1 **In situ immunostimulatory functions of DC in tissues**

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14 **Abstract**

15 Dendritic cells (DC) prime and orchestrate naïve T cell immunity in lymphoid

16 organs, but recent data also highlight the importance of DC-effector T cell

17 interactions in tissues. These studies suggest that effector T cells require a

18 second activating step *in situ* from tissue DCs in order to become fully

19 competent for effector functions and/or proliferation and survival. DC

20 stimulation of effector T cells within tissues has evolved as a mechanism to

21 ensure that T cells are activated to their full potential only at the site of ongoing

22 infection. Here we propose that under conditions of uncontrolled inflammation

23 and release of tissue antigens, the same DC-dependent checkpoint

24 perpetuates a destructive response and immunopathology.

25

Control of effector T cell function in tissues – a role for DC?

The immune system has evolved to ensure rapid and protective immunity against multiple pathogens while at the same time avoiding excessive damage to normal tissues. This careful balancing act requires exquisite control by multiple activating and regulatory checkpoints, many of which invoke the involvement of dendritic cells (DCs) that migrate to, or are resident within, secondary lymphoid organs. In the steady state, DCs laden with self or harmless environmental antigens traffic at low rates to draining lymph nodes (LN). Under these conditions, DC populations induce deletion of self-reactive T cells or expansion of regulatory T cells (Treg) within the secondary lymphoid organs [1]. In contrast, during infection and exposure to pathogen-associated molecular patterns, activated DC process microbial antigens within affected tissues and traffic to LN where they interact with naïve T cells, initiating a program of proliferation and effector T cell (Teff) differentiation [2]. As the infection is cleared by the ensuing effector response, the number of antigen-loaded DCs that enter draining LN falls. Furthermore, DC are killed by activated cytotoxic T lymphocytes (CTL) or their functions are modulated by naturally-occurring or inducible Treg, ensuring the primary response is self-limiting [3-6]. While this process of induction and counter-regulation acts to avoid the priming of an excessive T cell response, there are several reasons to consider that additional levels of control are needed outside lymphoid organs. For example, because there is a delay between DCs acquiring antigen in the infected tissue and initiation of naïve T cell activation, there exists the risk that effector cells armed with a full repertoire of harmful cytokines will induce an excessive response relative to the falling levels of

51 infection within the tissues due to activation of innate immune mechanisms.

52 Conversely, Teff accessing tissues must also overcome multiple inhibitory

53 influences, including exposure to co-inhibitory ligands (e.g. programmed

54 death ligand (PD-L)-1) and suppression by peripheral tissue Treg before they

55 can execute their functions [6]. In the absence of a mechanism to control the

56 balance between effector and inhibitory responses, T cells recruited to the

57 tissues may be unable to clear residual infection. Thus, existing models that

58 invoke the role of DCs solely within the afferent phase may lack the scope for

59 fine-tuning the immune response according to precise levels of infection.

60 Recent data highlighting the importance of DC-T cell interactions for effector

61 function in tissues, suggests that DCs control an additional checkpoint in the

62 efferent phase of the response. Thus, DCs outside lymphoid organs may be

63 required to shift the balance away from regulation and towards immunity,

64 specifically at sites infected by pathogens. In this way, DCs may also control

65 Teff function, such that T cells only produce potentially damaging immune

66 mediators in situations where the pathogen has not already been cleared by

67 other immune responses. In this Opinion we propose that this checkpoint

68 becomes deregulated under conditions of inflammation and release of tissue

69 antigens, and therefore that immunopathology in autoimmune disease or

70 graft-versus-host disease (GVHD) is driven *in situ* by DCs that drive

71 uncontrolled Teff activation in target organs.

72

73 **DCs at sites of infection and inflammation.**

74 The development of inducible murine models of DC ablation [7] or depletion

75 of phagocytic cells such as monocytes by injection of clodronate-coated

4

76 liposomes [8] have enabled more precise definition of the role of DCs *in vivo.*

77 In particular, the specific depletion of different DC populations at defined time

78 points has allowed investigators to ask detailed questions about the role of

79 DC-T cell interactions *in situ* in tissues. In these systems, the cell type-

80 specific expression of a high affinity diphtheria toxin (DT) receptor (DTR)

81 renders DCs exquisitely sensitive to killing by injection of DT [7].

