

1 **Antibodies and protection in systemic *Salmonella* infections: do we still**
2 **have more questions than answers?**

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15 **Abstract**

16 *Salmonella* causes grave systemic infections in humans and other animals and provides a paradigm for
17 other diseases where the bacteria have both intracellular and extracellular lifestyles.

18 New generations of vaccines rely on the essential contribution of the antibody responses for their
19 protection. The quality, antigen specificity and functions associated with antibody responses to this
20 pathogen have been elusive for a long time. Recent approaches that combine studies in humans and
21 genetically manipulated experimental models, and exploit awareness of the location and within-host life
22 cycle of the pathogen, are shedding light on how humoral immunity to *Salmonella* operates. However,
23 this area of research remains full of controversy and discrepancies.

24 The overall scenario indicates that antibodies are essential for resistance against systemic *Salmonella*
25 infections and can express the highest protective function when operating in conjunction with cell-
26 mediated immunity. Antigen specificity, isotype profile, Fc-gamma receptor usage and complement
27 activation are all intertwined factors that still arcanelly influence antibody-mediated protection to
28 *Salmonella*.

29 **Introduction**

30 Several serovars of *Salmonella enterica* cause systemic diseases in humans and other animals. The global
31 estimated burden of typhoid fever (serovar Typhi) is over 21M illnesses and 200,000 deaths with sustained
32 high incidence in Southeast Asia and endemic/epidemic occurrence increasingly reported in Africa (1-6).
33 Paratyphoid fever (serovars Paratyphi A, B and C) has an estimated 5.4M illnesses worldwide (3). Invasive
34 non-typhoidal *Salmonella* (iNTS) serovars (*e.g.* Typhimurium and Enteritidis) are a leading cause of lethal
35 sepsis and severe relapsing infections in young children and immune-compromised individuals, especially
36 in countries of the sub-Saharan African region (6-12) with an estimated 3.8M illnesses leading to 680,000
37 deaths annually and very high case-fatality ratios (20%) (7, 11). Antimicrobial resistance (AMR) is an
38 increasing problem in tackling many bacteria including *Salmonella* (7, 11, 13-15).

39

40 **Why is it important to gain a better understanding of antibody-mediated immunity to *Salmonella*?**

41 There is extensive international consensus on the urgent need for better and affordable vaccines against
42 systemic *Salmonella* infections. Vaccination has the potential for a high economic and health impact in
43 fighting AMR infections (16-19). Several classes of vaccines against systemic *Salmonella* disease have been
44 considered in the past decades (20, 21). These vary in their ability to induce protective cell-mediated and
45 humoral immunity with, broadly speaking, live attenuated vaccines being more efficient than non-living
46 preparations at eliciting Th1 type T-cell immunity known to contribute to host resistance to this bacterium
47 (22, 23).

48 Despite the superior protective activity shown in animal models, live *Salmonella* vaccines can cause lethal
49 infections in immune-compromised hosts (24-27). Mutants of *Salmonella*, including those that have been
50 considered so far as vaccine candidates, retain virulence and can rapidly kill immune-suppressed mice

51 (24-27). Mice that lack T-cell functions (25, 27) and mice co-infected with malaria are very susceptible to
52 infection with live *Salmonella* including mutants that have been considered as vaccine candidates (28,
53 29). Efforts to identify single gene mutations for the development of live vaccine candidates that would
54 be completely safe (totally unable to grow to high numbers) in severely immune-deficient animals have
55 been unsuccessful (24). This raises some concerns for the use of live vaccines in endemic areas with a
56 higher incidence of immune-suppressive conditions. For example, HIV and malaria are co-morbidities that
57 make humans more susceptible to systemic *Salmonella* infection leading to severe, often fatal disease,
58 and therefore pose dangers to the use of live vaccines (30, 31). These co-morbidities are widespread and
59 epidemiologically co-localize with areas of the developing world where there is a high incidence of
60 epidemic or endemic systemic salmonellosis and therefore where anti-*Salmonella* vaccines are most
61 needed (9, 20, 31, 32).

62 Mainly for safety reasons, non-living vaccines are currently being considered as prime candidates for
63 immunization against *Salmonella* diseases. The protective ability of these vaccines relies largely on the
64 induction of antibodies. If we are to use non-living vaccines as tools against systemic salmonellosis, it is
65 therefore essential to rationally optimize the antibody responses induced by these preparations. This
66 knowledge would also be immensely useful to understand how those comorbidities that impair antibody-
67 mediated functions increase susceptibility to disease and to design vaccine strategies that can at least in
68 part reverse these immune suppressing conditions. This will need to be based on a clearer understanding
69 of the qualitative and functional features of the protective antibody response against *Salmonella*.

70 This article will briefly outline the factors that influence the protective efficacy of the antibody response
71 to systemic *Salmonella* infections and will embed antibody functions in the context of the location and
72 spread of the bacteria during the infection process. The mini-review will also highlight the interactions

73 and dual requirement of T and B-cell mediated immunity both in the engenderment of antibody and T-
74 cell responses and in the expression of *in vivo* resistance to the pathogen.

75

76 **How can antibodies protect against a pathogen that has an intracellular lifestyle?**

77 The relative importance of antibodies and cellular immunity in host resistance to systemic *Salmonella*
78 infections has been a matter of debate for a long time.

79 The controversy initially originated from uncertainties in the location of the bacteria within the tissues of
80 an infected host. It is undisputable that in many animal species, including humans, *Salmonella* resides
81 inside phagocytes and grows within these cells using an armoury of genes and effectors to foster its
82 replication and evade intracellular killing (24, 33-40). Evidence from the mouse model on the role of the
83 phagocyte innate resistance gene *Ity* (now known as *Scla11a1*) in the control of bacterial division *in vitro*
84 and *in vivo* as well as studies where the bacteria could be seen to grow within cultured phagocytic cells
85 corroborated these views (41-44). However, evidence for extracellular growth and lack of intracellular
86 replication had also been produced, albeit based on electron microscopy studies where infections were
87 allowed to progress to very high, *pre-mortem*, bacterial numbers in the tissues. In these studies
88 intracellular bacteria were seen to undergo various stages of degradation inside macrophages and
89 granulocytes and therefore it was inferred that *Salmonella* was more likely to be an extracellular pathogen
90 and its virulence directly related to its antiphagocytic property (45).

91 The debate was further fuelled by immunological studies in both experimental animals and humans. On
92 the one hand evidence was provided for the protective role of antibodies being limited to the very early
93 stages of parenteral experimental infections, when the bacteria have not yet reached an intracellular
94 location, with no detectable effect on their later growth in the tissues (46, 47); on the other hand the

95 protective effect of whole-cell non-living typhoid vaccines, that induce mainly humoral immunity, and Vi
96 vaccines that do not directly contain *Salmonella*-specific T-cell antigens was undeniable (48-53). However,
97 protection by passive transfer of antibodies or T-cells alone and by non-living vaccines was seen only in
98 host-pathogen interactions where the infection is naturally mild and sublethal, such as challenging
99 resistant mice with either virulent or weakly virulent strains or susceptible mice with weakly virulent
100 strains (46, 50).

101 These discrepant views on the relative importance of humoral and cellular immunity to *Salmonella* were
102 largely reconciled by studies showing that passive transfer of both antibodies and T-cells, but neither
103 alone, could protect recipient innately susceptible animals against fatal systemic *Salmonella* disease (54).
104 More recently the essential role of both antibodies and T-cells in host resistance to *Salmonella* has been
105 corroborated by additional evidence obtained from human studies. For example, clinical observations
106 indicate that lack of antibodies at young age correlates with the incidence of iNTS in African children (55,
107 56), despite these children acquire *Salmonella*-specific CD4 Th1 cell immunity very early in life; only when
108 antibodies are developed in addition to T-cell immunity full protection is achieved (57). This resistance is
109 then abrogated by comorbidities that can affect either cellular or antibody-mediated functions (29, 58-
110 62). For example, HIV that has a profound effect of CD4⁺ T-cell immunity and malaria that impairs
111 phagocyte functions increase susceptibility to systemic *Salmonella* infections such as iNTS. A similar
112 increase in susceptibility is seen in patients with deficiencies in cytokines such as IFN γ and IL12 and in
113 their receptors leading to lack of activation of phagocytes and likely also T-cell immunity (9, 27, 63-67).

114

115

116 ***Salmonella*: a bacterium with a complex intra and extracellular pathogenesis. Where do antibodies**
117 **target *Salmonella*?**

118 *Salmonella* infections are normally acquired by the oral route and reach the blood after invading the
119 gastrointestinal tract (68). In the blood, the bacteria can be found either as extracellular bacteria or
120 associated with CD18⁺ cells (69) (Figure 1, A). Extracellular *Salmonella* in the blood can be targeted by
121 antibodies that enhance their uptake and killing by resident phagocytes of the spleen and liver (46, 70,
122 71) and can potentially lyse them *via* activation of the complement classical pathway (56). From the blood,
123 *Salmonella* reach an intracellular location within phagocytes of the liver, spleen and bone marrow (70, 72,
124 73) (Figure 1, B). Early in infection *Salmonella* reaches mainly splenic red pulp (F4/80⁺, MSR-A^{low}) and
125 marginal zone macrophages (MSR-A⁺) (74). In the liver, *Salmonella* localizes preferentially in the resident
126 Kupffer cells. During the infection bacteria can also be found in dendritic cells or B-cells (36, 74-77). As the
127 infection progresses and bacteria reside and possibly grow intracellularly (Figure 1, C), inflammatory cells
128 infiltrate the initial unicellular foci of infection, to form multicellular pathological lesions surrounded by
129 normal tissue (Figure 1, D). These lesions contain polymorphonuclear phagocytes (PMNs) during the first
130 few days of infection, later replaced by inflammatory macrophages (36, 78).

131 Once *Salmonella* have homed inside host cells, their intracellular location would render the bacteria
132 inaccessible to antibodies, making it difficult to envisage a role of humoral immunity in protection.
133 Detailed analysis of intracellular bacterial densities over the course of experimental infections in mice
134 have shed light on how and where the bacteria can become vulnerable to antibodies. In fact, microscopic
135 observation of immune-labelled and/or fluorochoime-expressing intracellular bacteria in the spleen and
136 liver revealed that the majority of infected phagocytes contain very low intracellular numbers at any time
137 of the infection, irrespective of overall the net growth of the bacteria in the organs (77). The infection

138 process is underpinned both by intracellular growth/survival and by an increase in the number of infected
139 cells and multicellular pathological lesions and not by increases in the number of visible intracellular
140 bacterial per phagocytes (24, 77, 79) (Figure 1, D). This is due to the continuous re-distribution of the
141 bacteria from infected host cells to uninfected ones, and in the spread of individual microorganisms to
142 new infection foci (Figure 1, E), mediated by the *Salmonella* Type three secretion system (T3SS) encoded
143 by the *Salmonella* Pathogenicity Island 2 (SPI-2) (80, 81). *Salmonella* infections are therefore dispersive
144 processes where bacteria have an intracellular phase of growth and an extracellular one of spread.

