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Osmotic regulation of cytokine synthesis *in vitro*

(osmolarity/stress)

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ABSTRACT **These studies were undertaken to investigate the therapeutic mechanism of saturated solutions of KI, used to treat infectious and inflammatory diseases. The addition of 12–50 mM KI to cultured human peripheral blood mononuclear cells resulted in 319–395 mosM final solute concentration and induced interleukin (IL)-8 synthesis. Maximal IL-8 production was seen when 40 mM salt was added (375 mosM) and was equal to IL-8 induced by endotoxin or IL-1α. However, there was no induction of IL-1α, IL-1β, or tumor necrosis factor to account for the synthesis of IL-8; the effect of KI was not due to contaminating endotoxins. Hyperosmolar NaCl also induced IL-8 and increased steady-state levels of IL-8 mRNA similar to those induced by IL-1α. IL-8 gene expression was elevated for 96 hr in peripheral blood mononuclear cells incubated with hyperosmolar NaCl. In human THP-1 macrophagic cells, osmotic stimulation with KI, NaI, or NaCl also induced IL-8 production. IL-1 signal transduction includes the phosphorylation of the p38 mitogen-activated protein kinase that is observed following osmotic stress. Using specific blockade of this kinase, a dose–response inhibition of hyperosmolar NaCl-induced IL-8 synthesis was observed, similar to that in cells stimulated with IL-1. Thus, these studies suggest that IL-1 and osmotic shock utilize the same mitogen-activated protein kinase for signal transduction and IL-8 synthesis.**

We began these studies to investigate whether KI modulated host defenses, particularly cytokine production. Compounds containing iodine, especially KI, have been used therapeutically for over 100 years to treat inflammatory and infectious diseases such as asthma, gout, gangrene, tertiary syphilis, and mycobacterial and fungal infections (reviewed in ref. 1). Oral KI is currently the treatment of choice for cutaneous sporotrichosis, although KI possesses no intrinsic antibacterial activity. It therefore seemed reasonable that KI could affect host phagocytic cells. In preliminary studies, we reported that the addition of 40 mM KI to cultured human peripheral blood mononuclear cells (PBMC), resulting in a final osmolarity of 375 mosmol/liter (mosM), induced interleukin (IL)-8 synthesis (2). As these studies expanded, we observed that similar concentrations of NaI or NaCl were equally effective in inducing IL-8 synthesis and, therefore, hyperosmolarity rather than the presence of iodide was likely stimulating the production of IL-8.

Mammalian cells are exposed to hyperosmotic conditions in the distal tubule of the kidney, during hemo- or peritoneal dialysis, when serum sodium rises as a consequence of dehydration, and when infused with hypertonic saline (3). In the present studies, we have examined the effect of hyperosmotic conditions on cytokine production. During the course of these studies, it was reported that mammalian cells phosphorylate proteins in response to 200 mM NaCl (400 mosM) (4, 5). Moreover, phosphorylation of a specific p38 mitogen-activated protein (MAP) kinase, homologous to the hyperosmolarity glycerol (HOG)1 stress-associated protein in yeasts, was reported (4). This hyperosmolar-responsive p38 MAP kinase is identical to that phosphorylated following exposure to endotoxin, tumor necrosis factor (TNF), or IL-1 (4, 6, 7). We now show that a simple and primitive stress condition such as hyperosmolarity provides a complete but specific signal for IL-8 gene expression and synthesis in primary human monocytic cells. IL-1, TNF, and lipopolysaccharide (LPS) also induce IL-8 gene expression and synthesis in these cells via receptor-mediated events; however, the post-receptor signal transduction by IL-1, TNF, and LPS appears to utilize pathways common to those used by cellular stress (4–8).