82 A prerequisite for any putative DC-specific checkpoint that activates Teff is

83 that sufficient DC numbers are maintained in infected tissues. Non-lymphoid

84 DC populations have become increasingly well characterized, and can be

85 defined in general by expression of the integrins CD11b and CD103 (for

86 comprehensive reviews see [9, 10]). According to the DC paradigm, DC

87 activation is concomitant with migration out of the tissue to draining LN, and

88 as such most DC research has focused on the role of DCs in lymphoid organs.

89 However, significant numbers of DCs do remain in infected and/or inflamed

90 tissue, and these cells may become refractory to subsequent activating

91 trafficking stimuli, thus maintaining tissue DC numbers [11]. In addition,

92 recruitment of DC precursors will rapidly replenish those activated DC

93 populations exiting the tissues, with the outcome that inflamed tissues often

94 contain higher numbers of DCs than in the steady state. For example,

95 CD11c$^+$ DCs accumulate in Leishmania- and herpes simplex virus (HSV)-

96 infected skin [12, 13], and in the lungs of influenza-infected mice [14]. The

97 majority of DCs recruited into inflamed or infected tissues are derived from

98 Ly6C^{high} monocytes, that have differentiated into CD11b$^+$ DC-like cells [15,

99 16]. These cells are rapidly recruited from the bone marrow in response to

100 infection or inflammation [17], and provide a large supply of monocyte-

101 derived, or inflammatory, DCs that may out-number other tissue-resident DCs

102 [18] and dominate up-take of the infectious agent for T cell priming in draining

103 LN [19]. Autoimmune diseases are often associated with an influx of large

104 numbers of inflammatory DCs into the target tissue. For example, DCs

105 accumulate at the sites of intestinal inflammation in patients with inflammatory

106 bowel diseases [20], and psoriatic skin contains a high frequency of

107 inflammatory DCs [21]. These DCs may shape the local immune environment

108 by the secretion of pro-inflammatory cytokines and chemokines [22, 23], and

109 can directly cause tissue damage via production of the effector molecules

110 TNFα and iNOS (TipDC) [24]. However, the rapid recruitment of monocyte-

111 derived DC to inflamed tissues means that they may also become the

112 dominant DC population to interact with incoming Teff *in situ* at the site of

113 infection [9].

114 This shift to inflammatory DC populations may however not be true for all

115 tissues. For example, epidermal Langerhans cells (LCs) turn over very slowly

116 with repopulation from a localized precursor population [10]. Unlike DCs in

117 other tissues, LCs remain the dominant DC population in the inflamed

118 epidermis [25], where local proliferation *in situ* may maintain cell numbers

119 [26]. Indeed, monocytes are only recruited to the epidermis under conditions

120 of severe inflammation and LC death, for example by UV-irradiation [10].

121

122 **DC-T cell interactions at sites of infection.**

123 Teff home to diseased tissues, where they eliminate pathogens via direct

124 killing of infected cells and through production of chemokines and cytokines,

125 which recruit and activate immune defense mechanisms by other cells.

126 Studies over the last decade have demonstrated that Teff function is
127 enhanced as they enter peripheral tissues, suggesting that interactions with
128 tissue cells may be important in influencing the final repertoire of effector
129 functions induced. For example, influenza-specific Teff were found to
130 undergo robust proliferation after entry into the lung [27]. Furthermore,
131 adoptively transferred T cells that had been primed *in vivo* [28] or *in vitro* [29]
132 were demonstrated to migrate to tissues and produce higher levels of effector
133 cytokines than those that had trafficked to LN. Similarly, CD4$^+$ T cells in the
134 lungs of mice infected with *Cryptococcus neoformans* displayed a more
135 activated phenotype, and produced more IFNγ, than those in draining LN [30].
136 Until recently though, the precise involvement of DCs in this response had not
137 been investigated.

