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A physical/psychological and biological stress combine to enhance endoplasmic reticulum stress

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ABSTRACT

The generation of an immune response against infectious and other foreign agents is substantially modified by allostatic load, which is increased with chemical, physical and/or psychological stressors. The physical/psychological stress from cold-restraint (CR) inhibits host defense against *Listeria monocytogenes* (LM), due to early effects of the catecholamine norepinephrine (NE) from sympathetic nerves on β1-adrenoceptors (β1AR) of immune cells. Although CR activates innate immunity within 2 h, host defenses against bacterial growth are suppressed 2–3 days after infection (Cao and Lawrence 2002). CR enhances inducible nitric oxide synthase (iNOS) expression and NO production. The early innate activation leads to cellular reduction-oxidation (redox) changes of immune cells. Lymphocytes from CR-treated mice express fewer surface thiols. Splenic and hepatic immune cells also have fewer proteins with free thiols after CR and/or LM, and macrophages have less glutathione after the in vivo CR exposure or exposure to NE in vitro. The early induction of CR-induced oxidative stress elevates endoplasmic reticulum (ER) stress, which could interfere with keeping phagocytized LM within the phagosome or re-encapsuling LM by autophagy once they escape from the phagosome. ER stress-related proteins, such as glucose-regulated protein 78 (GRP78), have elevated expression with CR and LM. The results indicate that CR enhances the unfolded protein response (UPR), which interferes with host defenses against LM. Thus, it is postulated that increased stress, as exists with living conditions at low socioeconomic conditions, can lower host defenses against pathogens because of oxidative and ER stress processes.

1. Introduction

Stress responses have evolved to increase an organism's chance of survival, by strengthening certain defense mechanisms required for "fight or flight" responses, but not without a price. It is known that an organism's susceptibility to infection is increased by stress; the aim of our study was to elucidate the cellular mechanisms involved in this sympathetic nervous system regulation of immunity to *Listeria monocytogenes* (LM). Cold (4 °C) restraint (CR) has been a model to elicit a physical stress response and a psychological stress response (Paré and Glavin, 1986; Glavin et al., 1994). Physical and psychological stresses are associated with lower socioeconomic status and poorer health (Adler and Rehkopf, 2008). Previously, it was reported that CR for 1 h induces factors, mainly from 6-hydroxydopamine-sensitive nerves of BALB/c mice, and that these neurotransmitters diminish immunologic defenses against the intracellular bacterium LM (Cao et al., 2002). CR interferes with the bacteriostatic/bactericidal response 2–3 days after infection, which was observed as an increased LM burden in the liver and spleen of BALB/c mice (Cao and Lawrence, 2002). Previous reports indicate that CR induces release of catecholamine, which signals via β1-adrenoceptors (β1AR) to impair host-defenses by altering innate immune mechanisms on immune cells from BALB/c or FVB/N mice (Cao et al., 2003a; Emeny et al., 2007).The current in vivo, ex vivo and in vitro study utilized the mouse model to investigate the mechanisms associated with the CR-induced effects.

Within the first 48–72 h of an LM infection, innate immune-defenses orchestrate the host-pathogen response and optimally prime Th1 adaptive immune cells to begin regulating the elimination of the bacteria. CR administered just before LM infection delays this response, prolonging and increasing the bacterial burden (Cao and Lawrence, 2002; Emeny et al., 2007). Peak levels of bacteria usually occur around day 3 under normal experimental conditions; the CR-stressed mice also exhibit peak LM burden on day 3 in the liver and spleen, but between days 2 and 3, there is less control of bacterial propagation (Cao and Lawrence, 2002). This early delay in bacterial killing indicates that CR interferes with the cellular and molecular mechanisms that normally control the intracellular propagation of bacteria and/or which facilitate the switch from innate to adaptive processes. We, therefore, performed

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a series of experiments to investigate oxidative and intracellular changes that might be implicated in stress-induced immunologic suppression immediately following CR treatment and then subsequently for 48 h after LM infection.

2. Materials and Methods

2.1. Mice, cold-restraint (CR) treatment, and Listeria monocytogenes infection

All experiments were performed using mice housed at the AAALAC-approved Wadsworth Center animal facility, in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines. All mice were maintained on a 12 h light/dark cycle with lights on from 7 AM to 7 PM. Although all experimental mice were 6–12 weeks of age, experimental comparisons always used mice within 2–3 weeks of age, which have no significant immune differences due to age. BALB/c mice were acquired from Taconic (Germantown, NY). $\beta1AR^{-/-}$ and $\beta2AR^{-/-}$ mice, bred on the FVB/NJ (FVB) background, were kindly provided by Dr. B. Kobilka (Stanford Univ). Wild-type (WT)-FVB mice were obtained from Jackson Laboratory (Bar Harbor, ME). For CR treatment, control mice were left in their original cages undisturbed, while mice subjected to CR were individually restrained in well-ventilated plastic 60-ml syringes at 4 °C for 1 h in the dark. CR represents a physical and a psychological stress. CR was performed between 8 and 11 AM on day 0; the mice were infected with LM immediately after CR treatment. Mice were intravenously injected with a sub-lethal dose of LM ($2–3 \times 10^3$ colony-forming units (CFU)/i.v. injection for FVB and $3.5–5 \times 10^3$ CFU/i.v. for BALB/c mice). The experiments were approved by the Wadsworth Center's IACUC.

