Institut Pasteur
Microbes and Infection 15 (2013) 911–919
www.elsevier.com/locate/micinf

Original article

Epithelial entry rather than the ensuing systemic immune response determines the pathogenicity of two *Salmonella enterica* serovar Typhimurium strains in a mouse model

Rikke Brandt [a], Anne Petersen [b,1], Susanne Brix [a], Tine Rask Licht [b], Hanne Frøkiær [c,*]

[a] *Center for Biological Sequence Analysis, Department of Systems Biology, Technical University of Denmark, Building 224, DK-2800 Kgs. Lyngby, Denmark*
[b] *Department of Microbiology and Risk Assessment, The National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark*
[c] *Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, Copenhagen University, Ridebanevej 9, DK-1871 Frederiksberg C, Denmark*

Received 3 May 2013; accepted 6 August 2013
Available online 24 August 2013

Abstract

Most studies of *Salmonella enterica* serovar Typhimurium infection focus only on the pathogenicity of one strain. We investigated whether differences in pathogenicity of two wild-type *S.* Typhimurium strains; DT120 and SL1344, were related to gut invasion or the resulting immune response.

Oral administration of a ten-fold lower number of SL1344 (10^6 CFU) as compared to DT120 (10^7 CFU) resulted in higher bacterial counts in liver and lymph nodes, and led to massive neutrophil infiltration of the spleen, while DT120 administration did not. In contrast, administration of the same dose (10^3 CFU) of the two strains intravenously resulted in the same levels of bacteria and neutrophils in spleen and bone marrow. Oral administration of SL1344 led to an increase in neutrophil apoptosis in both spleen and the bone marrow and four out of five mice died before Day 8, while in DT120 mice, no increase in neutrophil apoptosis was observed and all mice survived until Day 8. This study reveals that two wild-type *S.* Typhimurium strains, despite evoking highly comparable immune responses upon intravenous injection, exhibit diverse pathogenicity in mice and thus suggests that differences in their invasiveness and survival during gut passage determines the success of the ensuing immune response.

Keywords: *S.* Typhimurium; Neutrophils; Epithelial entry

1. Introduction

Salmonella are gram-negative facultative intracellular anaerobes, estimated to cause an annual 1.3 billion cases of disease worldwide [1]. Clinical symptoms range from gastroenteritis to severe systemic typhoid fever and bacteremia, the latter two caused by bacterial translocation from the gastrointestinal tract into circulation. A recent retrospective study of data from cases of human *Salmonella* infections during a 10 year period revealed that although the identified serotypes were genetically closely related, they differed significantly in pathogenic potentials, as outcome of salmonellosis was serotype-specific [2]. Understanding the relative risks associated with and within different serotypes for invasive infection is of major importance for public health.

Direct comparison of the pathogenicity of different *Salmonella* strains needs to be studied in animal models, and mouse models are widely used to study S*almonella* translocation and effects hereof on the immune system. Recently, it was demonstrated that *S.* Typhimurium, represented by *S.* Typhimurium 14028, was among the most virulent among 32

* Corresponding author. Tel.: +45 33353150.
E-mail address: hafr@sund.ku.dk (H. Frøkiær).
[1] Present address: Novo Nordisk A/S, Department 1190, Biopharm QC Support, Brogaardsvej 66, DK-2820 Gentofte, Denmark.

1286-4579/$ - see front matter © 2013 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.micinf.2013.08.004

strains tested in BALB/c mice [3]. In susceptible mice like BALB/c and C57/Bl6, oral infection with *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium, ST) leads to a systemic typhoid-like illness, and much of the knowledge and understanding of *Salmonella* pathogenesis and host defense mechanisms has been deduced from these models [4].