MATERIALS AND METHODS

Materials. Crystalline KI, NaI, and NaCl (Aldrich, catalogue nos. 22,991-1, 20,410-2, and 20,443-9, respectively) were dissolved in sterile pyrogen-free water (Abbott) at a concentration of 2 M. These solutions were subjected to autoclaving for 1 hr. Glycerol (Sigma) was dissolved in sterile pyrogen-free water (Abbott) at a concentration of 4 M. Recombinant human IL-1α (Hoffmann–LaRoche), IL-1 receptor antagonist (IL-1Ra, Synergen, Boulder, CO), and TNF soluble receptor p75-Fc chimera (TNFsRp75-Fc, Immunex) were used. These recombinant proteins did not contain measurable endotoxin (<100 pg/mg) by the *Limulus* amebocyte lysate assay with a sensitivity of 20 pg/ml (Associates of Cape Cod). LPS (B055) was purchased from Sigma and polymyxin B (PmxB) was obtained from Pfizer. The cytokine synthesis inhibitor SB 203580 (from John C. Lee and Peter R. Young, SmithKline Beecham) was dissolved to a stock concentration of 40 mM in dimethyl sulfoxide. The protein tyrosine kinase inhibitor herbimycin A (Calbiochem-Nova Biochem) was dissolved to a stock concentration of 1.0 mM in dimethyl sulfoxide. RPMI 1640 tissue culture medium (Sigma) containing 10 mM L-glutamine, 24 mM $NaHCO_3$ (Mallinckrodt), 10 mM Hepes (Sigma), 100 units of penicillin per ml, and 100 μg of streptomycin per ml (Irvine Scientific), at pH 7.4, was subjected to ultrafiltration to remove exogenous cytokine-inducing substances (9). The RPMI medium has a final osmolarity of 310 mosM.

PBMC. Human blood was drawn into heparinized (10 units/ml; Elkins-Sinn, Cherry Hill, NJ) syringes from healthy adult male volunteers. PBMC were separated by centrifugation on Ficoll (Sigma) and Hypaque (Winthrop Breon Laboratories, New York) gradients as described (10). The cells were washed with pyrogen-free saline (Abbott) and resuspended in RPMI medium at 5.0×10^6 cells per ml with 2% (vol/vol) heat-inactivated human AB serum unless stated otherwise. Five hundred microliters of cell suspension was added to round-bottom 12 × 75 mm polypropylene tubes (Falcon). For

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Abbreviations: IL, interleukin; IL-1Ra, IL-1 receptor antagonist; LPS, lipopolysaccharide; MAP, mitogen-activated protein; PBMC, peripheral blood mononuclear cells; PmxB, polymyxin B; RT, reverse transcription; TNF, tumor necrosis factor.

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cells stimulated with salts or glycerol, 450 μl of RPMI was added to 500 μl of cells followed by the addition of 50 μl of either salt or glycerol solution. The salt or glycerol solution was first diluted in sterile water to 20 times the desired final concentrations in the cell cultures. For example, to add 40 mM NaCl for a final osmolarity of 375 mosM, a stock of 2 M NaCl was diluted in water to 800 mM and 50 μl was added to 950 μl of the cells in RPMI medium. The final calculated osmolarity accounts for the 5% dilutional effect of water. LPS or IL-1α was diluted in RPMI medium and added to cells. IL-1Ra, TNFsRp75-Fc, SB 203580, or herbimycin A was incubated with cells 1 hr prior to the addition of stimulants. Unless indicated, cultures were incubated for 24 hr at 37°C in 5% CO_2, after which time they were frozen (−70°C) and thawed (37°C) for three cycles prior to radioimmunoassay (RIA) for cytokines.

THP-1 Cells. THP-1 cells were obtained from American Type Culture Collection and grown in polystyrene T-75 culture flasks (Corning) suspended in ultrafiltered RPMI medium containing 10% Serum Plus (JRH Biosciences, Lenexa, KS). When density reached ≈1 × 10^6 cells per ml, cells were resuspended in fresh RPMI medium with 10% Serum Plus at a concentration of 5 × 10^6 cells per ml. Five hundred microliters was added to polypropylene tubes (Falcon). Cells were stimulated and incubated as described above for PBMC.