138 Depletion of DCs, or their precursors, after T cell priming exacerbates
139 infection with influenza [31] or HSV-2 [18]. In the absence of antigen-
140 presenting DCs, pathogen (tetramer)-specific T cells do not proliferate and
141 survive [31-33], or are not reactivated to produce IFNγ [18, 34], in order to
142 mediate a protective response. This interaction has been shown to be
143 antigen-specific [31] and require co-stimulatory signals from DC in influenza-
144 infection models [33, 34]. CD11b$^+$ inflammatory DCs, including Tip DCs,
145 migrate into the lungs of influenza-infected mice [14, 35], where they present
146 antigen to Teff [35], and are therefore strong candidates to activate the
147 protective T cell response in this model. Monocyte-derived DCs were also
148 characterized as the DC population which induced IFNγ-production by
149 recruited T cells in HSV-2-infected mice [18]. These reports have suggested
150 that antigen-specific interactions between tissue DCs and T cells are required

151 to activate full Teff function at the site of infection. However, in these studies,

152 interactions between Teff and different populations of tissue DCs was inferred

153 using *ex vivo* DC-restimulation assays [18], or add-back of specific DC

154 populations to DC-depleted mice [31], which do not necessarily reflect the

155 cellular interactions which occur *in vivo*. Notably, DC populations distinct from

156 the alveolar DC subset that were depleted by treatment with clodronate

157 liposomes, were required to rescue Teff function [31]. Three further studies

158 have investigated the outcome of the interaction between DCs and effector T

159 cells at the site of inflammation, either by directly analyzing T cell cytokine

160 production *ex vivo* without restimulation by DCs, or using multi-photon

161 imaging to track Teff in real time. Depletion of DTR-expressing DCs by

162 injection of DT was used to show that DCs in the dermis of mice that had been

163 immunized with protein and adjuvant, or in the lungs of influenza-infected

164 mice, were required to induce antigen-specific IFNγ production by T cells

165 recruited to the inflamed/infected sites [34, 36]. CD11b$^+$ DCs were also

166 recruited into the central nervous system (CNS) of lymphocytic chorio-

167 meningitis virus (LCMV)-infected mice, where they formed stable long-lived

168 contacts with incoming T cells. These interactions resulted in the proliferation

169 of T$_{eff}$ *in situ* in the meninges [37]. Collectively, these studies demonstrate

170 that DC-T cell interactions in tissues enhance T cell function. The research

171 to date has either focused on investigating the direct augmentation of T cell

172 cytokine production by DCs at a single cell level, or the indirect enhancement

173 of Teff function due to proliferation *in situ* at the infection site. New studies

174 are required to directly compare whether both scenarios occur within an

175 infected tissue, or whether the interaction with DCs outside lymphoid organs

176 has different effects on Teff function, perhaps depending on the local

177 environment and the signals delivered by activating DCs. It has been

178 postulated that tissue antigen presenting cell (APC)-T cell interactions take

179 place within discrete areas of inflamed or infected tissues, that may facilitate

180 rapid activation of effector memory T cells upon secondary infection [38, 39].

181 These sites include tertiary lymphoid structures such as those found in the

182 lung, which are required for the maintenance of chronic immunity [40].

183 However, whether discrete regions within the tissue are required to foster

184 interactions between Teff and DCs has not been carefully addressed.

185

186 **Do DC- Teff interactions perpetuate disease?**

187 During the development of autoimmune disease, tissue-resident DCs will

188 migrate to draining LN to initiate the primary response. Priming is perpetuated

189 as incoming inflammatory DCs subsequently acquire tissue antigens released

190 by auto-reactive CTL, and migrate in turn to draining LN [41, 42]. In

191 experimental models of autoimmunity however, depletion of DCs ameliorates

192 tissue destruction independently of T cell priming [25, 43, 44]. Thus, DTR

193 models of DC/LC depletion have been used to show that activated CD4+ T

194 cells interact with kidney DCs to produce cytokines *in situ* and recruit

195 autoreactive CTL [43], while CD8+ T cells are activated to enhance effector

196 function, and therefore tissue damage, by epidermal LCs [25]. In the

197 MRL.Fas mouse model of systemic lupus erythematosus DC were recently

198 shown to be required for the proliferation of, and increased IFNγ-production

199 by, Teff in LN, and tissue immunopathology was less severe in the constitutive

