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Albumin selectively inhibits TNFα-induced expression of vascular cell adhesion molecule-1 in human aortic endothelial cells

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Abstract

Objective: Leukocyte adhesion to, and transmigration across, the vascular endothelium are critical initiating steps in inflammation and atherosclerosis. We hypothesized that albumin, the major plasma protein, acts as an anti-inflammatory agent towards endothelial cells. **Methods and Results:** To test the hypothesis, we studied the effects of bovine serum albumin (BSA) on TNFα-induced expression of adhesion molecules in cultured human aortic endothelial cells (HAEC). We found that incubation of HAEC for 16 h with BSA (0.5–5%, w/v) dose-dependently inhibited TNFα-induced mRNA and protein expression of vascular cell adhesion molecule-1 (VCAM-1), but not intercellular adhesion molecule-1 nor E-selectin. Yeast recombinant human serum albumin exerted similar inhibitory effects on VCAM-1 expression, whereas γ-globulin was ineffective. BSA also significantly inhibited TNFα-induced adhesion of monocytic THP-1 cells to HAEC in a dose-dependent manner. Furthermore, BSA strongly inhibited activation and nuclear translocation of the transcription factor, nuclear factor-κB (NF-κB). For example, the physiologically relevant concentration of 5% BSA inhibited NF-κB activation by 90±7%, VCAM-1 mRNA and protein expression by 81±4 and 80±13%, respectively, and THP-1 adhesion by 73±9% (n=3). The inhibitory effect of BSA on TNFα-induced VCAM-1 expression was not attenuated by inhibition of intracellular GSH synthesis. **Conclusions:** Our data show that physiological concentrations of albumin selectively inhibit TNFα-induced upregulation of VCAM-1 expression and monocyte adhesion, most likely by inhibiting NF-κB activation in a GSH-independent manner.

Keywords: Atherosclerosis; Cytokines; Endothelial receptors; Gene expression; Signal transduction

1. Introduction

Leukocyte recruitment to the arterial wall plays a critical role in inflammation and atherosclerosis [1,2] and requires the coordinated expression of cellular adhesion molecules on the endothelium, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin [2,3]. The expression of these adhesion molecules is induced by various stimuli, including tumor necrosis factor-α (TNFα), interleukin-1β, bacterial endotoxin and certain reactive oxygen species [2,3]. Therapeutic agents that block endothelial activation and leukocyte–endothelial interactions can also markedly inhibit inflammatory responses in vivo [3–5].

Epidemiological studies have consistently shown that reduced levels of albumin, the most abundant protein in human plasma and other extracellular fluids, is associated with increased morbidity and mortality [6]. This association holds true, in particular, for mortality from cardiovascular diseases, and even after adjustment for known coronary risk factors [7,8]. Albumin has numerous physiological functions, which include maintenance of blood osmotic pressure and capillary permeability, and transportation of fatty acids, metals, bilirubin, and other biological molecules and xenobiotics [9]. However, the physiological roles of albumin, particularly in inflamed tissue, are not fully understood.

One of the cardinal features of inflammation is an increase in vascular permeability, allowing large amounts of albumin to reach the interstitium and epithelial surfaces

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of organs [10]. An acute increase in tissue albumin is likely to exert beneficial effects, for example by binding metal ions and providing antioxidant protection [11]. Recently, it has been shown that albumin inhibits endothelial apoptosis [12], modulates arachidonic acid release and membrane fluidity [13], affects cellular redox signaling [14], and protects against myocardial and neuronal injury from ischemia and reperfusion [15–17].

In the present study, we hypothesized that albumin acts as a primary anti-inflammatory constituent of plasma by inhibiting endothelial activation by inflammatory cytokines. To address this hypothesis, we investigated whether albumin exerts a dose-dependent reduction in the ability of human aortic endothelial cells (HAEC) to upregulate adhesion molecule expression.

2. Methods

2.1. Materials

Bovine serum albumin (BSA) fraction V solution (30%, w/v), human γ-globulin and D,L-buthionine-*S*,*R*-sulfoximine (BSO) were purchased from Sigma (St. Louis, MO, USA). Purified recombinant human serum albumin (rHSA) prepared in the yeast *Pichia pastoris* was obtained from New Century Pharmaceuticals (Huntsville, AL, USA). Recombinant TNFα was purchased from Boehringer Mannheim Biochemica (Stuttgart, Germany). The investigation conforms to the principles outlined in the Declaration of Helsinki.

2.2. Cell culture

2.2.1. Human aortic endothelial cells

HAEC were obtained from Clonetics (San Diego, CA, USA). Cells were cultured at 37 °C in a humidified 95% air–5% CO_2 atmosphere, using 1% calf skin gelatin (Sigma)-coated 75 cm^2 flasks (Costar, Cambridge, MA, USA) and endothelial cell growth medium (Clonetics). After passage five, HAEC were switched to M199 medium (Sigma) supplemented with 20% fetal calf serum (FCS, Life Technologies, Grand Island, NY, USA; endotoxin test: <0.125 units/ml), 100 ng/ml streptomycin, 100 IU/ml penicillin, 250 ng/ml fungizone, 1 mmol/l glutamine (Life Technologies) and 1 ng/ml human recombinant basic fibroblast growth factor (bFGF, Boehringer Mannheim). Confluent cultures were harvested using 0.05% trypsin–0.02% EDTA (Sigma) and plated at a split ratio of 1:3 in 75 cm^2 flasks. All cells used in this study were of passages seven or eight.

2.2.2. Human monocytic THP-1 cells

Human monocytic THP-1 cells were purchased from the American Type Culture Collection (Rockville, MD, USA) and grown in RPMI-1640 medium (Sigma) containing 10% FCS, 100 μg/ml streptomycin, 100 IU/ml penicillin, 250 ng/ml fungizone, 1 mmol/l glutamine and 50 μmol/l 2-mercaptoethanol, and subcultured at a 1:5 ratio three times per week.