RIA. RIAs for IL-1α, IL-1β, IL-6, TNFα, and IL-8 were as described (11–15). The IL-1β RIA detects <10% of precursor IL-1β.

IL-8 mRNA. Ten milliliters of PBMC in 1% human serum was cultured as described above at 2.5 × 10^6 per ml. After 4 hr at 37°C, cells were centrifuged, and total RNA was isolated using Tri-Reagent (Molecular Research Center, Cincinnati). After precipitation in ethanol, the RNA was dissolved in Formazol (Molecular Research Center) and stored at −70°C until used. Ten micrograms of RNA was electrophoresed in an agarose/formaldehyde gel (1.0% agarose/6.6% formaldehyde) at 25 V. RNA was transferred onto a nitrocellulose membrane (16) (Hybond-N; Amersham), cross-linked by UV light, and prehybridized for 4 hr at 42°C. The hybridization solution was then removed and fresh hybridization solution containing 20–30 × 10^6 cpm of ^{32}P-labeled IL-8 cDNA probe was added. The IL-8 probe was a 500-bp fragment of the human IL-8 cDNA (kind gift from Theresa Pizarro, University of Virginia, Charlottesville). The probe was labeled with α-^{32}P [3000 Ci/mmol (1 Ci = 37 GBq); New England Nuclear] using random priming (Boehringer Mannheim), followed by chromatographic separation (Nunctrap Kit, Stratagene). After overnight hybridization, membranes were washed twice at 45°C for 30 min in 1× SSC/0.1% SDS and then in 0.2× SSC/0.1% SDS, before exposure to film for 21–24 hr.

IL-8 Reverse Transcription Polymerase Chain Reaction (RT-PCR). After extraction in Tri-Reagent, RNA was precipitated in ethanol and then dissolved in water. One hundred nanograms was subjected to RT as suggested by the manufacturer (GeneAmp RNA PCR kit, Perkin–Elmer). The DNA was then amplified by the PCR using complementary IL-8 oligonucleotide primers for 25 cycles in a thermocycler (Perkin–Elmer). The sequence of the 5′ primer was 5′-ATGACTTCCAAGCTGGCCGTGGCT-3′, and that of the 3′ primer was 5′-TCTCAGCCCTCTTCAAAAACTTCTC-3′ (Perkin–Elmer). For amplification of glyceraldehyde-3-phosphate dehydrogenase, the same protocol was employed using the 5′ primer 5′-TGAAGGTCGGAGTCAACGGATTTGGT-3′ and the 3′ primer 5′-CATGTGGGCCATGAGGTCCACCAC-3′. The amplified products were electrophoresed into a 2.0% agarose gel containing 0.5 μg of ethidium bromide per ml. Each PCR experiment was controlled by the substitution of water for sample RNA during RT; this control was then amplified for IL-8 cDNA. In each case, we observed no amplified products, indicating absence of contamination during PCR.

Statistics. Data are shown as means ± SEM. Group means are compared by analysis of variance (ANOVA) using Fisher's least significant difference.

RESULTS

KI Induces IL-8 in PBMC in the Absence of Endotoxin Contamination. Incubation of PBMC with LPS at 10 pg/ml or with the addition of 12.5–50 mM of KI resulted in production of IL-8 (Fig. 1). In parallel cultures, KI solutions or LPS in RPMI culture medium was incubated with or without PmxB. PmxB blocked LPS-induced IL-8. On the other hand, the lack of reduction in IL-8 by PmxB demonstrates that the effect of KI was not due to endotoxin contamination.

Specific Induction of IL-8 in PBMC by High Molar KI. Incubation of PBMC with IL-1α at 10 ng/ml or KI added at 2.5–40 mM resulted in dose-dependent production of total IL-8 (Fig. 2). The maximal induction was seen with the addition of 40 mM KI, which was similar to that induced by IL-1α. At 80 mM KI, no IL-8 production was observed. In the same cultures, there was a small but statistically not significant increase in IL-1α synthesis compared to unstimulated control cultures (data not shown). In separate experiments, there was no detectable increase in IL-6 over unstimulated control cells in response to increasing concentrations of KI (0.8–100 mM KI, data not shown).