2.2. Serum preparation and measurement of nitric oxide

Peripheral blood was collected into 1.7-ml Eppendorf tubes from BALB/c mice. After clot formation (overnight at 4 °C), serum was collected following centrifugation. Nitrite levels were measured with sera from BALB/c mice (n = 3/group/time point) without CR and at 2, 6 and 24 h after CR using the Griess assay (Green et al., 1981). Griess reagent (100 μl) (a 1:1 mixture of 1% sulfanilamide (*p*-aminobenzenesulfonamide) in 5% H_3PO_4 and 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride in H_2O) (Sigma-Aldrich, St. Louis, MO) was added (*v*/*v*) to diluted serum and standard ($NaNO_2$, Sigma) in 96-well plates. Plates were incubated at room temperature for 10 min, and absorbance was measured at 550 nm in a plate reader and concentration was determined from the standard curve.

2.3. Detection of surface levels of protein sulfhydryls on peripheral blood lymphocytes of WT-FVB, $\beta1AR^{-/-}$ and $\beta2AR^{-/-}$ FVB mice

Following CO_2 anesthesia, whole blood was obtained by cardiac puncture from control mice and mice subjected to CR stress (n = 4–6) and placed into heparinized tubes.Blood (50 μl) was washed twice with 0.1% NaN_3 in PBS, stained for analysis of surface sulfhydryls (R-SH) withstained for analysis of surface sulfhydryls (R-SH) with 20 μM AlexaFluor488 maleimide (Invitrogen, Carlsbad, CA) in 200 μl PBS for 15 min, on ice, in the dark, and washed twice with PBS.

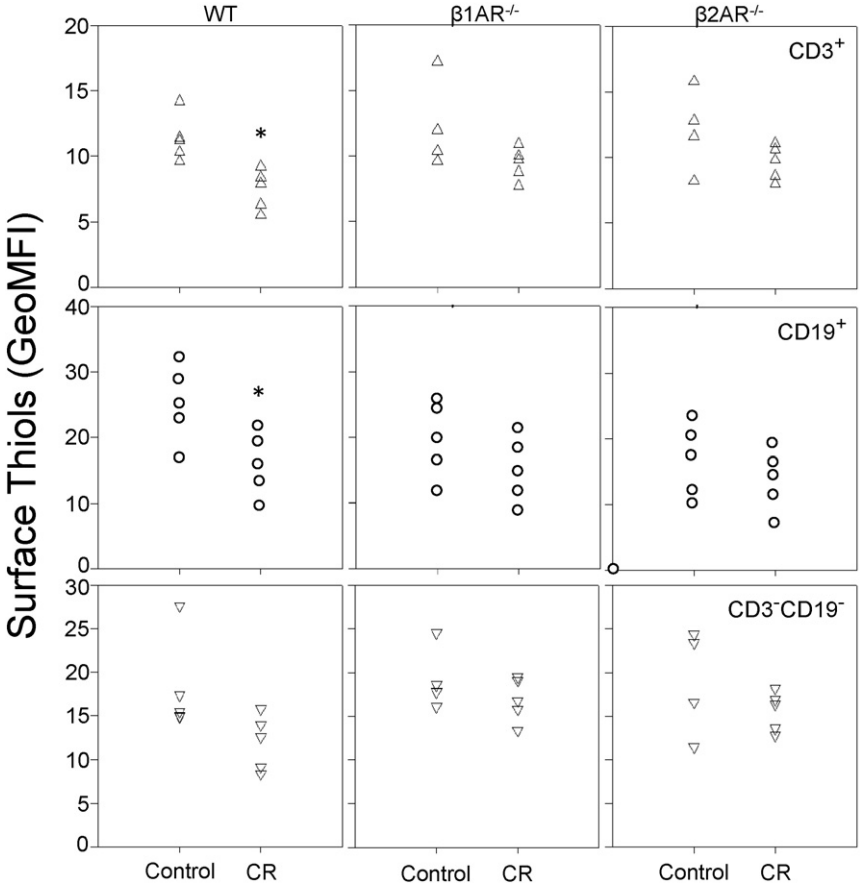

Fig. 1. CR affects cellular thiols. Flow cytometric analysis of surface thiols (sulfhydryls) on blood lymphocytes immediately after CR with the impermeant fluorescent thiol specific probe (AlexaFluor488-maleimide). CD3 and CD19 were used to assess gated lymphocytes ($CD45^{hi}/SSC^{low}$) after CR treatment of WT, $\beta1AR^{-/-}$ and $\beta2AR^{-/-}$ FVB/NJ mice (n = 4–6). Results are presented as geometric mean fluorescence intensity (GeoMFI). A significant ($p < 0.05$) loss of surface thiols after stress treatment is noted (*) for T and B cells from WT-FVB mice by one-way ANOVA and Tukey's post hoc test.