Hyperglycemic Levels of Glucose Inhibit Interleukin 1 Release from RAW 264.7 Murine Macrophages by Activation of Protein Kinase C*

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Diabetic patients with hyperglycemia (high blood glucose) have frequent and persistent bacterial infections linked to significantly diminished bactericidal activity and macrophage function. Interleukin-1 (IL-1), released primarily from activated macrophages, is a key mediator of effective host defense against microorganisms. We observe that hyperglycemic levels of D-glucose (8–20 mM) inhibit the release of IL-1 by lipopolysaccharide-stimulated RAW 264.7 murine macrophage cells. An inhibitor of glucose transport and metabolism, 2-deoxyglucose, prevents this inhibition of IL-1 release. High levels (8–20 mM) of fructose and mannose (but not galactose or l-glucose) also inhibit the release of IL-1 activity, suggesting that metabolism is required for IL-1 inhibition. Immunoprecipitation and activity measurements demonstrate that high glucose levels block the release of IL-1 but do not inhibit IL-1 production. High glucose levels (20 mM) increase protein kinase C (PKC) activity, and inhibitors of PKC block the inhibitory effects of glucose. Phorbol 12-myristate 13-acetate, an agonist of PKC, mimics glucose-inhibited induction of IL-1 release. These results demonstrate that high glucose levels inhibit IL-1 release (but not production) by RAW 264.7 murine macrophages, and this inhibition is mediated by PKC activation. These studies suggest that persistent infections in hyperglycemic patients may be due to an inhibition of IL-1 release from macrophages.

A major complication in diabetic patients is the frequent occurrence and long duration of bacterial infections (1–3). Three lines of evidence establish high blood glucose levels as a key mediator of decreased bactericidal activity: 1) a strong correlation between high blood glucose levels and the prevalence of infection (4), 2) reversal of high infection rate by normalizing blood glucose levels (5), and 3) decreased bactericidal activity by isolated macrophages upon exposure to high glucose levels (6).

High glucose concentrations in dialysis fluid correlate with an impairment of anti-bacterial defense in the peritoneal cavity of rats (7) and in a decrease of interleukin 1 (IL-1) release (8). IL-1, a key mediator of the immune response and effective host defense against microorganisms, is produced by macrophages in response to stimulants including bacterial products such as lipopolysaccharide (LPS). Activated macrophages have a high rate of glucose uptake (GLUT1 transporter) and glucose utilization (9–11). The proinflammatory cytokines IL-1α and IL-1β are expressed as 33-kDa precursor proteins that are cleaved intracellularly by cysteine proteases (12–14). The synthesis and release of IL-1, although often stimulated by the same factors, are regulated differently (15). Both IL-1α and IL-1β are expressed without leader sequences and are not secreted via the classic endoplasmic reticulum-Golgi apparatus as are many other cytokines (16). We recently reported that IL-1 is released from macrophages by a novel mechanism mediated by nitric oxide and cGMP (17), but the full mechanism of IL-1 secretion is still unknown.

In this study, we demonstrate that hyperglycemic glucose levels inhibit IL-1 release but not production by activated RAW 264.7 murine macrophages. This inhibition requires metabolism, is fully reversible upon removal of high glucose, and is mediated by protein kinase C. These findings may offer insight into the causes of diminished bactericidal activity in diabetics and suggest possible interventions for infections.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human IL-1β was obtained from Cistron (Pine Brook, NJ). Monoclonal hamster anti-murine IL-1α and IL-1β were kind gifts from Dr. David Chaplin (Washington University). CMRL-1066 tissue medium was obtained from Life Technologies, Inc. Laboratories. LPS (Escherichia coli serotype 0111:B4), PMA, t-glucose, D-mannose, D-fructose, and D-galactose were from Sigma.

Nitrite Determination—Nitrite release was determined by mixing 50 μl of culture medium with 50 μl of Griess reagent (1 part 0.1% naphthalene-2-sulfonic acid, disodium salt, 1% sulphuric acid, and 1% 1-sulfonaphthalein sodium salt, and 1 part N-(1-naphthyl)ethylenediamine dihydrochloride). After incubation for 10 min at room temperature, absorbance was determined at 540 nm.