There was no role for intermediate production of IL-1 or TNF in the stimulation of IL-8 due to KI. As shown in Fig. 2, IL-1Ra completely prevented IL-1α-induced IL-8 but had no effect on the induction of IL-8 by the addition of 40 mM of KI. When cells were incubated with IL-1Ra plus TNFsRp75-Fc (10 μg/ml each) for 1.0 hr, followed by addition of KI at 40 mM, no reduction in KI-induced IL-8 was observed (data not shown, $n = 3$).

Effect of NaCl. To study the specificity of the induction of IL-8 by KI, NaCl was used as a stimulant. As shown in Fig. 3, the addition of NaCl induced an increase in IL-8 synthesis similar to that observed using KI. Adding 40 mM NaCl to PBMC induced approximately as much IL-8 as did IL-1α, whereas at 80 mM there was less. Similar to results with KI, PmxB did not suppress NaCl-induced IL-8 (data not shown). The synthesis of mature 17,000 molecular weight IL-1β was not

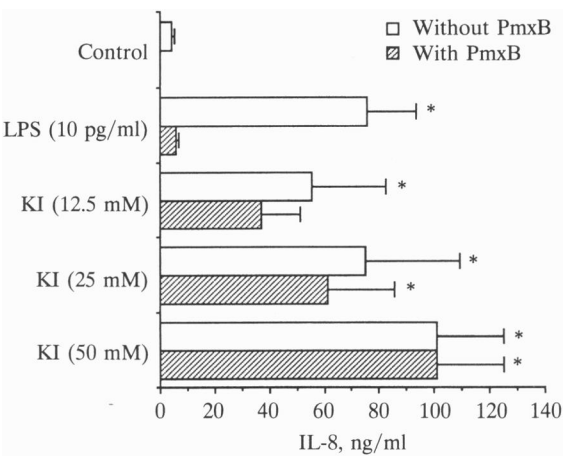

FIG. 1. Effect of KI on synthesis of IL-8 by PBMC. PBMC from three donors were incubated in medium (Control), in the presence of LPS (10 pg/ml), or with addition of increasing concentrations of KI as depicted. Parallel control medium, LPS, and KI solutions were preincubated with PmxB (final concentration, 2.5 μg/ml) for 30 min before being added to PBMC. After 24 hr PBMC cultures were assayed for total IL-8. Mean IL-8 levels ± SEM are shown. *, $P < 0.05$ compared to control. There was no statistically significant difference in IL-8 production in PBMC stimulated with KI in the presence or absence of PmxB.

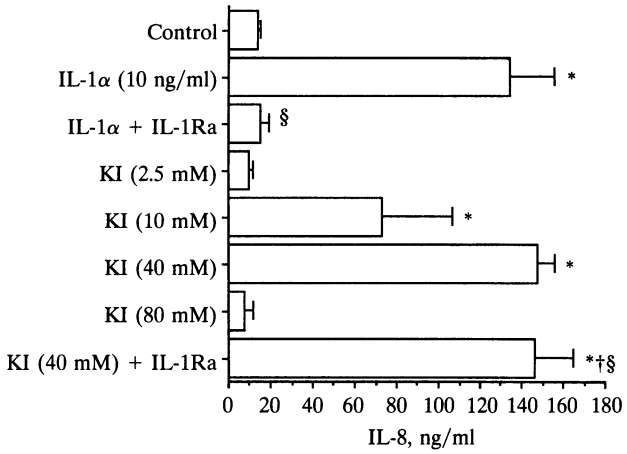

FIG. 2. Lack of IL-1 or TNF in KI-induced IL-8 in PBMC. PBMC from three donors were incubated in medium (Control), with IL-1α (10 ng/ml), or with KI at the concentrations indicated. IL-1Ra was added 1 hr prior to addition of IL-1α or 40 mM KI as shown. Mean IL-8 levels ± SEM are depicted. *, $P < 0.05$ compared to control; †, $P > 0.05$ compared to KI at 40 mM; §, IL-1Ra at 10 μg/ml.

detected (<115 pg per 2.5×10^6 PBMC, $n = 4$). Trypan blue exclusion was used to assess cytotoxic effects of high molar salts. After 4 and 24 hr at 37°C in IL-1α (10 ng/ml) or with NaCl (0.625–80 mM), there was no increase in trypan blue uptake compared to cells in isotonic culture medium. In these cultures, viability was >95%.

