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SCIENCE DIRECT
Developmental and Comparative Immunology 30 (2006) 942–953
Developmental & Comparative Immunology
www.elsevier.com/locate/devcompimm

Differential reactive oxygen and nitrogen production and clearance of *Salmonella* serovars by chicken and mouse macrophages

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Received 14 September 2005; received in revised form 18 November 2005; accepted 4 December 2005
Available online 5 January 2006

Abstract

The objective of the present study was to compare the uptake and killing of *Salmonella* serovars by murine and avian macrophage cell lines. We used *Salmonella enterica* serovars Enteritidis (SE338) and Typhimurium (SR11) for this study. Uptake of green fluorescent protein-labeled bacteria was measured using flow cytometry. Cell sorting and plating of viable infected macrophages demonstrated that bacterial clearance was significantly better with J774A.1 compared with HD11 cells. HD11 cells produced significantly higher amounts of nitric oxide (NO) than J774A.1 cells upon infection with SE338 and SR11, whereas J774A.1 cells exhibited greater superoxide production with SR11. Treatment of HD11 cells with recombinant chicken interferon gamma in the absence of bacteria enhanced NO production but did not induce increased levels synergistically with bacteria. Interferon treatment did not influence phagocytosis or increase killing by HD11 cells. Published by Elsevier Ltd.

Keywords: *Salmonella*; Chicken macrophages; Mouse macrophages; J774A.1; HD11; Nitric oxide; Superoxide; Flow cytometry

1. Introduction

Salmonella enterica are facultative intracellular bacteria of which serovar Typhimurium (ST) and serovar Enteritidis (SE) have a broad host range, including the capacity to cause human infections. Infection of humans by these organisms usually occurs by food-borne transmission. Human infection with SE is primarily caused by consumption of contaminated raw or partially cooked shell eggs while ST is implicated in contamination of chicken meat and of a variety of other foods [1–6]. When ingested by humans, SE and ST initially infect intestinal mucosal cells causing a transient diarrhea but rarely become systemic. In avian and murine hosts the infections can become systemic. In the mouse, this occurs as a result of bacteria translocating across the mucosa to sub-mucosal tissues including Peyer's patch lymphoid structures [7]. There, *Salmonellae* are taken up by phagocytes including monocytes and macrophages [8]. As facultative intracellular pathogens, they are able to persist within these cells and become disseminated to spleen, liver and other tissues, as monocytes circulate in blood and lymphatics [9]. In susceptible mice, systemic ST infection with a virulent strain

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0145-305X/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.dci.2005.12.001

results in a typhoid-like illness that can result either in the death of the host or clearance of the infection with a resulting sterile immunity, usually within 1–3 weeks [10]. The immune response in mice involves innate, cell-mediated and antibody components [7,8,10,11]. In chickens infected orally with SE, a similar colonization of organs occurs and includes the reproductive organs of hens leading to contamination of shell eggs [12]. In contrast to mice, colonization of the chicken gastrointestinal tract and tissues by SE often occurs without signs of overt clinical symptoms; furthermore, egg production of hens is not affected by SE infection [2]. Infection can persist for up to 18 weeks in laying hens. Experimental infection of chickens with SE results in both cell-mediated and antibody responses that are ultimately not effective in clearing the infection [13,14]. Induction of measurable immune protection with live, attenuated and heat-killed vaccines, suggests that in the absence of vaccination, the immune response has limited effectiveness [15]. This species-specific pathogenesis may be due to a variety of factors influencing differential host–pathogen interaction, including differences in interaction with components of cell-mediated and innate immunity including phagocytes, which play a central role in pathogenesis in both mouse and chicken. The ability of *Salmonellae* to survive within host cells is essential for the establishment of systemic infection [16]. With respect to the mouse model, *Salmonella* pathogenicity island encoded type III secretion system genes play a major role in host cell invasion and survival in macrophages in both ST and SE [17,18]. Activation of macrophages by inflammatory mediators such as interferon gamma and the importance of reactive oxygen and nitric oxide (NO) in killing of *Salmonellae* by primary mouse macrophages and macrophage cell lines, including J774A.1 is well established [7,8,10]. The in vitro interaction of avian phagocytic cells with *S. enterica* serovars has more recently been investigated. The activation by IL-2 of chicken heterophils, professional phagocytes analogous to mammalian polymorphonuclear cells, induces IL-8 and IL-18 mRNA following phagocytosis of SE [19]. Enhanced heterophil activation is associated with increased resistance and with cytokine mRNA expression [20]. In studies with the species-specific *S. enterica*, serovar Pullorum, splenic macrophages were found to play a role in persistent infection by harboring bacteria for 40 weeks after infection [21]. Macrophages from *Salmonella*-resistant chickens were also found to kill serovar Gallinarium more efficiently than macrophages from *Salmonella*-susceptible chickens, suggesting an important role for macrophages in this genetically based resistance [22]. In a recent study, we investigated the effect of recombinant chicken interferon-γ (rchIFN-γ) on the infection of primary chicken macrophages isolated from peripheral blood with ST and SE. ST showed an increased ability to survive in primary macrophages and interferon treatment caused increased cellular necrosis in combination with infection [23]. The ability to conduct these studies was inhibited by difficulty in obtaining and maintaining consistent populations of primary macrophages in large enough numbers. The use of a chicken macrophage cell line provided a means to overcome this problem. In previous studies, the avian monocyte–macrophage cell line HD11 showed increased bactericidal activity in vitro as well as enhanced production of cytokines and NO following exposure to CpG oligodeoxynucleotides [24,25] and was therefore a candidate for this purpose.

While there have been comparisons of *Salmonella* serovars in mouse and human macrophage cell lines [26] as well as in vivo comparisons in both mouse and chicken experimental infection [27], no studies have specifically focused on comparing SE and ST interaction with mouse and chicken macrophages. In this study, we have conducted in vitro infection studies with mouse and chicken macrophage cell lines focused on cellular measurement of uptake and survival of ST and SE using flow cytometry and cell sorting. The use of green fluorescent protein (GFP)-labeled SE and ST in combination with flow cytometry has proven to be a valuable approach, allowing for a more quantitative analysis of the dynamics of macrophage–bacteria interactions in vitro [24,28]. NO and superoxide production was also measured. In addition, the effect of recombinant IFN-γ on these parameters in chicken macrophages was also assessed. The results will shed light on the unique features of *S. enterica* infection in mice and chickens that dictate the differential response of mouse and chicken macrophages following infection.

2. Materials and methods

2.1. Cell lines

The murine monocytic cell line J774 A.1 (American type culture collection, Rockville, MD, USA)