Phorbol 12-myristate 13-acetate (PMA) was purchased from Calbiochem (La Jolla, CA). Calpoxin was from LC Laboratories (Woburn, MA). [35S]Methionine trans-label (1000 Ci/ mmol) and [y-32P]ATP were from Amersham Corp. Sodium nitrite was from Fisher. All other reagents were purchased from commercially available sources.

Cell Culture—RINm5F, an insulin-secreting cell line (18, 19), and RAW 264.7, a murine macrophage cell line (20, 21), were obtained from ATCC and cultured at the Washington University Tissue Culture Support Center. Cells were removed from T-75 flasks by treatment with 0.05% trypsin, 0.02% EDTA at 37 °C. The cells were washed and added to microwell plates at concentrations of 2 × 10⁶ cells/200 μl of CMRL-1066 supplemented with 10% heat-inactivated fetal bovine serum, 2 μM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 5.5 mM glucose (complete CMRL). The cells were incubated for 24 h after plating at 37 °C in 95% air, 5% CO₂, and the medium was replaced prior to initiation of experiments. Tissue culture medium was buffered with 25 mM HEPES to maintain a pH of 7.4 during incubation of RAW 264.7 cells.

IL-1 Bioactivity—RINm5F assay: IL-1 activity was determined as described previously (22). Briefly, cell-free culture medium obtained from LPS-activated RAW 264.7 cells was added to RINm5F cells (2 × 10⁶ cells/200 μl) at a final dilution of 1:100. Concurrently, a standard curve was performed using hIL-1α at concentrations between 0.1 and 1 unit/ml (10–100 pg). After incubation for 24 h at 37 °C in 95% air, 5% CO₂, the culture medium was collected, and nitrite levels were determined. IL-1 concentrations were extrapolated from the standard curve.

Nitrite Determination—Nitrite release was determined by mixing 50 μl of culture medium with 50 μl of Griess reagent (1 part 0.1% naph-
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thielythelenediamine dihydrochloride in H 2O plus 1 part 1.32% sulfanilamide in 60% acetic acid (23)) in a 96-well microtiter plate. The absorbance at 540 nm was measured on a TiterTek Multiskan MCC/340 plate reader, and nitrite concentrations were calculated from a standard curve using NaNO 2 concentrations between 0.1 and 10 mM.

Immunoprecipitation of IL-1—RAW 264.7 cells (1 × 10 6 cells in 1 ml) in CMRL methionine-deficient medium (9 parts CMRL without methionine; 1 part CMRL containing methionine) were activated with LPS (1 μg/ml) in 2 or 20 mM glucose and pulsed for 12 h with 150 μCi of [ 35S]methionine trans-label. The cells were washed with CMRL and incubated for an additional 12 h (total 24 h) at 37 °C in 95% air, 5% CO 2 . The supernatant was collected, and cells were removed by centrifugation at 200 × g for 1 min. The cells were washed in PBS and lysed in 0.15 M NaCl, 1% Nonidet plus protease inhibitors (aprotinin, 1 μg/ml; leupeptin, 1 μg/ml; phenylmethylsulfon fluoride, 0.1 mM; iodoacetamide, 1 mM; EDTA, 0.1 mM) by sonication, and the lysates were clarified by centrifugation at 10,000 × g for 30 min at 4 °C. Protease inhibitors were also added to the supernatant. Both lysate and supernatant were preclreated with 20 μl of protein-A-Sepharose. Equivalent fractions of each sample were immunoprecipitated for 2 h by addition of anti-murine IL-1α (final dilution 1:1000). Protein A-Sepharose was added for 1 h, and the beads were washed three times with PBS plus 1% Nonidet plus 0.2% SDS and finally PBS and then boiled in SDS-polyacrylamide gel electrophoresis sample buffer. Samples were resolved by 15% SDS-polyacrylamide gel electrophoresis and visualized by fluorography.

PKC Activity—PKC activity was determined as described previously (24). Briefly, RAW 264.7 cells (2 × 10 6 cells/200 μl) were incubated for 4 or 24 h at 37 °C in 95% air, 5% CO 2 and collected and washed with PBS. The cells were permeabilized with 50 μg/ml digitonin in buffer (40 μl): 137 mM NaCl, 5.4 mM KCl, 10 mM MgCl 2, 0.3 mM Na 2 PO 4, 0.4 mM KPO 4, 25 mM β-glycerophosphate, 5.5 mM glucose, 5 mM EGTA, 1 mM CaCl 2, and 20 mM Hepes, pH 7.2, with 1 mM [γ- 32P]ATP and 100 μM PKC specific substrate VRKRTTLRL. After 10-min incubation at 30 °C, the reaction was stopped with trichloroacetic acid (final 5%) and spotted on a phosphocellulose filter (Whatman P-81) and washed with 75 mM phosphoric acid and 75 mM NaPO 4, pH 7.5. The filters were counted in a Packard 1500 Tri-carb scintillation counter.

