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Dendritic cell recovery post-lymphodepletion: a potential mechanism for anti-cancer adoptive T cell therapy and vaccination

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Abstract

Adoptive transfer of autologous tumor-reactive T cells holds promise as a cancer immunotherapy. In this approach, T cells are harvested from a tumor-bearing host, expanded in vitro and infused back to the same host. Conditioning of the recipient host with a lymphodepletion regimen of chemotherapy or radiotherapy before adoptive T cell transfer has been shown to substantially improve survival and anti-tumor responses of the transferred cells. These effects are further enhanced when the adoptive T cell transfer is followed by vaccination with tumor antigens in combination with a potent immune adjuvant. Although significant progress has been made toward an understanding of the reasons underlying the beneficial effects of lymphodepletion to T cell adoptive therapy, the precise mechanisms remain poorly understood. Recent studies, including ours, would indicate a more central role for antigen presenting cells, in particular dendritic cells. Unraveling the exact role of these important cells in mediation of the beneficial effects of lymphodepletion could provide novel pathways toward the rational design of more effective anti-cancer immunotherapy. This article focuses on how the frequency, phenotype, and functions of dendritic cells are altered during the lymphopenic and recovery phases post-induction of lymphodepletion, and how they affect the anti-tumor responses of adoptively transferred T cells.

Keywords

Adoptive T cell transfer; Cancer; Chemotherapy; Dendritic cells; Lymphodepletion; Tumor; Vaccination

Introduction

The success of anti-tumor immunity depends on generation of effector T cells that can differentiate into functional long-lived memory cells [62,86,102]. The current inability to fully elucidate the critical factors involved in the generation of effective T cell responses has hindered the successful development of effective cancer vaccine therapy. Defining

approaches that can accentuate anti-tumor T cell responses would help developing potential immunotherapeutic protocols in the clinical setting. Due to the limited numbers and prevalent dysfunction of the tumor-reactive T cells found in a tumor-bearing host, studies have been focusing on how to correct the functions of these cells while effectively increasing their numbers. Adoptive T cell transfer is a potential approach in which several intrinsic factors affecting T cells harvested from tumor bearing host can be improved in vitro [34]. Extrinsic factors induced by preconditioning of the recipient host with cytoreductive regimens, such as chemotherapy (e.g. cyclophosphamide; CTX) or sublethal total body irradiation (TBI) can also significantly improve the anti-tumor efficacy of adoptive T cell therapy in particular when the latter is followed by active vaccination [5,42,51,115,118,139]. In the following sections, we will discuss how the application of these cytoreductive regimens mediate the beneficial effects of the adoptive T cell therapy by focusing on the potential roles of dendritic cells (DCs) in the vaccination setting.

Adoptive T cell transfer system in cancer setting

Adoptive transfer of autologous tumor-reactive T cells in the clinical setting consists of harvesting T cells from the patient's own peripheral blood, draining lymph nodes, or tumor bed (tumor infiltrating lymphocytes, TIL) followed by their expansion in vitro using polyclonal stimulation with anti-CD3 and anti-CD28 mAbs in the presence of IL-2 as a growth factor. The cells are then infused back to the same patient blood with administration of IL-2 to improve cell engraftment and survival [62]. Most of the earlier clinical studies of adoptive T cell therapy are based on the use of T cells expanded in vitro for several cycles before their adoptive transfer [26,73,77,109,111]. Although the use of these cells for adoptive transfer can mediate melanoma regression with an objective response rate of about 34%, the response rates in these studies were not better than treatment with cytokines or cancer vaccines [110]. Interestingly, recent studies clearly demonstrated that adoptively transferred T cells that had expanded in vitro for several cycles of expansion are considered terminally differentiated (exhausted), expressing the phenotype of effector memory T cells (T_{EM} ; $CD62L^{low}CCR7^{low}CD127R^{low}CD44^{high}$). Although these T_{EM} cells can express rapid effector functions in vitro, they show poor survival, trafficking, and persistence in vivo [62,86,102]. The limited in vivo anti-tumor responses of terminally differentiated T_{EM} cells were attributed to their acquisition of the phenotype of senescent cells and the loss of lymph node homing receptors, in particular CD62L and CCR7. These exhausted phenotypes limit their responses to vaccination with tumor antigens and accordingly their anti-tumor efficacy [62]. By contrast, antigen stimulation of T cells in vitro for a short period can generate early effector cells with central memory phenotype (T_{CM} ; $CD62L^{low}CCR7^{low}CD127R^{low}CD44^{high}$) with better survival, persistence, and anti-tumor efficacy than their T_{EM} counterparts upon their adoptive transfer into a lymphodepleted host [26,73,77,109,111]. The advantage of generation of T_{CM} cells over T_{EM} cells in vitro has been attributed to the higher expression levels of CD62L homing molecule in the early effector cells with T_{CM} phenotype than in the late effector cells with T_{EM} phenotype [61,62]. This homing receptor facilitates T cells to traffic to lymph nodes and their crosstalk with antigen presenting cells [57,108], resulting in the differentiation of T cells into effector cytolytic cells capable of combating cancer. Importantly, the type of cytokine added to T cells during their antigen stimulation in vitro can shape their T_{CM} versus T_{EM} phenotypes. For instance, IL-12 [24] and IL-15 favor T cells, which sustain the expression of CD62L and differentiate into a T_{CM} phenotype, while IL-2 favors differentiation of cells into a T_{EM} phenotype [61]. It would seem that these cytokines, besides their ability to modulate the magnitude of CD62L expression, can induce distinct intrinsic mechanisms in T cells that affect the antigen-specific responses in vivo. These intrinsic mechanisms of T cells have been reviewed before [34,62,63,83,95,112,120,135] and are beyond the scope of this article.