2.3. Experiments

HAEC were grown in 75-cm^2 flasks, petri dishes (100 mm) or 48- or 96-well tissue culture plates (Costar) until confluence was reached, using RPMI-1640 medium (Sigma) supplemented with 5% heat-inactivated FCS, 100 ng/ml streptomycin, 100 IU/ml penicillin, 250 ng/ml fungizone, 1 mmol/l glutamine and 1 ng/ml human recombinant bFGF. Cells were then washed once and incubated for 16 h in RPMI medium containing 5% FCS without or with different concentrations of BSA, rHSA or human γ-globulin. Thereafter, the cells were washed once and incubated with TNFα (100 U/ml) in the same medium with or without BSA, rHSA or human γ-globulin. Incubations were terminated after 5, 12, and 12 or 24 h, respectively, for subsequent determination of E-selectin, ICAM-1 and VCAM-1 protein levels. In other experiments, cells were treated for 16 h with different concentrations of BSA, washed once, and incubated with TNFα (100 U/ml) and BSA for 1 h for subsequent determination of NF-κB activation; 2 h for adhesion molecule mRNA levels; or 12 h for THP-1 monocyte adhesion. Cell morphology and viability, as determined by phase contrast microscopy and the MTT Assay Kit (Boehringer Mannheim), were unchanged under all experimental conditions used.

2.4. Measurement of adhesion molecules

2.4.1. Cell ELISA

ELISA was performed on HAEC monolayers in flat-bottom 96-well plates. Briefly, at the conclusion of the incubations, the cells were washed twice with cold PBS and fixed for 15 min at 4 °C with 0.1% glutaraldehyde (Sigma) in PBS. Plates were incubated at 37 °C for 1 h with 5% skim milk in PBS before adding a primary antibody to either E-selectin (R&D Systems, Minneapolis, MN, USA), ICAM-1 (R&D Systems) or VCAM-1 (DAKO, Carpinteria, CA, USA) and incubation over night at 4 °C. The plates were then washed three times with 0.1% Tween 20 in PBS and incubated for 1 h with a horseradish peroxidase-conjugated goat anti-mouse IgG secondary antibody (Amersham, Piscataway, NJ, USA). The expression of E-selectin, ICAM-1 and VCAM-1 was quantitated by the addition of the peroxidase substrate *o*-phenylenediamine dihydrochloride (Sigma). The absorbance of each well was measured at 492 nm in a microplate spectrophotometer (Molecular Devices, Palo Alto, CA, USA).

2.4.2. Flow cytometry

Flow cytometric analysis was used to determine surface

expression of VCAM-1. HAEC were washed three times with HBSS and removed from the culture dishes by treatment with 0.05% trypsin–0.02% EDTA. The cells were collected by centrifugation, washed once with phenol red free RPMI-1640 medium and re-suspended in RPMI medium containing 5% FCS. A fluorescein isothiocyanate (FITC)-conjugated mouse IgG anti-human VCAM-1 monoclonal antibody (R&D Systems) was added to the cell suspension at a final concentration of 3 μg/ml, followed by incubation for 30 min at 4 °C. Unbound antibody was removed by centrifugation at 1500×*g* for 5 min. Labeled cells were then analyzed using a XL-MCL flow cytometer (Coulter, Hialeah, FL, USA). At least 10,000 cells were analyzed. Background binding was detected using FITC-conjugated IgG isotype controls (R&D Systems).

2.5. Measurement of monocytic THP-1 cell adhesion to HAEC

Adhesion of THP-1 cells to confluent HAEC in 48-well plates was measured as described [18]. Briefly, the cells were washed twice with RPMI-1640 medium, and THP-1 cells (1×10^5/ml) were layered over HAEC and incubated for 1 h at 37 °C. Thereafter, the cells were washed three times with HBSS and fixed with 1% glutaraldehyde in PBS. The adherent THP-1 cells in each well were counted in five separate high power fields (40× phase contrast objective) under a microscope and expressed as adherent THP-1 cells per high power field.

2.6. Northern blot analysis

Total cellular RNA was isolated from HAEC using TRIzol Reagent (Life Technologies). The final RNA pellet was resuspended in 10–15 μl 0.5% SDS and the concentration determined by absorbance at 260 nm. Northern blot analysis was performed as described [19]. RNA samples were electrophoresed on a 1.2% agarose gel followed by capillary transfer of the fractionated RNA to a Duralon-UV membrane (Stratagene, La Jolla, CA, USA). RNA blots were hybridized with 10^6 cpm/ml of the ^{32}P-labeled oligonucleotide probes for either human E-selectin, VCAM-1 or ICAM-1 (R&D Systems), or cDNA for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Sigma) overnight at 57 °C in a hybridization oven. Blots were washed, air dried and exposed to Hyperfilm X-ray films (Amersham) at −80 °C.

2.7. Electrophoretic mobility shift assay (EMSA)

Nuclear extracts were prepared and EMSA was performed as described [20]. Five micrograms of nuclear extract was incubated with the ^{32}P-labeled double-stranded NF-κB oligonucleotide consensus sequence, 5′-AGTTGAGGGGACTTTCCCAGGC-3′ (Santa Cruz Biotechnology), at room temperature for 30 min. The incubation mixture included 1 μg of poly(dI-dC) in a binding buffer consisting of 25 mmol/l HEPES, pH 7.9, 0.5 mmol/l EDTA, 0.5 mmol/l DTT, 1% NP-40, 5% glycerol and 50 mmol/l NaCl. The DNA–protein complex was electrophoresed on 5% nondenaturing polyacrylamide gels in 0.5× Tris/borate EDTA buffer. The specificity of binding was examined by incubating nuclear extracts prepared from TNFα-treated cells with 2–4 μg of antibodies against either the p65 or p50 subunit of NF-κB (Santa Cruz Biotechnology) for 30 min at room temperature before addition of the ^{32}P-labeled oligonucleotide. Specificity was further tested by competition with a 100-fold excess of unlabeled competitor oligonucleotide. Radioactive bands were detected by autoradiography at −80 °C.

