Edinburgh Research Explorer

Direct manipulation of T lymphocytes by proteins of gastrointestinal bacterial pathogens

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11 **Abstract**

12 Gastrointestinal bacterial infection represents a significant threat to human health, as well

13 as a burden on food animal production and welfare. Although there is advanced knowledge

14 about the molecular mechanisms underlying pathogenesis, including the development of

15 immune responses to these pathogens, gaps in knowledge persist. It is well established that

16 gastrointestinal bacterial pathogens produce a myriad of proteins that affect the

17 development and effectiveness of innate immune responses. However, relatively few

18 proteins that directly affect lymphocytes responsible for humoral or cell-mediated immunity

19 and memory have been identified. Here, we review factors produced by gastrointestinal

20 bacterial pathogens that have direct T cell interactions and what is known about their

21 functions and mechanisms of action. T cell interacting bacterial proteins that have been

22 identified to date mainly target three major T cell responses: activation and expansion,

23 chemotaxis or apoptosis. Further, the requirement for more focused studies to identify and

24 understand additional mechanisms used by bacteria to directly affect the T cell immune

25 response and how these may contribute to pathogenesis is highlighted. Increased

26 knowledge in this area will help to drive development of better interventions in prevention

27 and treatment of gastrointestinal bacterial infection.

28

29 **Introduction**

30   Gastrointestinal bacterial infection represents a significant threat to human health

31 and welfare, with an estimated 900 million illnesses resulting in over 500 000 deaths in a

32 single year, according to the World Health Organisation (1). Although much is known about

33 the molecular mechanisms underlying persistence, pathogenesis and protection, significant

34 effort is still required to devise effective intervention strategies. Bacterial immune evasion

35 methods include expression of surface polysaccharides to resist complement-mediated

36 killing and opsonisation, enzymes to detoxify reactive oxygen species in phagosomes,

37 escape from phagosomes and in the case of intracellular bacterial pathogens, interference

38 in cellular antigen-presentation and innate immune responses by proteins secreted by Type

39 III or Type IV secretion systems (2). The role of Type III secretion in gastrointestinal bacterial

40 pathogens is covered by many high quality reviews (eg. 3), and therefore is not the focus of

41 this review. Relatively fewer mechanisms have been identified whereby bacteria are able to

42 directly affect lymphocytes during infection.   Further, understanding the full role of these

43 bacterial proteins and their T cell interactions during infections, any specificity for T cells

44 subsets, and proof that they are able to directly meet T cells in the body is crucial to

45 establish biological importance. However, such evidence is often lacking, and a number of

46 barriers to understanding these aspects exist.

47   Here, we review the known molecules and strategies that contribute to the direct

48 subversion or dampening of the adaptive T cell response in gastrointestinal bacterial

49 infection. In addition, we discuss the challenges and aspirations of identifying these

50 mechanisms.

51

52 **T cell distribution in the intestine**

3

53   An understanding of the importance of T-cell targeting strategies by bacteria that

54   infect the intestine requires an understanding of the lymphoid architecture, distribution of

55   gut resident T cells, the ability of T cells to be recruited to this tissue and the nature of the

56   downstream immune response that is triggered upon infection. T cell responses in the

57   intestine are mainly governed by the gut-associated lymphoid tissue (GALT), which is similar

58   to other secondary lymphoid tissues in the body, and in mammals, consists of the

59   mesenteric lymph nodes (mLNs) and Peyer's patches (PP), the appendix and multiple

60   smaller isolated lymphoid follicles studding the intestinal wall. In addition, there are isolated

61   immune cells scattered in the lamina propria (LP) and throughout the epithelium of the

62   intestine (reviewed in 4) that contribute to intestinal immune responses (summarized in

63   Figure 1). T cell responses in the gut can be initiated from several sites. Generally in an

64   intestinal immune response, intravascular naïve T cells home to the GALT (specifically to the

65   PP and mLN), where they can meet their cognate ligand in the context of the Major

66   Histocompatibility Complex (MHC) and become activated. These T cells are then able to exit

67   the lymphoid tissue via the lymphatic vessels and enter the circulation to home back to the

68   intestinal LP (reviewed in 5) where they are able to carry out their functions. Within the

69   mucosa, dendritic cells in the LP sample antigens and migrate to the PP and mLN where they

