In situ immunostimulatory functions of DC in tissues

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Abstract

Dendritic cells (DC) prime and orchestrate naïve T cell immunity in lymphoid organs, but recent data also highlight the importance of DC-effector T cell interactions in tissues. These studies suggest that effector T cells require a second activating step *in situ* from tissue DCs in order to become fully competent for effector functions and/or proliferation and survival. DC stimulation of effector T cells within tissues has evolved as a mechanism to ensure that T cells are activated to their full potential only at the site of ongoing infection. Here we propose that under conditions of uncontrolled inflammation and release of tissue antigens, the same DC-dependent checkpoint perpetuates a destructive response and immunopathology.

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

infection within the tissues due to activation of innate immune mechanisms. Conversely, Teff accessing tissues must also overcome multiple inhibitory influences, including exposure to co-inhibitory ligands (e.g. programmed death ligand (PD-L)-1) and suppression by peripheral tissue Treg before they can execute their functions [6]. In the absence of a mechanism to control the balance between effector and inhibitory responses, T cells recruited to the tissues may be unable to clear residual infection. Thus, existing models that invoke the role of DCs solely within the afferent phase may lack the scope for fine-tuning the immune response according to precise levels of infection. Recent data highlighting the importance of DC-T cell interactions for effector function in tissues, suggests that DCs control an additional checkpoint in the efferent phase of the response. Thus, DCs outside lymphoid organs may be required to shift the balance away from regulation and towards immunity, specifically at sites infected by pathogens. In this way, DCs may also control Teff function, such that T cells only produce potentially damaging immune mediators in situations where the pathogen has not already been cleared by other immune responses. In this Opinion we propose that this checkpoint becomes deregulated under conditions of inflammation and release of tissue antigens, and therefore that immunopathology in autoimmune disease or graft-versus-host disease (GVHD) is driven *in situ* by DCs that drive uncontrolled Teff activation in target organs.

DCs at sites of infection and inflammation.

The development of inducible murine models of DC ablation [7] or depletion of phagocytic cells such as monocytes by injection of clodronate-coated

liposomes [8] have enabled more precise definition of the role of DCs *in vivo*. In particular, the specific depletion of different DC populations at defined time points has allowed investigators to ask detailed questions about the role of DC-T cell interactions *in situ* in tissues. In these systems, the cell type-specific expression of a high affinity diphtheria toxin (DT) receptor (DTR) renders DCs exquisitely sensitive to killing by injection of DT [7].

A prerequisite for any putative DC-specific checkpoint that activates Teff is that sufficient DC numbers are maintained in infected tissues. Non-lymphoid DC populations have become increasingly well characterized, and can be defined in general by expression of the integrins CD11b and CD103 (for comprehensive reviews see [9, 10]). According to the DC paradigm, DC activation is concomitant with migration out of the tissue to draining LN, and as such most DC research has focused on the role of DCs in lymphoid organs. However, significant numbers of DCs do remain in infected and/or inflamed tissue, and these cells may become refractory to subsequent activating trafficking stimuli, thus maintaining tissue DC numbers [11]. In addition, recruitment of DC precursors will rapidly replenish those activated DC populations exiting the tissues, with the outcome that inflamed tissues often contain higher numbers of DCs than in the steady state. For example, $CD11c^{+}$ DCs accumulate in Leishmania- and herpes simplex virus (HSV)-infected skin [12, 13], and in the lungs of influenza-infected mice [14]. The majority of DCs recruited into inflamed or infected tissues are derived from $Ly6C^{high}$ monocytes, that have differentiated into $CD11b^{+}$ DC-like cells [15, 16]. These cells are rapidly recruited from the bone marrow in response to infection or inflammation [17], and provide a large supply of monocyte-

derived, or inflammatory, DCs that may out-number other tissue-resident DCs [18] and dominate up-take of the infectious agent for T cell priming in draining LN [19]. Autoimmune diseases are often associated with an influx of large numbers of inflammatory DCs into the target tissue. For example, DCs accumulate at the sites of intestinal inflammation in patients with inflammatory bowel diseases [20], and psoriatic skin contains a high frequency of inflammatory DCs [21]. These DCs may shape the local immune environment by the secretion of pro-inflammatory cytokines and chemokines [22, 23], and can directly cause tissue damage via production of the effector molecules TNFα and iNOS (TipDC) [24]. However, the rapid recruitment of monocyte-derived DC to inflamed tissues means that they may also become the dominant DC population to interact with incoming Teff *in situ* at the site of infection [9].