2.8. Statistical analysis

Data are reported as mean±S.D. One-way ANOVA or Student's unpaired *t*-test were used for statistical analysis of the raw data (e.g., OD_{492} values), and significance was accepted at the $P<0.05$ level. Data in Figs. 1, 2, 4, 5 and 6 are expressed as '% of TNFα stimulation', which was calculated as follows:

(value for TNFα plus BSA-treated cells/value for TNFα-treated cells) × 100%

Percent inhibition was calculated as follows:

[1 − (value for TNFα plus BSA-treated cells − value for unstimulated cells/value for TNFα-treated cells − value for unstimulated cells)] × 100%

3. Results

3.1. BSA selectively inhibits TNFα-induced expression of VCAM-1

As assessed by cell ELISA, cultured HAEC did not constitutively express E-selectin, but treatment with TNFα (100 U/ml) caused its temporary expression. Maximum levels of E-selectin were observed after 4 h of incubation, decreased levels at 8 h and return to non-detectable, basal levels by 24 h. VCAM-1 also was not constitutively expressed by HAEC, but was strongly induced following 6 h of incubation with TNFα, reaching maximum levels by 12 h. In contrast, ICAM-1 was expressed constitutively by HAEC, and was strongly upregulated by TNFα, with maximum levels reached after 12 h of incubation. Both VCAM-1 and ICAM-1 protein levels plateaued between 12 and 48 h of incubation (data not shown).

To investigate the effect of albumin on adhesion molecule expression, HAEC were treated for 16 h with increasing concentrations of BSA (0.5, 1, 2 and 5%, w/v) followed by incubation with TNFα (100 U/ml) for up to

24 h. As shown in Fig. 1A, cell ELISA analysis showed that VCAM-1 protein expression induced by TNFα was inhibited by BSA in a dose-dependent manner. For example, 5% BSA significantly inhibited TNFα-induced VCAM-1 expression by 89.8±2.6 and 97.4±0.5% ($n=3$) after 12 and 24 h of incubation, respectively. In contrast, TNFα-induced expression of E-selectin and ICAM-1 were not affected by BSA (Fig. 1A). The inhibitory effect of BSA on cell surface VCAM-1 expression was further verified by flow cytometric analysis. As shown in Fig. 1B and C, basal surface expression of VCAM-1 was low, with the anti-VCAM-1 signal equivalent to that of the isotype control. Treatment of the cells with TNFα induced a more than 20-fold increase in VCAM-1 expression, which was dose-dependently inhibited by BSA (Fig. 1B and C). For example, 5% BSA inhibited TNFα-induced VCAM-1 expression by 81.9±3.0 and 80.3±13.3% ($n=3$) after 12 and 24 h of incubation, respectively (Fig. 1B).

The specificity of the inhibitory effect of BSA was further investigated by comparison with γ-globulin, a major plasma protein unrelated to albumin. As shown in Fig. 2, γ-globulin (1, 2 or 5%) did not inhibit VCAM-1