Statistical analysis was performed using a Scheffe's F-test posthoc analysis (Statview V4.0, Abacus Concepts).

RESULTS

Effect of Glucose Levels on the Release of Nitric Oxide and IL-1 Activity by RAW 264.7 Cells—RAW 264.7 cells (2 × 10 6/200 μl) were exposed to LPS (1 μg/ml) plus varied glucose concentrations (0–20 mM) for 24 h. Nitrite and IL-1 in the spent medium were measured at 24 h. Glucose dose-dependently stimulated nitric oxide production (measured as the stable oxidation product, nitrite) (Fig. 1), reaching maximum levels at 10 mM glucose. Under conditions of very low glucose levels (0–1 mM), LPS-induced IL-1 activity was not detected over control levels. However, at glucose concentrations corresponding to in vivo basal blood glucose levels (2–6 mM), IL-1 activity release was 10-fold over nonstimulated control levels. A sharp decline in the release of IL-1 activity occurs with a further rise in glucose levels and IL-1 release is almost completely inhibited at 10–20 mM, which corresponds to hyperglycemic blood glucose levels.

Glucose Inhibition of IL-1 Release Is Blocked by 2-Deoxyglucose—To determine whether this inhibition of IL-1 release required glucose transport and/or metabolism, 2-deoxyglucose was added to LPS-stimulated RAW 264.7 cells for 24 h (Fig. 2). The compound, 2-deoxyglucose, competes with glucose for the GLUT-2 transporter, and phosphorylation of 2-deoxyglucose by hexokinase effectively inhibits glucose flux through the glycolytic pathway. At 5 mM glucose, LPS induced a 10-fold increase in the release of IL-1 activity, but IL-1 release was completely inhibited at 15 mM glucose. 2-Deoxyglucose (5 mM) blocked the inhibitory effect of 15 mM glucose.

Effects of Related Hexoses on IL-1 Activity Released by RAW 264.7 Cells—The ability of 2-deoxyglucose to block the inhibitory effects of glucose on IL-1 release suggests that metabolism is required for inhibition. To determine whether other metabolized sugars could inhibit IL-1 release, the effect of hexoses closely related to glucose were added to LPS-stimulated RAW 264.7 cells in the presence of 5 or 15 mM glucose (Fig. 3). L-Glucose and D-galactose, hexoses that are not metabolized by these cells, had little effect on IL-1 release at concentrations up to 20 mM. D-Fructose, moderately metabolized by macrophages, dose-dependently inhibited IL-1 release, but higher concentrations were required for complete inhibition than for D-mannose, which is metabolized at a similar rate to glucose.

Reversal of Glucose Inhibition of IL-1 Release—To determine whether the inhibitory effect of high glucose on IL-1 release is reversible, RAW 264.7 cells were stimulated with LPS for 24 h in the presence of 0–20 mM glucose (Fig. 4). As shown previously in Fig. 1, IL-1 release was maximal at 2–6 mM glucose but was attenuated in the absence of glucose (0 mM) and at 8–20 mM glucose. Following the 24-h incubation, RAW cells were washed and incubated with fresh medium containing 5 mM glucose for an additional 24 h. IL-1 release from cells initially incubated in the absence of glucose (0 mM) was still attenuated...
IL-1 release, RAW 264.7 cells were stimulated with LPS (1 μg/ml) for 24 h in the presence of 5 mM D-glucose plus the indicated concentrations of L-glucose, D-galactose, D-fructose, or D-mannose. IL-1 activity in the medium was measured by the RINm5F bioassay. Results are the average ± S.E. of three individual experiments containing three replicates per condition.

