Role of *Salmonella* surface components in immunomodulation of inflammatory mediators

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Abstract

*Salmonella enterica* serovar Typhimurium and its surface components were assessed for their inflammatory potential by footpad oedema test using plethysmometer. Inflammation was found to be the highest when outer membrane proteins (OMPs) were used as inflammmagen followed by lipid associated protein-lipopolysaccharide complex (LAP-LPS) and lipopolysaccharides (LPS). Inflammation produced by OMPs was found to be comparable to that by carrageenan (a known positive inflammmagen). However, injection of L-histidine (an antioxidant) prior to administration of carrageenan or *Salmonella enterica* serovar Typhimurium inhibited the inflammation, which indicated the involvement of oxidants during inflammatory response. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and nitric oxide (NO) production by peritoneal macrophages from infected mice exhibited a significant increase as compared to those of the immunized mice. In contrast, glutathione production was found to be the maximum in the macrophages taken from OMPs-immunized mice followed by LAP-LPS and LPS alone. The biochemical studies correlated well with histopathological studies of intestinal tissue of animals from various groups. Based upon these parameters, inflammation seems to be modulated by OMPs and LAP-LPS, which may be because of the protein moieties present in the components. Hence, immunization with protein moieties having L-histidine or L-histidine-like structures may suggest an alternative to the potential therapeutic values of anti-inflammatory drugs. Thus the results of this study form the basis for evaluating these antigens (either alone or in combination with polysaccharides) for preventive intervention rather than therapeutic. (Mol Cell Biochem 270: 167–175, 2005)

Key words: *Salmonella*, OMPs, LAP-LPS, L-histidine, inflammation, free radicals

Introduction

Inflammation is a basic host defense process, which occurs in response to noxious stimuli and to local injury. Amongst these, *Salmonella enterica* serovar Typhimurium is a leading causative agent of gastroenteritis in humans. In Salmonellosis, the ensuing inflammatory response of the intestinal mucosa has long been associated with *Salmonella* virulence [1]. Infiltrating inflammatory cells participate and ensue the destruction of the invading microbe by release of nonenzymatic mediators such as oxygen and nitrogen metabolites as well as enzymatic mediators such as elastase, cathepsin and collagenase [2]. Reactive oxygen species (ROS) such as superoxides (O$_2^-$) and hydroxyl radicals (·OH) propagate through a series of chain reactions and attack the polyunsaturated fatty acids present in the membrane. These radicals can also cause damage to DNA and proteins [3]. The other inflammatory mediator, nitric oxide (NO) is a gaseous free radical produced in biological systems. This regulates a diverse array of physiological functions and acts as an inter and extracellular

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messenger in most mammalian organs. Many types of cells produce NO during enzymatic conversion of L-arginine to L-citrulline by NO synthase (NOS). NO has been demonstrated to have a beneficial effect in host defense mechanisms against various pathogenic bacteria and protozoa [4–6]. The uncontrolled release of these cytotoxic substances [7] and proinflammatory mediators including cytokines (tumor necrosis factor and interleukins) [8, 9] by the migrating cells may damage the host tissues as well. Therefore, under such conditions, it is necessary to manage the hyper inflammation to useful level, to change the clinical manifestation of the disease.

The development of tissue injury depends upon the balance between the generation of toxic radicals and the tissue antioxidant status [10]. As a defense mechanism, all types of cells are endowed with enzymatic and nonenzymatic antioxidants, which fight against the toxic free radical species. One potential approach is the use of L-histidine or N-acetyl-L-cysteine, a precursor of glutathione, which maintain a favorable redox environment [11] and are effective in scavenging hydroxyl radicals. Several authors have reported an inverse correlation between the levels of GSH and lipid peroxides (LPOs). It seems that GSH apart from being a substrate for some antioxidant enzymes could be an effective free radical scavenger directly [12]. The alternative possible approach is to modulate the release of these mediators through immunoprophylaxis. It is known that LPS, Vi antigens and outer membrane proteins have an inflammatory potential, which results in the release of cytokines [13, 14]. These have also been reported to interact with the host immune cells efficiently [15–18]. Therefore, in the present study, the potential of Salmonella surface components in modulating the inflammatory response following immunization has been discussed.

**Materials and methods**

**Chemicals**

All the chemicals used in the present study were of analytical grade and were procured from standard firms.

**Bacterial strain**

*Salmonella enterica* sero var Typhimurium (1402/84) was procured from Central Research Institute, Kasauli (HP), India. The strain was checked biochemically and serologically prior to storage and use. It was maintained in 10% glycerol broth and also stored as lyophilized ampules.

**Animal model**

Balb/C mice each weighing 15–25 g (8–10 weeks old) and Wistar rats weighing 150–200 g were obtained from Central Animal House, Panjab University, Chandigarh, India. The animals were fed on commercially available diet and were given water ad libitum. Care and use of animals was followed in accordance with guidelines of the institutional ethical committee.

**Extraction of outer membrane proteins (OMPs)**

To study the expression of stress-induced proteins, OMPs were prepared by the method as described earlier [19]. Briefly, bacterial growth was harvested at 10000 rpm for 20 min. The pellet obtained was suspended in 20 mM Tris–HCl buffer (pH 7.6) containing 2 mM MgCl₂. Cells were washed twice and suspended in the same buffer containing 2 mM phenyl methyl sulphonyl fluoride (PMSF, Sigma, USA). Bacterial cells were disrupted by sonication using ultratip lab-sonic system (10 cycles of 30 s with 1 min interval in between) and undisrupted material was removed by low speed centrifugation (3000 × g, 4°C, 20 min). Supernatant was then ultracentrifuged (Beckam Coulter, Optima™ XL-100 K Ultracentrifuge, USA) at 100,000 × g for 60 min at 4°C and the pellet was suspended in 1% sodium lauryl sarcosyl (ICN, USA) in 20 mM Tris–HCl buffer (pH 7.6). After incubation for 2 h at 37°C, detergent insoluble OMP fraction was collected by ultracentrifugation at the same speed. The recovered outer membrane (OM) fraction was suspended in 20 mM Tris buffer containing 2 mM PMSF and stored at −20°C.

**Extraction of lipid associated protein-lipopolysaccharide complex (LAP-LPS) and lipopolysaccharides (LPS)**

For LAP-LPS preparation, conventional phenol extraction procedure of Westphal and Jann [20] as modified by Morrison and Leive [21] was used. For making LPS preparation, pronase was added to LAP-LPS preparation. The LPS content in both the preparations was measured by determining the 2-keto-3-deoxyoctonate (KDO) levels.

**Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)**

Protein content of various preparations (OMPs, LPS and LAP-LPS) was estimated by the method of Lowry *et al.* [22]. SDS-PAGE [23] was carried out to see the expression of protein and lipid profiles.