This shift to inflammatory DC populations may however not be true for all tissues. For example, epidermal Langerhans cells (LCs) turn over very slowly with repopulation from a localized precursor population [10]. Unlike DCs in other tissues, LCs remain the dominant DC population in the inflamed epidermis [25], where local proliferation *in situ* may maintain cell numbers [26]. Indeed, monocytes are only recruited to the epidermis under conditions of severe inflammation and LC death, for example by UV-irradiation [10].

DC-T cell interactions at sites of infection.

Teff home to diseased tissues, where they eliminate pathogens via direct killing of infected cells and through production of chemokines and cytokines, 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

has different effects on Teff function, perhaps depending on the local environment and the signals delivered by activating DCs. It has been postulated that tissue antigen presenting cell (APC)-T cell interactions take place within discrete areas of inflamed or infected tissues, that may facilitate rapid activation of effector memory T cells upon secondary infection [38, 39]. These sites include tertiary lymphoid structures such as those found in the lung, which are required for the maintenance of chronic immunity [40]. However, whether discrete regions within the tissue are required to foster interactions between Teff and DCs has not been carefully addressed.

Do DC- T_{eff} interactions perpetuate disease?

During the development of autoimmune disease, tissue-resident DCs will migrate to draining LN to initiate the primary response. Priming is perpetuated as incoming inflammatory DCs subsequently acquire tissue antigens released by auto-reactive CTL, and migrate in turn to draining LN [41, 42]. In experimental models of autoimmunity however, depletion of DCs ameliorates tissue destruction independently of T cell priming [25, 43, 44]. Thus, DTR models of DC/LC depletion have been used to show that activated $CD4^+$ T cells interact with kidney DCs to produce cytokines *in situ* and recruit autoreactive CTL [43], while $CD8^+$ T cells are activated to enhance effector function, and therefore tissue damage, by epidermal LCs [25]. In the MRL.Fas mouse model of systemic lupus erythematosus DC were recently shown to be required for the proliferation of, and increased IFNγ-production by, Teff in LN, and tissue immunopathology was less severe in the constitutive absence of DCs [44]. However, interactions between DC and Teff in

peripheral tissues were not addressed in this study. Within the CNS, and in accord with the LCMV study already discussed [37], MHC II^+ APC form long-lasting contacts with Teff that were in the process of crossing pial vascular walls [45]. In this elegant study, which exploited cytofluorometry to directly analyze effector cytokine production at the single cell level by parenchymal T cells *in situ*, it was found that APC-T cell interactions result in the activation of pro-inflammatory cytokines, chemokines and metalloproteases which facilitated entry of CTL deep into the CNS parenchyma to cause clinical disease [45]. Taken together, these studies implicate DC-Teff interactions in murine models of autoimmunity.

It was also recently demonstrated that resident rather than recruited DC can license Teff under certain conditions. This conclusion was derived from experiments dissecting the mechanism of cutaneous injury induced by allogenic T cells following bone marrow transplant. Using a tractable model of cutaneous GVHD, in which inflammation is induced by topical application of a Toll-like receptor agonist, and depletion of LC from Langerin-DTR recipients, it was found that tissue injury was reduced in the absence of LC, despite recruitment of CTL into the epidermis [25]. Although primed Teff were cytotoxic against hemtopoietic cells, they required the presence of epidermal host LC to up-regulate transcription of effector molecules once in the epidermis [25]. These data suggest that LCs can also control the Teff function under certain conditions. However, LCs were not required for this step in models of dermatitis or subcutaneous vaccination, where $CD11b^+$ DCs were the major protagonists. Therefore, a key question for future studies is to determine whether licensing is a ‘default’ mechanism of any DC population

that is within a tissue at the time of Teff infiltration or a unique property of individual subsets.

Concluding remarks.