145

146 **Is the dispersiveness of the infection a plausible explanation for the role of antibodies in protection**
147 **against *Salmonella*?** It would be reasonable to postulate that in a dispersive infection process, cell-
148 mediated immunity enhances the antimicrobial functions of phagocytes, therefore affecting the fate of
149 the intracellular bacteria (82-86); antibodies would opsonise the extracellular bacteria in transit between
150 cells and target them to activating cellular receptors, thus increasing the antimicrobial activity of
151 otherwise naive phagocytes at new infection foci (87, 88). Antibodies would therefore be expected to
152 have a major impact on the process.

153 However, we know from a large body of data that antibodies alone, in the absence of cell-mediated
154 immunity, are unable to modify the net growth rate of *Salmonella* in the spleen and liver of an infected
155 experimental animal (21, 46, 50). For example, adoptive transfer of *Salmonella* specific immunoglobulins
156 or immunisation with non-living vaccines surprisingly does not affect the growth curve of the bacteria *in*
157 *vivo*. An effect of antibodies is visible only in the first few hours of the infection where the initial kill of
158 the inoculum is enhanced (46). This early protective effect of antibodies is more evident when
159 experimental animals are challenged *via* the intraperitoneal route, where antibodies most likely

160 accelerate capture of the bacteria by peritoneal phagocytes and therefore abort extracellular growth in
161 the peritoneal cavity. The use of molecularly tagged and therefore individually traceable, *Salmonella*
162 populations, combined with mathematical modelling and statistical analysis of data has further confirmed
163 the inability of antibody to significantly affect the growth and spread of bacteria in the body. This research
164 approach has confirmed that, in the early stages of the infection, bacteria spread from cell to cell within
165 each organ, later followed by systemic spread of bacterial populations between distant body sites
166 coinciding with the appearance of bacteria in the blood (89). Using this system, it became clear that only
167 live vaccines (which induce both humoral and cell-mediated immunity) would be able to control
168 bacteraemia and restrain growth and spread of the bacteria in the body. Conversely, mice where
169 antibodies were the only vaccine-mediated effector mechanisms (i.e. mice immunised with killed vaccines
170 or depleted of T-cells after immunisation) would not be able to control the spread of the bacteria or abort
171 bacteraemia (71).

172

173 **Antigen specificity and protection.** *Salmonella* infections induce antibody responses against a large array
174 of surface, periplasmic and cytoplasmic antigens in humans and other infected animals. Immuno-reactive
175 antigens have been detected using ELISA, immunoblotting and protein microarrays (90-94). These include
176 lipopolysaccharide (LPS) antigens, porins, lipoproteins, fimbriae, flagella, heat shock proteins and in some
177 serovars, the Vi surface polysaccharide antigen (95, 96). Several antigens have been identified as targets
178 of the protective antibody response. The O-antigen, consisting of sugar repeats exposed on the bacterial
179 surface, is a prime target of protective antibodies with some epitopes being more protective than others.
180 Immunodominant serovar-specific O-antigens (e.g. O:4, O:9) determine to a large extent the specificity of
181 protection between serovars and induce antibodies that are more protective than the ones directed

182 against O-antigens shared among different *Salmonella* serogroups (e.g. O:12) (97-100). The Vi antigen is
183 immunogenic and was shown to confer protective immunity in human volunteers and in field trials, both
184 as a native polysaccharide and as a protein conjugate (49, 51, 53, 101-103).

185 The functional role of the antibody response to porins is still not fully clarified. Antibody responses against
186 some porins, but not others, are protective in animal models (104, 105). For example, antibodies to the
187 trimeric OmpD porin, but not the monomeric and abundant OmpA protein, protect mice against
188 parenteral challenge. Furthermore, OmpD is a candidate antigen for iNTS vaccines (20, 105, 106). The
189 reasons for the different protective ability of OmpA and OmpD have been elegantly postulated to be due
190 to different accessibility of antibodies to OmpD at the bacterial surface. Both OmpA and OmpD are located
191 in the outer membrane of *Salmonella* in a position potentially shielded by the LPS-O side chain. However,
192 OmpD creates a larger footprint than OmpA in the O-antigen layer, sufficient for a single IgG can gain
193 access to the most-exposed surface loop epitopes of OmpD (105). It remains unclear how antibodies to
194 OmpD mediate SBA given that at least two Ig need to come together for activation of the classical pathway
195 of the complement. The conclusion that OmpA is poorly accessible by IgG also clashed with data showing
196 its binding by human recombinant IgG specific for a mimotope inserted in OmpA (107).

197 Antibodies to flagellin are detectable in infected individuals and targeting this antigen can mediate
198 opsono-phagocytosis (87, 93).

199

200 **Which is the main protective mechanism of anti-*Salmonella* antibodies?**

201 Following their binding to the bacterial surface, antibodies can exert antibacterial efficacy mainly in two
202 ways. Either by increasing phagocytosis of bacteria *via* targeting the bacteria to specific receptors on the
203 surface of immune cells; or by direct bactericidal activity mediated by the activation of the classical

204 complement pathway (serum bactericidal activity, SBA). Both these mechanisms are likely to operate in
205 *Salmonella* infections. Early studies showed that clearance of *Salmonella* from the blood of mice is
206 dramatically accelerated by immune serum suggesting a role for antibodies in the enhancement of
207 phagocytosis (70). More recently, evidence for an enhancement of opsono-phagocytosis by *Salmonella*
208 antibodies has been corroborated by *in vitro* systems. Sera from humans vaccinated with live attenuated
209 *S. Typhi* enhance opsono-phagocytosis *in vitro* with IgG playing a major role(108). Engagement of
210 opsonised bacteria with the activating Fc-gamma receptors, on the surface of murine or human
211 phagocytes results in increased uptake of the bacteria, increased production of reactive oxygen
212 intermediates (ROI) and enhanced antibacterial functions of the infected cells (87, 88, 107). Opsonization
213 drives increases in both the number of phagocytes that ingest bacteria and in the efficiency of each
214 individual cell to ingest *Salmonella*, as indicated by the higher intracellular numbers per phagocyte of
215 opsonized *versus* non-opsonized bacteria (88, 107). Antibodies have also shown to be essential for optimal
216 phagocytosis of iNTS strains by peripheral blood cells from Africans (55).

217 Antibody-dependent, complement-mediated SBA correlates with susceptibility to iNTS in African children
218 (56) and therefore SBA has been often taken as an indication of the functional activity of antibodies when
219 testing new vaccines against iNTS (109). However, the role of SBA as a true mechanism of antibody-
220 mediated protection must be evaluated with caution. Studies that used human blood and sera to compare
221 the kinetics of SBA and phagocytosis of iNTS clinical isolates, show that phagocytosis allows bacteria to
222 escape the blood and establish intracellular infection before they are killed by the complement membrane
223 attack complex (110). This would indicate that opsono-phagocytosis is likely to be the prevailing
224 antimicrobial mechanism mediated by anti-*Salmonella* antibodies. Furthermore, no correlation was found

225 between protection afforded by live vaccination and SBA in a controlled human typhoid challenge
226 model(111).

227

228 **Complement or Fc-gamma receptors?**

229 The complement system and Fc-gamma receptors (FcγRs) can potentially play a crucial role in antibody-
230 mediated immunity against *Salmonella* diseases. However, their relative importance is unclear.

231 Complement-mediated SBA can result in bacterial killing, but, as discussed above, it is unclear whether
232 this mechanism is relevant for immunity to *Salmonella*. Complement appears to be essential for antibody
233 dependent-opsonophagocytosis of iNTS strains by human blood phagocytes and for the production of
234 reactive oxygen intermediates and bacterial killing (55). However, binding of antibody-opsonized
235 *Salmonella* to FcγR on human and murine cells in the absence of complement also enhances phagocytosis
236 and ROI-mediated bacterial killing, with the activating FcγRI playing a major role (88, 107).

237 *In vivo* studies also provide evidence for a role for both complement and FcγR, their relevance depending
238 on the experimental model used. When mice lacking FcγRI, II, III and IV (FcγR KO), or mice lacking the
239 complement C3 component (C3 KO), or all four FcγR and C3 (FcγR/C3 KO), were passively immunized with
240 anti-LPS O4 IgG2a monoclonal antibodies and subsequently infected parenterally with *Salmonella*
241 Typhimurium, only FcγR KO animals showed a significant reduction in the bacterial loads in the liver,
242 spleen and mesenteric lymph nodes (112), at a level similar to the ones observed in wild-type control
243 animals. In this model therefore, the role of complement prevails on that of FcγRs. However, when mice
244 lacking FcγRI, II, III and mice lacking C3 were immunised with a live attenuated vaccine and later
245 challenged orally with virulent *Salmonella*, only the FcγR-deficient mice succumbed to the infection (113)
246 indicating a prevailing role of FcγR.

247 In summary, evidence for a role of both FcγR and complement has been provided, but firm conclusions
248 on their relative importance are difficult to draw due to discrepancies between experimental conditions,
249 models and host species.

250

251 **Does quality of the antibody response matter?**

252 The qualitative traits of the antibody response have a great effect on its function and potency. The isotype
253 profile has effects on the binding of antibodies to FcγR receptors and on efficiency of complement
254 activation. This in turn has effects on SBA, opsono-phagocytosis and on the enhancement of the
255 intracellular antibacterial mechanisms of phagocytes.

256 Virtually all classes of Ig are produced in response to infection with *Salmonella*. As expected, IgM appear
257 early after infection and usually followed by IgG and IgA (92, 95, 114-117). Different isotype profiles are
258 seen following natural infection or vaccination with live or non-living vaccines (22, 118). Some non-living
259 vaccines can induce isotype-switched responses to *Salmonella* polysaccharide antigens indicating that the
260 T-cell responses induced by these preparations may not be able to mediate the suppression of bacterial
261 growth in the tissues, but are sufficient to support isotype switching of the Ig response. For example, IgG
262 responses are detected following vaccination with Vi-conjugate vaccines (51, 119, 120); interestingly
263 outer membrane vesicle vaccines (OMV) are capable of inducing highly effective IgG2a and IgG2b in mice
264 (109); live vaccines induce high titers of all IgG subclasses including higher levels of IgG2a (22).