With few exceptions, the addition of 40 mM NaCl consistently induced IL-8 production in 75 separate experiments using PBMC from 11 different donors. A mean fold increase of 3.4 (range, 2.1–11.3; $P < 0.001$) over control cells was observed in 79% of the experiments; an increase of <2.0-fold was observed in the remaining 21% of experiments. The optimal concentration of added NaCl varied between individuals from 20 to 60 mM. In 6 experiments using 5 donors, 80 mM NaCl induced less IL-8 than did lower concentrations.

PBMC were also cultured with increasing concentrations of an ineffective osmole, glycerol (5, 20, 80, or 160 mM). After 24 hr, no increase in IL-8 over control was detected ($n = 3$). An increase in IL-8 production was observed in THP-1 cells. After 24 hr, a 4.4-fold increase over unstimulated cells was measured

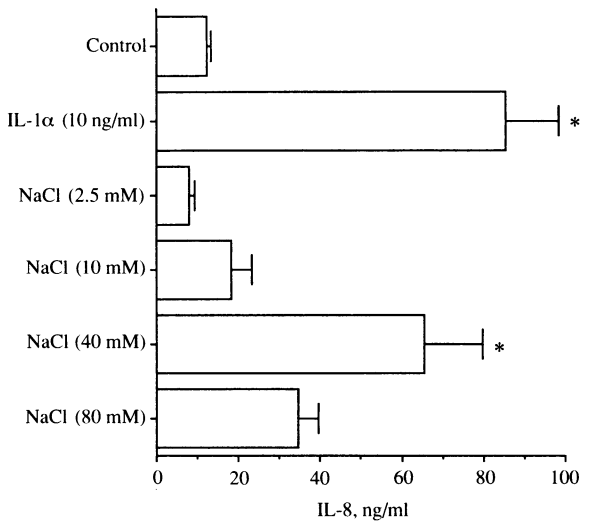

FIG. 3. Effect of NaCl on synthesis of IL-8 by PBMC. PBMC from five donors were incubated in the presence of medium alone (Control), with IL-1α (10 ng/ml), or with increasing concentrations of NaCl as shown. Mean IL-8 levels ± SEM are shown. *, $P < 0.05$ compared to control.

with the addition of 33.3 or 40 mM KI ($P < 0.05, n = 4$). Similar results were observed using NaI or NaCl as stimulants.

Osmotic Stress Increases Levels of IL-8 mRNA in PBMC. PBMC were incubated in the presence of medium (control), IL-1α, or increasing concentrations of NaCl. After 4 hr, steady-state mRNA for IL-8 was determined. Parallel cultures were continued for 24 hr and assayed for IL-8 protein. As shown in Fig. 4, IL-1α induced a 4-fold increase in IL-8 synthesis compared to control cells. The addition of 40 mM NaCl increased IL-8 synthesis 5-fold compared to control. An increase in steady-state level of IL-8 mRNA was observed in cells incubated with IL-1α or 40 mM NaCl (375 mosM). At 80 mM, the addition of NaCl was associated with less IL-8 mRNA, and there was no significant increase in IL-8 synthesis over that of control. At 20 mM and 10 mM NaCl, IL-8 synthesis and mRNA were correspondingly less than those at 40 mM. However, there was substantial IL-8 mRNA present in the control cells (310 mosM). In several experiments, we observed that the presence of 1% human serum can be a stimulant for IL-8 gene expression but not synthesis.

