Accessibility of O Antigens Shared between *Salmonella* Serovars Determines Antibody-Mediated Cross-Protection

Anees Ahmed, Ajay Suresh Akhade, and Ayub Qadri

Pathogenic *Salmonella* serovars produce clinical manifestations ranging from systemic infection typhoid to invasive nontyphoidal *Salmonella* disease in humans. These serovars share a high degree of homology at the genome and the proteome level. However, whether infection or immunization with one serovar provides protection against other serovars has not been well studied. We show in this study that immunization of mice with live typhoidal serovar, *Salmonella* Typhi, generates cross-reactive immune responses, which provide far greater resistance against challenge with nontyphoidal serovar *Salmonella* Enteritidis than with another nontyphoidal serovar, *Salmonella* Typhimurium. Splenic T cells from these immunized mice produced similar levels of IL-2 and IFN-γ upon ex vivo stimulation with Ags prepared from *S.* Enteritidis and *S.* Typhimurium. In contrast, Abs against *S.* Typhi interacted with live intact *S.* Enteritidis but did not bind intact *S.* Typhimurium. These pathogen-reactive Abs were largely directed against oligosaccharide (O)-antigenic determinant of LPS that *S.* Typhi shares with *S.* Enteritidis. Abs against the O determinant, which *S.* Typhi shares with *S.* Typhimurium, were present in the sera of immunized mice but did not bind live intact *Salmonella* because of surface inaccessibility of this determinant. Similar accessibility-regulated interaction was seen with Abs generated against *S.* Typhimurium and *S.* Enteritidis. Our results suggest that the ability of protective Abs elicited with one *Salmonella* serovar to engage with and consequently provide protection against another *Salmonella* serovar is determined by the accessibility of shared O Ags. These findings have significant and broader implications for immunity and vaccine development against pathogenic *Salmonellae*. *The Journal of Immunology*, 2020, 205: 438–446.

Pathogenic *Salmonellae* are a cause of high morbidity and mortality in many countries around the world. The clinical outcomes produced by these pathogens include systemic infection typhoid and paratyphoid, localized gastroenteritis, and invasive nontyphoidal *Salmonella* (iNTS) disease (1). Typhoid produced by *Salmonella enterica* serotype Typhi (*Salmonella* Typhi) accounts for 27 million cases with 217,000 deaths annually (2). iNTS disease caused by *Salmonella* Typhimurium and *Salmonella* Enteritidis in immunocompromised individuals has an annual global burden of around 90 million cases with 155,000 deaths annually (3). Currently, there are no vaccines available for human use against iNTS disease (4). The genomes of typhoidal and nontyphoidal *Salmonella* serovars share a high degree of homology (5, 6), despite which these serovars produce different clinical manifestations in humans and exhibit extreme host specificity (7). *S.* Typhi produces typhoid almost exclusively in humans. In contrast, *S.* Typhimurium, which causes only self-limiting localized gastroenteritis in normal human subjects, produces a systemic infection in mice that is analogous to human typhoid. This experimental model of *Salmonella* infection has been extensively used to understand human typhoid (8, 9). Studies carried out in this model have shown that both Abs and cell-mediated immune responses are required for optimal immunity against *Salmonella* (10–14). The degree of similarity between different *Salmonella* serovars particularly in cell surface–associated molecules (15, 16), which are targets of Abs, suggests that the immune responses elicited upon infection or immunization with one *Salmonella* serovar might be cross-reactive and capable of imparting immunity against related serovars. However, there have not been any detailed investigations on the nature of such cross-reactive responses and their relevance to immunity. Studies (not very conclusive) in humans with currently available *S.* Typhi–derived live oral vaccine Ty21a have not indicated that this vaccine can provide protection against closely related serovars such as *Salmonella* Paratyphi, *S.* Typhimurium, or *S.* Enteritidis, the reasons for which have not been elucidated.

In the current study, we show that immunization of mice with *S.* Typhi generates T and B cell responses that exhibit differential capabilities to provide protection against two closely related nontyphoidal *Salmonella* serovars. Mice immunized with *S.* Typhi did not produce Abs capable of binding live intact *S.* Typhimurium, yet cross-reactive T cells significantly prevented *S.* Typhimurium replication in the spleen in a mouse model of infection but could not prevent death of infected animals. In contrast, immunization with *S.* Typhi not only brought about better bacterial clearance but also enabled survival of animals in a model of *S.* Enteritidis infection. The latter was mainly due to the presence of Abs that bound live intact *S.* Enteritidis. This cross-reactivity was dependent on the expression of accessible oligosaccharide (O) Ags that *S.* Typhi shares with *S.* Enteritidis. These results suggest that surface-accessible O Ag–dependent determinants may be major targets of protective anti-*Salmonella* Abs. These findings have relevance in designing effective vaccines against infections with *Salmonellae*.

Hybridoma Laboratory, National Institute of Immunology, New Delhi 110067, India

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Address correspondence and reprint requests to Dr. Ayub Qadri, National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi 110067, India. E-mail address: ayub@nii.ac.in

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Abbreviations used in this article: iNTS, invasive nontyphoidal *Salmonella*; O, oligosaccharide; XR, *Salmonella* Rough.

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