Clinical Immunology (2007) **124**, 221–233

available at www.sciencedirect.com

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Galectin-9 protects mice from the Shwartzman reaction by attracting prostaglandin E_2-producing polymorphonuclear leukocytes

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Received 30 March 2007; accepted with revision 24 April 2007
Available online 8 June 2007

KEYWORDS
Rodent;
Neutrophils;
Endotoxin;
Shwartzman reaction;
Sepsis;
Suppression;
Galectin-9

Abstract Galectins play a crucial role in the modulation of innate and adaptive immunity. Here we show that galectin-9 (Gal-9) exhibits an anti-inflammatory role in LPS-induced inflammation. Intraperitoneal LPS injection enhances Gal-9 levels as well as promotes the production of pro-inflammatory cytokines, e.g., TNF-alpha, IFN-gamma and IL-12. We found that Gal-9 administration results in the protection of mice from the Shwartzman reaction, and Gal-9-deficient mice became susceptible to the Shwartzman reaction, thus implying the anti-inflammatory activity of Gal-9 against LPS-induced inflammation. Indeed, Gal-9 treatment together with LPS suppresses production of these pro-inflammatory cytokines, while it rather enhances than suppresses IL-4 and IL-10 production. We also found that LPS-induced elevation of TNF-alpha, IFN-gamma, and IL-12 does not occur in Gal-9 transgenic mice. Moreover, Gal-9 induces Gr-1^+ cell; probably polymorphonuclear leukocyte (PMN), as well as infiltration in to the peritoneal cavity, causing us to hypothesize PMNs are involved in Gal-9-mediated suppression. The fact that Gal-9 does not suppress LPS-induced TNF-alpha, IFN-gamma and IL-12 production in neutropenic mice, and that it does not protect those mice from the Shwartzman reaction, confirms the involvement of PMN in regulation. PMN attracted by Gal-9 produce PGE_2, which LPS-induced TNF-alpha production from the peritoneal macrophages is suppressed, while PMNs

Abbreviations: Gal, galectin; PMN, polymorphonuclear leukocyte; Mϕ, macrophage; DC, dendritic cells; PB, peripheral blood; PEC, peritoneal exudate cells; PLF, peritoneal lavage fluid.

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1521-6616/$ – see front matter
doi:10.1016/j.clim.2007.04.015

attracted by casein produce less PGE_2 and fail to suppress LPS-induced TNF-alpha production. Our data suggest that Gal-9 regulates LPS-induced inflammation and protects mice from the Shwartzman reaction by attracting PGE_2-producing PMN.

Introduction

Galectins are soluble metal-independent lectins bound to β-galactoside-containing glycoconjugates exhibiting a variety of biological activities, such as cell adhesion, proliferation [1–3], apoptosis [4,5], and cell-cycle progression [6]. Galectins are expressed by a variety of immune and inflammatory cells besides epithelial cells and exhibit pleiotropic functions such as pro- and anti-inflammatory functions [7–9].

Galectin-9 (Gal-9) has been originally classified as an eosinophil chemoattractant, inducing superoxide production and prolonging cell survival [10,11]. More recently, it has been shown that Gal-9 negatively regulates Th1 type immunity as a Tim-3 ligand [12]. We have also shown that Gal-9 expression is up-regulated by IFN-γ and IL-1β in various cell types [1,13,14], and that it induces maturation of immature dendritic cells (DC), suggesting that Gal-9 plays a crucial role in both innate and adaptive immunity [15]. As noted, Gal-1, Gal-3 and Gal-8 exhibit several important functions in PMN, the effector cells in the host's immune response against bacterial infection and inflammation, such as induction of superoxide and adhesion to endothelial cells [16–22]. Polymorphonuclear leukocytes (PMNs) have, thus, been long believed to play a major regulatory role in infectious disease by producing cytokines and chemokines, such as TNF-α, IL-1β, IL-8, IL-10, GM-CSF, RANTES, and MIP-1α by LPS stimulation [23].

