Histamine antagonizes TNF signaling by stimulating TNF receptor shedding from the cell surface and Golgi storage pool

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Summary

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, TNFR1 shedding and mobilization of TNFR1 molecules from the Golgi in cultured human EC. The Golgi pool of TNFR1 serves both to replenish cell surface receptors and as a source of shed receptor. Histamine-induced shedding is blocked by TAPI, an inhibitor of TNF-α converting enzyme (TACE), and through H1 receptor via a MEK-1/p42,p44 MAP 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 causes shedding of TNFR1 and diminishes TNF-mediated induction of endothelial adhesion molecules. These results both clarify relationships among TNFR1 populations and reveal a novel anti-inflammatory activity of histamine.

Keywords: Endothelial cell /autacoid /TNF-α converting enzyme / sTNFR1 / Human
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Introduction

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 CD120a) or 75 (TNFR2 or CD 120b) kD(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 IκB kinase (IKK), various MAP kinases (including cJun 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 IκB proteins, such as IκBα, 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 (TRADD) to the cytoplasmic DD of the ligand-occupied receptor molecule. Although the majority of TNFR1 molecules are located within the Golgi apparatus, TRADD 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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Both types of TNF receptors can be released from the cell surface by the actions of a metalloproteinase called TNF alpha 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, since sTNFRs 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- or anti-inflammatory depending on whether it acts on an effector (e.g. macrophage) or target (e.g. endothelial) cell, releasing ligand or receptors, respectively. Pharmacological studies have suggested that TACE activity in cells may be regulated by several mechanisms. For example, TNFR shedding in many cell types can be initiated by phorbol esters, implicating a role for PKC, the target of phorbol ester action(7;14;15). Shedding of amyloid precursor protein from HEK293 cells, which is also mediated by TACE, is blocked by inhibitors of MEK-1, the activator of p42 and p44 MAP kinases(16). In this case, PKC may lie upstream of MEK-1. Salicylates, at concentrations that induce apoptosis, trigger TNFR shedding from EC via a pathway blocked by an inhibitor of p38 MAP kinase(17). It is unclear whether these differences are agonist-specific, cell-specific or both.
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Physiological activators of TACE in EC are unknown. Histamine is a vasoactive autacoid, released by activated human mast cells or basophils, that produces a rapid but transient EC response. Two well described effects of histamine are EC contraction, resulting in loss of permselectivity and subsequent development of edema, and EC synthesis of vasodilators, such as PGI$_2$ and NO(18). Histamine-mediated vascular leak and vasodilation underlie the classic “wheal and flare” response of allergy. Histamine also stimulates regulated secretion of stored EC proteins, such as von Willebrand factor and surface translocation of others such as P-selectin(19). Previous studies have shown that TNF pretreatment potentiates some histamine responses, such as vasodilator synthesis, but not others such as von Willebrand factor secretion(20). Histamine acts through trimeric G protein coupled receptors (H1, H2, H3 or H4) and may elicit calcium transients, protein kinase C activation and MAP kinase activation(21). Histamine does not activate IKK in EC and may actually inhibit activation of NFkB via calcium-dependent production of NO(22).

In the present study, we have investigated the effect of histamine on TNFR1 expression in human EC. We find that this agent causes both mobilization of receptor from the Golgi pool and shedding of receptor into the medium. This action appears to utilize H1 type receptors and is mediated by a MEK-1/p42, p44 MAP kinase pathway. These responses correlate with transiently diminished TNF-mediated endothelial activation, identifying a new function for histamine and supporting the hypotheses that the Golgi receptor pool is a reservoir for both cell surface and shed receptors.
Materials and Methods

