding, and also by direct observation of the mobilization of transfected receptor in response to histamine. In EC shedding of TNFR1 is also regulated by the expression of aminopeptidase regulator of TNFR1 shedding (ARTS-1), a protein that binds specifically to the extracellular domain of TNFR1, and increases shedding of TNFR1 but not TNFR2 (37). Histamine increases shedding of both TNFR1 and TNFR2, indicating that its action could not be fully explained by a direct effect on ARTS-1, but expression of ARTS-1 in EC could contribute to the increased shedding of TNFR1.

Shedding of TNFR1 was increased by both PMA and histamine, and PMA-induced shedding of TNFR1 could be inhibited by a PKC inhibitor, supporting the observation that TACE can be activated by protein kinase C (15). However, our results demonstrate that histamine acts through a PKC-independent pathway. It has been reported that nerve growth factor-induced β-amylod precurs protein shedding is regulated by a MEK-1/1/LAPK pathway that can be activated by multiple first and second messengers in both a PKC-dependent and -independent manner (38, 39). Shedding induced by histamine was partially blocked by a selective MEK-1 inhibitor PD98059, which suggested at least part of the shedding induced by histamine was initiated through a MEK-1/1/LAPK pathway. In contrast, experiments using SB202810 suggest that p38MAPK was not involved. An NO donor has been shown to be able to activate TACE (40). In our system, shedding induced by either PMA or histamine was not affected by the nitric-oxide synthase antagonist L-NMMA, although it is possible that other reactive oxygen species, which can activate TACE (41), may be involved. Thus, there may be several enzymatic cascades leading to activation of TACE and shedding of cell surface receptors, and different signaling pathways may be activated by different stimuli (15, 42).

Several observations suggest that histamine limits TNF responses through a direct effect on TNF receptor shedding. The effect of histamine on both TNFR1 cell surface expression and TNF-induced IkBo degradation was transient, with both effects occurring over the same time period. This is also consistent with the report that TNF induces IkBo degradation predominantly through TNFR1 (43). In addition, the effect of
Histamine on TNF-induced IκBα degradation was lost if receptor shedding was prevented by the TACE inhibitor TAPI. Finally, histamine had no effect on IL-1-induced IκBα degradation. The timing of exposure of cells to histamine in relation to TNF is likely to be a key determinant of the effect on TNF responses, and may explain why histamine does not inhibit...

**Fig. 6.** Effect of histamine on TNFR1 expression in human skin grafts using immunogold electron microscopy. a, keratinocytes show positive staining for cytokeratin (A), and the pattern is not altered by histamine injection (B). In saline-treated skin grafts, immunogold labeling of TNFR1 is seen predominantly in a peri-nuclear pattern (arrowhead) (E) with occasional particles seen on the cell surface (arrow); after histamine injection, the majority of immunogold labeled TNFR1 is found on cell membranes and in intercellular spaces (F). Boxed areas in C and D show low power images of E and F. (n, nucleus; c, cytoplasm; ics, intercellular space. Original magnification: A, ×7,100; B, ×3,000; C and D, ×3,000; E, ×38,500; and F, ×18,000.) b, TNFR1 gold particles were counted at ×3,000 magnifications in 10 fields containing on average 8 keratinocytes. More gold particles were located in the membrane and extracellular regions of histamine compared with saline-treated grafts (p < 0.05). c, in saline-treated skin grafts, immunogold labeling of TNFR1 in endothelial cells is predominantly in a peri-nuclear pattern (B). After histamine injection, the majority of immunogold labeled TNFR1 is found on cell membranes and in intercellular spaces (D). Boxed areas in A and C show lower power images with the regions of B and D boxed. (n, nucleus; l, lumen. Original magnification: A and C, ×2,400; B, ×38,500; and D, ×55,000).
Histamine Antagonizes TNF Signaling

Histamine exerts multiple regulatory effects during the development of an immune inflammatory response (46). In cultured EC the effects of histamine on cell contraction and release of vasodilators are accompanied by pro-inflammatory effects, which include increased expression of P-selectin and release of vasodilators are accompanied by pro-inflammatory effects, which include increased expression of P-selectin and the effects of histamine on TNF responses appears to be through histamine increases RNA for both ICAM-1 (a) and E-selectin (b) in a dose dependent manner from 0–1000 ng in human skin grafts. Pretreatment with histamine reduced induction of ICAM-1 and E-selectin across this concentration range. Data are expressed as fold induction using ICAM-2 as a control gene for ICAM-1, and GAPDH as a control gene for E-selectin.