265 The isotype profile of the antibody response impacts on its protective function. The efficacy of individual
266 subclasses has been studied in murine and human systems and this area of research is not devoid of
267 controversy. In murine studies, polyclonal and monoclonal IgM as well as IgA to *Salmonella* polysaccharide
268 antigens, were found to be highly protective and in some cases more protective than IgG (100, 121, 122).

269 Conversely, opsonization of *Salmonella* with O4-specific IgA, IgG1, IgG2a, or IgG2b, but not IgM
270 monoclonal antibodies, resulted in cell-dependent bacterial killing *in vitro*. In *in vivo* passive immunization
271 studies, IgG2a and IgG2b O4-specific monoclonal antibodies provided higher functional activity than IgA,
272 IgM and IgG1 by decreasing the bacterial load in the blood and tissues (123).

273 A role for IgA in protection against *Salmonella* is shown by the increased susceptibility to infection of
274 polymeric immunoglobulin receptor (pIgR^{-/-}) knock-out mice, which are unable to bind and actively
275 transport dimeric IgA to the mucosae (124) and by the protective ability of IgA monoclonal antibodies *in*
276 *vivo* (125).

277 The relative potency of individual isotypes can be dependent on the antigen that is targeted. In fact,
278 differently for what seen in the case of antibodies against the LPS-O antigen, it has been shown that mice
279 lacking IgG1, but not lacking IgG2a are substantially less protected after immunization with the OmpD
280 porin than wild-type controls. Immunization with OmpD was maintained in t-bet deficient mice that do
281 not produce IgG2a. This is consistent with IgG1 having an important role for protection after immunization
282 with OmpD, but IgG2a being less important (126).

283 The relative potency of human IgG was studied in an *in vitro* system where OmpA and flagella were tagged
284 with a foreign CD52 mimotope (TSSPSAD). The bacteria were opsonized with a panel of humanized
285 recombinant CD52 antibodies that share the same antigen-binding V-region, but have constant regions of
286 different subclasses. This work revealed that, although opsonization with all the IgG subclasses increase
287 *Salmonella* uptake by human phagocytes, differences in potency can easily be revealed. IgG3 resulted in
288 the highest level of bacterial uptake and the highest average bacterial load per infected cell, which was
289 closely followed by IgG1, then IgG4 and lastly IgG2. Phagocytosis mediated by IgG1, IgG3 and IgG4 had a
290 higher dependency on FcγRI than FcγRIIA, whereas IgG2-mediated phagocytosis required FcγRIIA more

291 than FcγRI (87, 107). Therefore, both the subclass of human IgG and the type of FcγR that is available for
292 antibody binding affects the function of anti-*Salmonella* antibodies.

293 Some IgG subclasses can be detrimental to the antimicrobial function of the antibody response. In fact,
294 the inability of blood from HIV patients to kill *Salmonella* is due to an inherent inhibitory effect of anti-LPS
295 antibodies. This inhibition is dependent on high concentrations of antibodies and strongly associated with
296 IgA and IgG2 anti-LPS antibodies, possibly related to the poor ability of IgA and IgG2 to activate
297 complement, and deposition of complement at sites where it cannot insert in the bacterial membrane
298 (127).

299

300 **Crosstalk between B-cells and T-cells and quality traits of the antibody response.**

301 T-cell responses can be detected in animals and humans following infection with live *Salmonella*(128-
302 130). Isotype switching and production of anti-protein antibodies requires the presence of T-cells (27,
303 118); for example, T-cell deficient athymic mice produce mainly IgG3 and IgM antibodies to
304 lipopolysaccharide, whereas euthymic mice can produce IgM, IgG1, IgG2a, IgG2b, and IgG3 anti-LPS and
305 anti-*Salmonella* protein antibodies (27). Similarly, some classes of non-living vaccines only elicit a
306 restricted isotype repertoire (22). The reciprocal interaction between T- and B-cells is essential for the
307 development of both humoral and cell-mediated immunity to *Salmonella* (Figure 2). In the absence of
308 functional B-cells, the onset of T-cell immunity is impaired, albeit not abrogated in mice (131-134).
309 Cytokine production and antigen presentation via MHC Class II molecules from B-cells are essential for
310 activation of Th1 and Th17 responses to *Salmonella*, as shown by studies in bone marrow chimera mice
311 where only B-cells are deficient in selected immunological functions (135). Interestingly B-cells can
312 engender T-cell responses *via* engagement of innate immune receptors early in infection and *via* specific

313 activation of the antigen-specific B-cell receptor later in the disease. These functions instigate a loop
314 where T-cell activation in turn contributes to the isotype profile of the response. In fact, antigen-specific
315 IgG2c primary response are dependent on MyD88 signaling to B cells, while other Ig classes are not (IgG1
316 and IgG3) or much less so (IgG2b, IgA). Lack of MyD88 signaling in B-cells of chimeric mice results in
317 impairment of development of IFN γ effector T cells, a likely contributory factor in the lack of IgG2c (136).

318

319 **Key considerations for the development of *Salmonella* Vaccines.**

320 Antibodies are an essential component of the protective immune response to *Salmonella*. An ideal vaccine
321 would therefore be the one able to induce both antibody responses and protective cellular immunity.

322 The induction of some level of T-cell immunity is essential whichever the choice of vaccine. T-cell
323 immunity induced by natural infection or live vaccines is sufficient to support the isotype switching and
324 affinity maturation of the antibody response, but also to mediate the suppression of the growth of virulent
325 bacteria when either resistant or susceptible animals are re-infected. On the contrary, T-cell immunity
326 induced by some non-living vaccines is sufficient to support isotype switching and anti-protein responses
327 (109, 118), but not protective cellular responses. Why this level or type of T-cell immunity is unable to
328 activate phagocytes and curtail bacterial net growth in the tissues, still remains one of the main
329 unanswered questions in bacterial vaccinology.

330 Antibodies alone can have an impact on the very early stages of the infection, when they enhance
331 bacterial killing before *Salmonella* have reached an intracellular location within phagocytes (46, 71);
332 however they have no effect on the net growth of bacteria in the tissues and surprisingly on the control
333 of bacteraemia (9, 46, 60, 71, 127, 137). Therefore, it is likely that in individuals who do not have specific
334 Th1 memory, vaccine-induced antibody responses protect by preventing the establishment of the

335 infection following transmission of the pathogen. The situation would be different in populations where
336 background cellular immunity is present. For example, those vaccines that induce mainly antibody
337 responses would be more effective in disease-endemic areas, where a background of cellular immunity is
338 already present in the population, likely due to low grade pre-exposure to the pathogen and/or cross-
339 reactive antigens of other micro-organisms. Interestingly, young African children develop Th1 immunity
340 to *Salmonella* very early in life, but remain susceptible to iNTS until they acquire *Salmonella*-specific
341 antibodies (57); the aim of an effective iNTS vaccine for children would therefore be the induction of
342 antibodies early in life. A lower protective efficacy of Vi vaccines against typhoid fever has been detected
343 in volunteers in a controlled human challenge study compared to field studies, further suggesting the
344 possibility that a different immunological exposure background due to the geographical area may affect
345 vaccine efficacy(102, 103, 138).

346 The development of both safer live attenuated vaccines and non-living vaccines would be desirable; the
347 former would be more suitable for travelers where elicitation *de novo* of both T- and B-cell immunity is
348 required; the latter vaccines would be suitable for use in endemic areas where, as discussed earlier,
349 increased safety is a prerequisite and induction of antibodies would suffice.

350 Optimization of the immune response is important especially for non-living vaccines whose efficacy is
351 likely to be entirely based on antibodies. The importance of the isotype profile in relation to antigen
352 specificity and function has been touched upon above with some representative examples.

353 Greater efforts are needed to optimize antibody responses induced by vaccines to be used in areas where
354 immune suppressive co-morbidities co-localize geographically with the target disease. An example is
355 provided by malaria. This disease has a dual effect on humoral immunity. Firstly, malaria can suppress the
356 acquisition of anti-*Salmonella* antibodies (59, 139). Secondly, malaria can impair complement levels (61)

357 and suppress the antimicrobial functions of phagocytes (28, 58, 140), therefore potentially not allowing
358 the expression of antibody-mediated resistance (55, 88). More research is needed to identify vaccine
359 solutions that can overcome these problems. In fact, iNTS vaccines for Africa must induce immune
360 responses optimized to confer resistance in the presence of multiple and varying underlying
361 comorbidities.

362 The optimal choice of antigens is still debatable and several single-antigen vaccines are being developed
363 and/or are in use. For example, the vaccines based on the Vi polysaccharide are immunogenic and induce
364 protective serum responses. However, these vaccines include a single antigen that is not essential for the
365 virulence of the bacterium (Vi negative variants of *S. Typhi* are capable of causing disease (141)) and is
366 down-regulated within the tissues soon after infection (142). Multi-antigen vaccines would probably be a
367 wiser choice than single-antigen ones and would also offer the possibility to induce antibody responses
368 that can potentially protect against a large number of *Salmonella* serovars. For example, low-reactogenic
369 outer membrane vesicle-based vaccines that contain multiple structural antigens are very immunogenic
370 and elicit highly functional isotype-switched antibody responses against a variety of polysaccharide and
371 protein determinants (109, 143).

372

373 **Conclusions.**

374 The overall scenario that emerges from decades of research in experimental animals and in humans
375 indicates that antibodies are certainly essential for resistance against systemic *Salmonella* infections and
376 can express the highest level of protective functions when operating in conjunction with T-cell mediated
377 immunity. Antigen specificity, isotype profile, FcγR receptor usage and complement activation (Figure 3)
378 are intertwined factors that have great influence on antibody-mediated protection to *Salmonella*.