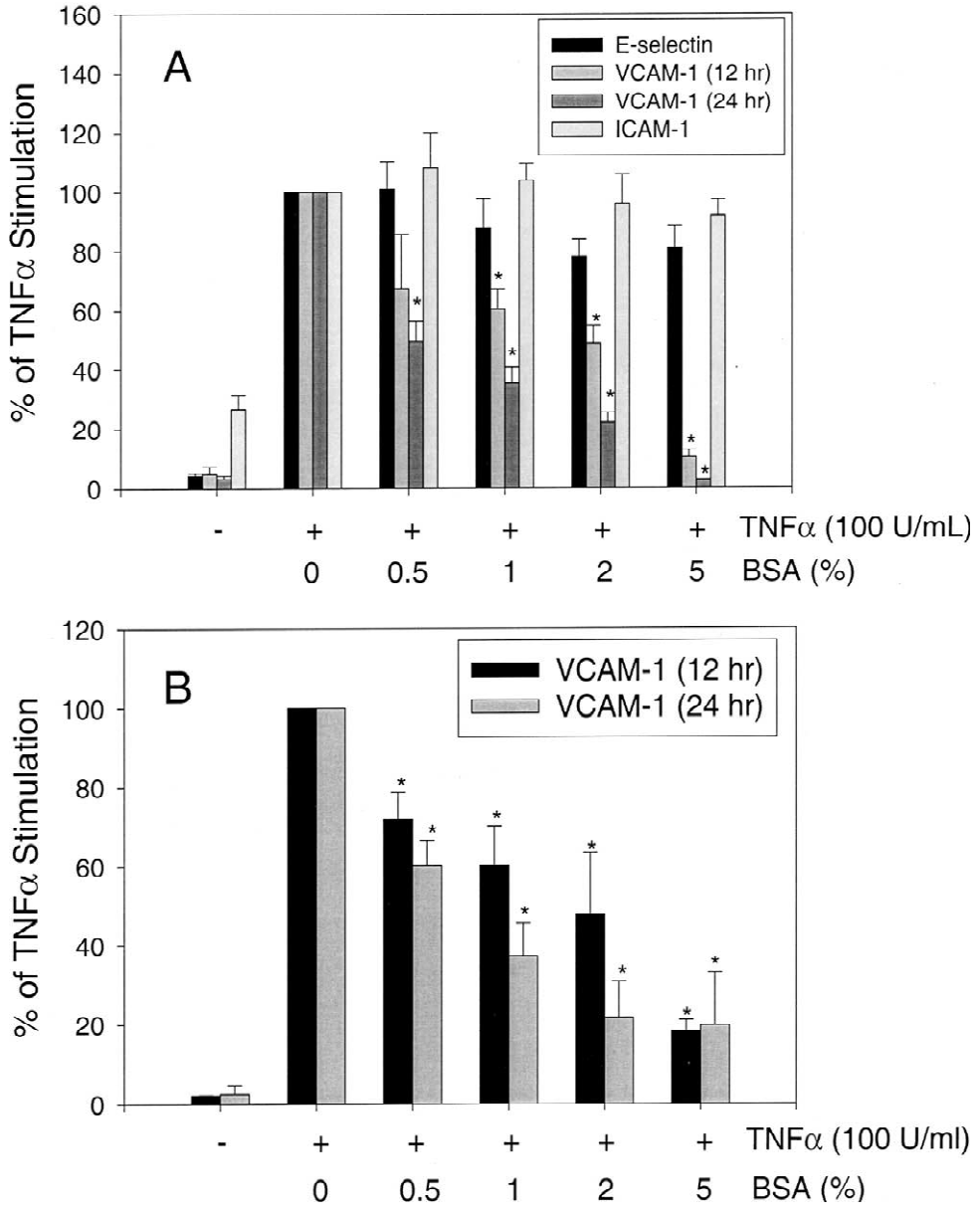

Fig. 1. BSA dose-dependently inhibits TNFα-induced VCAM-1 protein expression in HAEC. HAEC were incubated as described in Methods without (0%) or with different concentrations of BSA (0.5, 1, 2 and 5%, w/v) for 16 h, followed by stimulation with TNFα (100 U/ml) for up to 24 h. Adhesion molecules were measured by both cell ELISA (A) and flow cytometry (B). Histograms for VCAM-1 expression after 12 h of incubation with TNFα are shown in (C), using a three-decade $\log^{10}$ fluorescence scale against the number of gated cells or 'events' with corresponding fluorescence values. Data shown are mean values±S.D. of three independent experiments. *$P<0.05$ compared with TNFα alone (0% BSA).

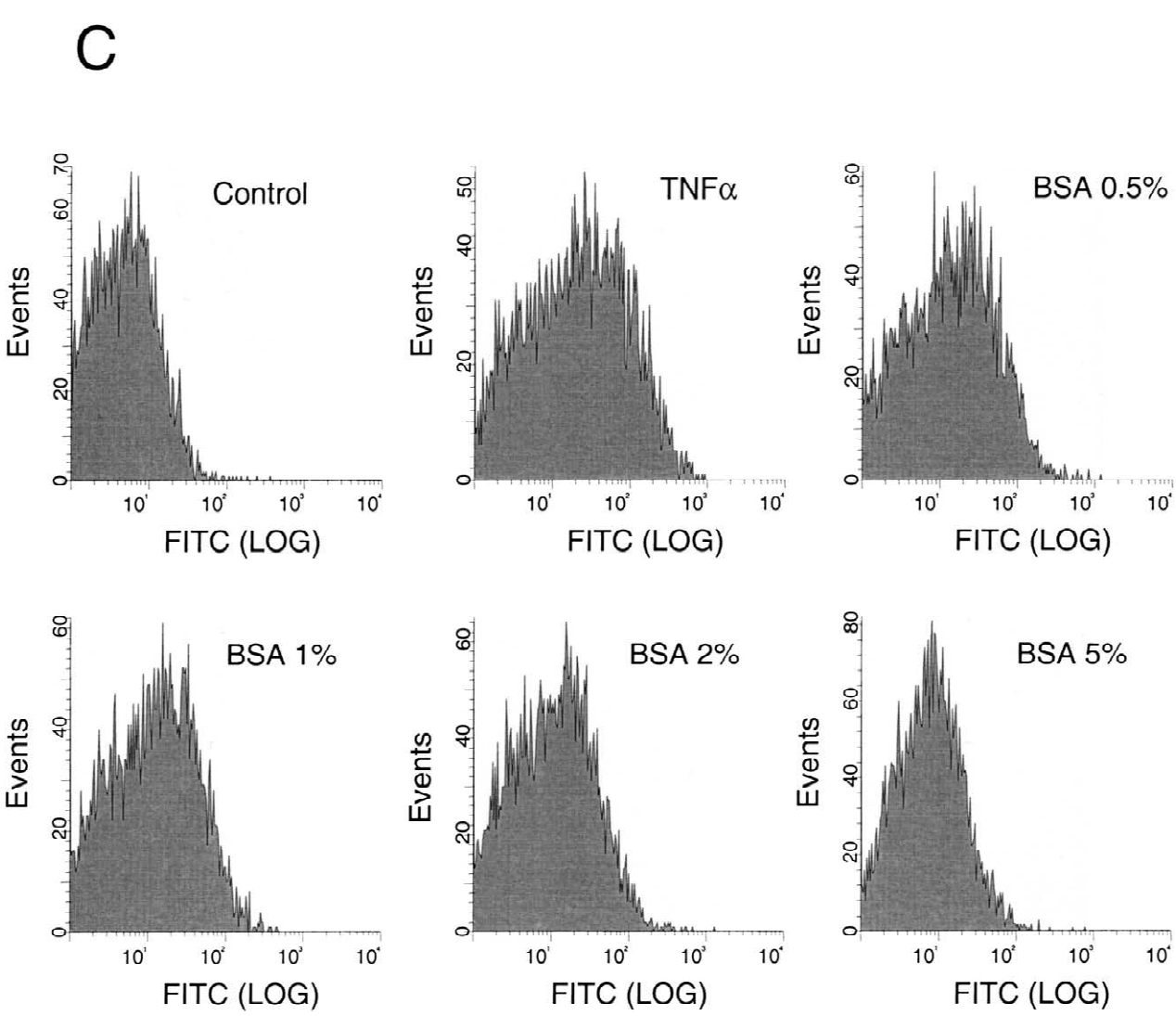

Fig. 1. (*continued*)

expression in HAEC. In contrast, rHSA (0.5, 1 or 2%) strongly inhibited VCAM-1 expression in cells incubated with TNFα for 12 or 24 h (Fig. 2), but had no effect on ICAM-1 and E-selectin expression (data not shown).

