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Research paper

Altered monocyte and macrophage numbers in blood and organs of chickens injected i.v. with lipopolysaccharide

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ABSTRACT

Lipopolysaccharide (LPS) is a Gram-negative bacteria cell wall component that activates monocytes and macrophages to produce nitric oxide (NO) from inducible nitric oxide synthase. Nitric oxide production in the plasma of chickens peaks 5–6-h post-i.v. LPS injection reflecting iNOS activation. To determine monocyte responsiveness after an i.v. LPS injection, a time course study was conducted examining the concentrations among peripheral blood leukocytes post-i.v. LPS injection in male and female chickens, the proportions among peripheral mononuclear leukocyte (PBMC; containing lymphocytes, thrombocytes, and monocytes) populations isolated from the blood samples collected at various times post-i.v. LPS treatment, and the ability of monocytes to produce NO with and without further LPS stimulation in vitro using the PBMC NO production assay. Additionally, monocyte extravasation activity was determined by analyzing macrophage proportions after the i.v. LPS injection in spleen, lung, and liver tissues. Blood was collected from male and female chickens at 0 h (pre-LPS injection control) and at 1, 3, 6, 24, and 48 h post-LPS injection, and additionally, at 72 h from female chickens. Tissues were collected 0, 1, 6, and 48 h post-i.v. LPS injection from male chickens. Monocyte concentrations dropped substantially by 1 h in both males and females. In males, monocyte concentrations returned to control concentrations by 6 h and increased at 24- and 48-h post-LPS injection, whereas in females, monocyte concentrations recovered more slowly, returning to near control concentrations by 24–48-h and increasing above control levels by 72 h. Lipopolysaccharide stimulated NO production by PBMC cultures established from blood samples obtained at various times post-LPS injection in vivo followed the same pattern as monocyte concentrations in the blood. Hence, NO concentrations within PBMC cultures were dependent upon the number of monocytes that were in the PBMC cultures isolated at different times post-i.v. LPS injection. Furthermore, macrophage proportions in spleen tissues responded similarly to monocyte concentrations in the blood, decreased in lung tissue, and varied widely in liver tissue throughout 48 h after an LPS injection. Monocytes and other leukocytes may attach to the endothelium post-i.v. LPS injection preventing the monocytes from entering the needle during blood collection resulting in what seems to be leukopenia

Abbreviations: NO, nitric oxide; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; WBC, white blood cells; PBMC, peripheral blood mononuclear cells; PBS, Dulbecco's phosphate buffered saline; PE, phycoerythrin; FSC, forward scatter; SSC, side scatter; FL-1, fluorescence intensity between 515 and 545 nm; FL-2, fluorescence intensity between 564 and 606 nm; KUL01+, monocytes/macrophages; CD3+, T lymphocytes; Bu-1+, B lymphocytes; DAB, 3,3′-diaminobenzidine tetrahydrochloride; T/B ratio, T lymphocyte proportions divided by B lymphocyte proportions.

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in blood and in PBMC cultures attenuating NO production in PBMC cultures. Furthermore, monocyte differentiation and recruitment from the bone marrow is a likely contributor to the reconstitution and rise of monocyte concentrations in blood samples post-i.v. LPS injection.

1. Introduction

Lipopolysaccharide, or endotoxin, is a cell wall component of Gram-negative bacteria that initiates the production and the release of vasoactive factors including nitric oxide (NO) from innate immune leukocytes. Nitric oxide is a potent vasodilator that is synthesized from L-Arg by endothelial nitric oxide synthase (eNOS) or inducible nitric oxide synthase (iNOS). Endothelial cells release NO in transient bursts following eNOS activation in response to LPS injection acutely modulating vasoconstriction (Albrecht et al., 2003; Martinez-Lemus et al., 2003; Deem, 2004; Wideman and Chapman, 2004; Bowen et al., 2006a,b). Inducible nitric oxide synthase activation in monocytes and macrophages yields copious amounts of NO (Hussain and Qureshi, 1997, 1998; Pulido et al., 2000; Dil and Qureshi, 2002a,b). Nitric oxide production peaks in chicken plasma 5 h after an i.v. LPS injection reflecting iNOS activation in vivo (Chapman and Wideman, 2006; Bowen et al., 2007). Additionally, i.v. administered bacterial endotoxin induces leukopenia within 1 h in mammalian circulating blood (Brunning et al., 1964; Verghese et al., 1980; Richardson et al., 1989). Leukopenia occurs within 1 h following i.v. LPS injection and typically recovers to normal concentrations by 5–8-h post-injection (Brunning et al., 1964; Richardson et al., 1989). Lipopolysaccharide exposure induces innate immune cell proliferation in mice within 48 h causing the mouse to become non-responsive to the bacteria for a time (Moeller et al., 1978).