Osmotic Stress Induces Prolonged Steady-State IL-8 mRNA. We next examined the effect of hyperosmolarity on IL-8 gene expression in the absence of serum. As shown in Fig. 5*A*, there was a small amount of IL-8 synthesized by control cells incubated in RPMI medium without serum. IL-8 reached a maximal level of 2.7 ng/ml at 24 hr. This was accompanied by a transient increase in gene expression for IL-8 as shown by the RT-PCR product. Of note, the 4-hr time point contained a significant amount of IL-8 PCR product, similar to the elevated levels of mRNA observed at the 4-hr time point by Northern analysis (Fig. 4). However, this transient IL-8 gene expression in control cells was not associated with significant IL-8 synthesis measured at 24 hr (2.7 ng/ml). In contrast (Fig. 5*B*), in the presence of an additional 40 mM NaCl, IL-8 synthesis continued to increase above that of control to a maximum level of 23.7 ng/ml at 96 hr. This increase was accompanied by a sustained level of IL-8 gene expression throughout the time course. Amplification of glyceraldehyde-3-phosphate dehydrogenase present in the RT product of these

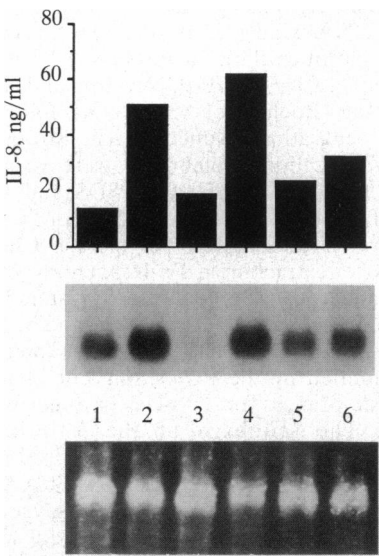

FIG. 4. Increased IL-8 mRNA synthesis in PBMC following exposure to NaCl. PBMC (25×10^6) were cultured in medium containing 1% serum either alone (lane 1), with 10 ng of IL-1α per ml (lane 2), or with the addition of NaCl at 80, 40, 20, or 10 mM (lanes 3–6, respectively). IL-8 synthesis was measured after 24 hr of incubation. In matching aliquots, total RNA was isolated after 4 hr and assessed for the presence of IL-8 mRNA (shown below the protein data). Ethidium bromide-stained rRNAs are shown for each condition in the bottom line.

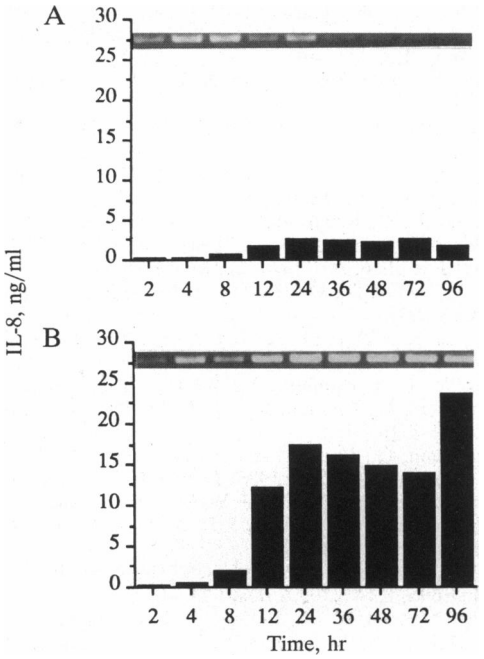

FIG. 5. Time course of IL-8 gene expression in response to NaCl. PBMC (5×10^6) were cultured without (*A*) or with (*B*) an additional 40 mM NaCl in the absence of serum. At the indicated time points, cultures were assayed for IL-8 production. RNA was extracted from cell pellets of parallel cultures and assayed for IL-8 mRNA by PCR. The ethidium bromide-stained bands of PCR product corresponding to the indicated time points are shown at the top of each graph.

samples revealed that unequal sample loading did not account for IL-8 gene expression described above.