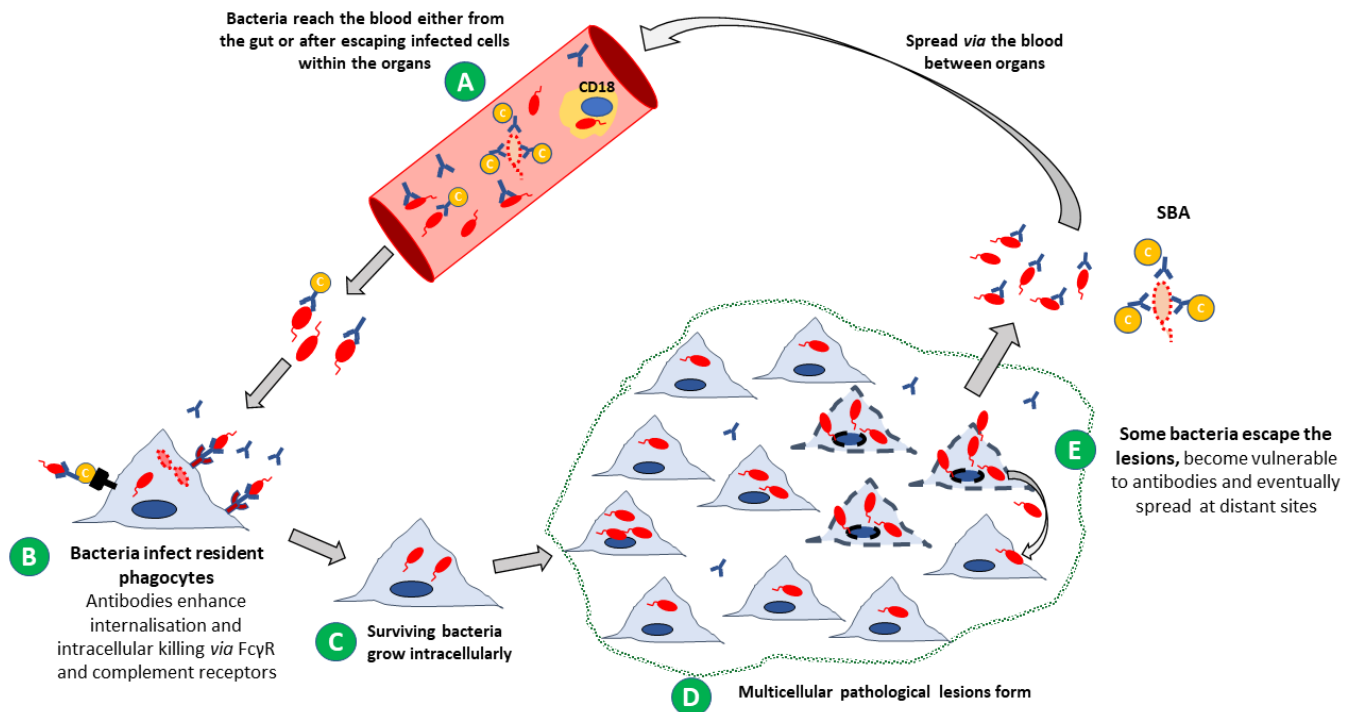
379 There is still a large deal of discrepancy between findings and conclusions from different studies over
380 several decades and this makes rational vaccine design very difficult.

381 To improve current vaccines and design new ones in the future it will be necessary to shed light on the
382 mechanisms that underpin both the development of antibody responses and their effector functions. It
383 will also be necessary to learn how to tailor antibody responses to situations where other components of
384 the immune system might be impaired, as often seen in endemic areas where immune-suppressive co-
385 morbidities geographically coincide with systemic *Salmonella* infections. Different vaccines and antibody
386 responses may be needed for travellers and residents in endemic areas.

387

388 Figures

Figure 1



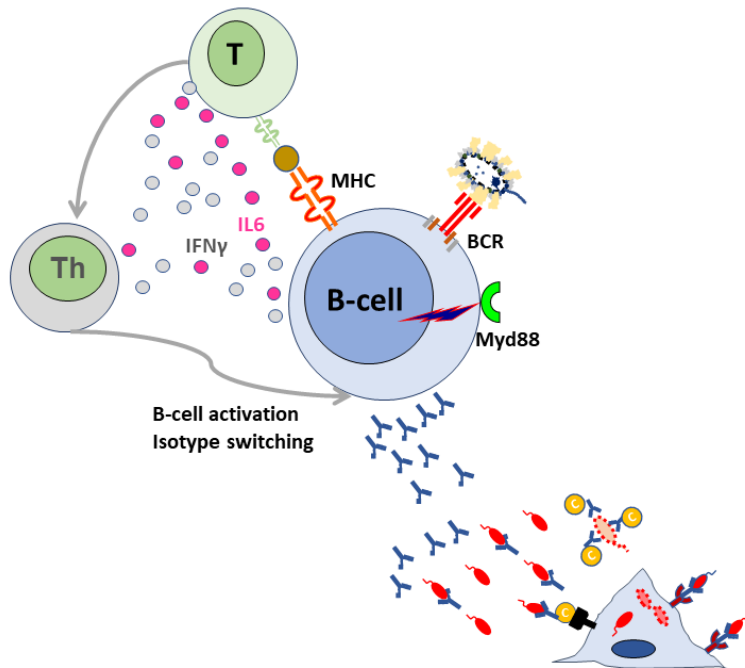
389

390 **Figure 1. The complex pathogenesis of *Salmonella*: an intracellular antibody-refractive growth phase**

391 **and an extracellular antibody-susceptible phase of spread. A)** After invading the gut, *Salmonella* reach

392 the blood where can be targeted by antibodies. Some bacteria in the blood are lysed by antibody- and
393 complement-dependent serum bactericidal activity (SBA). **B)** Bacteria opsonised by antibodies are
394 engulfed and killed more efficiently by resident phagocytes as soon as they reach the tissues (*e.g.* spleen,
395 liver, bone marrow). **C)** Those bacteria that resist killing establish unicellular initial infection foci (infected
396 phagocytes). Surviving bacteria grow mainly in an intracellular location. **D)** The initial single-cell infection
397 foci become spatially separated multicellular pathological lesions due to the infiltration of
398 polymorphonuclear phagocytes (PMNs) and later mononuclear cells. In this intracellular location bacteria
399 are inaccessible to antibodies. Bacteria grow intracellularly within the multicellular infection foci, but their
400 numbers within each phagocyte remain low due to the spread of the infection to infection of new host
401 cells. **E)** Some bacteria escape the lesions, become vulnerable to antibodies and eventually spread at
402 distant sites. When the bacteria are released from infected cells, they might undergo three different fates:
403 I) be targeted by antibodies, and killed *via* complement-mediated SBA or opsonophagocytosis; II) rapidly
404 infect neighbouring cells within the same lesion; III) travel to distant sites in the body to establish new
405 unicellular infection foci.

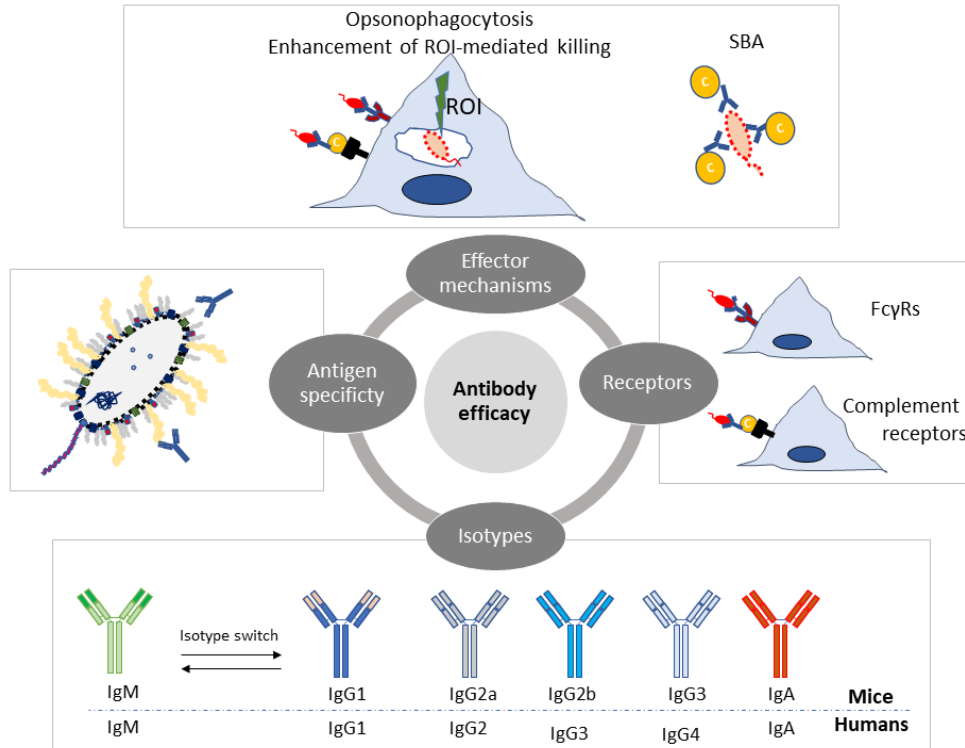
Figure 2



406

407 **Figure 2. B-cells and T-cells crosstalk impact on quality of the antibody response.** MyD88 signalling and
408 recognition of bacterial antigens *via* B-cell Receptor (BcR) leads to B-cell cell activation and antigen
409 presentation to naïve T-cells *via* MHC Class II molecules. IL6 and IFN γ from B-cells mediate the
410 development of Th immunity. Th immunity in turn triggers activation and maturation of B-cells and induce
411 isotype switching of the antibody response.

Figure 3



412

413 **Figure 3. Antibody-mediated protection to *Salmonella* is underpinned by a complex interplay between**
414 **qualitative traits and effector mechanisms.** Antigen specificity, isotype profile, FcγR receptor usage,
415 complement activation, and different effector mechanisms (opsonophagocytosis, killing *via* reactive
416 oxygen intermediates (ROI) and serum bactericidal activity (SBA)) are intertwined factors that influence
417 antibody-mediated protection to *Salmonella*.

418 **References.**

419

- 420 1. Breiman RF, Cosmas L, Njuguna H, Audi A, Olack B, Ochieng JB, Wamola N, Bigogo GM, Awiti G,
421 Tabu CW, Burke H, Williamson J, Oundo JO, Mintz ED, Feikin DR. 2012. Population-based
422 incidence of typhoid fever in an urban informal settlement and a rural area in Kenya:
423 implications for typhoid vaccine use in Africa. PLoS One 7:e29119.

