Elevated Monocyte Interleukin-6 (IL-6) Production in Immunosuppressed Trauma Patients. I. Role of FcγRI Cross-linking Stimulation

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This study demonstrates that immunodepressed trauma patients' monocytes produce elevated interleukin-6 to adherence, bacterial, and cytokine stimulation compared to immunocompetent trauma patients' or normals' monocytes, suggesting their in vivo preactivation possibly mediated by the hyperimmunoglobulinemia which characterizes these patients. Furthermore, stimulation of monocytes through cross-linking their FcγRI induces and augments interleukin-6 (IL-6) production to subsequent stimulation both in trauma patients' (P < 0.001) and in normals' (P < 0.001) monocytes. As we reported earlier, immunodepressed trauma patients have an increased proportion of FcγRI-bearing monocytes in their total monocyte population and here we show that those FcγRI+ monocytes produce significantly elevated interleukin-6, suggesting a relationship between elevated monocyte interleukin-6 production and FcγRI triggering. Interleukin-6 induction by FcRI stimulation is not mediated solely by FcRI-induced MØ tumor necrosis factor alpha, IL-1α, or IL-1β production and is independent of MØ prostaglandin E2 levels. Therefore, FcRI stimulation-induced elevated MØ IL-6 might contribute to the increased immunoglobulin levels posttrauma.

KEY WORDS: Tumor necrosis factor alpha; interleukin-1; prostaglandin E2; immunosuppression.

INTRODUCTION

Aberrant monocyte (MØ) functions, including elevated prostaglandin E2 (PGE2), decreased antigen presenting capacity, and increased tumor necrosis factor alpha (TNFα) levels, are pivotal in mediation of posttrauma immunosuppression contributing to septic shock after severe injury (1-7). In addition, both elevated spontaneous IgG production and elevated serum and urinary IL-6 levels are characteristics of immunodepressed trauma patients (8-13). IL-6 is produced by a variety of cell types, including monocytes, and is responsible for the terminal differentiation of B cells (14-19). Therefore, elevated IL-6 levels may contribute to trauma patients' development of excessive circulating IgG (8, 9, 19, 20). Human peripheral blood MØ constitutively express the IL-6 gene and MØ IL-6 production is induced by several stimuli such as adherence, bacterial stimuli, and TNFα (14, 15, 21, 22). Since many of these well-characterized inducers for MØ IL-6 production occur at high concentrations in the post-trauma MØ microenvironment, it lends support to the contention that augmented MØ IL-6 production may be responsible for elevated circulating IL-6 levels. In this study, we evaluated IL-6 production by MØ from patients with major injuries during the postinjury period and investigated possible mechanisms to explain its posttrauma elevation.

Circulating IgGs can bind, cross-link, and activate MØ expressing FcγRI receptors to produce several cytokines, including TNFα (23-26). We have previously shown that immunodepressed trauma patients experience an increase in the numbers of the MØ subpopulation which expresses high densities of the type I, Fc-gamma receptor (FcγRI+ MØ) (27-30). MØ activation through the FcγRI could occur in trauma patients who have bacteremia and increased circulating immunoglobulin levels (6, 9). Stimulation through FcγRI cross-linking has been suggested to induce MØ IL-6 production,
implying that increased IL-6 could augment circulating IgG levels initiating a vicious cycle maintaining increased IL-6 levels in immunosuppressed postinjury patients (31). Therefore, we assessed IL-6 production in response to FcγRI cross-linking and to subsequent bacterial and lymphokine stimulation in M0 from trauma patients. Since cross-linking of M0 FcγRI also induces PGE2 production (25, 26) and PGE2 can regulate the production of certain monokines, the relationship between elevated M0 IL-6 and PGE2 was assessed in the trauma patients.

METHODS

Blood Donors

A total of 23 patients admitted to the University of Massachusetts Medical Center Trauma Unit, Worcester, and Worcester City Hospital Burn Unit was assessed in this study. This included nine trauma patients with major injuries and an injury severity score >25 (eight male, one female). The 14 burn patients had greater than 20% burn injury calculated as percentage of the total body surface with second- to third-degree burns (12 male and 2 female were studied). The patients' ages ranged from 20 to 75; the average age was 41 years. Of the total 23 trauma and burn patients included in the study, 17 became immunodepressed (as evidenced by depressed mitogen responses and septic episodes) and 4 of these succumbed to sepsis.

Normal controls were tested along with each patient. Volunteers from laboratory and hospital staff at the University of Massachusetts Medical Center (ages 20–58) served as normal controls. Informed consent was obtained from all patients and controls.

Reagents

Iscove's modified Dulbecco's medium (IMDM) was obtained from Gibco Laboratories (Grand Island, NY) and fetal bovine serum (FBS) from Hyclone (Logan, UT). Endotoxin contamination was less than 12 pg/ml in the culture medium and FBS. Polymixin B sulfate was purchased from Gibco Laboratories (Grand Island, NY). Human recombinant interferon-gamma (IFNγ) was purchased from Collaborative Research Inc. (Bedford, MA). Muramyl dipeptide (MDP) was a generous gift from Ciba-Geigy (Basel, Switzerland). Indomethacin was obtained from Sigma (St. Louis, MO). Human recombinant IL-6 was a generous gift from Dr. S. Clark (Genetics Institute, Cambridge, MA). Polyclonal anti-TNFα neutralizing antibody was obtained from Genzyme (Boston, MA). Anti-human interleukin-1α and -1β polyclonal rabbit antiserum was purchased from Cistron Biotechnology (Pine Brook, NJ).

Monocyte Separation and Stimulation

Mononuclear cells separated by Ficoll-Hypaque density centrifugation were depleted from T cells by rosetting with neuraminidase-treated sheep red blood cells (SRBC) (2, 26). M0 purity after adherence to microexudate-treated surface was >95% by fluorescein isothiocyanate (FITC)-labeled OKM5 staining. M0 subpopulations were separated by rosetting the M0 with anti-RH-coated human O, Rh(D)+ erythrocytes as previously described (2, 26). This rosetting technique has been shown to provide cross-linking of the high-affinity, Fc-gamma receptors (FcγRI) with subsequent M0 stimulation as well as to enrich M0 for the rosetting, high-density FcγRI-bearing M0 subpopulation (FcγRI-positive M0) (26, 32). Rosetting, FcγRI-positive M0 were freed of erythrocytes by lysing. Nonrosetting M0 represented the FcγRI-negative M0 subpopulation. Since the nonrosetting M0 subpopulation bears very low-density or no FcγRI (FcγRI-negative M0), these M0 do not receive FcγRI stimulation during rosetting. The whole M0 population, the FcγRI+ M0, and the FcγRI- M0 subpopulations were cultured at a 1 × 10⁶/ml concentration in IMDM containing 15% FBS, 50 U/ml penicillin-G, 50 μg/ml streptomycin, 50 μg/ml gentamycin, 2.5 μg/ml fungizone, 4 mM L-glutamine, 1 mM Na pyruvate, and 1% MEM nonessential amino acids (Hazelton Labs, Lenexa, KS). M0 were stimulated with 20 μg/ml MDP alone or with a combination of 100 U/ml/10⁶ M0 of IFNγ, or 10⁻⁶ M indomethacin. IFNγ, indomethacin, anti-TNFα, and/or anti IL-1α, IL-1β neutralizing antibodies were added 4 hr prior to the addition of MDP. M0 supernates were collected after 16–18 hr of stimulation and kept frozen at −80°C until the monokine assays were performed. Adherent M0 were collected by EDTA treatment and scraping and recovered cells were kept frozen at 5 × 10⁶/ml concentration in PBS for further analysis.