**Materials:** Mouse monoclonal anti-human TNFR1, TNFR2, and control IgG, Quantikine human TNFR1 and TNFR2 ELISA kits; human recombinant TNF-α and human recombinant IL-1 were all purchased from R&D Systems Europe (Abingdon, U.K). Goat anti-mouse FITC-conjugated antibody was from DAKO (Glostrup, Denmark). Goat anti-human TACE antibody and rabbit anti-human IκB-α antibody were from Santa Cruz (Wiltshire, U.K). Horse anti-goat and goat anti-rabbit horseradish peroxidase-conjugated antibodies were from Vector Laboratories Ltd (Peterborough, U.K) and Bio-Rad (Hertfordshire, U.K) respectively. TAPI, a specific inhibitor of TACE, was purchased from Peptides International (Louisville, U.S.A). Proteinase inhibitor cocktail was from Roche Diagnostics Ltd (East Sussex, U.K). The ECL system was from Amersham Pharmacia Biotech UK Ltd (Buckingamshire, U.K). Bisindolylmaleimide, PD98059 and SB202810 were from Calbiochem (Nottingham, U.K). Sulfo-NHS-biotin and NeutrAvidin were from Pierce (Chester, U.K). Unless otherwise indicated, all reagents were from Sigma-Aldrich Company Ltd (Dorset, U.K).

**Cell Culture:** Human umbilical vein EC (HUVEC) were isolated from human umbilical cords and serially cultured in modified M199 culture medium, containing 20% v/v heat inactivated bovine fetal calf serum (FCS), 100 μg/ml heparin sodium salt, 30 μg/ml endothelial cell growth supplement, 2 mM L-glutamine, 60 U/ml penicillin and 0.5 μg/ml streptomycin at 37°C, in 5% CO₂ on gelatin-coated tissue culture plastic (Appleton Woods, U.K) as previously described(23). Cells were used at passages 2-4. Such cultures are free of detectable leukocytes by immunostaining for CD45.
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Measurement of Cell Surface TNF Receptor Expression by Flow Cytometry: HUVEC were seeded into 6-well tissue culture plate (1.5x10^5 cells per well), and 24 hours later the confluent cells were treated with histamine 100 µM for 0.5 to 16 hours. 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 non-enzymatic 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 minutes. Cells were then washed twice and incubated with secondary antibody for another 40 minutes on ice. EC were then washed three times and resuspended in 500 µl 2% paraformaldehyde in PBS. Fixed cells were analysed by flow cytometry using FACSCalibur machine (BD Biosciences, Oxford, U.K). Data were analyzed using WinMDI 2.8 software.

Detection of Soluble Receptors by ELISA: HUVEC were seeded into 6-well tissue culture plate as described above 24 hours before each experiment. Cells were then washed in media containing 10% heat inactivated FCS and then treated with histamine or PMA for one hour. 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 minutes before treatment with histamine or PMA. After treatment the media from each well were collected, centrifuged at 1500 rpm (380 g) for 5 minutes and the clarified supernatants were collected and stored at –20°C for 1 to 2 weeks until analyzed. ELISA assays for sTNFR1 and sTNFR2 were performed following the manufacturer’s instructions. Developed assay plates were read at wavelength 450 nm and 540 nm with TiterTek Multiscan plate reader and the results were calculated using a standard curve generated each time an assay was performed.
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*Cell Surface Labeling and Sample Preparation for TACE:* HUVECs grown to confluence in T75 flasks (3×10⁶) were washed twice in ice-cold PBS (PH8.0). The membrane impermeable biotinylaton reagent, NHS-SS-Biotin was added to a final concentration 0.5 mg/ml in PBS and the cells were incubated at 4°C for 30 minutes. The cells were then washed twice with ice-cold PBS and incubated with complete media at 37°C for 15 minutes. Cells were then treated with 100 µM histamine or 0.1 µM PMA for 30 minutes. After treatment, the supernatants were removed and the cells were then lysed using 25 mM Tris base, 135 mM NaCl, 2.6 mM KCl, 1% Nonidet P-40, protein inhibitor cocktail, 1 mM PMSF and 25 µM TAPI for 30 minutes. Lysates were centrifuged at 10,000 rpm for 5 minutes, and the clarified supernatant was transferred to tubes containing NeutrAvidin beads. After incubation for 1 hour 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/HCl, 15% sucrose, 4% SDS, 10 mM EDTA, 0.1 mg/ml bromophenol blue, 4% mercaptoethanol) for 3 minutes and analysed by immuno-blotting as described below.