Improving adoptive T cell therapy with lymphodepletion

Accumulating evidence now supports that induction of immune lymphodepletion in the recipient host by treatment with sublethal TBI or anti-cancer chemotherapeutic drugs, such as CTX and doxorubicin before adoptive transfer of in vitro-activated T cells can markedly improve the survival, persistence, and anti-tumor efficacy of the transferred T cells [33,34]. These studies reported a long-term engraftment of the infused T cells, which comprised a larger fraction of the patient repertoire. Although recent studies have explored some of the cellular and molecular mechanisms underlying the beneficial effects of lymphodepletion regimen to the homeostatic- and antigen-driven responses of the adoptively transferred T cells, the precise mechanisms behind these effects are poorly understood [105]. What has been defined includes the following.

- Enhanced engraftment and survival of the transferred T cells by creation of a space “niche” [63].
- A rapid induction of homeostatic cytokines [17,115,119,134].
- Elimination of regulatory CD4⁺CD25⁺ T (T_{reg}), NKT cells, and myeloid-derived suppressor cells [6,9,46,53,95].
- Depletion of endogenous cells that compete with the transferred T cells for cytokines “cytokine sink” [17,33,63].

Recent studies would suggest, however, that these mechanisms might not be the principal means by which lymphodepletion regimens augment adoptive immunotherapy, in particular in the presence of active vaccination [74,80,101,118,134]. Moreover, these mechanisms have been investigated during the lymphopenic phase, and few studies addressed the role of the cellular components that might be altered at the recovery phase, in which the host recovers from the induced lymphopenia; days 5–18. It could be postulated that some mechanisms related to the recognition of tumor antigen by the host cells, in particular dendritic cells (DCs), might play a critical role. Indeed, recent preclinical studies including ours have pointed to the active roles of DCs in mediation of the beneficial effects of lymphodepletion to adoptive T cell therapy [96,115]. In the following sections, we will discuss how the numbers and activation phenotype of DCs are altered during the lymphopenic and recovery phases after induction of lymphodepletion and how this alteration affects responses of adoptively transferred T cells.

Alteration in the frequency and phenotype of DCs after lymphodepletion

Rapid activation of DCs during the lymphopenic phase

DCs are the most potent professional antigen presenting cells and as such have been a promising target for the development of new cancer treatments [106]. The presence of physiological numbers of DCs is considered a crucial factor for both homeostatic and antigen-driven expansion of the peripheral pool size of memory T_{CM} and T_{EM} subsets [36,55,100]. Given this central role of DCs in shaping the quantity and quality of immune responses, we have been focusing our recent studies on understanding their roles in T cell responses in the context of lymphodepletion. Using the OT-1 and pmel-1 TCR transgenic mouse models, in which CD8⁺ T cells are engineered to recognize MHC class-I OVA (SIINFEKL) [23] and melanoma (gp100_{25–33}) [92] peptides, respectively, we have been addressing the role of DCs at the lymphopenic and recovery phases after CTX treatment. These models are based on the adoptive transfer of a few (1 million) trackable CD8⁺ T cells from a TCR transgenic mouse into a recipient mouse followed by vaccination. These models allow for visualization of the responses (expansion, contraction; activation, trafficking, function) of the antigen-specific CD8⁺ T cells (the donor cells) within a large population of

CD8⁺ T cells (host cells). This also allows for a side-by-side testing of the responses of the host innate immune cells, including DCs. Using these TCR CD8⁺ T cells with B16 melanoma, we have established that adoptive transfer of OT-1 CD8⁺ T cells at the lymphopenic phase after CTX treatment results in marked increases in post-vaccination T cell responses, including enhanced expansion, function, and delayed contraction of adoptively transferred CD8⁺ T cells; these effects were further augmented when peptide vaccination was combined with the TLR3 agonist poly(I:C) [118].

