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Vector-primed mice display hypo-responsiveness to foreign antigen presented by recombinant *Salmonella* regardless of the route of delivery

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Abstract

Our previous studies have shown that mice which have been orally primed with an attenuated *Salmonella* vector [*S. enterica* serovar Stanley] are hypo-responsive to foreign antigens later delivered orally by the same vector strain, responding with significantly impaired serum and intestinal antibody responses compared with those seen in unprimed controls. Initial vector priming of the gut-associated lymphoid tissue (GALT) is likely to result in impaired persistence of recombinant *Salmonella* later administered orally. Delivery of recombinant bacteria by the intra-peritoneal or intra-nasal route, to avoid exposure to a primed GALT, did not allow vector-primed recipients to mount normal antibody responses to the foreign pilus protein K88. The negative impact of vector priming could be largely overcome, however, if mice were exposed to the foreign protein just prior to priming with the vector strain. Using this strategy, vector-primed mice displayed normal gut IgA and intermediate serum IgG responses to K88 following oral administration of recombinant *Salmonella*. Our findings are compatible with the concept of epitopic suppression, in which failure to respond to the foreign vaccine antigen reflects the clonal dominance of B cells specific for epitopes associated with the vector strain.

Keywords: Recombinant *Salmonella*; Antibody responses; Epitopic suppression; Clonal dominance

1. Introduction

Research continues towards the development of an improved attenuated typhoid vaccine strain, one which retains the safety of the present Ty21a vaccine but which is sufficiently immunogenic to confer protection after a single dose [1,2]. In addition to its potential impact on the incidence of typhoid among inhabitants of (and travellers to) endemic regions, such a strain would also allow the implementation of the generic vaccine approach of using attenuated *Salmonella* to deliver heterologous protective antigens. The resulting multivalent vaccines would have the potential to alleviate suffering from infectious diseases in the developing world.

This vaccination strategy would be threatened, however, if recipients with pre-existing immunity to *Salmonella*—following environmental exposure or vaccination—were less responsive than naïve individuals to the foreign antigen(s) presented by the recombinant vaccine. The potential adverse consequences of pre-existing immunity to the vaccine vector first became apparent in experiments conducted in our laboratory about a decade ago [3]. Our initial and subsequent reports have shown that vector-primed mice are comparatively unresponsive to foreign antigens subsequently presented by the same vector strain, mounting significantly weaker serum and gut antibody responses than age-matched, unprimed controls [3–5]. Other studies in which this issue has been directly addressed have variously supported [6,7] or challenged [8,9] these findings. The *Salmonella* strain used as a vector and the nature of the foreign antigen are both important determinants of the significance of an initial priming infection, with respect to the induction of host hypo-responsiveness [4]. The potential impact of this problem remains unresolved and awaits clinical evaluation.

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The replication of recombinant Salmonella vaccines will be curtailed in hosts with pre-existing anti-vector immunity, effectively reducing the vaccine dose and the likelihood of a primary response to the passenger antigen. If the vector strain is non-invasive, such as the *Salmonella enterica* serovar Stanley (*S*. Stanley) strain used in our studies, host immunity will be expressed by the gut-associated lymphoid tissue (GALT). One aim of the present report was to ascertain whether vector-primed mice still display hypo-responsiveness to a passenger antigen if the recombinant vaccine bacteria are administered by other routes, to circumvent any detrimental impact of a primed GALT on vaccine persistence. Both intra-nasal (IN) and intra-peritoneal (IP) vaccination of vector-primed mice has been performed. The former route is potentially applicable to humans whose environmental priming might have resulted in primed GALT.

We have previously suggested [3,4] that the inability of vector-primed mice to respond to a passenger antigen expressed by recombinant Salmonella is analogous to the phenomenon of epitopic suppression, a term first applied to the failure of carrier-primed mice to respond to hapten following injection of haptenated carrier. Studies by LeClerc and colleagues [10,11] suggested that epitopic suppression reflected intra-molecular antigenic competition resulting from clonal dominance. Carrier priming triggers clonal expansion of B cells specific for various epitopes presented by the carrier protein. Upon injection of haptenated carrier, the greatly increased frequency of vector-specific B cells, with their propensity for efficient antigen-presentation, effectively limits the availability of the conjugate to B cells potentially responsive to hapten. Other studies suggest that epitopic suppression is not restricted to responses to simple chemical haptens. Analogous suppression of responses to viral [12] or transplantation antigens [13] has been observed in primed mice, and to foreign peptide delivered by a protein carrier to primed humans [14].

In the present context, if the hypo-responsiveness induced by vector priming is indeed a reflection of the clonal dominance of vector-specific B cells, it might be possible to prevent induction of the hypo-responsive state by also priming the mice to the passenger antigen. Evaluating this possibility, by exposing mice to the K88 fimbrial protein just prior to vector priming, was the second aim of this report.

2. Results

2.1. The significance of primed GALT for expression of hypo-responsiveness in vector-primed mice

Initial experiments indicated that a direct comparison of GALT colonisation by recombinant Salmonella in control and vector-primed hosts would not be feasible. Since in our model these animals are ca. 20 weeks of age when orally dosed with K88-expressing *S*. Stanley (*S*. Stanley-K88), we first examined recovery of recombinant bacteria from unimmunised mice of this age. Although such mice reliably respond with strong primary anti-K88 responses, recovery of bacteria from Peyer's patches was highly variable, making it impractical to use these mice as positive controls against which to evaluate the impact of a primed GALT on vaccine persistence. It was, therefore, necessary to assess indirectly the extent to which this might underlie the hypo-responsiveness of vector-primed hosts.

2.2. IP vaccination of vector-primed mice

Groups of vector-primed and control mice were sub-divided and immunised IP with either of two doses of *S*. Stanley-K88. A preliminary dose–response experiment had shown the higher dose to consistently elicit strong serum IgG anti-K88 responses; the lower dose contained 30-fold fewer bacteria and was the lowest which consistently elicited a serum response. By analogy with the system in which mice are primed with a protein carrier [10], a lower dose of recombinant bacteria might be more likely to reveal epitopic suppression.

Sera were collected at intervals and antibody responses to K88 and vector lipopolysaccharide (LPS) determined by ELISA. As shown in Fig. 1, animals which had been orally primed with the vector strain were unable to respond to the K88 antigen presented by the recombinant bacteria, even though these were introduced systemically. Marginal responses were detected in only two of twelve vector-primed recipients ($P < 0.001$ for days 24, 40, 60 at each dose of vaccine). Unprimed mice mounted IgG responses in proportion to the dose of vaccine organisms administered (Fig. 1).

Anti-LPS responses were as expected, with primed animals showing stronger responses than those seen in controls (Fig. 1). As with oral immunisation [3–5], IP administration of recombinant bacteria elicited anti-LPS responses which were weaker and slower to develop than the anti-K88 responses.

2.3. IN vaccination of vector-primed mice

Recent reports have shown that immune responses to foreign antigens expressed by recombinant *Salmonella* can be observed after IN immunisation of adult mice [15,16]. A preliminary dose–response study revealed that IN administration of *S*. Stanley-K88 elicited serum IgG responses to the passenger antigen over a four log range of doses (not shown). On the basis of these data two doses of vaccine were selected, and administered IN to control and (orally) vector-primed mice. Blood and faecal pellet [FP] samples were collected at intervals and IgG and IgA responses to K88 determined by ELISA.