*IκB-α Degradation Assay:* HUVEC were grown to confluence in 6-well plates and then treated with or without 100 µM histamine for various time points. The media containing shed receptors was then removed and complete media with or without 50 uints/ml TNF or 1 ng/ml IL-1 was added for 15 minutes. (For the experiment of effect of TAPI, 25 µM of TAPI was added half an hour before histamine treatment). Cells were then washed with ice cold PBS twice and lysed in 25 mM Tris base, 135 mM NaCl, 2.6 mM KCl, 1% Nonidet P-40, protein inhibitor cocktail and 1 mM PMSF for 30 minutes. Samples were centrifuged and the supernatants were collected and boiled.
in sample buffer (75 mM Tris/HCl, 10% sucrose, 0.2 mg/ml bromophenol blue, 2% SDS) for 3 minutes prior to analysis by immuno-blotting as described below. Protein concentration was determined using BCA protein assay kits (Pierce, Chester, U.K).

**Immuno-blotting:** Proteins (25 µg) in sample buffer were separated by SDS polyacrylamide gel electrophoresis and then transferred to nitrocellulose membrane and immunoblotted (5). Polyclonal anti-TACE and anti-IκB-α antibodies were used at a dilution of 1:500 and detected by enhanced chemiluminescence using ECL according to the manufacturer’s instructions. Serial dilution of samples for immuno-blotting confirmed that the density of bands was within the linear range of detection.

**Confocal Immunofluorescence or Fluorescence Microscopy:** HUVEC grown to confluence on coverslips were treated with 0.75 µl/ml of Golgi Probe (Cambridge Bioscience, Cambridge, UK) for 30 minutes, and then treated with or without 100 µM histamine or 0.1 µM PMA for one hour at 37°C before fixation and staining. EC were fixed by adding 1 ml of 2% paraformaldehyde in PBS to the 1 ml of complete growth media in which the treatments were performed. This, and all subsequent steps were performed at room temperature. After fixing for 2 minutes cells were washed three times with PBS/1%BSA. Where indicated, fixed EC were permeabilized by incubating in 0.1% Triton X-100 for 1 minute and then washed twice with PBS/1%BSA. Cells were then incubated with mouse monoclonal anti-hTNFR1 in PBS/1%BSA for 1 hour. After washing three times with PBS/1%BSA, EC were incubated with secondary FITC-conjugated antibody for 45 minutes. EC were washed twice with PBS/1%BSA and once in PBS, and coverslips were mounted in Citifluor
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(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 (gfp-TNFR1) were introduced into HUVEC by transient transfection. In brief, HUVEC were grown to 70% confluence on 100 mm diameter plastic culture plates were transfected approximately 18 hours after passage with gfp-TNFR1(24) using a modified DEAE-dextran protocol as previously described (25). Transfection efficiencies typically were between 15% and 25%. 24 h after transfection cells were plated onto fibronectin-coated glass-bottom culture plates (MatTek, Ashland, MA). After 24 h replicate wells were either pretreated with or without 25 µM TAPI (Peptides International), and then mock treated or exposed to 100 µM histamine for the indicated times and imaged live using a Zeiss Confocal microscope running LSM 510 software.

Effects of histamine on human skin: The in vivo effects of histamine on human skin were examined using immunodeficient (SCID/beige) C.B-17 mice stably engrafted with two 1 cm² split thickness grafts as previously described (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 (Histatrol, composed of histamine base 0.1 mg/ml and histamine phosphate 0.275 mg/ml, Center Laboratories, Port Washington NY) or 10
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µl 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 minutes prior to TNF (R&D Sytems, Minneapolis, MN) administration. Two mice at each dose (0, 3, 30, 100, 300, 1000 ng) of TNF were injected subcutaneously in the scapular region, well separated from the graft site. Animals were euthanised and skin grafts were harvested 6 hours after TNF injection. Harvested grafts were snap frozen in liquid nitrogen and stored at –80 °C until assay for mRNA content (see below).