Consistent with other studies that showed space-independent enhanced immune responses of adoptively transferred CD4⁺ T cells after CTX treatment [80], the enhanced effects of CTX preconditioning to CD8⁺ T cells were not felt to be mediated by creation of a “space niche” since infusion of up to 200 million wild type naïve cells immediately after CTX treatment did not block its beneficial effects [118]. Interestingly, however, the beneficial effect of CTX was associated with a rapid activation of DCs in the liver and spleen during the early phase of lymphopenia (days 1–4) and were dependent on both the presence of CD11b-expressing cells and intact IFN- α/β signaling pathways. Given that, DCs express CD11b and are capable of producing IFN- α/β , these results suggested a possible role for DCs during the lymphopenic phase in mediation of the adjuvant effects of CTX preconditioning regimen. Indeed, earlier studies have reported that mouse interdigitating DCs isolated 2 days after CTX treatment showed an enhanced accessory function compared with the control DCs [70], and that follicular DCs harvested from lymph nodes during the lymphopenic phase post-CTX treatment retained exogenous antigen for long time and were capable of inducing a better antibody responses [98]. Similar to CTX, TBI also induces a rapid activation of DCs coincided with augmented T cell responses [18,28,69,141]. Human studies also showed a rapid activation of DCs after myeloablative allogeneic hematopoietic stem cell transplantation [47,69]. These studies would indicate to the rapid activation of DCs as a potential mechanism contributing to the enhanced anti-tumor responses to vaccination with different antigen formulations during the lymphopenic phase [16,37,67,104,115,118,125,131].

How activation of DCs is induced early after lymphodepletion

DCs have been reported to be required for the lymphopenia-driven homeostatic (i.e. in absence of antigen priming) proliferation of naïve and memory CD8⁺ T cells [36,138]. Furthermore, the antigen-specific responses of adoptively transferred T cells into a lymphodepleted host was found to be likely due to an enhanced MHC Class I-restricted antigen presentation by elements of the transplanted bone marrow [134]. Taken together, these studies would suggest that DCs post-induction of lymphodepletion would play an important role in the enhanced anti-tumor immune responses. In the following sections, we will discuss studies that would suggest this role of DCs at the lymphopenic and recovery phases post-lymphodepletion. Although it is not clear how lymphodepletion regimens induces the rapid DC activation and how DCs are possibly contributing to lymphodepletion-induced enhanced effects on T cell responses, several mechanisms are possible:

- A massive apoptosis of the host cells as well as tumor cells occurs rapidly after induction of lymphodepletion [11,118]. In this setting, cells undergoing apoptosis are rapidly and specifically recognized by phagocytic cells, in particular DCs. After recognition, apoptotic bodies are silently removed by phagocytosis which can result in activation of DCs [49,59,126].
- The induction of tumor cell apoptosis and the release of endogenous TLR agonists such as heat-shock proteins and uric acid can act through TLRs and thus induce DC maturation [31,113]. Uric acid, at least in its crystal form can activate DCs and augment tumor rejection [48,122,123]. Dying tumor cells induced by local

irradiation of tumor can also release the high-mobility-group box 1 alarmin protein, which can bind to TLR4 expressed by DCs and increase the efficiency of tumor antigen processing and presentation [7].