3.2. BSA inhibits TNFα-induced monocytic THP-1 cell adhesion to HAEC

Since VCAM-1 is essential for the interaction of endothelial cells with monocytes, we determined whether BSA affects monocyte adhesion to HAEC. Incubation of confluent HAEC with 100 U/ml TNFα for 12 h caused a more than 10-fold increase in adhesion of monocytic THP-1 cells, compared to adhesion of THP-1 cells to unstimulated HAEC (Fig. 3). This increase in HAEC adhesiveness was dose-dependently reduced by pre-treatment of the cells for 16 h with increasing concentrations of BSA (Fig. 3). For example, 5% BSA inhibited THP-1 adhesion by 73.0±8.6% ($n=3$).

3.3. BSA selectively inhibits TNFα-induced upregulation of VCAM-1 mRNA levels

To further define the mechanism by which BSA inhibits TNFα-induced VCAM-1 expression, mRNA levels of E-selectin, VCAM-1 and ICAM-1 were measured. As shown in Fig. 4, treatment of HAEC for 2 h with TNFα (100 U/ml) resulted in strong induction of mRNA levels of VCAM-1. However, pre-treatment of the cells with BSA for 16 h significantly inhibited TNFα-induced upregulation of VCAM-1 mRNA expression in a dose-dependent manner (Fig. 4). Densitometric analysis of the Northern blots showed that, at a concentration of 5% BSA, TNFα-induced VCAM-1 message levels were decreased by 81±4% ($n=3$) (Fig. 4B). In contrast, BSA had no significant effect on TNFα-induced upregulation of E-selectin and ICAM-1 mRNA levels in HAEC (data not shown). These results indicate that BSA selectively blocks TNFα-induced VCAM-1 gene transcription in endothelial cells.

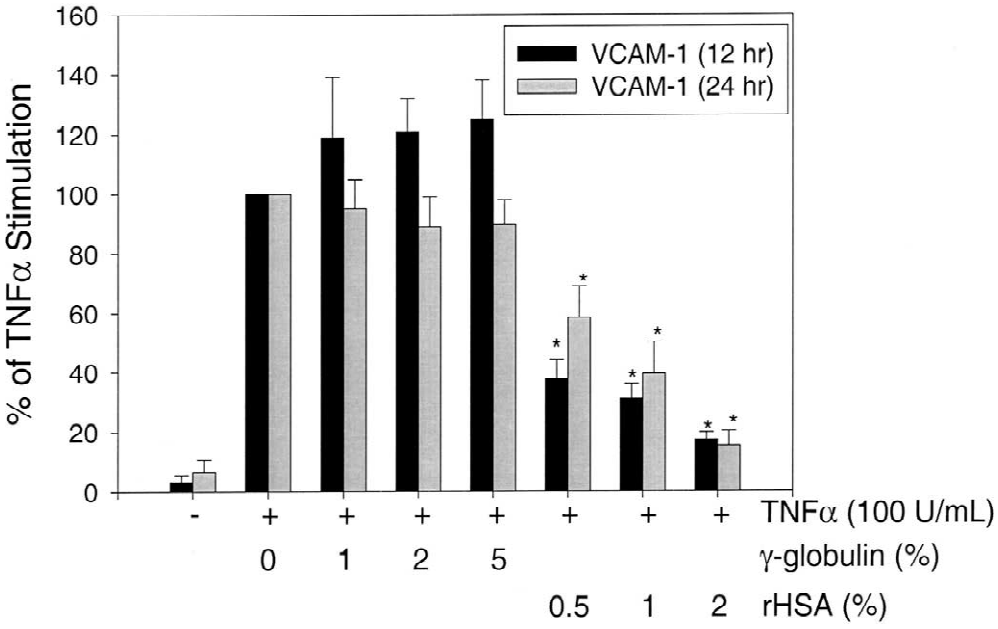

Fig. 2. rHSA, but not γ-globulin, inhibits TNFα-induced VCAM-1 protein expression in HAEC. HAEC were incubated as described in Methods without (0%) or with different concentrations of γ-globulin (1, 2 and 5%, w/v) or rHSA (0.5, 1 and 2%, w/v) for 16 h, followed by stimulation with TNFα (100 U/ml) for 12 or 24 h. VCAM-1 was measured by cell ELISA. Data shown are mean values±S.D. of four separate incubations, and representative of three independent experiments. *$P<0.05$ compared with TNFα alone (0% γ-globulin or rHSA).

3.4. BSA inhibits TNFα-induced NF-κB activation

As nuclear translocation of the transcription factor NF-κB is required for the transcriptional induction of endothelial cell adhesion molecules [21], we evaluated the effects of BSA on TNFα-induced NF-κB activation. As expected, TNFα alone strongly induced NF-κB activation, as determined by EMSA (Fig. 5). However, pre-treatment of HAEC with 1, 2 or 5% BSA dose-dependently inhibited TNFα-induced NF-κB activation by 33±11, 65±8 and 90±7% ($n=3$), respectively, as quantitated by densitometry (Fig. 5B). The specificity of the protein–DNA complex for the NF-κB sequence was demonstrated by successful competition with excess unlabeled NF-κB con-