- 424 2. Crump JA, Luby SP, Mintz ED. 2004. The global burden of typhoid fever. *Bull World Health Organ*
425 82:346-53.
- 426 3. Crump JA, Mintz ED. 2010. Global trends in typhoid and paratyphoid Fever. *Clin Infect Dis*
427 50:241-6.
- 428 4. Lutterloh E, Likaka A, Sejvar J, Manda R, Naiene J, Monroe SS, Khaila T, Chilima B, Mallewa M,
429 Kampondeni SD, Lowther SA, Capewell L, Date K, Townes D, Redwood Y, Schier JG, Nygren B,
430 Tippet Barr B, Demby A, Phiri A, Lungu R, Kaphiyo J, Humphrys M, Talkington D, Joyce K,
431 Stockman LJ, Armstrong GL, Mintz E. 2012. Multidrug-resistant typhoid fever with neurologic
432 findings on the Malawi-Mozambique border. *Clin Infect Dis* 54:1100-6.
- 433 5. Neil KP, Sodha SV, Lukwago L, S OT, Mikoleit M, Simington SD, Mukobi P, Balinandi S, Majalija S,
434 Ayers J, Kagirita A, Wefula E, Asiimwe F, Kweyamba V, Talkington D, Shieh WJ, Adem P, Batten
435 BC, Zaki SR, Mintz E. 2012. A large outbreak of typhoid fever associated with a high rate of
436 intestinal perforation in Kasese District, Uganda, 2008-2009. *Clin Infect Dis* 54:1091-9.
- 437 6. Crump JA, Heyderman RS. 2014. Invasive *Salmonella* infections in Africa. *Trans R Soc Trop Med*
438 *Hyg* 108:673-5.
- 439 7. Crump JA, Sjolund-Karlsson M, Gordon MA, Parry CM. 2015. Epidemiology, Clinical Presentation,
440 Laboratory Diagnosis, Antimicrobial Resistance, and Antimicrobial Management of Invasive
441 *Salmonella* Infections. *Clin Microbiol Rev* 28:901-37.
- 442 8. Feasey NA, Dougan G, Kingsley RA, Heyderman RS, Gordon MA. 2012. Invasive non-typhoidal
443 salmonella disease: an emerging and neglected tropical disease in Africa. *Lancet* 379:2489-99.
- 444 9. Gordon MA. 2008. *Salmonella* infections in immunocompromised adults. *J Infect* 56:413-22.

- 445 10. Gordon MA, Graham SM, Walsh AL, Wilson L, Phiri A, Molyneux E, Zijlstra EE, Heyderman RS,
446 Hart CA, Molyneux ME. 2008. Epidemics of invasive *Salmonella enterica* serovar enteritidis and *S.*
447 *enterica* Serovar typhimurium infection associated with multidrug resistance among adults and
448 children in Malawi. *Clin Infect Dis* 46:963-9.
- 449 11. Kariuki S, Gordon MA, Feasey N, Parry CM. 2015. Antimicrobial resistance and management of
450 invasive *Salmonella* disease. *Vaccine* 33 Suppl 3:C21-C29.
- 451 12. Reddy EA, Shaw AV, Crump JA. 2010. Community-acquired bloodstream infections in Africa: a
452 systematic review and meta-analysis. *Lancet Infect Dis* 10:417-32.
- 453 13. Cooke FJ, Wain J. 2004. The emergence of antibiotic resistance in typhoid fever. *Travel Med*
454 *Infect Dis* 2:67-74.
- 455 14. Harish BN, Menezes GA. 2011. Antimicrobial resistance in typhoidal salmonellae. *Indian J Med*
456 *Microbiol* 29:223-9.
- 457 15. Klemm EJ, Shakoor S, Page AJ, Qamar FN, Judge K, Saeed DK, Wong VK, Dallman TJ, Nair S, Baker
458 S, Shaheen G, Qureshi S, Yousafzai MT, Saleem MK, Hasan Z, Dougan G, Hasan R. 2018.
459 Emergence of an Extensively Drug-Resistant *Salmonella enterica* Serovar Typhi Clone Harboring a
460 Promiscuous Plasmid Encoding Resistance to Fluoroquinolones and Third-Generation
461 Cephalosporins. *MBio* 9.
- 462 16. Baker SJ, Payne DJ, Rappuoli R, De Gregorio E. 2018. Technologies to address antimicrobial
463 resistance. *Proc Natl Acad Sci U S A* 115:12887-12895.
- 464 17. Bloom DE, Black S, Salisbury D, Rappuoli R. 2018. Antimicrobial resistance and the role of
465 vaccines. *Proc Natl Acad Sci U S A* 115:12868-12871.

- 466 18. Klugman KP, Black S. 2018. Impact of existing vaccines in reducing antibiotic resistance: Primary
467 and secondary effects. *Proc Natl Acad Sci U S A* 115:12896-12901.
- 468 19. Tagliabue A, Leite LCC, Leroy OY, Rappuoli R. 2019. Editorial: A Global Perspective on Vaccines:
469 Priorities, Challenges and Online Information. *Front Immunol* 10:2556.
- 470 20. MacLennan CA, Martin LB, Micoli F. 2014. Vaccines against invasive *Salmonella* disease: current
471 status and future directions. *Hum Vaccin Immunother* 10:1478-93.
- 472 21. Mastroeni P, Chabalgoity JA, Dunstan SJ, Maskell DJ, Dougan G. 2001. *Salmonella*: immune
473 responses and vaccines. *Vet J* 161:132-64.
- 474 22. Harrison JA, Villarreal-Ramos B, Mastroeni P, Demarco de Hormaeche R, Hormaeche CE. 1997.
475 Correlates of protection induced by live Aro- *Salmonella typhimurium* vaccines in the murine
476 typhoid model. *Immunology* 90:618-25.
- 477 23. Kotlarski I, Pope M, Doherty K, Attridge SR. 1989. The in vitro proliferative response of lymphoid
478 cells of mice infected with *Salmonella enteritidis* 11RX. *Immunol Cell Biol* 67 (Pt 1):19-29.
- 479 24. Grant AJ, Oshota O, Chaudhuri RR, Mayho M, Peters SE, Clare S, Maskell DJ, Mastroeni P. 2016.
480 Genes Required for the fitness of *Salmonella enterica* serovar Typhimurium During Infection of
481 Immunodeficient gp91-/-phox Mice. *Infect Immun* doi:10.1128/IAI.01423-15.
- 482 25. Hess J, Ladel C, Miko D, Kaufmann SH. 1996. *Salmonella typhimurium* aroA- infection in gene-
483 targeted immunodeficient mice: major role of CD4+ TCR-alpha beta cells and IFN-gamma in
484 bacterial clearance independent of intracellular location. *J Immunol* 156:3321-6.
- 485 26. Muotiala A. 1992. Anti-IFN-gamma-treated mice--a model for testing safety of live *Salmonella*
486 vaccines. *Vaccine* 10:243-6.

- 487 27. Sinha K, Mastroeni P, Harrison J, de Hormaeche RD, Hormaeche CE. 1997. Salmonella
488 typhimurium aroA, htrA, and aroD htrA mutants cause progressive infections in athymic (nu/nu)
489 BALB/c mice. *Infect Immun* 65:1566-9.
- 490 28. Lokken KL, Mooney JP, Butler BP, Xavier MN, Chau JY, Schaltenberg N, Begum RH, Muller W,
491 Luckhart S, Tsolis RM. 2014. Malaria Parasite Infection Compromises Control of Concurrent
492 Systemic Non-typhoidal Salmonella Infection via IL-10-Mediated Alteration of Myeloid Cell
493 Function. *PLoS Pathog* 10:e1004049.
- 494 29. Roux CM, Butler BP, Chau JY, Paixao TA, Cheung KW, Santos RL, Luckhart S, Tsolis RM. 2010. Both
495 hemolytic anemia and malaria parasite-specific factors increase susceptibility to Nontyphoidal
496 Salmonella enterica serovar typhimurium infection in mice. *Infect Immun* 78:1520-7.
- 497 30. Feasey NA, Everett D, Faragher EB, Roca-Feltrer A, Kang'ombe A, Denis B, Kerac M, Molyneux E,
498 Molyneux M, Jahn A, Gordon MA, Heyderman RS. 2015. Modelling the Contributions of Malaria,
499 HIV, Malnutrition and Rainfall to the Decline in Paediatric Invasive Non-typhoidal Salmonella
500 Disease in Malawi. *PLoS Negl Trop Dis* 9:e0003979.
- 501 31. Gordon MA. 2011. Invasive nontyphoidal Salmonella disease: epidemiology, pathogenesis and
502 diagnosis. *Curr Opin Infect Dis* 24:484-9.
- 503 32. Scott JA, Berkley JA, Mwangi I, Ochola L, Uyoga S, Macharia A, Ndila C, Lowe BS, Mwarumba S,
504 Bauni E, Marsh K, Williams TN. 2011. Relation between falciparum malaria and bacteraemia in
505 Kenyan children: a population-based, case-control study and a longitudinal study. *Lancet*
506 378:1316-23.

- 507 33. Garvis SG, Beuzon CR, Holden DW. 2001. A role for the PhoP/Q regulon in inhibition of fusion
508 between lysosomes and Salmonella-containing vacuoles in macrophages. *Cell Microbiol* 3:731-
509 44.
- 510 34. Helaine S, Thompson JA, Watson KG, Liu M, Boyle C, Holden DW. 2010. Dynamics of intracellular
511 bacterial replication at the single cell level. *Proc Natl Acad Sci U S A* 107:3746-51.
- 512 35. Shea JE, Beuzon CR, Gleeson C, Mundy R, Holden DW. 1999. Influence of the Salmonella
513 typhimurium pathogenicity island 2 type III secretion system on bacterial growth in the mouse.
514 *Infect Immun* 67:213-9.
- 515 36. Richter-Dahlfors A, Buchan AMJ, Finlay BB. 1997. Murine salmonellosis studied by confocal
516 microscopy: Salmonella typhimurium resides intracellularly inside macrophages and exerts a
517 cytotoxic effect on phagocytes in vivo. *J Exp Med* 186:569-80.
- 518 37. Vazquez-Torres A, Xu Y, Jones-Carson J, Holden DW, Lucia SM, Dinauer MC, Mastroeni P, Fang
519 FC. 2000. Salmonella pathogenicity island 2-dependent evasion of the phagocyte NADPH
520 oxidase. *Science* 287:1655-8.
- 521 38. Fields PI, Swanson RV, Haidaris CG, Heffron F. 1986. Mutants of Salmonella typhimurium that
522 cannot survive within the macrophage are avirulent. *Proc Natl Acad Sci U S A* 83:5189-93.
- 523 39. Gordon MA, Gordon SB, Musaya L, Zijlstra EE, Molyneux ME, Read RC. 2007. Primary
524 macrophages from HIV-infected adults show dysregulated cytokine responses to Salmonella, but
525 normal internalization and killing. *AIDS* 21:2399-408.
- 526 40. Gordon MA, Kankwatira AM, Mwafulirwa G, Walsh AL, Hopkins MJ, Parry CM, Faragher EB,
527 Zijlstra EE, Heyderman RS, Molyneux ME. 2010. Invasive non-typhoid salmonellae establish

528 systemic intracellular infection in HIV-infected adults: an emerging disease pathogenesis. Clin
529 Infect Dis 50:953-62.

530 41. Harrington KA, Hormaeche CE. 1986. Expression of the innate resistance gene *Ity* in mouse
531 Kupffer cells infected with *Salmonella typhimurium* in vitro. *Microb Pathog* 1:269-74.

532 42. Hormaeche CE. 1979. The natural resistance of radiation chimeras to *S. typhimurium* C5.
533 *Immunology* 37:329-32.