- The rapid induction of inflammatory cytokines such as IFN- α [38,45,54,88] can act as danger signals [79,81,113,119]. These signals would result in activation of DCs, their maturation, and migration to lymph nodes, a critical step required for optimal antigen-specific T cell responses [10]. We and others have reported induction of several inflammatory cytokines, in particular type I IFNs, post-CTX treatment [17,107,117,128,136,141], and that the absence of type I IFNs in vivo abrogated the beneficial effects of CTX to the anti-tumor responses of adoptively transferred immune cells [101,118].
- The release of LPS (a TLR4 ligand) due to the TBI- or chemotherapy-induced damage in the integrity of mucosal barriers in the intestinal tract and the translocation of microbial products [1,85]. This microbial translocation would lead to induction of inflammatory cytokines [17,117,119] and a rapid activation of DCs and the associated enhanced effector immune responses [69,141]. In line with this notion, exogenous LPS can substitute for the endogenous TBI-induced LPS for augmentation of the anti-tumor responses of CD8⁺ T cells to active vaccination when they were adoptively transferred to immune cell (NK and CD4⁺ T) cell-ablated recipient mice [96]. Similarly, we have found that addition of the TLR3 agonist poly(I:C) to OVA vaccination during the lymphopenic phase after CTX preconditioning markedly augmented CD8⁺ T cell expansion, coinciding with significant activation of DCs in the spleen and liver [118].
- Lymphodepletion removes the endogenous lymphocytes that compete with the donor T cells for the access to the host DCs, allowing the transferred T cell to crosstalk with a relatively larger pool of antigen-bearing DCs [58].
- Killing of significant numbers of tumor cells and the release of tumor antigens from the dead cells, which can be cross-presented to T cells by DCs [43]. The enhanced effects on immune-mediated tumor regression of radiotherapy and CTX therapy have been found to be due to the disruption of the stroma-tumor network within the tumor bed cells [52,140], coinciding with induction of the expression of adhesion molecules, such as P-selectin, enhancing infiltration of immune effectors into the tumor stroma [39,40,113]. The disruption of the stroma-tumor network would result in the release of tumor antigen that can be picked up and presented by the stromal cells themselves or the localized DCs for cytolytic effector T cells. The possibility of the cross presentation of the antigens released from the dead tumor cells would explain the enhanced anti-tumor effects of adoptively transferred T cells into a CTX-preconditioned host in the absence of active vaccination [9,15,17,129] or even in absence of adoptive T cell transfer [8,12–14,16,37,131]. It would also explain the anti-tumor efficacy of vaccination with naïve (i.e. with no antigen loading) DCs [20], where the antigens released from the dead tumor cells can be further cross presented by the exogenous DCs.

Therefore, it appears that the rapid induction of an inflammatory and apoptotic milieu by lymphodepletion can provide an environment, which is conducive toward the induction of activated DCs. The inflammatory milieu induced by lymphodepletion, however, is transient and diminishes during the recovery phase. Therefore, the suggested role of DCs would be transient and can be effective only when vaccination is performed during lymphopenia. In general, the optimization of increases in the numbers, activation, survival, and the anti-tumor responses of the adoptively transferred T cells into a lymphodepleted host have been found to require antigen boosting [74,89], which is often performed during the recovery

from lymphopenia. Since this time frame would be after the normalization of the activation state of the DCs logic would indicate the presence of additional mechanism(s) that would be in effect during the rebound phase. As discussed in the following section, there is increasing evidence that DCs develop an augmented presence during the rebound phase and could, at least in part, be one factor contributing to the augmented T cell responses to antigen boosting at the recovery from lymphodepletion.

Expansion of DCs during the recovery phase

While we were investigating the role of DCs in mediation of the beneficial effects of CTX-induced lymphopenia to adoptive T cell therapy, we observed substantial increases in both the relative and absolute numbers of DCs with myeloid (CD11c^{high}CD11b^{high}Ly6G^{low}B220^{low}) and immature (CD40^{low}CD80^{low}CD86^{low}) phenotype during the recovery phase after CTX-induced lymphodepletion [115]. Despite their immature phenotype, these CTX-expanded DCs were functional based on the phagocytosis and in vivo and in vitro antigen presenting function assays. Recent studies in human showed also increases in the numbers of DCs during the recovery phase in the peripheral blood of cancer patients receiving a combinatorial treatment of CTX and the growth factors G-CSF or GM-CSF [35,103,130]. Although it was not clear in these studies whether the increase in the frequency of DCs is solely due to the effects induced by CTX or the growth factors, our results demonstrate that CTX per se is capable of inducing DC expansion in the peripheral blood in a murine model. This notion is consistent with the capability of CTX to induce mobilization of hematopoietic stem cells (CD34⁺) from bone marrow to circulation [71,82,87,133]; these CD34⁺ showed higher differentiation rate into DCs when cultured in vitro with growth factors [35]. Our in vitro studies also demonstrated higher tendency of BM from CTX-treated mice to generate DCs in vitro [116]. Furthermore, the anti-tumor T cell response and protective immunity in mice that received TBI and immune reconstitution was found to be associated with significant increases in the number of lymph node DCs, which were further enhanced after vaccination [75]. Additionally, one recent study showed that the beneficial effects of CTX to the anti-tumor effects of T cells associate with DC turnover in spleen, liver, and tumor site. These newly recruited DCs were suggested to be originated from proliferating early DC progenitors and secreted more IL-12 and less IL-10 compared to those from untreated tumor-bearing animals. They were also fully capable of priming T cell responses and ineffective in inducing expansion of T_{reg} cells [104]. In the same vein, we also found that post-CTX expansion of DCs was associated with proliferation of DCs in bone marrow during the lymphopenic phase and in the blood and spleen during the recovery phase [114]. Interestingly, CTX induced a dynamic surge in the expression of growth factors and chemokines in bone marrow, where CCR2 and Flt3 signaling pathways were critical for DC expansion [114]. Taken together, it could be suggested that the beneficial effects of lymphodepletion, such as those induced by CTX, to enhance the antitumor potency of T cells extend beyond the well-documented cytotoxicity and lymphodepletion and include the augmented presence of DCs during the recovery phase. This observation of DC rebounding at certain time points after induction of lymphodepletion indicate that in addition to the rapid activation of DCs following the induction of lymphopenia, increases in the numbers of these cells also occur at the recovery phase. This would form a foundation for a new rationale design for immunotherapeutic strategies post-lymphodepletion.