To confirm that high glucose levels inhibit IL-1 release but not synthesis, immunoprecipitation of intra- and extracellular IL-1α was performed (Fig. 7). LPS-activated RAW 264.7 cells were pulse-labeled with [35S]methionine for 12 h in the presence of 2 or 20 mM glucose. The cells were then washed, fresh chase medium added, and the incubation continued for a total of 24 h. At 24 h, a large amount of extracellular IL-1α was detected at 2 mM glucose but not 20 mM glucose or in unstimulated samples (Fig. 7). IL-1α, mainly in the form of precursor protein, was detected intracellularly at 20 mM glucose with a lesser amount of protein at 2 mM glucose, suggesting that metabolically labeled intracellular IL-1α stores were depleted by release at 2 mM glucose in LPS-stimulated cells. These data combined with the findings in Fig. 6b indicating that elevated glucose concentrations of 10 and 20 mM do not alter intracellular IL-1 activity, add further support to the conclusion that high glucose levels inhibit IL-1 release but not synthesis. In our previous studies (17) more than 90% of IL-1 activity released by activated RAW 264.7 cells at 24 h was due to IL-1α. Neither IL-1β or precursor IL-1β were detected above control levels in parallel pulse labeling studies to assess IL-1β processing and release (data not shown), probably due to the low level of IL-1β.

**Effects of High Glucose Levels on IL-1 Production and Release**—That high glucose levels exert inhibitory effects at an early time point suggests that high glucose may be inhibiting the induction or synthesis of IL-1. To determine whether high glucose attenuates the release of bioactive IL-1 by inhibiting LPS-induced synthesis of IL-1, both intracellular and extracellular IL-1 activity was measured (Fig. 6). Following a 24-h incubation in the presence of 0, 5, 10, or 20 mM glucose, extracellular IL-1 released by RAW cells into the medium was assayed for activity (Fig. 6a). As demonstrated previously, 0, 10 and 20 mM glucose exhibited a strong inhibitory effect on the release of IL-1 activity. To measure intracellular activity, the cells were collected by centrifugation, washed, lysed by sonication, and assayed for IL-1 activity (Fig. 6b). While intracellular IL-1 activity was attenuated in the absence of glucose (0 mM), glucose at 10 and 20 mM failed to inhibit IL-1 activity as compared with 5 mM glucose. These data suggest that high glucose has little effect on IL-1 synthesis but has a strong inhibitory effect on the release of IL-1.

**Time Dependence of Glucose Inhibition of IL-1 Release**—To determine when high glucose levels must be present to inhibit IL-1 release, RAW 264.7 cells were stimulated with LPS (1 μg/ml) in the presence of 5 mM glucose for 24 h. Glucose was added at the indicated times during the incubation for a final concentration of 15 mM (Fig. 5). When added during the first 10 h of a 24-h incubation, 15 mM glucose almost completely inhibited IL-1 release. Addition of glucose after 10 h resulted in only partial (11–14 h) or no (15–24 h) inhibition, demonstrating that the inhibitory action of high glucose occurs within the first 10 h of the incubation.

**Results are the average ± S.E. of three individual experiments containing three replicates per condition.**

**Fig. 3.** Metabolized hexoses inhibit IL-1 release from RAW 264.7 cells. RAW 264.7 cells were activated with LPS (1 μg/ml) for 24 h in the presence of 5 mM D-glucose plus the indicated concentrations of L-glucose, D-galactose, D-fructose, or D-mannose. IL-1 activity in the medium was measured by the RINm5F bioassay. Results are the average ± S.E. of three individual experiments containing three replicates per condition.

**Fig. 4.** Glucose inhibition of IL-1 release is reversed by removal of high glucose. RAW 264.7 cells were activated with LPS (1 μg/ml) in the presence of 0–20 mM glucose as indicated for 24 h, and the medium was assayed for IL-1 activity. Following the initial 24-h incubation, the cells were washed with fresh medium containing 5 mM glucose, incubated for another 24 h, and IL-1 activity was measured. Results are the average ± S.E. of three individual experiments containing three replicates per condition.