200 absence of DCs [44]. However, interactions between DC and Teff in

201 peripheral tissues were not addressed in this study. Within the CNS, and in

202 accord with the LCMV study already discussed [37], MHC II+ APC form long-

203 lasting contacts with Teff that were in the process of crossing pial vascular

204 walls [45]. In this elegant study, which exploited cytofluorometry to directly

205 analyze effector cytokine production at the single cell level by parenchymal T

206 cells *in situ*, it was found that APC-T cell interactions result in the activation

207 of pro-inflammatory cytokines, chemokines and metalloproteases which

208 facilitated entry of CTL deep into the CNS parenchyma to cause clinical

209 disease [45]. Taken together, these studies implicate DC-Teff interactions in

210 murine models of autoimmunity.

211 It was also recently demonstrated that resident rather than recruited DC can

212 license Teff under certain conditions. This conclusion was derived from

213 experiments dissecting the mechanism of cutaneous injury induced by

214 allogenic T cells following bone marrow transplant. Using a tractable model

215 of cutaneous GVHD, in which inflammation is induced by topical application

216 of a Toll-like receptor agonist, and depletion of LC from Langerin-DTR

217 recipients, it was found that tissue injury was reduced in the absence of LC,

218 despite recruitment of CTL into the epidermis [25]. Although primed Teff were

219 cytotoxic against hemtopoietic cells, they required the presence of epidermal

220 host LC to up-regulate transcription of effector molecules once in the

221 epidermis [25]. These data suggest that LCs can also control the Teff function

222 under certain conditions. However, LCs were not required for this step in

223 models of dermatitis or subcutaneous vaccination, where CD11b+ DCs were

224 the major protagonists. Therefore, a key question for future studies is to

225 determine whether licensing is a 'default' mechanism of any DC population

226 that is within a tissue at the time of Teff infiltration or a unique property of

227 individual subsets.

228

229 **Concluding remarks.**

230 Recent data highlight the importance of DC-T cell interactions in tissues to

231 enhance protective immunity against infection. We propose that Teff are

232 licensed by DCs *in situ*, and that this step provides an important checkpoint

233 to activate maximal effector function at sites of infection. DCs may be

234 licensed by interaction with pathogen-derived molecules [46], or CD4 T cells

235 [47] and as a result persist in an altered state that is equipped to activate

236 effector T cell responses. Here, we suggest that DCs may themselves

237 license, and therefore modulate, CD4$^+$ and CD8$^+$ Teff function. In this context,

238 the term licensing describes an interaction between tissue DCs and recruited

239 T cells that leads to enhanced Teff function. This may be due to a

240 combination of augmented production of effector cytokines, chemokines and

241 other molecules, and/or local expansion of Teff due to enhanced proliferation

242 and/or survival. Under conditions of inflammation and release of tissue

243 antigens, this checkpoint could result in the aggravation of a dysregulated T

244 cell response, whereby DCs drive the continued proliferation and activation of

245 tissue-destructive T cells (Figure 1). During immune responses to pathogens

246 the licensing of Teff by DCs will be limited over time as fewer T cells are

247 primed in draining LN, and pathogen-derived antigens are cleared from the

248 tissue. At this point tissue-specific regulatory mechanisms such as exposure

249 to PD-L1, and suppression by Treg will dominate immune responses in the

250 tissue to ensure that any autoreactive T cells activated during the anti-

11

251 pathogen response are not licensed by DC at the infected site. In addition,

252 DC may also directly license Treg function [36]. However, during

253 autoimmunity these immunosuppressive responses are often impaired, for

254 example due to inhibition of Treg function [48]. In this context the unchecked

255 augmentation of self-reactive Teff function by DC will further drive T cell-

256 mediated immunopathology.