Besides its induction of myeloid DC expansion, CTX can also expand other myeloid cells. Several previous studies, including ours, have reported increases in the numbers of cells expressing the phenotype (Gr-1⁺CD11b⁺) of the myeloid-derived suppressor cells (MDSC) in the peripheral blood and spleen [3,4,97,118]. Our most recent studies showed that this MDSC expansion start to gradually decrease prior the peak of DC expansion (Fig. 1).

Consistent with the previous studies, we have observed recently that cancer patients treated with chemotherapy containing CTX harbor high number of MDSC, which are capable of suppressing T cell responses in vitro [25]. Previous clinical studies also showed that CTX-induced MDSC expansion could limit T cell responses [3,4,97]. We found in our preclinical studies, however, that MDSC expansion by CTX does not abrogate its beneficial effects to adoptively transferred T cell responses to peptide vaccination [115,118]. Because vaccination, in particular, in the presence of a potent adjuvant such TLR agonists, can induce post-vaccination inflammatory mediators, it can be suggested that the presence of an inflammatory microenvironment may induce activation of MDSC and drive their differentiation into beneficial T cell activators or at least in part block their suppressor function. A previous study also described a role of MDSC in mediating the antitumor effects of CTX by a nitric oxide-dependent mechanism [97]. This notion is consistent with recent data in the literature showing that treatment of Gr-1⁺CD11b⁺ cells, isolated from immunocompromised animals or patients, with stimuli such as GM-CSF/IL-4, and all-*trans*-retinoic acid, can block the suppressive activity of these cells and drive their differentiation to mature DCs [2,32,93,94]. Moreover, Gr-1⁺CD11b⁺ cells from tumor-bearing mice have been found to acquire the phenotype characteristic to DCs (CD11c^{high}) upon their adoptive transfer into naïve, but not tumor-bearing, recipient mice [64,76]. Therefore, it might be feasible that application of strategies that can induce simultaneous activation of both DCs and MDSC expanded post-chemotherapy favor the creation of an overall stimulatory rather than inhibitory host microenvironment. Alternatively, the immunosuppressive activities of the expanded MDSC by CTX and the tumor can be blocked by targeting their regulatory pathways, including increased production of reactive oxygen species, the metabolism of the amino acid L-arginine by arginase I and nitric oxide synthetase 2, and the high levels of indoleamine-2,3-dioxygenase (IDO). Drugs such as the IDO inhibitor 1-methyl-*d*-tryptophan (d-1mT) and the multi-targeted receptor tyrosine kinase inhibitors sunitinib have been found to block the suppressor function of MDSC [22,30,64,65,84,137].