Equal Inhibition of IL-8 Synthesis Induced by IL-1α or NaCl by SB 203580. SB 203580 is a pyridinyl imidazole that binds to and blocks the activity of two related human p38 MAP kinases (17). One is identical to the human p38 MAP kinase activated by LPS, hyperosmolarity (18), or IL-1 (7). Preincubation of PBMC with SB 203580 inhibited IL-8 induction by either IL-1α or 40 mM NaCl (375 mosM, Fig. 6). This inhibition was significant between 0.06 M and 2 μM SB 203580 for IL-1α-induced IL-8 ($IC_{50} = 0.13$ μM, $P < 0.01$). In PBMC stimulated with NaCl, SB 203580 also inhibited IL-8 synthesis between 0.03 M and 2 μM ($IC_{50} = 0.08$ μM, $P < 0.05$). As shown, no notable difference was observed in the inhibitory curves using either IL-1α or NaCl as stimulant. Herbimycin A also inhibited NaCl-induced IL-8 synthesis, which was similar to the inhibition of IL-1-induced IL-8 ($n = 4$, $IC_{50} = 0.04$ μM). In separate experiments, control dimethyl sulfoxide concentrations did not decrease IL-1α- or NaCl-induced IL-8 production.

DISCUSSION

We show that osmotic stress increases *in vitro* IL-8 gene expression and synthesis in primary human mononuclear cells and the human THP-1 macrophagic cell line. IL-8 synthesis was observed using three different salts (KI, NaI, and NaCl) and suggests that the original observation of KI-induced IL-8 (2) was due to osmotic stress rather than to the presence of the iodide species. Of particular interest was the observation that KI or NaCl stimulated synthesis of IL-8 but not IL-1α, IL-1β, TNFα, or IL-6. Since the RPMI culture medium itself contained 310 mosM of solute, the addition of 40 mM KI or NaCl results in a stimulatory osmolarity of 375 mosM. At this osmolarity, there was as much IL-8 induced as that induced by IL-1α or LPS, two well-established stimulators of IL-8 gene expression and synthesis (19). The stimulatory effect of these

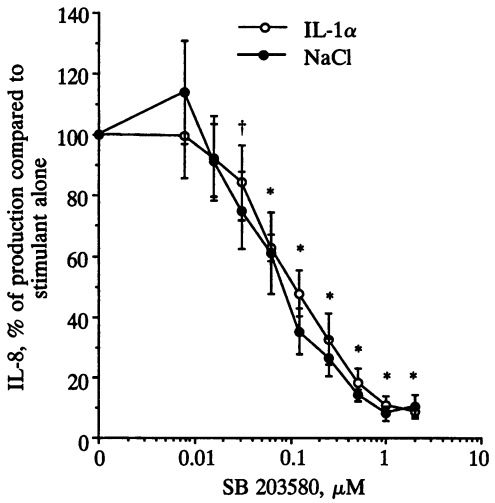

FIG. 6. Inhibition of IL-8 synthesis by SB 203580. PBMC from five donors were incubated for 24 hr with IL-1α (10 ng/ml) or NaCl (375 mosM final solute osmolarity) as stimulants. SB-203580 was added to PBMC aliquots 1 hr prior to the addition of either stimulant. The highest dimethyl sulfoxide concentration used in these experiments was 0.005%, which was without effect. Data are expressed as the percent change from addition of stimulant alone (100%). Mean IL-8 levels ± SEM are shown. *, $P < 0.05$ compared to either stimulant added alone; †, $P < 0.05$ compared to NaCl.

salt concentrations on IL-8 synthesis was not due to contaminating endotoxins and did not require an intermediate production of IL-1 or TNF, as the presence of specific inhibitors of IL-1 (IL-1Ra) and TNF (TNFsRp75) did not diminish IL-8 production. Several years ago, we examined the effect of adding 20 or 40 mM sodium acetate to human PBMC suspended in tissue culture medium (350 or 390 mosM). Although sodium acetate increased production of bioactive IL-1, the addition of 20 or 40 mM NaCl did not affect IL-1 production (20).