Similar to observations in mammals, leukopenia was also demonstrated in the peripheral circulation of broiler chickens after an i.v. LPS injection (Wang et al., 2003; Shini et al., 2008). Shini et al. (2008) reported that an i.v. LPS injection induces stress in chickens, as reflected by elevated plasma corticosterone level and an elevated heterophil/lymphocyte ratio within 1 h post-LPS. Additionally, macrophages isolated from the peritoneal cavity of chickens that were pretreated with LPS did not express iNOS after a second exposure to LPS in vitro, and macrophages were shown to be pretranscriptionally non-responsive to LPS (Cheng et al., 1996; Dietert and Golemboski, 1998). However, a more detailed time analysis of chicken white blood cell (WBC) profiles and functional abilities following LPS injection is needed. In fact, it is unknown whether monocytes remaining in the circulation after an i.v. LPS injection continue to produce NO ex vivo without further LPS stimulation and whether in vivo LPS-activated monocytes can be further stimulated with LPS in vitro to produce NO or are non-responsive to LPS.

Peripheral mononuclear leukocyte (PBMC; include monocytes, lymphocytes, and thrombocytes in chickens) cultures prepared from whole blood allow the assessment of NO production by monocytes in response to LPS-stimulated iNOS activation in vitro (Bowen et al., 2006b; Wideman et al., 2006). The PBMC NO production assays can be established quickly and, as shown by flow cytometric analysis of NO production by individual cells using DAF-FM diacetate labeling, monocytes are the only PBMC population in the LPS-stimulated PBMC cultures that actively produce NO in response to LPS (Bowen et al., 2006b).

Using the PBMC NO production assay we conducted a preliminary study to examine NO production by monocytes in PBMC cultures prepared from blood samples of chickens that had been injected i.v. with LPS 5 and 48 h previously. The 5 h PBMC collection time-point was chosen because it is the time-point when plasma NO concentrations are known to be maximally elevated following i.v. LPS administration, whereas the 48 h time point reflected the time post-LPS injection when plasma NO concentration had returned to non-detectable levels (Bowen et al., 2007). Based on this preliminary study, it appeared that monocytes in PBMC cultures prepared 5 h after i.v. LPS administration exhibited attenuated NO production in response to LPS-stimulation in vitro, when compared to monocytes in PBMC cultures established at 48 h after an i.v. LPS injection. Further analysis of the proportions of monocytes in the 5 and 48 h PBMC suspension revealed greatly reduced percentages of monocytes in the 5 h PBMC suspensions when compared to the 48 h PBMC suspensions. Hence it appeared that the attenuated NO production by the 5 h compared to the 48 h PBMC cultures in response to in vitro LPS stimulation may be due to lower proportions of monocytes in the 5 h PBMC preparation rather than to attenuated responsiveness of the monocytes to LPS stimulation.

The objectives of this study were to further examine the effects of i.v. LPS administration in chickens. Specifically, a time course study was conducted to examine the concentrations and proportions among peripheral blood leukocytes post-i.v. LPS injection, determine the proportions among PBMC populations isolated from the blood samples collected at various times post-i.v LPS treatment, and to assess the ability of monocytes to produce NO with and without further LPS stimulation in vitro using the PBMC NO production assay. Additionally, considering reports of leukopenia in the peripheral circulation following i.v. LPS administration in chickens, the distribution of leukocytes in spleen, lung and liver was also examined.

2. Materials and methods

2.1. Chicken maintenance

Young-adult chickens from the MHC-matched ($B^{101/101}$) egg-type Brown line and Light Brown Leghorn