**Fig. 5.** Time dependence of inhibition of IL-1 release by glucose. RAW 264.7 cells were activated with LPS (1 μg/ml) for 24 h in the presence of 5 mM D-glucose. Glucose (10 mM, for a final concentration of 15 mM) was added at the indicated times during the incubation. IL-1 activity in the medium was measured by the RINm5F bioassay. Results are the average ± S.E. of three individual experiments containing three replicates per condition.
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PKC Inhibitors Block Glucose Inhibition of IL-1 Release—Recently, several studies have implicated PKC activation by glucose in diabetic complications due to hyperglycemia (24–27). To determine whether PKC activation plays a role in high glucose inhibition of IL-1 release, we tested the effects of PKC inhibitors. LPS-stimulated RAW 264.7 cells were incubated with 5–20 mM glucose for 24 h, and IL-1 activity released into the medium was measured. Selective PKC inhibitors, chelerythrine (10 μM, IC₅₀ = 0.6 μM for PKC (28)) and calphostin C (1 μM, IC₅₀ = 0.05 μM for PKC; incubation under lighted conditions (29)), a nonselective inhibitor, H7 (100 μM, IC₅₀ = 6 μM for PKC (30)), and a selective PKA inhibitor, H89 (1 μM, IC₅₀ = 0.048 μM for PKA (31)) were added at 4 h post-LPS stimulation to minimize effects on LPS signaling. High glucose (10–20 mM) inhibited IL-1 release in controls lacking kinase inhibitors and with the PKA inhibitor, H89 (Fig. 8). However, the specific PKC inhibitors, chelerythrine and calphostin C, and the non-specific PKC inhibitor, H7, blocked the inhibitory effects due to high glucose without affecting IL-1 release at 5 mM glucose. In related experiments, it was determined that the presence of PKC inhibitors was required during the initial 12 h of the incubation to block the inhibitory effects of high glucose (data not shown).

PKC Agonist Mimics Glucose Inhibition of IL-1 Release—To confirm that PKC activation can mediate the inhibition of IL-1 release, the effects of a PKC agonist, PMA, on IL-1 release were analyzed. RAW 264.7 cells were incubated with LPS (5 mM glucose), LPS (15 mM glucose), or LPS (5 mM glucose + PMA 100 nM) for 24 h (Fig. 9). PMA, like high glucose, almost completely inhibited the release of IL-1, and this inhibition was blocked by addition (at 4 h) of 10 μM chelerythrine.

PKC Activity in RAW 264.7 Cells—The activity of PKC was measured in RAW cells at 4 and 24 h post-LPS stimulation (Fig. 10). At 4 h, the presence of 20 mM glucose increases PKC activity overall levels at 5 mM glucose (Fig. 10a). As a positive control, PMA (100 nM) also increases measured activity. At 24 h (Fig. 10b), the presence of high glucose actually decreases activity, suggesting a down-regulation of PKC activity following an initial increase.

DISCUSSION

Diabetics have more frequent infections and of longer duration than non-diabetics (1, 2) due to a decrease in bactericidal activity linked to hyperglycemia (4). In this study, we demonstrate that high glucose inhibits the release of IL-1, a key mediator of the immune response and effective defense against microorganisms (32), from murine macrophages. IL-1 release from RAW 264.7 murine macrophages is maximal at 2–6 mM glucose (−basal blood glucose levels), but is inhibited at higher concentrations (8–20 mM) corresponding to hyperglycemia in diabetes (Fig. 1). At very low glucose levels (0–1 mM), IL-1 release and production are inhibited (Figs. 1 and 6), confirming a report by Orlinski and Newton (33) that a minimal level of glucose is required for IL-1 synthesis.

In contrast to a decrease in IL-1 release, nitric oxide production by RAW 264.7 cells increases with higher glucose levels.
This increase in nitric oxide synthase activity was also observed by Sharma et al. (34) and was mediated by glucose activation of PKC.

The inhibitory effect on IL-1 release by high glucose levels requires metabolism as 2-deoxyglucose, a glucose transport and metabolism inhibitor, blocks the glucose effect (Fig. 2). Related hexoses that are metabolized by macrophages, fructose and mannose, mimic the inhibitory effect of glucose, but non-metabolized sugars, L-glucose and galactose, have no effect on IL-1 release (Fig. 3).

The inhibition of IL-1 release by high glucose levels is fully reversible at 24 h by removal of excess glucose (Fig. 4), indicating that glucose effects are transient and are not toxic to the cells. This reversibility is consistent with the recovery of infection fighting ability in hyperglycemics upon normalization of blood glucose (5).