70   are able to prime and present antigen to naïve CD4+ and CD8+ T cells, which clonally

71   expand. These T cells may become memory cells, which accumulate over time in the LP. The

72   LP is mainly enriched for the $CD4^+$ T_{reg} and T_h17 cells. In contrast, the intraepithelial

73   lymphocyte (IEL) resident T cell population is mainly composed of both T cell receptor (TCR)

74   αβ and TCR γδ (TCR αβ are generally considered "conventional T cells", TCR γδ cells are

75   often considered non-conventional atypical T cells), both mainly CD8ββ isoform. However,

76   IELs appear to lack some typical T cell surface molecules such as CD2 (adhesion molecule),

77   CD28 (activating co-receptor) and Thy-1 (pan T cell marker of human and mouse cells).

78   These cells are considered "activated, yet resting" and are different compared to peripheral

79   T cells (which express CD4 or CD8αβ isoform; reviewed in (4)).

80   Although there is not a large literature describing direct effects of gastrointestinal

81   bacterial pathogens on T cells, a picture of the general strategies used to alter T lymphocyte

82   function is emerging (summarised in Figure 2). The effects identified can be broadly

83   assigned to three groups: those that affect T cell activation and proliferation, those that

84   affect chemotaxis and those that cause elimination of T cells, with most of the strategies

85   identified thus far falling into the first category. Little duplication of specific strategies

86   across species of intestinal bacteria has been identified so far, and the mechanisms and

87   observations described are derived from a relatively small number of bacterial species.

88   **Proteins that affect T cell activation and proliferation**

89   A number of bacterial proteins have been identified that act to interfere with signalling

90   cascades in T cell activation and expansion, which are outlined below. These proteins are

91   often soluble, diffusible factors, and can act externally to the T cell, as well as intracellularly.

92   *Superantigens*

93   Perhaps the earliest, well known and characterised bacterial factor with T cell-

94   affecting activity is superantigen. Barber observed superantigen activity in *Staphylococcus*

95   in 1914, and identified the cause to be a microorganism-derived toxin (6). Since then, there

96   have been numerous studies characterising the activity of superantigens. One of the

97   hallmarks of superantigens is their ability to activate a large population of T cells at very low

98   concentrations (7, 8). At a basic level, superantigens are able to cross-link a relatively large

99   number of T cells to antigen-presenting cells (APC) compared to normal antigen-driven

100   activation, inducing wide spread non-antigen-specific activation of T cells, ultimately leading

101   to clonal deletion and anergy, thus suppressing a productive T cell response (9).

102   Superantigens are effective because they bind outside the peptide-binding groove of MHC,

103   they are not MHC restricted and activation does not rely on antigen internalisation and

104   processing. In addition, they specifically require the TCR β chain, not the Vα-Vβ chain

105   pairing required in conventional antigen recognition by the TCR (reviewed in 8).

106   Although most well described in *Staphylococcus*, superantigens have been identified

107   in other bacteria, including the pathogens *Yersinia enterocolitica* (10), and *Yersinia*

108   *pseudotuberculosis* (11)*,* the latter of which most often causes a self-limiting gastrointestinal

109   infection. However, strains of *Y. pseudotuberculosis* have also been reported to infect the

110   gut and cause Far East Scarlet-like Fever (reviewed in 16), and many strains associated with

111   this pathogenic infection express the superantigen *Y. pseudotuberculosis*-derived mitogen A

112   (YPMa). Strains deficient in YPMa have been demonstrated to have decreased

113   pathogenicity, however, growth of the bacteria was unaffected in the major immune organs

114   after oral infection, so YPMa may have more pronounced effects in systemic infection (13),

115   and a more recent study has linked the toxic activity of YPMa to activation of a hepatotoxic

116    $CD4^+$ T cell subset (14).