TNF responses when administered simultaneously with TNF (44, 45).

Histamine exerts multiple regulatory effects during the development of an immune inflammatory response (46). In cultured EC the effects of histamine on cell contraction and release of vasodilators are accompanied by pro-inflammatory effects, which include increased expression of P-selectin and IL-8, both of which are stored in Weibel-Palade bodies (47) and can act in concert, in vitro, to promote the leukocyte binding and transmigration. However, in vivo the principal response to histamine is increased vascular permeability and vasodilatation without recruitment of leukocytes. Our studies have shown a modest inhibitory effect of histamine on TNF responses but demonstrate a marked inhibitory effect of histamine on EC responses to TNF in vivo. In cultured cells the effect of histamine on TNF responses appears to be through loss of cell surface receptors rather than an inhibitory effect of shed soluble receptors. However, neutralization of TNF by shed receptors may contribute to more dramatic loss of EC responsiveness to TNF observed in vivo following histamine treatment of human skin. Our ultrastructural studies suggest that keratinocytes may be a major source of sTNFR in this context. Histamine caused a marked accumulation of extracellular TNFR1 in human skin engrafted onto SCID mice and diminished up-regulation of the endothelial cell-specific gene E-selectin in response to TNF. The predominant cell types, which displayed evidence of TNFR1 mobilization and shedding in engrafted skin, were keratinocytes, although mobilization of TNFR1 also occurred in EC.

Soluble TNF receptors are emerging as important regulators of inflammatory disease. Soluble TNF receptor fusion proteins suppress inflammation in experimental models of inflammation, and a soluble TNFR2.Fc hybrid molecule has entered clinical practice as an anti-inflammatory agent (11). In kidney, EC are the major cell type expressing TNFR1 (26), and our studies identify EC as a potentially important source of sTNFR1. The role for soluble TNFR1 as a physiological inhibitor of inflammatory responses is supported by the observation that patients with mutations in the gene encoding TNFR1, which disrupt extracellular cysteines and impair cleavage and shedding of the receptor develop a periodic fever syndrome known as TRAPS (TNF receptor-associated periodic syndrome). This syndrome is characterized by attacks of fever, sterile peritonitis, arthralgia, myalgia, skin rash, and/or conjunctivitis (48).

In summary, the effect of histamine on mobilization of Golgi-associated TNFR1 and receptor shedding both clarifies the relationships among cell surface, Golgi-associated, and shed TNFR1 molecules and reveals a novel mechanism through which histamine may limit the capacity of TNF to elicit an inflammatory response in an evolving allergic reaction. They also point to EC as a major source of sTNFR1 and that the Golgi pool of TNFR1 molecules may serve as an endogenous pool of anti-inflammatory reagents.