Electron microscopy of skin grafts: Human skin graft tissue was dissected in pieces of less than 1 mm in thickness and fixed by immersion in 2% formaldehyde (J.T. Baker, Philipsburg, NJ) in 0.1 M PIPES buffer, pH 7.6 for 1.5 hours at 4ºC. The tissue was processed for freeze-substitution and low temperature embedding for immunogold electron microscopy as previously described(26). In brief tissue was cryo-protected in 30% propylene glycol for one hour at 4ºC, and frozen in melting propane cooled in liquid nitrogen, substituted against methanol containing 0.1% uranyl acetate at -90ºC for 24 hours at -70ºC for 24 hours and at -50ºC for 24 hours. The tissue was then impregnated with Lowicryl HM 20 over a period of 3 days and the resin was polymerised by ultraviolet irradiation at a temperature of -50ºC. Ultrathin sections 70 nm in thickness were cut on a Leica Ultracut-S (Leica Vienna) ultramicrotome and mounted on Formvar-coated grids.
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Immunogold Labeling for Electron Microscopy: The grids were incubated, section down, for half and hour at room temperature in blocking buffer containing 10% fetal calf serum (FCS) in TBS to suppress non-specific antibody binding. Excess blocking buffer was removed and they were incubated overnight, at room temperature, with either, mouse anti-hTNFR-1 or mouse anti-hCytokeratin (MNF116, Dako, UK) at 1:5 dilution in blocking buffer. Omission of primary antibody and use of isotype-specific primary antibody or non-immune serum were used as negative controls. After rinsing extensively with TBS, the grids were incubated with goat anti-mouse conjugated with either 1 nm- or 20nm-collidal gold particles (British Biocell International Ltd, Cardiff, UK) diluted 1:100 in the blocking solution for one hour at room temperature. Following thorough rinse in TBS, grids labeled with 1 nm-colloidal gold were incubated with silver enhancement solution (British Biocell International Ltd, Cardiff UK) for 4 minutes and washed in deionised water. All grids were then contrast stained with uranyl acetate and lead citrate for 15 seconds each. They were then viewed in a Philips 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 × 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 Trizol (Invitrogen, Carlsbad, CA) and homogenized using a polytron tissue grinder until smooth. Samples were further processed according to the manufacturer’s
instructions, modified by centrifugation of the homogenate at 12,000 g at 4°C for 10 minutes 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 Perkin Elmer [ABI-PE]; Foster City, CA) following the manufacturer’s instructions. Random hexamers were used as primers to transcribe 700ng 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 BioRad iCycler IQ Multi-color Real-Time Detection System (BioRad; Hercules, CA) as previously described (50). Briefly, a solution of 2x TaqMan Universal PCR Master Mix (ABI-PE) containing primers and probes were prepared and aliquoted into individual wells of iCycler iQ PCR Plates (BioRad) and cDNA as added to give a final volume of 25 µl. Conditions for PCR reactions included 2 minutes at 50°C, 10 minutes at 95°C and 50 cycles of denaturation at 95°C for 15 seconds, 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 baseline is the CT. Low CT values reflect a high copy number and visa 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.
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for ICAM-1. E-selectin was normalized to GAPDH. Primers for ICAM-1 were purchased from ABI-PE. 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 were:

E-selectin forward: CATGGAGACCATGCAGTGTA,
E-selectin reverse: GGATTTGTCACAGCATCACA;
ICAM-2 forward: CTGACTGTGGCCCTCTTCAC,
ICAM-2 reverse: CACGTGTACCTCGAATACCTTCTC;
GAPDH forward: GAAGGTGAAGGTCGGAGTC,
GAPDH reverse: GAAGATGGTGATGGGATTTC.

Probes were purchased from ABI-PE with 6-carboxyfluescein 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.
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Results

Effects of Histamine on Endothelial Cell Surface TNF Receptor Expression and Shedding

As previously reported, cultured HUVEC express TNFR2, and to a lesser extent TNFR1 on their cell surface(2). Treatment of confluent EC monolayers with histamine (100 \( \mu \)M) for 30 minutes 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 one hour, while the recovery of TNFR2 was slower (Table 1).

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 first hour 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 2). 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 induced by PMA could be inhibited by the TACE inhibitor TAPI (7 and Table 3). To determine whether histamine induced shedding involves TACE we pre-treated HUVEC with TAPI 25 \( \mu \)M for 30 minutes. TAPI by itself increased the cell surface levels of both TNFR1 and TNFR2, consistent
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with a basal rate of TACE-mediated shedding (Table 3). TAPI treatment completely prevented the shedding caused by histamine. TAPI prevented the reduction of TNFR1 on the cell surface induced by histamine (Table 3), further supporting the link between receptor loss and shedding. These results suggest that histamine increases receptor loss through TACE-mediate shedding, although it is possible that other TAPI-sensitive sheddase may be involved.