534 43. Hormaeche CE. 1979. Genetics of natural resistance to salmonellae in mice. *Immunology* 37:319-
535 27.

536 44. Hormaeche CE. 1979. Natural resistance to *Salmonella typhimurium* in different inbred mouse
537 strains. *Immunology* 37:311-8.

538 45. Hsu HS. 1989. Pathogenesis and immunity in murine salmonellosis. *Microbiol Rev* 53:390-409.

539 46. Collins FM. 1974. Vaccines and cell-mediated immunity. *Bacteriol Rev* 38:371-402.

540 47. Mackaness GB, Blanden RV, Collins FM. 1966. Host-parasite relations in mouse typhoid. *J Exp*
541 *Med* 124:573-83.

542 48. Acharya IL, Lowe CU, Thapa R, Gurubacharya VL, Shrestha MB, Cadoz M, Schulz D, Armand J,
543 Bryla DA, Trollfors B, et al. 1987. Prevention of typhoid fever in Nepal with the Vi capsular
544 polysaccharide of *Salmonella typhi*. A preliminary report. *N Engl J Med* 317:1101-4.

545 49. Levine MM, Ferreccio C, Black RE, Tacket CO, Germanier R. 1989. Progress in vaccines against
546 typhoid fever. *Rev Infect Dis* 11 Suppl 3:S552-67.

547 50. Eisenstein TK, Killar LM, Sultzer BM. 1984. Immunity to infection with *Salmonella typhimurium*:
548 mouse-strain differences in vaccine- and serum-mediated protection. *J Infect Dis* 150:425-35.

- 549 51. Szu SC, Stone AL, Robbins JD, Schneerson R, Robbins JB. 1987. Vi capsular polysaccharide-protein
550 conjugates for prevention of typhoid fever. Preparation, characterization, and immunogenicity in
551 laboratory animals. *J Exp Med* 166:1510-24.
- 552 52. Thiem VD, Lin FY, Canh DG, Son NH, Anh DD, Mao ND, Chu C, Hunt SW, Robbins JB, Schneerson
553 R, Szu SC. 2011. The Vi conjugate typhoid vaccine is safe, elicits protective levels of IgG anti-Vi,
554 and is compatible with routine infant vaccines. *Clin Vaccine Immunol* 18:730-5.
- 555 53. Szu SC. 2013. Development of Vi conjugate - a new generation of typhoid vaccine. *Expert Rev*
556 *Vaccines* 12:1273-86.
- 557 54. Mastroeni P, Villarreal-Ramos B, Hormaeche CE. 1993. Adoptive transfer of immunity to oral
558 challenge with virulent salmonellae in innately susceptible BALB/c mice requires both immune
559 serum and T cells. *Infect Immun* 61:3981-4.
- 560 55. Gondwe EN, Molyneux ME, Goodall M, Graham SM, Mastroeni P, Drayson MT, MacLennan CA.
561 2010. Importance of antibody and complement for oxidative burst and killing of invasive
562 nontyphoidal Salmonella by blood cells in Africans. *Proc Natl Acad Sci U S A* 107:3070-5.
- 563 56. MacLennan CA, Gondwe EN, Msefula CL, Kingsley RA, Thomson NR, White SA, Goodall M, Pickard
564 DJ, Graham SM, Dougan G, Hart CA, Molyneux ME, Drayson MT. 2008. The neglected role of
565 antibody in protection against bacteremia caused by nontyphoidal strains of Salmonella in
566 African children. *J Clin Invest* 118:1553-62.
- 567 57. Nyirenda TS, Gilchrist JJ, Feasey NA, Glennie SJ, Bar-Zeev N, Gordon MA, MacLennan CA,
568 Mandala WL, Heyderman RS. 2014. Sequential acquisition of T cells and antibodies to
569 nontyphoidal Salmonella in Malawian children. *J Infect Dis* 210:56-64.

- 570 58. Cunnington AJ, de Souza JB, Walther M, Riley EM. 2012. Malaria impairs resistance to Salmonella
571 through heme- and heme oxygenase-dependent dysfunctional granulocyte mobilization. *Nat*
572 *Med* 18:120-7.
- 573 59. Cunnington AJ, Riley EM. 2010. Suppression of vaccine responses by malaria: insignificant or
574 overlooked? *Expert Rev Vaccines* 9:409-29.
- 575 60. Gordon MA, Banda HT, Gondwe M, Gordon SB, Boeree MJ, Walsh AL, Corkill JE, Hart CA, Gilks CF,
576 Molyneux ME. 2002. Non-typhoidal salmonella bacteraemia among HIV-infected Malawian
577 adults: high mortality and frequent recrudescence. *Aids* 16:1633-41.
- 578 61. Greenwood BM, Brueton MJ. 1974. Complement activation in children with acute malaria. *Clin*
579 *Exp Immunol* 18:267-72.
- 580 62. Mooney JP, Lee SJ, Lokken KL, Nanton MR, Nuccio SP, McSorley SJ, Tsolis RM. 2015. Transient
581 Loss of Protection Afforded by a Live Attenuated Non-typhoidal Salmonella Vaccine in Mice Co-
582 infected with Malaria. *PLoS Negl Trop Dis* 9:e0004027.
- 583 63. Klemm EJ, Gkrania-Klotsas E, Hadfield J, Forbester JL, Harris SR, Hale C, Heath JN, Wileman T,
584 Clare S, Kane L, Goulding D, Otto TD, Kay S, Doffinger R, Cooke FJ, Carmichael A, Lever AM,
585 Parkhill J, MacLennan CA, Kumararatne D, Dougan G, Kingsley RA. 2016. Emergence of host-
586 adapted Salmonella Enteritidis through rapid evolution in an immunocompromised host. *Nat*
587 *Microbiol* 1:15023.
- 588 64. de Jong R, Altare F, Haagen IA, Elferink DG, Boer T, van Breda Vriesman PJ, Kabel PJ, Draaisma
589 JM, van Dissel JT, Kroon FP, Casanova JL, Ottenhoff TH. 1998. Severe mycobacterial and
590 Salmonella infections in interleukin-12 receptor-deficient patients. *Science* 280:1435-8.

- 591 65. Jouanguy E, Doffinger R, Dupuis S, Pallier A, Altare F, Casanova JL. 1999. IL-12 and IFN-gamma in
592 host defense against mycobacteria and salmonella in mice and men. *Curr Opin Immunol* 11:346-
593 51.
- 594 66. MacLennan C, Fieschi C, Lammas DA, Picard C, Dorman SE, Sanal O, MacLennan JM, Holland SM,
595 Ottenhoff TH, Casanova JL, Kumararatne DS. 2004. Interleukin (IL)-12 and IL-23 are key cytokines
596 for immunity against *Salmonella* in humans. *J Infect Dis* 190:1755-7.
- 597 67. Picard C, Fieschi C, Altare F, Al-Jumaah S, Al-Hajjar S, Feinberg J, Dupuis S, Soudais C, Al-Mohsen
598 IZ, Genin E, Lammas D, Kumararatne DS, Leclerc T, Rafii A, Frayha H, Murugasu B, Wah LB,
599 Sinniah R, Loubser M, Okamoto E, Al-Ghoniaim A, Tufenkeji H, Abel L, Casanova JL. 2002.
600 Inherited interleukin-12 deficiency: IL12B genotype and clinical phenotype of 13 patients from
601 six kindreds. *Am J Hum Genet* 70:336-48.
- 602 68. Carter PB, Collins FM. 1974. The route of enteric infection in normal mice. *J Exp Med* 139:1189-
603 203.
- 604 69. Vazquez-Torres A, Jones-Carson J, Baumler AJ, Falkow S, Valdivia R, Brown W, Le M, Berggren R,
605 Parks WT, Fang FC. 1999. Extraintestinal dissemination of *Salmonella* by CD18-expressing
606 phagocytes. *Nature* 401:804-8.
- 607 70. Biozzi G, Howard JG, Halpern BN, Stiffel C, Mouton D. 1960. The kinetics of blood clearance of
608 isotopically labelled *Salmonella enteritidis* by the reticuloendothelial system in mice.
609 *Immunology* 3:74-89.
- 610 71. Coward C, Restif O, Dybowski R, Grant AJ, Maskell DJ, Mastroeni P. 2014. The effects of
611 vaccination and immunity on bacterial infection dynamics in vivo. *PLoS Pathog* 10:e1004359.

- 612 72. Warren J, Mastroeni P, Dougan G, Noursadeghi M, Cohen J, Walport MJ, Botto M. 2002.
613 Increased susceptibility of C1q-deficient mice to *Salmonella enterica* serovar Typhimurium
614 infection. *Infect Immun* 70:551-7.
- 615 73. Dunlap NE, Benjamin WH, Jr., McCall RD, Jr., Tilden AB, Briles DE. 1991. A 'safe-site' for
616 *Salmonella typhimurium* is within splenic cells during the early phase of infection in mice. *Microb*
617 *Pathog* 10:297-310.
- 618 74. Salcedo SP, Noursadeghi M, Cohen J, Holden DW. 2001. Intracellular replication of *Salmonella*
619 typhimurium strains in specific subsets of splenic macrophages in vivo. *Cell Microbiol* 3:587-97.
- 620 75. Dunlap NE, Benjamin WH, Jr., Berry AK, Eldridge JH, Briles DE. 1992. A 'safe-site' for *Salmonella*
621 typhimurium is within splenic polymorphonuclear cells. *Microb Pathog* 13:181-90.
- 622 76. Yrlid U, Svensson M, Hakansson A, Chambers BJ, Ljunggren HG, Wick MJ. 2001. In vivo activation
623 of dendritic cells and T cells during *Salmonella enterica* serovar Typhimurium infection. *Infect*
624 *Immun* 69:5726-35.
- 625 77. Sheppard M, Webb C, Heath F, Mallows V, Emilianus R, Maskell D, Mastroeni P. 2003. Dynamics
626 of bacterial growth and distribution within the liver during *Salmonella* infection. *Cell Microbiol*
627 5:593-600.
- 628 78. Mastroeni P, Skepper JN, Hormaeche CE. 1995. Effect of anti-tumor necrosis factor alpha
629 antibodies on histopathology of primary *Salmonella* infections [published erratum appears in
630 *Infect Immun* 1995 Dec;63(12):4966]. *Infect Immun* 63:3674-82.
- 631 79. Shea JE, Hensel M, Gleeson C, Holden DW. 1996. Identification of a virulence locus encoding a
632 second type III secretion system in *Salmonella typhimurium*. *Proc Natl Acad Sci U S A* 93:2593-7.