117   *Lymphostatin*

118   Lymphostatin (LifA, Efa-1) is one of the largest known bacterial proteins at 365 kDa,

119   and is a putative glycosyltransferase, expressed by enteropathogenic *Escherichia coli* (EPEC)

120   and non-O157 enterohaemorrhagic *E. coli* (EHEC) (15). It has homology to the large

121   clostridial toxins A and B (TcdA/B) at the N terminal portion of the protein, where the

122   catalytic glycosyltransferase domain of TcdA/B resides (15, 16). The existence of a soluble

123   factor capable of inhibiting mitogen-activated lymphocyte proliferation and pro-

124   inflammatory cytokine expression was first described using crude bacterial lysates of the

125   prototype EPEC strain E3248/69 (17). Lymphostatin was then subsequently identified using

126   a cosmid library screen to identify the gene responsible for this activity, which was

127   confirmed by mutation of the *lifA* gene in EPEC (15). Recently, it was shown that

128   lymphostatin, like its clostridial homologues, is able to bind sugar moieties, in this case UDP-

129   N-acetyl glucosamine (UDP-GlcNAc), and has significant predicted structural homology

130   around the putative glycosyltransferase domain. A DXD motif within this domain is

131   necessary for UDP-GlcNAc binding and lymphostatin activity, however, formal evidence of

132   sugar transfer and the identity of the cellular target remain elusive (16). It has been

133   demonstrated that lymphostatin is capable of inhibiting all major T cell subsets. In addition,

134   lymphostatin has some activity against B cells, but not natural killer cells (18). Further, the

135   effects of lymphostatin on T cells appear to be long-lived, even in the absence of continued

136   incubation with the protein, preventing mitogenic activation for more than 18 hours after

137   transient exposure and withdrawal of the protein. Lymphostatin was also able to inhibit

138   antigen-specific proliferation of bovine T cells using *Theileria parva* antigens presented on

139   infected irradiated APCs to *T. parva* specific T cells as a model antigen system (18). These

140   findings suggest that lymphostatin might act to permanently de-sensitise T cells to stimulus,

141   possibly suppressing T cell responses and preventing or dampening a productive immune

142   response and delaying clearance of infection (18). It would appear that the effects of

143   lymphostatin interfere with signalling in a membrane proximal way, as inhibition was not

144   achieved in T cells stimulated with Phorbol 12-myristate 13-acetate (PMA)/ionomycin,

145   which bypass membrane signalling.

146   Lymphostatin is known to play an important role in intestinal colonization of calves

147   by non-O157 EHEC strains of multiple serogroups (19, 20) and of mice by *Citrobacter*

148   *rodentium* (21). However, attenuation is evident early after infection, before adaptive

7

149   responses may be expected to have developed. Alongside the ability to suppress T cell

150   activation, lymphostatin also appears to be associated with adhesion (22), possibly as a

151   consequence of effects on Type III secretion in some strains (19, 20). These results indicate

152   that lymphostatin may have additional roles in infection. There are a number of unresolved

153   questions regarding the activity of lymphostatin, including its cellular target of glycosylation.

154   Further, in O157:H7 strains of EHEC, where full-length lymphostatin is not expressed, there

155   is a putative homologue, ToxB, that also shows T cell inhibitory activity, as well as homology

156   at the N-terminal end of the molecule to TcdA/B (18). This suggests that lymphostatin and

157   lymphostatin-like molecules may be a family of proteins expressed by *E. coli* to control T cell

158   responses to infection.

159

160   *VacA*

161   *Helicobacter pylori* expresses the VacA vacuolating cytotoxin, which has direct

162   activity against T cells, specifically inhibiting T cell proliferation (23–25) as well as effects on

163   other cells, including phagocytes and epithelial cells (likely by a different mechanism;

164   reviewed in 23). VacA is a two domain protein, processed from a protoxin form, after

165   secretion via a Type Va system from the bacteria (reviewed in 24). Variation in the VacA

166   gene amongst different strains of *H. pylori* results in varying levels of toxicity among the

167   different variants (28). Like other toxins, VacA must be taken up by the cell in order to exert

168   its activity, and it has been shown that both domains are needed for proper uptake and

169   function of the toxin (29). The integrin CD18, expressed on the cell surface, has been

170   identified as being important for uptake of VacA in human T cells (30), mediated by Protein

171   Kinase C (PKC), and activation of the T cell is required to see the active endocytosis of VacA

172   in T cells (31). In addition, VacA is able to block calcium flux in the Jurkat T cell line (32), and

173   prevent IL-2 expression by blocking translocation of the transcription factor NF-AT (24, 25).

174   Overall, the data suggest that VacA targets previously activated T cells. Using *in vivo* studies

175   in mice, a null mutation of *vacA* was reported to impair initial colonization of mice by *H.*

176   *pylori*, however, once infection by the *vacA* mutant becomes established, the bacterial load

177   and extent of intestinal inflammation were similar to the parent strain (33). This effect is

178   independent of an effect on T cells, as mice T cells do not express a compatible receptor

179   that allows uptake of VacA (30).

