Evidence of a Plasma-Mediated "Window" of Immunodeficiency in Rats Following Trauma

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The etiology of immunodeficiency following trauma was investigated. Plasma collected from Fischer rats 1–8 hr following a 40% surface area thermal injury (TI) displays immunosuppressive activity (ISA). Peak ISA (4 hr) exceeded 90% inhibition of Con A3-induced proliferation of normal spleen cells. Splenic macrophage IL-1 secretion and NK activity are also inhibited by 4-hr TI plasma. Most importantly, these same cellular immune functions decline in rats by 4 hr following TI. After a further decline by 16 hr (IL-1 = 19.8% and NK activity = 40% of normal), these cellular immune functions rebound toward normal values by 2 days following TI. Thus, ISA in plasma is both temporally and functionally linked to the cellular immune defects observed. Sham-treatment rats display a similar, although less marked, pattern of plasma-linked transient cellular immune defects indicating a role for stress in these responses. ISA is abolished by mild heat (56°C for 30 min) and wholly contained in the >10-kD fraction of plasma. Together, these results provide evidence that previously unrecognized molecules in plasma induce a "window" of immunodeficiency early following trauma.

KEY WORDS: Trauma; stress; immunodeficiency; immunosuppression; immunoregulation.

INTRODUCTION

It is well documented (1–10) that immune competence declines following major injuries. It is widely postulated that there is a progressive loss of immune competence as overt signs of immunodeficiency, and sepsis, typically appear several days or later following major trauma (11–13). In line with these results, a variety of immunosuppressive factors has been reported (14–17) in the circulation in the days and weeks following major injuries of humans. In contrast to these results, marked changes in the plasma levels of many hormones, metabolites, and other potentially immunomodulatory factors occur within several hours of injury and most of these changes are short-lived (18–23). It seemed reasonable to postulate, therefore, that events occurring during the "shock phase" play a role in the initiation of the plasma and cell-mediated immune dysfunctions observed later.

Evidence presented shows that plasma collected from Fischer rats 1–8 hr following a 40% surface area thermal injury (TI) displays broad-based immunosuppressive activity (ISA) in vitro. The early and transient appearance of this ISA in plasma, as well as physicochemical properties of the responsible molecules, such as heat lability, suggests that it bears no apparent relationship to circulating inhibitory factors observed (14–17) later following trauma. As important, the rapid appearance of this ISA in plasma following TI coincides with a marked decline in cellular immune functions including macrophage IL-1 secretion and NK activity. However, like the plasma ISA these cellular immune defects are transient, rebounding toward normal values by 2 days following TI. Together these results suggest that there is an early plasmamediated "window" of immunodeficiency following major trauma rather than simply a progressive loss of immune competence as has often been postulated. Such a window may provide an explanation for other results (24, 25) showing that bacterial translocation through the intestinal wall tran-
siently increases following major trauma. In turn, immunodeficiency and infections observed later following major trauma may result, in part, from events occurring within several hours of injury. More generally, the marked decrease and rapid rebound in cellular immune functions observed provide evidence that the immune system, like other organ systems, is susceptible to "acute-phase" regulation following major trauma.

MATERIALS AND METHODS

Animals

Fischer 344 rats (CDF/CrLBR) were purchased from Charles River Laboratories, Wilmington, MA. These "VAF" rats are free of common rat viruses, bacteria, and parasites as assessed by routine serological testing. Rats were housed at Rhode Island Hospital in filter-top cages. Male rats (175-225 g) were used. C3H/HeJ mice were purchased from Jackson Laboratories, Bar Harbor, ME. Animals were routinely euthanized using CO2.

Thermal Injury

The procedure employed was similar to that reported by other investigators (21). Briefly, rats were anesthetized using pentobarbitol (70 mg/kg) and shaved on the dorsal and ventral surfaces. After verifying that full anesthesia had been achieved, rats were placed in an insulated holder (kindly provided by Dr. B. Pruitt, San Antonio, TX) that exposes 20% of the skin surface area. The dorsal and ventral surfaces were sequentially immersed in 95°C water for 10 and 3 sec, respectively, to achieve a 40% surface area scald injury. Immediately thereafter, rats were injected intraperitoneally with 10 ml of pyrogen-free physiologic saline for volume restitution. Sham-treatment rats were handled identically except for immersion in 37°C water. Rats were housed individually following treatment. Mortality resulting from this thermal injury procedure was less than 5% and was attributable mainly to overanesthetization.

Cell Culture

Spleens were finely diced on ice and pressed through a 40-gauge stainless-steel screen using a syringe plunger. Dissection medium consisted of Hank’s balanced solution containing 1% fetal bovine serum (FBS; Sterile Systems, Logan, UT), 10 mM morpholinopropane sulfonic acid (MOPS; Sigma St. Louis, MO), and penicillin/streptomycin. Unless indicated, tissue culture reagents were purchased from GIBCO Laboratories, Grand Island, NY. Cells were washed and resuspended in "complete" medium consisting of RPMI 1640 supplemented with 10% FBS, MOPS, antibiotics, 1 mM glutamine, and 5 × 10^-5 M 2-mercaptoethanol. The particular lot of FBS employed in this study contains 0.027 ng/ml endotoxin as reported by Sterile Systems and verified using a chromogenic limulus amebocyte lysate assay (Whittaker, M.A. Bioproducts, Walkersville, MD). Spleen cells were cultured at 10^6/ml in 200 μl in quadruplicate in round-bottom microtiter wells. For mitogen-induced proliferation, cells were cultured in 10 μg/ml Con A (Grade IV, Sigma, St. Louis, MO) or 1 μg/ml lipopolysaccharide (LPS; Difco Laboratories, Detroit, MI). Cells were cultured for 2 days at 37°C in 7% CO2 in air and pulsed for 18 hr with 0.5 μCi 3H-thymidine (5 Ci/mmol, NEN, Boston, MA). In order to measure 3H-thymidine uptake, cells were harvested using a PHD Cell Harvester (Cambridge Technology, Cambridge, MA) and counted using a standard channels ratio program. Variation between replicate wells was routinely less than 10%.

Cytokines

Recombinant human interleukin-1 alpha (rIL-1) was kindly provided by Dr. P. Lomedico (Hoffman-LaRoche, Nutley, NJ). Recombinant human interleukin-2 (rIL-2) was kindly provided by Dr. J. Farrar (Hoffman-La Roche, Nutley, NJ). Cytokines were stored frozen at -80°C until use.

Macrophage Enrichment/Culture

Unfractionated spleen cells (10^6) in 100 μl were incubated in round-bottom microtiter wells for 2 hr at 37°C. Nonadherent cells were removed by washing the wells four times with complete medium. Medium (200 μl) containing 1 μg/ml LPS was added and the macrophage-enriched populations were incubated for 20 hr at 37°C. Supernatants (75 μl) were removed into microtiter wells and kept frozen at -80°C until assayed for IL-1 content. If adherent cells were to be counted after adherence, spleen cells were incubated for 2 hr in 150-mm flat-bottom dishes, washed, and cooled at 4°C, and adherent cells were recovered by gentle scraping with a