- 633 80. Brown SP, Cornell SJ, Sheppard M, Grant AJ, Maskell DJ, Grenfell BT, Mastroeni P. 2006.
634 Intracellular demography and the dynamics of *Salmonella enterica* infections. *PLoS Biol* 4:e349.
- 635 81. Grant AJ, Morgan FJ, McKinley TJ, Foster GL, Maskell DJ, Mastroeni P. 2012. Attenuated
636 *Salmonella Typhimurium* lacking the pathogenicity island-2 type 3 secretion system grow to high
637 bacterial numbers inside phagocytes in mice. *PLoS Pathog* 8:e1003070.
- 638 82. Mastroeni P, Arena A, Costa GB, Liberto MC, Bonina L, Hormaeche CE. 1991. Serum TNF alpha in
639 mouse typhoid and enhancement of a *Salmonella* infection by anti-TNF alpha antibodies. *Microb*
640 *Pathog* 11:33-8.
- 641 83. Mastroeni P, Clare S, Khan S, Harrison JA, Hormaeche CE, Okamura H, Kurimoto M, Dougan G.
642 1999. Interleukin 18 contributes to host resistance and gamma interferon production in mice
643 infected with virulent *Salmonella typhimurium*. *Infect Immun* 67:478-83.
- 644 84. Mastroeni P, Harrison JA, Chabalgoity JA, Hormaeche CE. 1996. Effect of interleukin 12
645 neutralization on host resistance and gamma interferon production in mouse typhoid. *Infect*
646 *Immun* 64:189-96.
- 647 85. Mastroeni P, Villarreal-Ramos B, Hormaeche CE. 1992. Role of T cells, TNF alpha and IFN gamma
648 in recall of immunity to oral challenge with virulent salmonellae in mice vaccinated with live
649 attenuated aro- *Salmonella* vaccines. *Microb Pathog* 13:477-91.
- 650 86. Muotiala A, Makela PH. 1990. The role of IFN-gamma in murine *Salmonella typhimurium*
651 infection. *Microb Pathog* 8:135-41.
- 652 87. Goh YS, Armour KL, Clark MR, Grant AJ, Mastroeni P. 2016. Igg Subclasses Targeting the Flagella
653 of *Salmonella enterica* Serovar *Typhimurium* Can Mediate Phagocytosis and Bacterial Killing. *J*
654 *Vaccines Vaccin* 7.

- 655 88. Uppington H, Menager N, Boross P, Wood J, Sheppard M, Verbeek S, Mastroeni P. 2006. Effect of
656 immune serum and role of individual Fcγ receptors on the intracellular distribution and
657 survival of *Salmonella enterica* serovar Typhimurium in murine macrophages. *Immunology*
658 119:147-58.
- 659 89. Grant AJ, Restif O, McKinley TJ, Sheppard M, Maskell DJ, Mastroeni P. 2008. Modelling within-
660 host spatiotemporal dynamics of invasive bacterial disease. *PLoS Biol* 6:e74.
- 661 90. Brown A, Hormaeche CE, Demarco-de-Hormaeche R, Winther M, Dougan G, Maskell DJ, Stocker
662 BA. 1987. An attenuated *aroA* *Salmonella typhimurium* vaccine elicits humoral and cellular
663 immunity to cloned beta-galactosidase in mice. *J Infect Dis* 155:86-92.
- 664 91. Liang L, Juarez S, Nga TV, Dunstan S, Nakajima-Sasaki R, Davies DH, McSorley S, Baker S, Felgner
665 PL. 2013. Immune profiling with a *Salmonella Typhi* antigen microarray identifies new diagnostic
666 biomarkers of human typhoid. *Sci Rep* 3:1043.
- 667 92. Villarreal-Ramos B, Manser J, Collins RA, Dougan G, Chatfield SN, Howard CJ. 1998. Immune
668 responses in calves immunised orally or subcutaneously with a live *Salmonella typhimurium aro*
669 vaccine. *Vaccine* 16:45-54.
- 670 93. Brown A, Hormaeche CE. 1989. The antibody response to salmonellae in mice and humans
671 studied by immunoblots and ELISA. *Microb Pathog* 6:445-54.
- 672 94. Tran TH, Nguyen TD, Nguyen TT, Ninh TT, Tran NB, Nguyen VM, Tran TT, Cao TT, Pham VM,
673 Nguyen TC, Tran TD, Pham VT, To SD, Campbell JI, Stockwell E, Schultsz C, Simmons CP, Glover C,
674 Lam W, Marques F, May JP, Upton A, Budhram R, Dougan G, Farrar J, Nguyen VV, Dolecek C.
675 2010. A randomised trial evaluating the safety and immunogenicity of the novel single oral dose
676 typhoid vaccine M01ZH09 in healthy Vietnamese children. *PLoS One* 5:e11778.

- 677 95. Murphy JR, Baqar S, Munoz C, Schlesinger L, Ferreccio C, Lindberg AA, Svenson S, Losonsky G,
678 Koster F, Levine MM. 1987. Characteristics of humoral and cellular immunity to *Salmonella typhi*
679 in residents of typhoid-endemic and typhoid-free regions. *J Infect Dis* 156:1005-9.
- 680 96. Sztein MB. 2007. Cell-mediated immunity and antibody responses elicited by attenuated
681 *Salmonella enterica* Serovar Typhi strains used as live oral vaccines in humans. *Clin Infect Dis* 45
682 Suppl 1:S15-9.
- 683 97. Hormaeche CE, Mastroeni P, Harrison JA, Demarco de Hormaeche R, Svenson S, Stocker BA.
684 1996. Protection against oral challenge three months after i.v. immunization of BALB/c mice with
685 live Aro *Salmonella typhimurium* and *Salmonella enteritidis* vaccines is serotype (species)-
686 dependent and only partially determined by the main LPS O antigen. *Vaccine* 14:251-9.
- 687 98. Segall T, Lindberg AA. 1993. Oral vaccination of calves with an aromatic-dependent *Salmonella*
688 dublin (O9,12) hybrid expressing O4,12 protects against *S. dublin* (O9,12) but not against
689 *Salmonella typhimurium* (O4,5,12). *Infect Immun* 61:1222-31.
- 690 99. Svenson SB, Lindberg AA. 1981. Artificial *Salmonella* vaccines: *Salmonella typhimurium* O-
691 antigen- specific oligosaccharide-protein conjugates elicit protective antibodies in rabbits and
692 mice. *Infect Immun* 32:490-6.
- 693 100. Carlin NI, Svenson SB, Lindberg AA. 1987. Role of monoclonal O-antigen antibody epitope
694 specificity and isotype in protection against experimental mouse typhoid. *Microb Pathog* 2:171-
695 83.
- 696 101. Hale C, Bowe F, Pickard D, Clare S, Haeuw JF, Powers U, Menager N, Mastroeni P, Dougan G.
697 2006. Evaluation of a novel Vi conjugate vaccine in a murine model of salmonellosis. *Vaccine*
698 24:4312-20.

- 699 102. Jin C, Gibani MM, Moore M, Juel HB, Jones E, Meiring J, Harris V, Gardner J, Nebykova A,
700 Kerridge SA, Hill J, Thomaidis-Brears H, Blohmke CJ, Yu LM, Angus B, Pollard AJ. 2017. Efficacy
701 and immunogenicity of a Vi-tetanus toxoid conjugate vaccine in the prevention of typhoid fever
702 using a controlled human infection model of Salmonella Typhi: a randomised controlled, phase
703 2b trial. *Lancet* 390:2472-2480.
- 704 103. Shakya M, Colin-Jones R, Theiss-Nyland K, Voysey M, Pant D, Smith N, Liu X, Tonks S, Mazur O,
705 Farooq YG, Clarke J, Hill J, Adhikari A, Dongol S, Karkey A, Bajracharya B, Kelly S, Gurung M, Baker
706 S, Neuzil KM, Shrestha S, Basnyat B, Pollard AJ, Ty VACNST. 2019. Phase 3 Efficacy Analysis of a
707 Typhoid Conjugate Vaccine Trial in Nepal. *N Engl J Med* 381:2209-2218.
- 708 104. Kuusi N, Nurminen M, Saxen H, Valtonen M, Makela PH. 1979. Immunization with major outer
709 membrane proteins in experimental salmonellosis of mice. *Infect Immun* 25:857-62.
- 710 105. Dominguez-Medina CC, Perez-Toledo M, Schager AE, Marshall JL, Cook CN, Bobat S, Hwang H,
711 Chun BJ, Logan E, Bryant JA, Channell WM, Morris FC, Jossi SE, Alshayea A, Rossiter AE, Barrow
712 PA, Horsnell WG, MacLennan CA, Henderson IR, Lakey JH, Gumbart JC, Lopez-Macias C, Bavro
713 VN, Cunningham AF. 2020. Outer membrane protein size and LPS O-antigen define protective
714 antibody targeting to the Salmonella surface. *Nat Commun* 11:851.
- 715 106. Gil-Cruz C, Bobat S, Marshall JL, Kingsley RA, Ross EA, Henderson IR, Leyton DL, Coughlan RE,
716 Khan M, Jensen KT, Buckley CD, Dougan G, MacLennan IC, Lopez-Macias C, Cunningham AF.
717 2009. The porin OmpD from nontyphoidal Salmonella is a key target for a protective B1b cell
718 antibody response. *Proc Natl Acad Sci U S A* 106:9803-8.

- 719 107. Goh YS, Grant AJ, Restif O, McKinley TJ, Armour KL, Clark MR, Mastroeni P. 2011. Human IgG
720 isotypes and activating Fcγ receptors in the interaction of *Salmonella enterica* serovar
721 Typhimurium with phagocytic cells. *Immunology* 133:74-83.
- 722 108. Wahid R, Zafar SJ, McArthur MA, Pasetti MF, Levine MM, Sztein MB. 2014. Live oral *Salmonella*
723 *enterica* serovar Typhi vaccines Ty21a and CVD 909 induce opsonophagocytic functional
724 antibodies in humans that cross-react with *S. Paratyphi A* and *S. Paratyphi B*. *Clin Vaccine*
725 *Immunol* 21:427-34.
- 726 109. Micoli F, Rondini S, Alfini R, Lanzilao L, Necchi F, Negrea A, Rossi O, Brandt C, Clare S, Mastroeni
727 P, Rappuoli R, Saul A, MacLennan CA. 2018. Comparative immunogenicity and efficacy of
728 equivalent outer membrane vesicle and glycoconjugate vaccines against nontyphoidal
729 *Salmonella*. *Proc Natl Acad Sci U S A* 115:10428-10433.
- 730 110. Siggins MK, O'Shaughnessy CM, Pravin J, Cunningham AF, Henderson IR, Drayson MT,
731 MacLennan CA. 2014. Differential timing of antibody-mediated phagocytosis and cell-free killing
732 of invasive African *Salmonella* allows immune evasion. *Eur J Immunol* 44:1093-8.
- 733 111. Juel HB, Thomaidis-Brears HB, Darton TC, Jones C, Jones E, Shrestha S, Sie R, Eustace A, Galal U,
734 Kurupati P, Van TT, Thieu NTV, Baker S, Blohmke CJ, Pollard AJ. 2017. *Salmonella* Typhi
735 Bactericidal Antibodies Reduce Disease Severity but Do Not Protect against Typhoid Fever in a
736 Controlled Human Infection Model. *Front Immunol* 8:1916.
- 737 112. Rossi O, Coward C, Goh YS, Claassens JWC, MacLennan CA, Verbeek SJ, Mastroeni P. 2019. The
738 essential role of complement in antibody-mediated resistance to *Salmonella*. *Immunology*
739 156:69-73.