180

181   *YopH*

182   Another example of inhibition of T cell activation by interference in T cell signalling is

183   the YopH protein from *Yersinia*. YopH is expressed by *Yersinia spp.* that infect the gut (34),

184   including *Y. entercolitica* and *Y. pseudotuberculosis*, and has been characterised as a protein

185   tyrosine phosphatase (35). In *in vitro* studies, using T cell-like cell lines, YopH was able to

186   inhibit IL-2 production induced by antigen stimulation, the effects of which were upstream

187   of PMA/Ionomycin (36). It was apparent by Western Blotting that general tyrosine

188   phosphorylation of signalling molecules was inhibited. YopH has also been shown to exhibit

189   activity against B cell activation via the B cell receptor, with similar characteristics (36).

190   These effects were independently confirmed in primary human T cells (37). In T cells, YopH

191   is able to dephosphorylate the early signalling molecule Lck (38). Further, it has been shown

192   that YopH interacts with a number of adaptor molecules involved in early T cell receptor

193   signalling. Using a trapping mutant, YopH was shown to directly dephosphorylate

194   recombinant phosphorylated Lck in an *in vitro* activity assay, while not

195   dephosphosphorylating other associated adaptor molecules, indicating some specificity of

196   activity (39). This is an elegant mechanism, as an effect on relatively few molecules of Lck

197   would have a large impact on downstream signalling due to amplification through the

198   signalling cascade. These studies remain quite far removed from the complex *in vivo*

199   infection, so the implications of these activities are not entirely known. However, it has

200   been demonstrated *in vivo* that *yopH* deficient *Y. enterocolita* are drastically attenuated in

201   oral infection of C57BL/6 mice, although colonization of the small intestine persists until at

202   least 21 days post-infection (40). Colonization by the YopH mutant declined quickly after

203   infection (40). Further, in an intranasal infection model, a *yopH* deficient strain was less

204   effective at lung colonization (41). In both cases, reduced colonization was seen early in

205   infection before one might expect an adaptive response to have properly formed, and so it

206   remains unclear what role YopH/T cell interactions play in virulence. In addition to YopH,

207   *Yersinia* expresses an additional protein, invasin, which may allow *Yersinia* to subvert

208   lymphocytes, particularly T cells, to influence their motility and facilitate dissemination of

209   *Yersinia* to distal sites (42). These two proteins may function to simultaneously neutralize T

210   cell activation while keeping the cells intact to allow *Yersinia* infection, and redirection to

211   other sites within the body. An invasin homologue in EPEC and *Citrobacter rodentium*,

212   intimin (reviewed in 41), has been shown to interact with T cells, however, it is difficult to

213   separate its direct effects on lymphocytes from the vital role it plays in gut colonization

214   when interpreting its role *in vivo* (44, 45).

215

216   *Interference with metabolic activity*

217   T cells undergo rapid metabolic reprogramming on activation, one of the

218   requirements of which is a source extracellular amino acids (46). Import of amino acids such

219   as asparagine and glutamine is required to accommodate the increased metabolic load

220   induced by aerobic glycolysis during activation and proliferation of T cells (47). There are at

221   least two examples of proteins from gastrointestinal bacterial pathogens that appear to

222   inhibit T cell activation via limiting availability of extracellular amino acids.

223   In a recent publication, Floch and colleagues (48) reported that the *Campylobacter*

224   *jejuni* protein, gamma-glutamyl transpeptidase (GGT) was capable of inhibiting mitogenic

225   proliferation of T cells *in vitro*. Although GGT is known to be important in intestinal

226   colonization by *C. jejuni* in the chicken (49), little is known about its activity on T cells.