The apparent involvement of TACE in the histamine response prompted us to examine TACE expression and localization in EC. By immuno-blotting, TACE protein was found in both membrane and intracellular fractions of HUVEC (Fig 2). TACE exists in two forms of molecular weights 120kD and 100kD, which correspond to the pro-enzyme and the mature enzyme, respectively, as previously reported in human mononuclear cell lines(27). Both forms of the enzyme were observed in intracellular fractions whereas surface membrane-associated TACE was only detected as the mature form. Although PMA decreases cell surface TACE in mononuclear cells(28), the relative expression of the pro-enzyme and the mature form in different endothelial cell fractions was not affected by either histamine or PMA treatment. These data show that TACE is expressed in HUVEC but do not support a model in which TACE activation by histamine is controlled by translocation to the membrane.

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
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that can disrupt cellular protein transportation from the Golgi apparatus to the plasmic membrane(29). In one hour, Brefeldin A did not affect the amount of TNFR1 spontaneously shed into the media, but the cell surface level was reduced significantly (Table 4). 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 a 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 4). 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 pre-treatment 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 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 used these cells to observe translocation of the fluorescent-labeled receptor in real time. As shown in Fig 3d Panel A, mock treated cells show fluorescence localized to the perinuclear region consistent with receptor in the Golgi apparatus. Exposure of the cells to histamine
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(Panel B) leads to a time dependent dispersal of the receptor from 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 guanyl 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). Since the shedding of amyloid precursor protein (APP), 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 (p38MAPK) did not affect either PMA or histamine induced shedding (Fig 4). These results are consistent with the hypothesis that MEK/p42/44MAPK 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
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To investigate if the shedding of TNFR1 caused by histamine has any effect on TNF responses in HUVEC, TNF-induced degradation of IκBα was analyzed by immunoblotting. On its own histamine had no direct effect on the level of cellular IκBα at any time point from 0.5 to 12 hours, whereas TNF, as previously reported (1), induced rapid IκBα degradation (Fig 5a). Pretreatment with histamine for half an hour prior to addition of TNF diminished the extent of TNF-induced IκBα degradation: the effect 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 IκBα degradation (Fig 5b). IL-1 also induces IκBα degradation in HUVEC, but IL-1Rs are not subject to TACE-mediated shedding. To examine the specificity of the histamine effect, histamine pre-treated cells were tested for IL-1 responsiveness. At no time point did histamine pre-treatment show interference with IL-1 induced IκBα 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 sTNF receptors that are required to neutralize biological responses to TNF, which are approximately 5 ng/ml for sTNFR1 and 500 ng/ml for sTNFR2 (9). The concentration of sTNFRs in media after histamine treatment typically reached only 30-40pg/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 on to immunodeficient (SCID beige) mice. First we injected replicate grafts with either saline or histamine and examined the tissue 30 minutes later by immunelectron 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, while the distribution of cytokeratin was not altered (Fig 6a). Quantification of TNFR1 labeling by counting immuno-gold 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 pre-treatment on TNF responses in vivo we used quantitative RT-PCR to evaluate ICAM-1 and E-selectin mRNA induction. E-selectin is restricted in its expression to EC lining post-capillary venules. ICAM-1 is expressed by EC throughout the microvasculature. Although keratinocytes can also express ICAM-1, EC are the predominant cell types that respond to TNF. Injection of TNF produced a dose-dependent increase in transcripts encoding both E-selectin and ICAM-1. Pretreatment with histamine resulted in significant blunting of TNF induction of both E-selectin and ICAM-1 mRNA (Fig 7).
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Discussion

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 which 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 a 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
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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 mobilised 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 ARTS-1 (aminopeptidase regulator of TNFR1 shedding), 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 is 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 NGF induced β-APP shedding is regulated by a MEK-1/MAPK 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/MAPK 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 NOS
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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 IκBα degradation was transient, with both effects occurring over the same time period. This is also consistent with the report that TNF induces IκBα 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 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
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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 on to SCID mice, and diminished upregulation of the endothelial cell specific gene E-selectin in response to TNF. The predominant cell type, 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
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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.
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Reference List


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26-38


Boiani, N., Schooley, K. A., Gerhart, M., Davis, R., Fitzner, J. N., Johnson, R.