- 740 113. Menager N, Foster G, Ugrinovic S, Uppington H, Verbeek S, Mastroeni P. 2007. Fcγ receptors are crucial for the expression of acquired resistance to virulent *Salmonella enterica*
741 serovar Typhimurium in vivo but are not required for the induction of humoral or T-cell-
742 mediated immunity. *Immunology* 120:424-32.
- 744 114. Lindberg AA, Segall T, Weintraub A, Stocker BA. 1993. Antibody response and protection against
745 challenge in mice vaccinated intraperitoneally with a live aroA O4-O9 hybrid *Salmonella dublin*
746 strain. *Infect Immun* 61:1211-21.
- 747 115. Metcalf ES, O'Brien AD. 1981. Characterization of murine antibody response to *Salmonella*
748 typhimurium by a class-specific solid-phase radioimmunoassay. *Infect Immun* 31:33-41.
- 749 116. Forrest BD, LaBrooy JT, Beyer L, Dearlove CE, Shearman DJ. 1991. The human humoral immune
750 response to *Salmonella typhi* Ty21a. *J Infect Dis* 163:336-45.
- 751 117. Tagliabue A, Villa L, De Magistris MT, Romano M, Silvestri S, Boraschi D, Nencioni L. 1986. IgA-
752 driven T cell-mediated anti-bacterial immunity in man after live oral Ty 21a vaccine. *J Immunol*
753 137:1504-10.
- 754 118. Cunningham AF, Gaspal F, Serre K, Mohr E, Henderson IR, Scott-Tucker A, Kenny SM, Khan M,
755 Toellner KM, Lane PJ, MacLennan IC. 2007. *Salmonella* induces a switched antibody response
756 without germinal centers that impedes the extracellular spread of infection. *J Immunol*
757 178:6200-7.
- 758 119. Szu SC, Klugman KP, Hunt S. 2014. Re-examination of immune response and estimation of anti-Vi
759 IgG protective threshold against typhoid fever-based on the efficacy trial of Vi conjugate in
760 young children. *Vaccine* 32:2359-63.

- 761 120. Dahora LC, Jin C, Spreng RL, Feely F, Mathura R, Seaton KE, Zhang L, Hill J, Jones E, Alam SM,
762 Dennison SM, Pollard AJ, Tomaras GD. 2019. IgA and IgG1 Specific to Vi Polysaccharide of
763 Salmonella Typhi Correlate With Protection Status in a Typhoid Fever Controlled Human
764 Infection Model. *Front Immunol* 10:2582.
- 765 121. Saxen H, Makela O. 1982. The protective capacity of immune sera in experimental mouse
766 salmonellosis is mainly due to IgM antibodies. *Immunol Lett* 5:267-72.
- 767 122. Saxen H, Makela O, Svenson SB. 1984. Isotype of protective anti-Salmonella antibodies in
768 experimental mouse salmonellosis. *Infect Immun* 44:633-6.
- 769 123. Goh YS, Clare S, Micoli F, Saul A, Mastroeni P, MacLennan CA. 2015. Monoclonal Antibodies of a
770 Diverse Isotype Induced by an O-Antigen Glycoconjugate Vaccine Mediate In Vitro and In Vivo
771 Killing of African Invasive Nontyphoidal Salmonella. *Infect Immun* 83:3722-31.
- 772 124. Wijburg OL, Uren TK, Simpfendorfer K, Johansen FE, Brandtzaeg P, Strugnell RA. 2006. Innate
773 secretory antibodies protect against natural Salmonella typhimurium infection. *J Exp Med*
774 203:21-6.
- 775 125. Michetti P, Mahan MJ, Slauch JM, Mekalanos JJ, Neutra MR. 1992. Monoclonal secretory
776 immunoglobulin A protects mice against oral challenge with the invasive pathogen Salmonella
777 typhimurium. *Infect Immun* 60:1786-92.
- 778 126. Zhang Y, Dominguez-Medina C, Cumley NJ, Heath JN, Essex SJ, Bobat S, Schager A, Goodall M,
779 Kracker S, Buckley CD, May RC, Kingsley RA, MacLennan CA, Lopez-Macias C, Cunningham AF,
780 Toellner KM. 2017. IgG1 Is Required for Optimal Protection after Immunization with the Purified
781 Porin OmpD from Salmonella Typhimurium. *J Immunol* 199:4103-4109.

- 782 127. Goh YS, Necchi F, O'Shaughnessy CM, Micoli F, Gavini M, Young SP, Msefula CL, Gondwe EN,
783 Mandala WL, Gordon MA, Saul AJ, MacLennan CA. 2016. Bactericidal Immunity to Salmonella in
784 Africans and Mechanisms Causing Its Failure in HIV Infection. *PLoS Negl Trop Dis* 10:e0004604.
- 785 128. Salerno-Goncalves R, Pasetti MF, Sztein MB. 2002. Characterization of CD8(+) effector T cell
786 responses in volunteers immunized with Salmonella enterica serovar Typhi strain Ty21a typhoid
787 vaccine. *J Immunol* 169:2196-203.
- 788 129. Salerno-Goncalves R, Wyant TL, Pasetti MF, Fernandez-Vina M, Tacket CO, Levine MM, Sztein
789 MB. 2003. Concomitant Induction of CD4(+) and CD8(+) T Cell Responses in Volunteers
790 Immunized with Salmonella enterica Serovar Typhi Strain CVD 908-htrA. *J Immunol* 170:2734-41.
- 791 130. Villarreal B, Mastroeni P, de Hormaeche RD, Hormaeche CE. 1992. Proliferative and T-cell specific
792 interleukin (IL-2/IL-4) production responses in spleen cells from mice vaccinated with aroA live
793 attenuated Salmonella vaccines. *Microb Pathog* 13:305-15.
- 794 131. Mastroeni P, Simmons C, Fowler R, Hormaeche CE, Dougan G. 2000. Igh-6(-/-) (B-cell-deficient)
795 mice fail To mount solid acquired resistance to oral challenge with virulent salmonella enterica
796 serovar typhimurium and show impaired Th1 T-cell responses to salmonella antigens [In Process
797 Citation]. *Infect Immun* 68:46-53.
- 798 132. Ugrinovic S, Menager N, Goh N, Mastroeni P. 2003. Characterization and Development of T-Cell
799 Immune Responses in B-Cell-Deficient (Igh-6(-/-)) Mice with Salmonella enterica Serovar
800 Typhimurium Infection. *Infect Immun* 71:6808-19.
- 801 133. Mittrucker HW, Raupach B, Kohler A, Kaufmann SH. 2000. Cutting edge: role of B lymphocytes in
802 protective immunity against salmonella typhimurium infection [In Process Citation]. *J Immunol*
803 164:1648-52.

- 804 134. Nanton MR, Way SS, Shlomchik MJ, McSorley SJ. 2012. Cutting edge: B cells are essential for
805 protective immunity against Salmonella independent of antibody secretion. *J Immunol* 189:5503-
806 7.
- 807 135. Barr TA, Brown S, Mastroeni P, Gray D. 2010. TLR and B cell receptor signals to B cells
808 differentially program primary and memory Th1 responses to Salmonella enterica. *J Immunol*
809 185:2783-9.
- 810 136. Barr TA, Brown S, Mastroeni P, Gray D. 2009. B cell intrinsic MyD88 signals drive IFN-gamma
811 production from T cells and control switching to IgG2c. *J Immunol* 183:1005-12.
- 812 137. MacLennan CA, Gilchrist JJ, Gordon MA, Cunningham AF, Cobbold M, Goodall M, Kingsley RA,
813 van Oosterhout JJ, Msefula CL, Mandala WL, Leyton DL, Marshall JL, Gondwe EN, Bobat S, Lopez-
814 Macias C, Doffinger R, Henderson IR, Zijlstra EE, Dougan G, Drayson MT, MacLennan IC,
815 Molyneux ME. 2010. Dysregulated humoral immunity to nontyphoidal Salmonella in HIV-infected
816 African adults. *Science* 328:508-12.
- 817 138. Lin FY, Ho VA, Khiem HB, Trach DD, Bay PV, Thanh TC, Kossaczka Z, Bryla DA, Shiloach J, Robbins
818 JB, Schneerson R, Szu SC. 2001. The efficacy of a Salmonella typhi Vi conjugate vaccine in two-to-
819 five-year-old children. *N Engl J Med* 344:1263-9.
- 820 139. Greenwood BM, Bradley-Moore AM, Bryceson AD, Palit A. 1972. Immunosuppression in children
821 with malaria. *Lancet* 1:169-72.
- 822 140. Cunnington AJ, Njie M, Correa S, Takem EN, Riley EM, Walther M. 2012. Prolonged neutrophil
823 dysfunction after Plasmodium falciparum malaria is related to hemolysis and heme oxygenase-1
824 induction. *J Immunol* 189:5336-46.

- 825 141. Hornick RB, Greisman SE, Woodward TE, DuPont HL, Dawkins AT, Snyder MJ. 1970. Typhoid
826 fever: pathogenesis and immunologic control. *N Engl J Med* 283:686-91.
- 827 142. Janis C, Grant AJ, McKinley TJ, Morgan FJ, John VF, Houghton J, Kingsley RA, Dougan G,
828 Mastroeni P. 2011. In vivo regulation of the Vi antigen in Salmonella and induction of immune
829 responses with an in vivo-inducible promoter. *Infect Immun* 79:2481-8.
- 830 143. Rossi O, Caboni M, Negrea A, Necchi F, Alfini R, Micoli F, Saul A, MacLennan CA, Rondini S, Gerke
831 C. 2016. Toll-Like Receptor Activation by Generalized Modules for Membrane Antigens from
832 Lipid A Mutants of Salmonella enterica Serovars Typhimurium and Enteritidis. *Clin Vaccine*
833 *Immunol* 23:304-14.
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