Recently, it has been shown that there are several subsets of PMN through the analysis of their macrophage (Mϕ) modification activities [24], and that resident PMN may convert to pro-inflammatory or anti-inflammatory PMN in response to host circumstances. Furthermore, it has been shown that PMN produce an unidentified factor that inhibits the release of TNF-α and IL-6 from activated Mϕ [25], suggesting that PMNs are not a single and terminally differentiated population, but have a subset that also suppresses inflammation through Mϕ modification. The mechanistic details of this have yet to be clarified.

The purpose of the present investigation is to show a novel function of Gal-9 in the regulation of pro-inflammatory cytokine production in innate immunity.

Materials and methods

Mice

Male BALB/c and C57BL/6 mice were purchased from Japan SLC. BALB/c, C57BL/6, Gal-9-transgenic (BALB/c) and Gal-9-deficient (C57BL/6) mice were maintained on a 12:12-h light–dark cycle in a conventional animal facility at the Kagawa University. The animals were fed a standard laboratory diet and water ad libitum. All experimental procedures were approved by the Animal Care and Use Committee, conforming to the Guidelines for Animal Experimentation, Kagawa University.

Induction of LPS-induced inflammation and lethal Shwartzman reaction

Male BALB/c mice (8 weeks old, 23 to 24 g) were i.p. injected with 5 μg LPS with or without Gal-9. In some experiments, the effects of PGE_2 and PGE_2 receptor antagonist on LPS-induced inflammation were assessed. PGE_2 and PGE_2 receptor antagonists [26], such as antagonist for EP1 (ONO-8713), EP2 (AH6809) and EP4 (ONO-AE3-208), were kindly donated by Dr. Narumiya at Kyoto University. All antagonists were dissolved in DMSO and those antagonists (200 μg) were administered after dilution with PBS according to the methods previously described [27,28].

The Shwartzman reaction was induced in male BALB/c mice (8 weeks old) by i.p. priming injection of LPS (10 μg/mouse, *Escherichia coli* 0111:B4, Sigma) followed by i.v. challenge of LPS (120 μg/mouse) after 15 h. After LPS injection, the mice were monitored for 3 to 4 days. Control mice received an equivalent volume of PBS. In the case of C57BL/6 and Gal-9-deficient mice (8 weeks old, 23 to 24 g), LPS, 30 μg/mouse at priming and 200 μg/mouse at challenge, was used to induce the Shwartzman reaction.

Cell separation and culture

Gr-1^+ PMN from peritoneal exudates cells (PEC) were stained with allophycocyanin (APC)–anti-Gr-1, administered anti-APC magnetic beads (Miltenyi Biotec), and positively selected through MACS LS separation columns (Miltenyi Biotec). The purity of Gr-1^+ cells from PEC was about 95–97%. In the case of peripheral blood (PB) leukocytes, lymphocytes were first deleted from PB leukocytes by negative selection using both anti-Thy1.2 (Militenyi Biotec) and anti-B220 (Militenyi Biotec). Then, PB Gr-1^+ PMNs were enriched by positive selection using anti-Gr-1 as described above. The purity was more than 95%. PEC or PMN was cultured in RPMI 1640 medium (Sigma) supplemented with 10% FCS, 2 mM L-glutamine, 100 U/ml of penicillin and 100 μg/ml streptomycin. The PEC and PMN were cultured with or without 100 ng/ml LPS overnight.

Construction of the *Pichia pastoris* expression plasmid, expression and purification of Gal-9

The Gal-9 expression plasmid pAB1008 was constructed as follows: the mouse Gal-9 M ORF derived from pBKCMV (MGal-9) was provided with the *Eco*RI and *Not*I restriction sites at the 5′- and 3′-ends of the Gal-9 ORF, respectively, by PCR using the oligonucleotides 5′-GGGGAATTC (*Eco*RI) ATGGCT-