Glycerol is a cell membrane-permeable solute and therefore an example of an ineffective osmole. It increases tonicity but does not establish an osmotic gradient across cell membranes. Using twice the concentration of glycerol to achieve equivalent extracellular osmolarity as that of the dissociable salts, there was no increased synthesis of IL-8 above control cells. These findings emphasize the importance of an osmotic gradient across the membrane and indicate that hyperosmolarity *per se* is an insufficient signal to generate IL-8. In mammalian renal tubular cells, specific gene expression of *Egr-1*, c-*fos*, and the heat shock protein HSP70 follows the addition of 100 mM NaCl to the culture medium (510 mosM final osmolarity) (21). Addition of 200 mM glycerol (510 mosM final osmolarity) did not activate these genes (21), which is similar to our findings regarding IL-8 synthesis in PBMC.

In considering the similarities between gene expression induced by IL-1 and hyperosmotic stress, recent studies have focused on a primitive kinase cascade. Macrophagic cells exposed to LPS phosphorylate the p38 MAP kinase homologue of HOG1, which is required for yeasts to grow in hyperosmotic conditions (4). Activation of p38 kinase was also demonstrated in mammalian cells exposed to 200 mM NaCl (4). In other mammalian cells, c-*jun* kinase 1 (Jnk1) (another relative of HOG1 and a member of the MAP kinase family) was activated by osmotic stress (5). Heat shock- or osmotic stress-activated reactivating kinase is another homologue of HOG1 (8). Thus, similarities exist between stress and IL-1 receptor signaling in inducing gene expression and synthesis of IL-8. IL-8 gene expression takes place following activation of transcription factors AP-1, NF-κB, and NFIL-6 (22), and IL-1 also stimulates these transcription factors (23, 24). Of the

kinases activated by IL-1, attention has focused on the p38 and p54α kinases (6, 7). Therefore, it appears that the same phosphorylation of the p38 kinase takes place in cells exposed to either IL-1 or hyperosmolarity. In a similar fashion, the activation of jnk1 by hyperosmolar stress probably also takes place in IL-1-treated cells since p54α is related to p54γ, and p54γ is a truncated form of jnk1 (6).

The pyridinylimidazole compound SB 203580 primarily reduces the translation of LPS-induced cytokines in human cells. Using a compound related to SB 203580 as an affinity ligand probe, two intracellular proteins were purified and named cytokine-suppressive antiinflammatory drug binding proteins (CSBP) 1 and 2 (17). CSBP2 has an amino acid sequence that differs from murine p38 at only two residues (4). The recently cloned human p38 MAP kinase (18) is identical to CSBP2 (17). Since we observed similar inhibiting concentrations by SB 203580 of IL-8 synthesis induced by either IL-1 or NaCl, it is likely that p38 is also involved in the translation of IL-8 induced by either IL-1 or hyperosmolarity. Thus, despite the evolution of IL-1 and its receptor, the ancient stress-related MAP kinase cascade is still utilized by IL-1. In terms of kinase cascades, hyperosmotic stress and IL-1 in these cells activate a family of MAP kinases distinct from those activated by growth factors such as epidermal growth factor (7, 8). Therefore, IL-1 appears to be functionally linked with exogenous stressors such as osmotic shock, heat shock, chemical stress, and ultraviolet irradiation, any of which can activate the MAP stress pathway.

One can speculate that neutrophil-mediated host defenses are important in clinical conditions. In hemorrhagic shock in rats, decreased bacterial translocation from the gut was seen following resuscitation with hypertonic saline compared to infusions with isotonic saline (25). In rabbits, infusion of hypertonic saline resulted in enhanced immune function (26). In a prospective, randomized, double-blind clinical study, infusion of hyperosmotic saline in dextran (2400 mosM) in trauma patients achieved *in vivo* solute concentrations in the ranges we studied *in vitro* (343 $\pm$ 34 mosM). This resulted in improved outcome in a subset of patients compared to those receiving normal saline (3). Our findings may shed light on these clinical observations.

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