A time study revealed that high glucose (15 mM) is effective in inhibiting IL-1 release only during the first 10 h of a 24-h incubation (Fig. 5). Addition of elevated levels of glucose after 14 h had no effect. These early effects of glucose suggest a role in blocking LPS signaling and IL-1 induction and synthesis; however, measurement of intra- and extracellular IL-1 activity and protein suggest that IL-1 synthesis is not affected by high glucose (Figs. 6 and 7). Pulse labeling studies with LPS-activated RAW 264.7 cells demonstrate the recovery of proteolytically processed IL-1α in the culture medium following a 12-h chase in the presence of 2 mM, but not 20 mM, glucose. The absence of any precursor or processed IL-1α in the culture medium in the presence of 20 mM glucose under these pulse-chase conditions combined with the inability of 10 or 20 mM glucose to alter intracellular IL-1 activity further supports this conclusion that only IL-1 release and not synthesis is inhibited by high glucose concentrations.

Hyperglycemia is a major causal factor in the development of complications such as retinopathy, nephropathy, and vascular dysfunctions in diabetic patients, and these adverse effects have been attributed to activation of PKC via de novo synthesis of diacylglycerol (24–27). In our study, inhibitors of PKC (but not PKA) blocked the inhibitory effects of high glucose levels on IL-1 release (Fig. 8). An activator of PKC, PMA, mimicked the inhibitory effects of glucose (Fig. 9) and PKC activity at 4 h post-LPS stimulation was increased by 20 mM glucose (Fig. 10).

These results demonstrate that high glucose inhibits IL-1 release from RAW 264.7 cells by an early increase in PKC activity. While 20 mM glucose increases PKC activity at 4 h post-LPS stimulation, prolonged exposure (24 h) to high glucose concentration decreases PKC activity (Fig. 10). PKC activity does not appear to be required for IL-1 release as PKC inhibitors do not block IL-1 release (Fig. 8). Thus, this late decrease in PKC activity does not directly account for the inhibitory actions of glucose.

High glucose levels inhibit the production of cGMP (35–37), probably through a PKC-mediated mechanism as inhibitors of PKC abrogate glucose-induced reductions of cGMP. We recently reported that IL-1 release by macrophages is mediated by nitric oxide via increases in cGMP levels (17). IL-1 release (but not production) was blocked by inhibitors of both nitric oxide synthase and guanylate cyclase and restored upon addition of exogenous nitric oxide and cGMP. Thus, high glucose levels may inhibit IL-1 release by lowering cGMP levels and interfering with the secretion pathway. Studies are currently

FIG. 9. PKC agonists inhibit IL-1 release by RAW 264.7 cells. RAW cells were incubated with LPS (1 μg/ml) and glucose (15 mM) or PMA (100 nM) for 24 h. The glucose concentration in the LPS + PMA groups was 5 mM. Chelerythrine (10 μM) was added 4 h after LPS initiation. The cell-free medium was assayed for IL-1 activity. Results are the average ± S.E. of three individual experiments containing three replicates per condition.

FIG. 10. PKC activity in RAW 264.7 cells. Cells were stimulated with LPS in the presence of 5 or 20 mM glucose or 100 nM PMA. PKC activity was measured at 4 h (a) and 24 h (b) as described under "Experimental Procedures." Results are the average ± S.E. of three to four individual experiments containing three replicates per condition. Significant differences to the LPS + 5 mM glucose control are indicated by * (p < 0.01) and by # (p < 0.05).
underway to explore the role of cGMP in the glucose-induced inhibition of IL-1 release.

Alternatively, PKC may interfere with the IL-1 secretion pathway by phosphorylation of protein(s)-mediating secretion. The mechanism of IL-1 release is unique and relatively unknown as IL-1 is expressed without a leader sequence and is not secreted via the classic endoplasmic reticulum-Golgi apparatus as is the case with other cytokines (16). Further research in this area may identify potential targets for PKC phosphorylation and disruption of IL-1 secretion.

Although the overproduction of IL-1 contributes to a variety of inflammatory diseases, IL-1 is necessary for proper immune function. Blocking the IL-1 receptor with its receptor antagonist, IL-1Ra, or by antibody neutralization increases infection, suggesting that a sufficient level of endogenous IL-1 is required for normal host defense (32). It has been proposed that increased susceptibility of uncontrolled diabetics to infection may result from impaired macrophage function (5). Furthermore, Rayfield et al. (4) have reported a significant correlation between the prevalence of infections in poorly controlled diabetics and the glucose levels (8–20 mM) that media glucose concentrations comparable with uncon- 

Albeit both the PKC and the glucose effects are mediated by PKC activation. These studies may shed light on the reasons for the prevalence of infections in poorly controlled diabetics and suggest possible interventions for treatment of these infections.

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