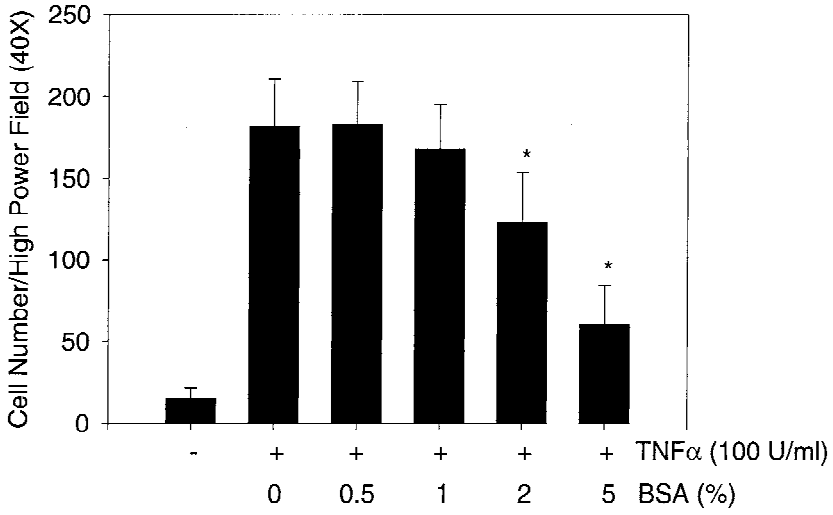

Fig. 3. BSA dose-dependently inhibits adhesion of monocytic THP-1 cells to TNFα-stimulated HAEC. HAEC were incubated as described in Methods without (0%) or with different concentrations of BSA (0.5, 1, 2 and 5%, w/v) for 16 h, followed by stimulation with TNFα (100 U/ml) for 12 h. Adhesion of THP-1 cells (1×10^5/ml) was measured as described in Methods and expressed as the average number of adherent THP-1 cells in five high power fields per well. Data shown are mean values±S.D. of three independent experiments. *$P<0.05$ compared with TNFα alone (0% BSA).

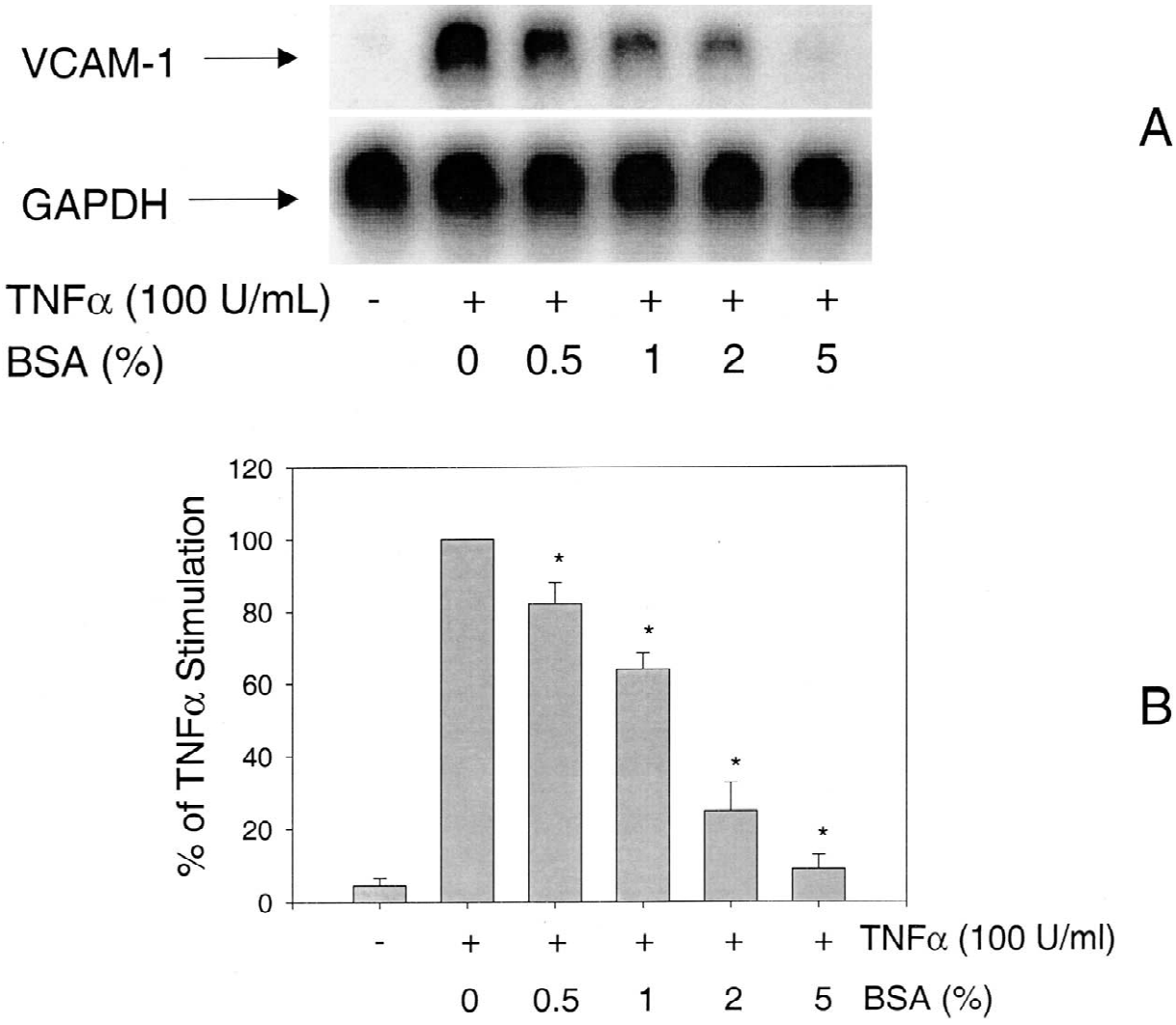

Fig. 4. BSA dose-dependently inhibits TNFα-induced upregulation of VCAM-1 mRNA levels in HAEC. HAEC were incubated as described in Methods without (0%) or with different concentrations of BSA (0.5, 1, 2 and 5%, w/v) for 16 h, followed by stimulation with TNFα (100 U/ml) for 2 h. VCAM-1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels were measured by Northern blot analysis. Data shown are representative of three experiments (A). The intensity of the bands was quantitated by densitometry and, after normalization to GAPDH, expressed as percentage of TNFα-induced VCAM-1 mRNA levels in cells incubated without BSA (B). *$P<0.05$ compared with TNFα alone (0% BSA).

sensus oligonucleotide, and by supershift of the band following incubation of the nuclear extracts with anti-NF-κB p65 or p50 antibodies (Fig. 5A).