REFERENCES

1. Madge, L. A., and Pober, J. S. (2001) Exp. Mol. Pathol. 70, 317–325
2. Slovik, M. R., De Luca, L. G., Fiers, W., and Pober, J. S. (1993) Am. J. Pathol. 143, 1724–1730
3. Ledgerwood, E. C., Pober, J. S., and Bradley, J. R. (1999) Lab. Invest. 79, 1041–1050
4. Bradley, J. R., Thiru, S., and Pober, J. S. (1995) Am. J. Pathol. 146, 27–32
5. Jones, S. J., Ledgerwood, E. C., Prins, J. B., Galbraith, J., Johnson, D. R., Pober, J. S., and Bradley, J. R. (1999) J. Immunol. 162, 1042–1048
6. Bennett, M., Macdonald, K., Chan, S. W., Lazio, J. F., Simari, R., and Wassberg, P. (1998) Science 282, 290–293
7. Reddy, P., Slack, J. L., Davis, R., Cerretti, D. P., Kozlosky, C. J., Blanton, R. A., Shows, D., Poschon, J. J., and Black, R. A. (2000) J. Biol. Chem. 275, 14674–14681
8. Hooper, N. M., Karran, E. H., and Turner, A. J. (1997) Biochem. J. 321, 265–279
9. Van Zee, K. J., Kohno, T., Fischer, E., Rock, C. S., Moldawer, L. L., and Lowry, S. F. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 4845–4849
10. Bajwa, A., and Pober, J. S. (2000) Curr. Opin. Immunol. 12, 479–486
11. Black, R. A., Rauh, C. T., Kozlosky, C. J., Poschon, J. J., Slack, J. L., Wolfson, M. F., Castner, B. J., Stocking, K. L., Reddy, P., Srinivasan, S., Nelson, N., Buiani, N., Scholey, K. A., Gerhart, M., Davis, R., Fitch, J. N., Johnson, R. S., Paxton, R. J., March, C. J., and Cerretti, D. P. (1997) Nature 385, 729–733
12. Mass, M. L., Jin, S. L., Mills, E. M., Bickett, D. M., Burkhat, W., Carter, H. L., Chin, W. C., Clow, W. C., Dulsbury, J. H., Hasler, D., Hoffman, C. R., Kost, T. A., Lamberts, M. H., Leesnitzer, M. A., McCaley, P., McGeenan, G., Mitchell, J., Moyer, M., Patel, G., Rocque, W., Overton, L. K., Schoenen, P., Seaton, T., Su, J. L., and Becherer, J. D. (1997) Nature 385, 729–733
13. Asako, H., Kurose, I., Wolf, R., DeFrees, S., Zheng, Z. L., Phillips, M. L., Pahel, G., Rocque, W., Overton, L. K., Schoenen, P., Seaton, T., Su, J. L., and Becherer, J. D. (1997) Nature 385, 729–733
14. Matsubara, J., Kato, K., and Kato, K. (1999) J. Biol. Chem. 274, 13643–13649
15. Bolz, S. S., and Pahel, U. (1997) Cardiovasc. Res. 36, 437–444
16. Anagnost, L., Kanose, I., Wolf, R., DeFrees, S., Zheng, Z. L., Phillips, M. L., Pahel, G., Rocque, W., Overton, L. K., Schoenen, P., Seaton, T., Su, J. L., and Becherer, J. D. (1997) Nature 385, 729–733
17. Madge, L. A., Spiers-Horning, M. R., and Pober, J. S. (1999) J. Biol. Chem. 274, 13644–13649
18. Bolz, S. S., and Pahel, U. (1997) Cardiovasc. Res. 36, 437–444
19. Asako, H., Kanose, I., Wolf, R., DeFrees, S., Zheng, Z. L., Phillips, M. L., Pahel, G., Rocque, W., Overton, L. K., Schoenen, P., Seaton, T., Su, J. L., and Becherer, J. D. (1997) Nature 385, 729–733
20. Zavoico, G. B., Evenstein, B. M., Schafer, A. I., and Pober, J. S. (1989) J. Immunol. 142, 3993–3998
21. Romans, A. J., and Dickens, J. M. (2001) Br. J. Pharmacol. 133, 1378–1386
22. Speicke, T., Darius, H., Koth, B., Huhner, F., and Liao, J. K. (1998) J. Leukocyte Biol. 63, 732–739
23. Bradley, J. R., Thiru, S., and Pober, J. S. (1995) Am. J. Pathol. 147, 627–641
24. Gaeta, M. L., Johnson, D. R., Kluger, M. S., and Pober, J. S. (2000) Lab. Invest. 80, 1185–1194
25. Karmann, K., Min, W., Fanslow, W. C., and Pober, J. S. (1996) J. Exp. Med. 184, 173–182
26. Al Lamki, R. S., Wang, J., Skepper, J. N., Thiru, S., Pober, J. S., and Bradley, J. R. (2001) Lab. Invest. 81, 1503–1515