257

258 Despite differences reported in the literature on the effects that this licensing

259 step has on Teff function, a consensus is emerging on the nature of the DC

260 that mediates this response. Thus, recruited inflammatory (CD11b^{+}) DCs

261 license enhanced Teff function in both infection and immunopathology (Figure

262 1). This is in accord with a role for these DCs during the effector phase of the

263 immune response, as recently suggested by others [9]. Many questions

264 remain about the nature of the interaction between DCs and Teff in tissues;

265 for example, more work is needed to understand to what extent MHC-T cell

266 receptor signaling alone is sufficient to activate enhanced effector proliferation

267 and/or function, and how different co-stimulatory or -inhibitory signals from

268 DC control Teff function [33, 34]. In addition, it is not known whether DCs

269 must be activated to license incoming T cells. DCs require pathogen-derived

270 signals in order to prime a naïve T cell response [49]. However, Teff will

271 potentially have different requirements, and inflammatory cytokine-driven

272 activation of tissue DCs may be sufficient for them to interact with, and

273 license, recruited T cells. More data is also needed to determine whether this

274 DC-dependent licensing step is specific for the primary response, or if it is

275 also required to re-stimulate memory T cells on secondary challenge by

276 pathogens (see Box 1). Dermal DCs have been shown to license cytokine

277 production by Treg in the skin [36] and an interesting possibility is that DCs

278 control the balance between effector and regulatory function *in situ* at the site

279 of infection. Understanding and targeting DC-licensing of T cells beyond

280 lymphoid organs may represent an important therapeutic step to both

281 enhance the function of pathogen- and tumor-specific T cells *in situ*, and limit

282 T cell-mediated pathology in autoimmunity and GVHD.

283

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425

426

427 **Legend**

428 <u>Figure 1. Licensing of effector T cell function by tissue DCs</u>

429 A. Following infection, monocytes will be rapidly recruited from the blood into

430 the infected tissue. Monocytes will differentiate into inflammatory DCs that

431 present infection-derived antigen to incoming effector T cells. This interaction

432 will result in proliferation and/or enhanced effector cytokine production by T

433 cells, ensuring that a sufficient T cell response is elicited to clear the infection.

434 Depletion of priming DCs and therefore effector T cells in draining LN, and a

435 reduction in the amount of pathogen-derived antigen present at the infected

436 site due to clearance of the invading organism, will limit the duration of the

437 response, with minimal damage to the surrounding tissue by Teff.

438 B. During the development of autoimmune disease, inflammatory DCs which

439 have been recruited to the inflamed tissue, will present self antigens to Teff.

440 This interaction will enhance effector function, leading to immunopathology

441 as autoreactive T cells attack cells in the surrounding tissue, and may also

442 produce chemokines and proteases to invade further into the tissue.

443 Continual recruitment of licensing DCs presenting tissue-derived antigen, and

444 therefore the persistent enhancement of T cell function perpetuates the cycle,

445 resulting in severe immunopathology in the target organ.

446

447   Box 1

448   **Activation of memory T cells by tissue DCs.**

449   Memory T cells can be divided into two populations, central memory T cells

450   (T_{CM}) that reside in LN, and effector memory T cells (T_{EM}) that circulate

451   through tissues, and are poised to react to secondary infections. DCs are

452   required to reactivate memory T cells after viral infection [50], and specific DC

453   populations may perform this function [51]. In parallel with the primary

454   response, a DC-dependent checkpoint could be important to activate T_{EM}

455   function only in those tissues exposed to secondary challenge by a pathogen.

456   Nonetheless, it is not known whether memory T cells that have interacted with

457   tissue DCs are more functionally active than those that have seen antigen on

458   other cells. Inflammatory DCs activated local proliferation of memory T cells

459   in response to HSV-1 infection [52], and both B cells and DCs (though not

460   monocyte-derived DCs) were also required to activate CD4$^+$ memory T cells

461   in a mucosal model of HSV-2 [18, 53]. Using a murine model of postoperative

462   ileus induced by surgical manipulation of the intestine, and DT-mediated

463   depletion of DTR$^+$ DC, it was recently shown that CD11b$^+$ CD103$^+$ DC

464   recruited into the inflamed tissue were required for differentiation of T helper

465   1-like memory T cells, and therefore drove the postoperative inflammatory

466   response [54]. More studies are still needed however to determine whether

467   tissue DCs specifically enhance memory T cell function upon restimulation

468   outside lymphoid organs, and whether the licensing DCs populations are the

469   same as those required during the primary response.