A., Lambert, M. H., Leesnitzer, M. A., McCauley, P., McGeehan, G., Mitchell,
J., Moyer, M., Pahel, G., Rocque, W., Overton, L. K., Schoenen, F., Seaton, T.,

151, 5631-5638


274, 13643-13649


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Figure legends

Figure 1. Histamine induces shedding of TNF receptors.

HUVEC were treated with histamine for the times indicated and the concentration of TNF receptors shed into the media measured as described in materials and methods. Untreated cells spontaneously shed soluble TNF receptors into culture media. By 24 hours the concentration of sTNFR1 reached about 160 pg/ml, while much lower concentrations of sTNFR2 were detected. Most of the increased shedding induced 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.

Figure 2. HUVEC expression of TACE.

Cell surface and intracellular forms of TACE were prepared as described in materials and methods and analysed by western blotting. The intracellular fraction shows two bands with molecular weight of 100KD and 120KD, representing the mature and pro-enzyme forms of TACE respectively. The membrane fraction contains only the mature 100KD form. Treatment with histamine for 30 minutes did not alter the relative expression of the pro-enzyme and mature form in different cell fractions.

Figure 3. Histamine and PMA cause mobilisation 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), whilst the distribution of the Golgi marker is
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unchanged. HUVEC transfected with gfp-TNFR1 show a Golgi pattern of fluorescence (d, panel A). Histamine caused mobilisation of gfp-TNFR1 (d, panel B).

Figure 4. Effect of inhibitors of NO synthase, PKC, p38MAP kinase and MEK-1 on shedding of TNFR1.

Shedding of TNFR1 was stimulated by histamine or PMA in the presence or absence of inhibitors as describe in material and methods. L-NMMA did not affect shedding caused by either histamine (p=0.24) or PMA (p=0.19) (a). The PKC inhibitor bisindolylmaleimide inhibited the shedding caused by PMA (p=0.001), but not by histamine (p=0.21) (b). The p38MAP kinase inhibitor SB202810 (25mM) has no effect on shedding caused by either agent (c). PD98059 (25mM), a selective inhibitor of MEK-1 partially inhibits shedding caused by both histamine (p=0.01) and PMA (p=0.04) (d). Data are average values from 3 separate experiments, where p<0.05 is statistically significant.

Figure 5. Effect of histamine on TNF-mediated I?Ba degradation.

HUVEC were treated with histamine for various time periods for upto 12 hours, and then treated with or without TNF (50 units/ml) or IL-1 for a further 15 minutes. Immunoblotting for I?Ba was performed on 20 µg cell lysates. Average values of results from 3 separate experiments are presented in arbitrary units of density. Histamine itself has no effect on I?Ba degradation by TNF or IL-1 at any time point, but it inhibits TNF induced I?Ba degradation at 0.5 hour’s time point while has no effect on IL-1 induced I?Ba degradation at any time point tested (a). This inhibition
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of TNF’s effect by histamine was blocked by pre-treatment with TAPI (b); data are average values from 2 experiments.

Figure 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 labelling 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 labelled TNFR1 is found on cell membranes and in intercellular spaces (F). Panels C and D show low power images with the regions of panels E and F boxed. (n, nucleus; c, cytoplasm; ics, intercellular space. Original magnification: A, × 7,100; B, × 7,100; C, D, × 3,000; E, × 38,500; 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 extra-cellular regions of histamine compared to saline treated grafts (p < 0.05).