Although the invasion and intestinal translocation of *Salmonella* serotypes associated with systemic illness have been vigorously studied in humans as well as in murine model systems, the precise mechanism and route is still being debated. The current view is that *Salmonella* cells, after oral ingestion, are able to reach deeper tissues by two major routes. One port of entry is through microfold cells overlaying Peyer's patches (PP) and solitary intestinal lymphoid tissues [5–7], or through villous microfold cells distributed in the mucosal epithelium [8]. The second port of entry involves uptake by CD18-expressing phagocytic cells, which are believed to carry bacteria directly into the blood stream and lymph, thus bypassing PPs [9]. Direct bacterial entry into the blood stream without simultaneous involvement of phagocytes has never been reported, but cannot be excluded. Which of these mechanisms that dominates may depend on the number of bacteria present in the gut as well as on the virulence of the specific strain [10], and both may in turn affect the immune response to the bacterium. Previous studies by Rescigno and colleagues [11] demonstrated that specific virulence genes in *S.* Typhimurium may affect the route of gut translocation and their translocation capacity. Specifically, oral administration of wild type *S.* Typhimurium SL1344 resulted in translocation of a higher number of bacteria to PP, mesenteric lymph nodes (mLN) and spleen, whilst administration of a ten-fold higher dose of the less invasive mutant InvA$^-$ caused low bacterial counts in PP, but comparable numbers in mLN and spleen [11]. Such differences in intestinal translocation properties may impact the type and extent of the primary immune response against the invading bacteria. To which extent the ability to translocate on one hand and the ability to disseminate within the host on the other determines the final outcome is, however, not clear.

Phagocytes such as macrophages (Mφs) and neutrophils play an important role in host survival upon *Salmonella* invasion [12–14] although some evidence suggests that neutrophils also play a prominent role in shuttling live microbes from tissues to draining lymph nodes, thereby promoting bacterial dissemination [15,16]. Neutrophils are short-lived cells that degranulate and undergo apoptosis upon bacterial phagocytosis [17]. Under healthy conditions, the great majority of murine neutrophils resides in the bone marrow (BM) [18], while during infection large numbers are released into blood circulation and directed to the periphery, where they control invading pathogens [19]. Induction of antibody-mediated neutropenia has revealed the important role of neutrophils in early killing of invading *Salmonella*, as neutropenic mice show increased bacterial burden on Day 1 post challenge [20]. Orally administered lethal doses of *Salmonella* were previously reported to involve heavy neutrophil infiltration of spleen, mLN and PP 5 days after infection correlating with bacterial burden [20,21]. The role played by neutrophils in the pathogenesis of *S.* Typhimurium and in relation to the divergent pathogenic potential of different strains is still not clear. Moreover, it remains to be disclosed whether neutrophil influx to infected organs causes increased granulopoiesis or neutrophil depletion in the bone marrow upon *S.* Typhimurium infection.

The host defense systems activated in response to invasion by different *S.* Typhimurium strains may well depend on the route of entrance as well as the number of translocating bacteria and their ability to proliferate in the intestine and within organs. In the present study, by using two different strains of *S.* Typhimurium, we addressed the role of route of entry in the pursuing immune response. We found that the two *S.* Typhimurium strains translocated differently in a mouse model and aimed to uncover whether the pathogenicity of these strains was related primarily to their ability to translocate across the intestinal barrier or to the systemic immune response induced in the host.

2. Materials and methods

2.1. Mice and challenge protocol

Eight weeks old conventional female BALB/c mice were purchased from Taconic Europe (Lille Skensved, Denmark) and housed in standard cages in an environmentally controlled facility with a 12-h light/dark cycle. During the study the temperature was kept at 22 ± 1 °C, relative humidity at 55 ± 5% and air was changed 8–10 times per hour. Mice were fed standard chow and water ad libitum. For oral infections, if not otherwise indicated, mice were infected with 10^7 CFU (*S.* Typhimurium DT120) or 10^6 CFU (*S.* Typhimurium SL1344) by gastric gavage. Mice were in all experiments injected in the morning and groups of mice to be compared directly, were injected at the same time. For intravenous injections, mice were injected with 10^4 CFU *S.* Typhimurium SL1344 or DT120 in the tail vein. Following challenge, mice were observed twice a day. If symptoms of severe disease (ruffled fur, altered behavior) developed, the mice were euthanized immediately, due to ethical considerations. Animal studies were performed under conditions approved by the Danish Animal Experiments Inspectorate (Council for Animal Experimentation) and by the in-house Animal Welfare Committee.

2.2. Salmonella strains and culture

S. Typhimurium SL1344 and DT120 strains resistant to nalidixic acid and chloramphenicol were kindly provided by Jens Bo Andersen, The National Food Institute, Technical University of Denmark. ST SL1344 has been described elsewhere [22] and ST DT120 was isolated from the lungs of a cow at the Danish Veterinary Laboratory. *S.* Typhimurium strains were grown in closed 50 ml tubes at 37 °C, 200 rpm overnight in 20 ml LB broth supplemented with 10 μg/ml chloramphenicol. For infections, overnight cultures were diluted appropriately in saline. The number of CFU in the inoculums was determined by plating on LB-agar plates supplemented with 10 μg/ml chloramphenicol.