227   However, it is tempting to extrapolate from what is known about a similar GGT that is

228   expressed by *H. pylori*. The GGT of *H. pylori* plays an essential role in colonization of the

229   gastric mucosa in mice (50). GGTs are N-terminal nucleophile hydrolases that play a role in

230   the degradation of glutathione, and GGTs across mammal and bacterial species often

231   exhibit a high protein sequence identity, with the GGT of *C. jejuni* clustering with

232   *Helicobacter spp.* (51). Treatment of mouse T cells with recombinant GGT from *H. suis*

233   inhibits CD3/CD28-stimulated proliferation in a concentration-dependent manner (52). In

234   human peripheral blood mononuclear cells, GGT also inhibits PMA/Ionomycin stimulated

235   proliferation, causing cell cycle arrest, inhibiting c-Myc and c-Raf (53). GGT more specifically

236   causes glutamine deprivation in the extracellular space of T cells, downregulating both c-

237   Myc and IRF4 which are sensitive to glutamine, and required for metabolic adaptation (54).

238   Overall, the data suggest that GGT is able to modulate the response of T cells in infection,

239   likely through control of the extracellular availability of glutamine, which is required during

240   activation.

241   A second example of a gastrointestinal bacterial protein that interferes with T cell

242   metabolism comes from *Salmonella enterica* serovar Typhimurium, which has been

243   reported to directly inhibit primary mouse T cells (55), and is thought to limit availability of

244   asparagine to T cells (56). When assessing a number of cell surface expressed molecules, no

245   difference was noted in levels of CD69, CD25α, CD44, and CD62L in cells infected with *S.*

246   Typhimurium compared to uninfected controls. However, in the presence of *S.*

247   Typhimurium*,* neither IL-2 nor IFN-γ were produced with CD3 cross-linking (both these

248   cytokines are up-regulated during T cell activation). Cytokine production was restored if the

249   cells were separated from the bacteria in a transwell arrangement, indicating that direct

250   contact with the bacteria was required for this effect. From here, the authors extended

251   their observations, again using *in vitro* methods with primary mouse cells, showing that *S.*

252   Typhimurium was able to down regulate surface expression the TCR β chain, resulting in

253   decreased gene expression, intracellular and surface protein, at least partially explaining the

254   mechanism targeted to inhibit T cell activation (57). Not only that, but this effect was only

255   observed in the presence of live bacteria, as treatment with heat-inactivated bacteria

256   abrogated this effect. The effects were shown to be unrelated to Type III secretion or the

257   bacterial virulence plasmid. It was later shown that the protein responsible for this was L-

258   asparaginase II (STM3106; *asnB*) (58). In a mouse model of bacterial persistence, the burden

259   of bacteria was lower in mutants lacking L-asparaginase II, suggesting that this molecule

260   may enable bacterial persistence by dampening the T cell-mediated immune response (58).

261   In contrast, in a screen of *S.* Typhimurium mutants in pigs, calves and chickens, a transposon

262   insertion was not attenuating in the gut, albeit within 3-4 days after oral infection (59).

263   Nonetheless, the characterization of the activity of the L-asparaginase II on T cells is a good

264   example of how bacterial subversion can lead to insight into basic host cell biology. In this

265   case these studies highlight the importance of asparagine as a nutrient in T cell metabolism

266   and activation (60).

267

268   **Interference in lymphocyte chemotaxis**

269   The majority of T cell-interacting bacterial proteins appear to mainly exert effects on

270   T cell activation, however, another strategy is to interfere with lymphocyte migration. For

271   example, *Shigella* exhibit the ability to directly invade T cells and cause an inhibition in their

272   chemokine-induced migration (61). *S. flexneri* are able to directly invade PMA-activated

273   CD4+ T cells, but not unstimulated, unactivated, primary CD4+ cells, with substantially

274   reduced responses to the chemoattractant CXCL12. CXCL12 signals through the chemokine

275   receptor CXCR4, the expression of which was not perturbed in these experiments (61). The

276   bacterial protein, IpgD, which can be secreted through the type III secretion apparatus (62),

277   has been implicated as being responsible for this activity, by acting on the pool of

278   intracellular phosphatidylinositol 4,5-bisphosphate (PIP2). Additionally, it would appear

279   that IpgD is able to act intracellularly in the absence of any other bacterial effectors (61).

280   These observations have been verified experimentally *in vivo* in mice, revealing that *S.*

281   *flexneri* target CD4+ T cells in the lymph node and confirming that invasion and migration

282   arrest occur *in vivo* (63). This discrimination between activated and non-activated T cells

283   could result in more specific targeting of activated T cells in the lamina propria rather than

284   the lymphoid follicles in the intestinal mucosa, thus targeting those cells that might actively

285   respond to infection. Further, a recent publication reported the ability of *Shigella* to inject

286   effectors into T cells in the absence of subsequent invasion, and suggest that the majority of

287   T cells are targeted by injection only, raising the possibility that the bacteria could use a "hit

288   and run" strategy to affect lymphocytes (64).