Fig. 7. Effect of histamine treatment on TNF response on human skin graft. Quantitative RT-PCR results demonstrate that TNF increases RNA for both ICAM-1 (a) and E-selectin (b) in a dose dependent manner from 0–1000 ng in human skin grafts. Pretreatment with histamine reduced induction of ICAM-1 and E-selectin across this concentration range. Data are expressed as fold induction using ICAM-2 as a control gene for ICAM-1, and GAPDH as a control gene for E-selectin.
27. Schlondorff, J., Becherer, J. D., and Blobel, C. P. (2000) *Biochem. J.* **347**, 131–138
28. Doedens, J. R., and Black, R. A. (2000) *J. Biol. Chem.* **275**, 14598–14607
29. Pelham, H. R. (1991) *Cell* **67**, 449–451
30. Krauss, S., and Brand, M. D. (2000) *FASEB J.* **14**, 2581–2588
31. Leung, D. Y., Pober, J. S., and Cotran, R. S. (1991) *J. Clin. Invest.* **87**, 1805–1809
32. Wershil, B. K., Wang, Z. S., Gordon, J. R., and Galli, S. J. (1991) *J. Clin. Invest.* **87**, 446–453
33. Furuta, G. T., Schmidt-Choudhury, A., Wang, M. Y., Wang, Z. S., Lu, L., Furlano, R. I., and Wershil, B. K. (1997) *Gastroenterology* **113**, 1560–1569
34. Gordon, J. R., and Galli, S. J. (1990) *Nature* **346**, 274–276
35. Walsh, L. J., Trinchieri, G., Waldorf, H. A., Whitaker, D., and Murphy, G. F. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 4220–4224
36. Dri, P., Gasparini, C., Menegazzi, R., Cramer, R., Alberi, L., Presani, G., Garbisa, S., and Patriarca, P. (2000) *J. Immunol.* **165**, 2165–2172
37. Cui, X., Hawari, F., Alsacry, S., Lawrence, M., Combe, C. A., Geng, W., Rouhani, A., Otsushi, M., Masaki, S., Sato, S., and Ohmori, K. (1996) *Cell. Immunol.* **171**, 285–288
38. Jolly-Tornetta, C., and Wolf, B. A. (2000) *Biochemistry* **39**, 15282–15289
39. Buxbaum, J. D., Ruedl, A. A., Parker, C. A., Cypress, A. M., and Greengard, P. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 4489–4493
40. Zhang, Z., Kolls, J. K., Oliver, P., Good, D., Schwarzenberger, P. O., and Zhang, Z., Oliver, P., Lancaster, J. R., Jr., Schwarzenberger, P. O., Joshi, M. S., Cork, J., and Kolls, J. K. (2001) *FASEB J.* **15**, 303–305
42. Arrighi, J., and Massague, J. (1995) *J. Cell Biol.* **128**, 433–441
43. McFarlane, S. M., Pashmi, G., Connell, M. C., Littlejohn, A. F., Tucker, S. J., Vandenabeele, P., and MacEwan, D. J. (2002) *FEBS Lett.* **515**, 119–126
44. Miki, I., Kusano, A., Ohta, S., Hanai, N., Otsushi, M., Masaki, S., Sato, S., and van Mourik, J. A., Romani, D. W., and Voorberg, J. (2002) *Histochem. Cell Biol.* **117**, 113–122
45. Murray, A. G., Petzelbauer, P., Hughes, C. C., Costa, J., Askenase, P., and Pober, J. S. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 9146–9150
46. Heide, C. A., Stevens, J., Livak, K. J., and Williams, P. M. (1996) *Genome Res.* **6**, 986–994