3.5. The inhibitory effect of BSA on VCAM-1 expression is not affected by BSO

Glutathione, which is present in human cells in millimolar concentrations and plays a pivotal role in cellular antioxidant defenses, has been implicated in the regulation of NF-κB activation [21]. Since albumin has been reported to increase intracellular GSH levels [14], we investigated the role of GSH in the inhibition of VCAM-1 expression by albumin. As shown in Fig. 6, pre-treatment of HAEC for 16 h with different concentrations of BSA in the presence of 200 μmol/l BSO, an irreversible inhibitor of γ-glutamyl-cysteinyl synthetase, did not abolish the inhibitory effect of BSA on TNFα-induced VCAM-1 expression. These data suggest that the inhibitory effect of BSA is not mediated by increased intracellular GSH levels.

4. Discussion

Several lines of evidence strongly suggest that decreased serum albumin levels are associated with increased morbidity and mortality [6], even within the normal, physiological range of 35–50 g/l albumin (3.5–5%, w/v) [22]. The estimated increase in the odds of death ranges from 24 to 56% for each 2.5 g/l (0.25%) decrement in serum albumin concentration [6]. The association may predict total and cause-specific mortality, in particular car-

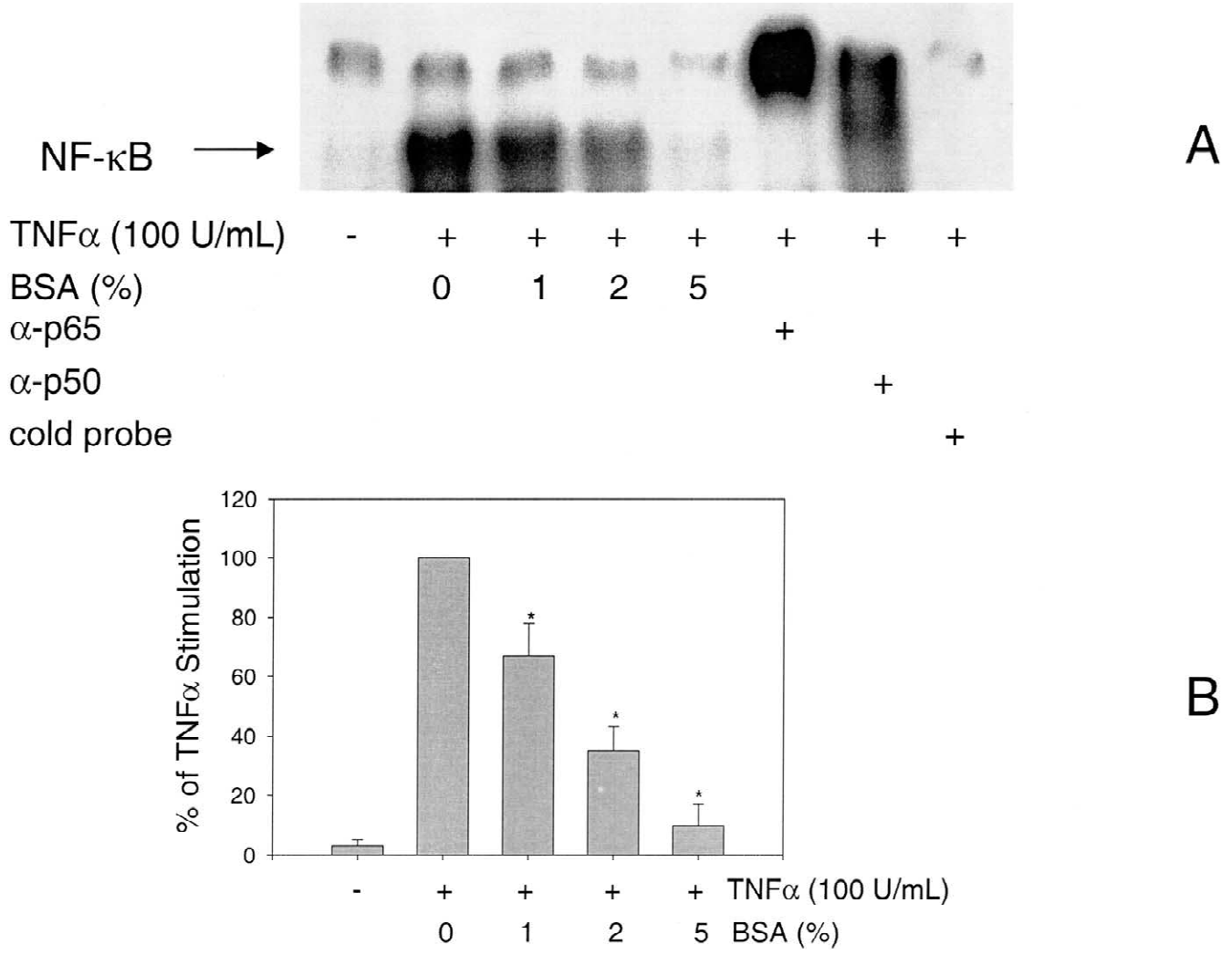

Fig. 5. BSA dose-dependently inhibits TNFα-induced NF-κB activation in HAEC. HAEC were incubated as described in Methods without (0%) or with different concentrations of BSA (1, 2 and 5%, w/v) for 16 h, followed by stimulation with TNFα (100 U/ml) for 1 h. NF-κB in nuclear extracts was measured by EMSA. Control lanes included a 100-fold excess of unlabeled NF-κB consensus oligonucleotide (cold probe) and antibodies against the p65 (α-p65) and p50 subunits of NF-κB (α-p50). Data shown are representative of three experiments (A). The intensity of the bands was quantitated by densitometry and expressed as percentage of TNFα-induced NF-κB activation in cells incubated without BSA (B). *$P<0.05$ compared with TNFα alone (0% BSA).

diovascular mortality [7]. A direct protective effect of albumin is suggested by the persistence of the association after adjustment for known coronary risk factors. In addition, many studies have demonstrated that low serum albumin is associated with poor outcome of numerous other diseases and inflammatory conditions, e.g. liver and renal diseases, certain types of cancer, burns, trauma, AIDS, septic shock and rheumatoid arthritis [9].