289

290   **Elimination of T cells**

291   A further strategy that is shared with more than one bacterial genus is seen with the

292   induction of apoptosis in T cells by the heat labile toxins, expressed by *E. coli* and *Vibrio*

293   *cholera*e, although they appear to have slightly different specificity and mode of action

294   between families and variants of the toxin. The heat labile toxins are structurally related

295   bacterial toxins that induce diarrhea in humans and animals (65). These toxins are

296   oligomers consisting of an A polypeptide bound to a pentameric array of B polypeptides.

297   The toxic effects are determined by the cell surface binding specificity of the B pentamers,

298   and the ADP ribosylating specificity of the A subunit (66). Cholera toxin (CT) produced by *V.*

299   *cholerae* binds to the ganglioside GM1 on epithelial cells via its B subunits, and when it is

300   trafficked to the cell cytosol, it catalyzes ADP ribosylation of adenylate cyclase, leading to

301   increased intracellular cAMP causing water secretion and diarrhea (67). However, it has

302   additional effects on other cells, including T cells. It was demonstrated some time ago that

303   CT was able to induce apoptosis in CD8+ T cells, although at that time the implications

304   during infection were unclear (68). More recently, it was confirmed that CT was able to

305   decrease the numbers of CD8 cells, and that this was not due to either a downregulation of

306   cell surface receptors, or selective proliferation of other cell types (69). Similarly, LTIIa from

307   *E.* coli is also able to deplete CD8+ T cells, likely by induction of apoptosis via cross-linking of

308   the ganglioside receptors, although this has not yet been explicitly demonstrated (69). In

309   mice injected with LT, transient induction of apoptosis mediated by glucocorticoids was

310   seen in all thymocyte subsets, although immature T cells were more affected than mature

311   cells (70). This effect was dependent on route of administration, and demonstrated that *in*

312   *vitro* treatment of cells did not entirely reflect the *in vivo* effects observed (70). In addition,

313   the maturation state of the T cell appears to determine the mechanism of apoptosis

314   triggered (71). Further, although CT does not appear to invoke apoptosis in CD4+ T cells, it

315   does appear to be able to inhibit activation, at least based on measurement of cell surface

316   expressed molecules (69).

317   There has been significant interest in using CT and LT as adjuvants in vaccination, and

318   understanding how it is able to steer T cell responses provides insight in how to better

319   improve vaccination, or to engineer non-toxic derivatives that are able to promote its

320   adjuvancy (72–75).

321   To our knowledge, there is only one other protein from a gastrointestinal bacterial

322   pathogen reported to have the ability to invoke apoptosis in T cells. This is the YpkA protein

323   of *Yersinia*, which is a multidomain protein with kinase activity. Expression of YpkA from a

324   mammalian expression vector transfected in Jurkat T cells induced significant apoptosis (76),

325   however, its role during infection is unclear.

326

327   **Concluding remarks**

328   It is evident from the examples above that gastrointestinal bacterial pathogens have

329   evolved diverse strategies to modulate lymphocyte function. However, the biological

330   significance of such activity during infection remains challenging to dissect, particularly for

331   factors that play additional roles in colonization. For such factors, the T lymphocyte

332   response to infection by a null mutant relative to the isogenic parent will be affected by the

333   magnitude and duration of exposure to bacterial antigens. One strategy to overcome this is

334   to use ligated intestinal loop models and recover intraepithelial lymphocytes exposed to

335   bacterial strains or their products *in situ* (e.g. (77)). Although, it can be challenging to

336   stimulate such cells to proliferate *ex vivo* and loop models often hold large numbers of

337   laboratory-cultured bacteria over the mucosa for a limited time, and thus do not simulate

338   the normal progression of gastrointestinal infection. It is noteworthy that attenuation of

339   mutants lacking some lymphocyte inhibitory factors is detected before one may anticipate

340   that adaptive responses have been generated, and further research is needed to understand

341   their impact on early pro-inflammatory responses and lymphocyte migration *in vivo*.

342   Further, for many of these proteins, little is known about their effects on T cells of specific

343   subsets and differentiation states. Knowledge of which might provide further insight into

344   their impact and timing of action during infection.