(c) In saline treated skin grafts, immunogold labelling of TNFR1 in endothelial cells is predominantly in a peri-nuclear pattern (B). After histamine injection, the majority of immunogold labelled TNFR1 is found on cell membrane and in intercellular spaces (D). panel A and C show lower power images with the regions of panel B and D boxed. (n, nucleus; l, lumen. Original magnification: A and C, × 2,400; B, × 38,500; D, × 55,000)
Figure 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 to 1000 ng in human skin grafts. Pre-treatment 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.
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### Tables

<table>
<thead>
<tr>
<th>Histamine treatment (Hour)</th>
<th>TNFR1</th>
<th>TNFR2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.79±0.4</td>
<td>2.82±0.2</td>
</tr>
<tr>
<td>0.5</td>
<td>1.16±0.5 **</td>
<td>1.75±0.1 *</td>
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<tr>
<td>1</td>
<td>1.58±0.1</td>
<td>2.11±0.3 *</td>
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<tr>
<td>2</td>
<td>1.48±0.6</td>
<td>2.08±0.2 *</td>
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<tr>
<td>4</td>
<td>1.56±0.5</td>
<td>2.15±0.4 *</td>
</tr>
<tr>
<td>16</td>
<td>1.63±0.6</td>
<td>2.72±0.3</td>
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</table>

Table 1. Histamine transiently reduces endothelial cell surface TNF receptor expression. HUVEC were treated with 100 µM histamine for the time indicated, and TNF receptor expression measured by FACS analysis as described in material and methods. Values are means ± SE from 3 separate experiments, corrected for background staining in each experiment. * p<0.05; ** p<0.01 compared to 0 time.
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<table>
<thead>
<tr>
<th>Treatment</th>
<th>sTNFR1 concentration (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>7.04±2.0</td>
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<tr>
<td>Diphenhydramine</td>
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<tr>
<td>Cimetidine</td>
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<td>Histamine</td>
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<tr>
<td>Histamine + Diphenhydramine</td>
<td>12.2±2.4</td>
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<tr>
<td>Histamine + Cimetidine</td>
<td>31.8±7.0</td>
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</table>

Table 2. Histamine induces shedding in a dose dependent manner through its H1 receptor. Levels of soluble TNFR1 in the culture media following treatment of HUVEC for one hour with histamine across the concentration range from 0 to 1000 µM were: 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 (1,000 µM). (Data are average values from two separate experiments with similar results). Pre-treatment with the H1 antagonist diphenhydramine (100 µM) or H2 antagonist cimetidine (100 µM) was performed for 15 minutes prior to treatment with histamine 100 µM for one hour. The H1 antagonist blocked the histamine induced shedding, whilst the H2 antagonist had no effect. Data are expressed as average values + SE from three experiments.
Table 3. 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 in materials and methods. Cell surface TNF receptor levels were measured by FACS analysis, and shed receptors measured by ELISA. TAPI blocked the reduction in the level of both cell surface receptors, which was induced by histamine. Data are mean ± SE in arbitrary units from three experiments. * 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).
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<table>
<thead>
<tr>
<th>Treatment</th>
<th>TNFR1 (arbitrary unit)</th>
<th>TNFR2 (arbitrary unit)</th>
<th>sTNFR1 in media (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>1.51±0.4</td>
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<td>Brefeldin A</td>
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<td>7.7±1.2</td>
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<td>Histamine</td>
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<td>1.1±0.5*</td>
<td>30.2±0.7</td>
</tr>
<tr>
<td>Histamine+brefeldin A</td>
<td>0.68±0.5*</td>
<td>0.95±0.5*</td>
<td>13.7±3.3</td>
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</tbody>
</table>

Table 4. 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 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 ± SE from three experiments. * P<0.05 compared to untreated.
Figure 1, Wang et al
Figure 2, Wang et al
Figure 3, Wang et al
Figure 4, Wang et al
Figure 5, Wang et al.
Cytokeratin

Saline

A

n

n

n

n

Histamine

B

n

n

n

TNFR1

Low power

C

n

n

n

D

n

n

n

High power

E

n

n

F

ics

c

n

ics

c

Figure 6a,
Wang et al
Figure 6b, Wang et al
Endothelial cells

Figure 6c, Wang et al
Figure 7, Wang et al
Histamine antagonizes TNF signaling by stimulating TNF receptor shedding from the cell surface and Golgi storage pool
Jun Wang, Rafia S. Al-Lamki, Hui Zhang, Nancy Kirkiles-Smith, Mary Lou Gaeta, Sathia Thiru, Jordan S. Pober and John R. Bradley

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