In the present study, we show for the first time that physiological concentrations of albumin selectively inhibit TNFα-induced VCAM-1 expression, monocyte adhesion and NF-κB activation in human aortic endothelial cells. These effects appear to be specific, since γ-globulin, a major serum protein unrelated to albumin, does not affect adhesion molecule expression. In contrast, recombinant human serum albumin strongly inhibits VCAM-1 expression, a finding that precludes serum contaminant(s) as the active agent(s) in our BSA preparations. Our data, therefore, indicate that albumin plays an essential role in inhibiting cytokine-induced endothelial activation. Since a recent, elegant in vivo study showed that VCAM-1, in contrast to ICAM-1, plays a critical role in the initiation of atherosclerosis [23], our data further suggest that increasing albumin levels may be an effective strategy to lower cardiovascular risk.

It should be noted that the inhibitory effects of BSA and rHSA on VCAM-1 expression were observed in the presence of 5% FCS, which was necessary to keep the cells viable during experiments. Assuming that FCS contains 5% albumin, 5% FCS would provide 0.25% albumin,

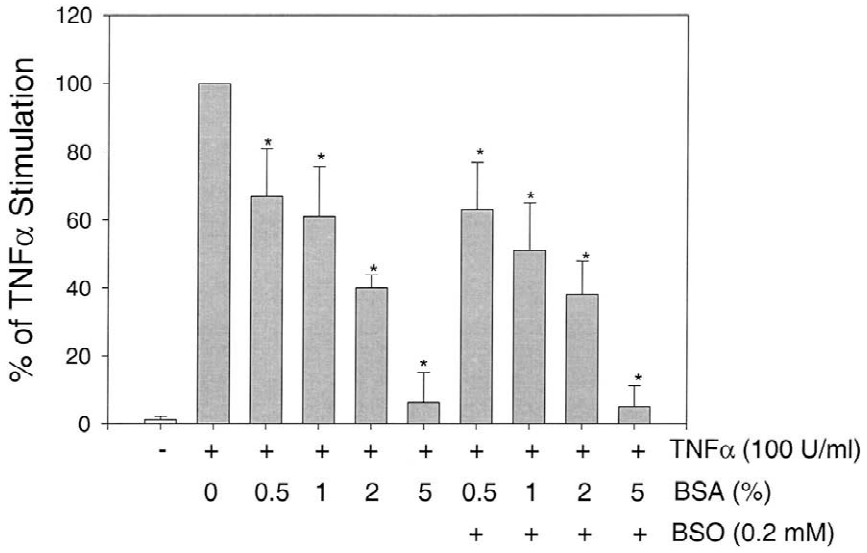

Fig. 6. BSO does not abolish the inhibitory effect of BSA on TNFα-induced VCAM-1 expression in HAEC. HAEC were incubated as described in Methods without (0%) or with different concentrations of BSA (0.5, 1, 2 and 5%, w/v) and BSO (200 μmol/l) for 16 h, followed by stimulation with TNFα (100 U/ml) for 12 h, again in the absence or presence of BSA and BSO. VCAM-1 protein levels were measured by cell ELISA. Data shown are mean values±S.D. of three independent experiments. *$P<0.05$ compared with TNFα alone (0% BSA).

which may exert a small effect on NF-κB activation and VCAM-1 expression. However, different concentrations of FCS (from 1% up to 20%) did not dose-dependently inhibit TNFα-induced adhesion molecule expression in our cell culture systems (data not shown), indicating that FCS albumin is much less effective than BSA and rHSA. Furthermore, in the absence of BSA or rHSA, i.e., in control cells incubated in the presence of 5% FCS, there was robust stimulation of adhesion molecule expression and NF-κB activation by TNFα (see, e.g., Figs. 4 and 5).

As indicated above, among the various biological mechanisms that may explain the beneficial effects of increased albumin levels in epidemiological and clinical studies, a direct protective effect of albumin has been proposed. One possibility is that albumin binds TNFα, thereby inhibiting interaction with its receptor(s). However, such a mechanism is unlikely to explain our data, as we observed selective inhibition by albumin of VCAM-1 expression, but not ICAM-1 and E-selectin.

There is substantial evidence that albumin exerts antioxidant effects, including scavenging of reactive oxygen species and binding of transition metal ions. Indeed, it has been suggested that albumin represents the major antioxidant in human plasma and other extracellular fluids [11,24–28]. Because endothelial VCAM-1 expression is known to be sensitive to the cellular redox environment [29], the observed inhibition by albumin may be related to its antioxidant functions [11,27]. We recently found that intracellular metal chelators strongly inhibit TNFα-induced adhesion molecule expression in HAEC [30]. Therefore, it is tempting to speculate that the inhibitory effect of albumin on VCAM-1 expression is related, at least in part, to its ability to bind metal ions [11], thereby inhibiting metal-mediated lipid peroxidation [31] or hydroxyl radical formation [32].

Another mechanism by which albumin may inhibit VCAM-1 expression is suggested by the recent observation that BSO abolishes the inhibitory effect of albumin on TNFα-induced NF-κB activation in human lung epithelial cells [14]. BSO is a specific inhibitor of γ-glutamylcysteinyl synthetase, the rate-limiting enzyme in cellular GSH synthesis. Thus, albumin may stimulate GSH synthesis, which in turn may inhibit NF-κB activation and VCAM-1 expression. However, we found that BSO did not abolish the inhibitory effect of BSA on TNFα-induced VCAM-1 expression, indicating that, in HAEC, changes in intracellular GSH levels are not mediating the effects of BSA. These results are consistent with our previous observations that manipulating cellular GSH levels in HAEC with BSO or the cellular cysteine delivery agents, L-2-oxo-4-thiazolidinecarboxylic acid and *N*-acetyl-L-cysteine, did not affect TNFα-induced adhesion molecule expression and NF-κB activation [33].

In conclusion, our data indicate that physiological concentrations of albumin selectively inhibit TNFα-induced VCAM-1 expression in human aortic endothelial cells. Further studies are necessary to investigate the molecular mechanisms underlying this observation and to determine the in vivo efficacy of albumin as an anti-inflammatory and potentially anti-atherogenic agent.

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