345   While some of the strategies outlined here rely on direct contact between the

346   pathogen and lymphocytes (e.g. via Type III secretion), in many cases inhibition relies on

347   diffusion of soluble proteins to meet their target cell type. Some of the factors described are

348   active in extremely low concentrations (e.g. lymphostatin acts in the femtomolar range;

349   (16)) and the extent to which lymphocytes in circulation are affected requires study. It is

350   evident from the ability of Shiga toxins to cause endothelial damage in kidney glomeruli that

351   proteins produced by gastrointestinal pathogens in the gut can act distally.

352   While the molecular basis of the activity of some lymphocyte inhibitory factors is

353   well understood (e.g. VacA, YopH, IpgD), for others a need exists to identify their cellular

354   targets and how their modification produces the observed phenotype. Such studies have

355   the potential to yield novel insights into both the basis of pathogenesis, but also the cellular

356   pathways and factors governing lymphocyte activation and function. With an understanding

357   of the mode of action of inhibitory factors, it may also become feasible to design new

358   treatments. For example, with the knowledge that *Helicobacter* may use γ-

359   glutamyltranspeptidase to restrict lymphocyte activation via interference in glutamate

360   metabolism, researchers have recently demonstrated that oral glutathione supplementation

361   can reduce gastric pathology and inflammation due to *H. suis* in gerbils (78). The extent to

362   which this is a consequence of altered T lymphocyte function requires further study.

363   It is striking that relatively little direct duplication of strategies to inhibit lymphocyte

364   function has been identified across bacterial genera. Nevertheless, the vast quantities of

365   sequence data now generated for pathogens will facilitate the identification of homologs of

366   lymphocyte inhibitory factors that may be relevant in other diseases and differ in

367   mechanism. For example, a family of proteins homologous to lymphostatin occur in diverse

368   *Chlamydia* species of veterinary and public health importance and share predicted

369   glycosyltransferase motifs (79).

370   In addition to evaluating the value of lymphocyte inhibitory factors as subunit

371   vaccines or as targets for novel inhibitors, merit exists in exploring the therapeutic potential

372   of such molecules for disorders associated with lymphocyte proliferation or activity. A

373   challenge of such will be ensuring specific targeting of pathology-associated lymphocytes

374   without deleterious effects on immune function.

375

ACKNOWLEDGEMENTS

379

FIGURES

381   FIGURE 1: GALT and T cell distribution in the intestine

382   Immune responses in the intestine are mainly controlled by gut associated lymphoid tissue

383   (GALT), including the Peyers patches, mesenteric lymph nodes and isolated lymphoid

384   follicles in the mucosa and lamina propria. The mucosa is also studded with intraepithelial

385   lymphocytes. Naïve T cells can be recruited from the circulation to lymphoid organs in the

386   intestines, where they can be activated.

387   FIGURE 2: Summary of T cell interacting bacterial proteins and their targets

388   The majority of bacterial proteins that interact with T cells are directed at modifying

389   activation/proliferation, however, there are some proteins that affect chemotaxis and

390   apoptosis. Where the key affected molecules are known, these are indicated, however, the

391   details of a number of molecules remain unknown. Bacterial protein names are bounded by

392   gray boxes. sAg= superantigen (*Staphylococcus)*, CT= cholera toxin(*Vibrio cholera*),

393   GGT=gamma glutamyl transferase (*H. pylori, C. jejuni*), IpgD= invasion plasmid gene D

394   (Shigella), LifA= lymphocyte inhibiting factor A (*E.* coli), LT= heat labile toxin (*E. coli)*,

395   STM3106=asparaginase (*Salmonella*), APC= antigen presenting cell, MHC= major

396   histocompatibility complex.

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636

Bacteria

M cells

Peyer's patch

Bacteria

Enterocytes

IEL

Dendritic cell presenting antigen

Naïve T cell

Bacterial products

Bacteria

Follicle

Dendritic cell sampling lumen

Gut lumen

Activated T cell

Lamina propria

MLN

B cell

Local circulation

APC

MHC

sAg

TCR

CT/LT

GM1

GGT

STM3106

Glutamine

YopH

Lck

P

Lck

LifA/ToxB

?

IpgD

PIP2

PIP5

CT

AC

ATP

cAMP

IL-2

VacA

Asparagine

Proliferation

Apoptosis

Chemotaxis

T cell