Recent data highlight the importance of DC-T cell interactions in tissues to enhance protective immunity against infection. We propose that Teff are licensed by DCs *in situ*, and that this step provides an important checkpoint to activate maximal effector function at sites of infection. DCs may be licensed by interaction with pathogen-derived molecules [46], or CD4 T cells [47] and as a result persist in an altered state that is equipped to activate effector T cell responses. Here, we suggest that DCs may themselves license, and therefore modulate, $CD4^+$ and $CD8^+$ Teff function. In this context, the term licensing describes an interaction between tissue DCs and recruited T cells that leads to enhanced Teff function. This may be due to a combination of augmented production of effector cytokines, chemokines and other molecules, and/or local expansion of Teff due to enhanced proliferation and/or survival. Under conditions of inflammation and release of tissue antigens, this checkpoint could result in the aggravation of a dysregulated T cell response, whereby DCs drive the continued proliferation and activation of tissue-destructive T cells (Figure 1). During immune responses to pathogens the licensing of Teff by DCs will be limited over time as fewer T cells are primed in draining LN, and pathogen-derived antigens are cleared from the tissue. At this point tissue-specific regulatory mechanisms such as exposure to PD-L1, and suppression by Treg will dominate immune responses in the tissue to ensure that any autoreactive T cells activated during the anti-

pathogen response are not licensed by DC at the infected site. In addition, DC may also directly license Treg function [36]. However, during autoimmunity these immunosuppressive responses are often impaired, for example due to inhibition of Treg function [48]. In this context the unchecked augmentation of self-reactive Teff function by DC will further drive T cell-mediated immunopathology.

Despite differences reported in the literature on the effects that this licensing step has on Teff function, a consensus is emerging on the nature of the DC that mediates this response. Thus, recruited inflammatory ($CD11b^+$) DCs license enhanced Teff function in both infection and immunopathology (Figure 1). This is in accord with a role for these DCs during the effector phase of the immune response, as recently suggested by others [9]. Many questions remain about the nature of the interaction between DCs and Teff in tissues; for example, more work is needed to understand to what extent MHC-T cell receptor signaling alone is sufficient to activate enhanced effector proliferation and/or function, and how different co-stimulatory or -inhibitory signals from DC control Teff function [33, 34]. In addition, it is not known whether DCs must be activated to license incoming T cells. DCs require pathogen-derived signals in order to prime a naïve T cell response [49]. However, Teff will potentially have different requirements, and inflammatory cytokine-driven activation of tissue DCs may be sufficient for them to interact with, and license, recruited T cells. More data is also needed to determine whether this DC-dependent licensing step is specific for the primary response, or if it is 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.
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Legend

Figure 1. Licensing of effector T cell function by tissue DCs

A. Following infection, monocytes will be rapidly recruited from the blood into the infected tissue. Monocytes will differentiate into inflammatory DCs that present infection-derived antigen to incoming effector T cells. This interaction will result in proliferation and/or enhanced effector cytokine production by T cells, ensuring that a sufficient T cell response is elicited to clear the infection. Depletion of priming DCs and therefore effector T cells in draining LN, and a reduction in the amount of pathogen-derived antigen present at the infected site due to clearance of the invading organism, will limit the duration of the response, with minimal damage to the surrounding tissue by Teff.

B. During the development of autoimmune disease, inflammatory DCs which have been recruited to the inflamed tissue, will present self antigens to Teff. This interaction will enhance effector function, leading to immunopathology as autoreactive T cells attack cells in the surrounding tissue, and may also produce chemokines and proteases to invade further into the tissue. Continual recruitment of licensing DCs presenting tissue-derived antigen, and therefore the persistent enhancement of T cell function perpetuates the cycle, resulting in severe immunopathology in the target organ.

Box 1

Activation of memory T cells by tissue DCs.

Memory T cells can be divided into two populations, central memory T cells (T_{CM}) that reside in LN, and effector memory T cells (T_{EM}) that circulate through tissues, and are poised to react to secondary infections. DCs are required to reactivate memory T cells after viral infection [50], and specific DC populations may perform this function [51]. In parallel with the primary response, a DC-dependent checkpoint could be important to activate T_{EM} function only in those tissues exposed to secondary challenge by a pathogen. Nonetheless, it is not known whether memory T cells that have interacted with tissue DCs are more functionally active than those that have seen antigen on other cells. Inflammatory DCs activated local proliferation of memory T cells in response to HSV-1 infection [52], and both B cells and DCs (though not monocyte-derived DCs) were also required to activate $CD4^+$ memory T cells in a mucosal model of HSV-2 [18, 53]. Using a murine model of postoperative ileus induced by surgical manipulation of the intestine, and DT-mediated depletion of DTR^+ DC, it was recently shown that $CD11b^+$ $CD103^+$ DC recruited into the inflamed tissue were required for differentiation of T helper 1-like memory T cells, and therefore drove the postoperative inflammatory response [54]. More studies are still needed however to determine whether tissue DCs specifically enhance memory T cell function upon restimulation outside lymphoid organs, and whether the licensing DCs populations are the same as those required during the primary response.