How expansion of DCs at recovery phase benefits adoptive T cell therapy

If DCs at the lymphopenic phase are important because of their activation state, how could an augmented presence of DCs during the recovery phase benefit T cell responses if they express immature phenotype? It could be postulated that inflammatory cytokines produced by effector cells after antigen priming at the lymphopenic phase induce the activation of DCs during their expansion and thus augment the immune response to antigen boosting. The beneficial effect of the inflammatory cytokine milieu on DC activation, however, is expected to be weak since its peak (days 5–7) of induction precedes the peak (days 10–14) of DC expansion. Therefore, either a second vaccination or provision of exogenous inflammatory adjuvant such as a TLR agonist at the recovery phase of CTX would be required to activate rebounding DCs (Fig. 2). This notion is consistent with our studies showing the minimal increases in the post-vaccination responses of OT-1 CD8⁺ cells when the antigen priming was performed at the time of DCs expansion in absence of exogenous adjuvant poly(I:C) administration in contrast to the marked increase in the post-vaccination CD8⁺ T cell responses when poly(I:C) was co-administered with peptide vaccination at the time of DC expansion [118]. The advantage of the provision of poly(I:C) could be attributed to its rapid induction of the inflammatory cytokines TNF- α , MCP-1, IFN- α , and IL-6 [114,115,118]. Indeed, induction of these cytokines was associated with the appearance of activated CCR7^{high}CD40^{high}CD80^{high} DCs in lymph nodes, coinciding with a significant decrease in the numbers of DCs in the peripheral blood. Given that CCR7 is essential for migration of activated DCs to lymph nodes [19], its up-regulation would suggest that the appearance of DCs in lymph nodes of CTX-treated mice after poly(I:C) treatment is, at least in part, due to their recruitment from the peripheral blood upon their maturation.

Using the pmel-1 mouse model, in which CD8⁺ T cells can recognize gp100_{25–33} peptide only in the presence of a potent adjuvant [92], we found that priming and boosting with gp100 peptide plus poly(I:C) treatment at the lymphopenic and recovery phases induced dramatic increases in CD8⁺ T cell responses and regression of B16 tumor growth than those obtained after vaccination at either phase or at both phases but in absence of poly(I:C) treatment [115]. Importantly, depletion of DCs before boosting resulted in a significant abrogation of CD8⁺ T cell responses, indicating to the importance of post-CTX expanded DCs. Similar requirement of DCs to antigen recall responses has been reported in the viral setting [19]. Taken together, it appears that expansion of a large pool of DCs per se can slightly benefit the CD8⁺ T cell responses, but can dramatically augment the responses if an inflammatory signal capable of maturing DCs is concomitantly induced. This would explain why prime-boost vaccination with peptide post-chemotherapy or irradiation is only effective when exogenous adjuvant is co-administered during vaccination [16,41,60,67,^{74,90},91,99,113,115]. It would also explain the enhanced T cell responses after induction of expansion of endogenous DCs with mobilizing factors, in particular Flt3L, followed by vaccination in combination of the TLR7 agonist imiquimod [121] and TLR9 agonist CpG ODN [29,56].

DC rebounding post-lymphodepletion may benefit ex vivo DC vaccines

The expansion of endogenous DCs during recovery from lymphodepletion could also benefit ex vivo DC-based vaccination. Recent studies have shown that single vaccination with antigen-loaded DCs immediately after TBI- or CTX-induced lymphopenia is not sufficient to induce significant tumor-specific T cell responses, unless a second DC vaccination is performed after 7–10 days [21,74,89]. Because DCs are already expanded during the second DC vaccination, it could be postulated that endogenous DCs provide a help to the exogenous DCs. Exogenously administered DCs migrate to draining lymph nodes, leading to a strong anti-tumor response. Migration of DCs to draining lymph nodes, however, is limited when the draining lymph nodes are saturated with the migrated cells [27,50,68,89]. Conditioning lymph nodes, for example with pre-injection of naïve unpulsed DCs or inflammatory cytokines, such as TNF- α , can significantly enhance the migration and retention of exogenous DCs, resulting in a robust anti-tumor immunity [78]. Therefore, several scenarios could be postulated to explain how expansion of endogenous DCs post-lymphodepletion augments vaccination with exogenous DC vaccine.

- Induction of lymphopenia per se creates an immune niche in the lymph nodes, enhancing their capacity to recruit higher numbers of endogenous DCs upon creation of the inflammatory microenvironment and their activation. This would explain the enhanced survival and migration of exogenous DCs administered after chemotherapy [20].
- Migration of endogenous DCs into lymph nodes would condition the latter to further increase their saturation (ceiling) capability for the exogenous DCs, resulting in arrival of higher numbers of antigen-bearing DCs. Since the magnitude of the antigen-specific T cell responses correlates with the numbers of DCs in the lymphoid tissues, in particular lymph nodes [27], arrival of more numbers of antigen-bearing DCs along with the arrival of adoptively transferred T cells would limit the antigen competition for the antigen recognition and results in higher frequency of the antigen-specific T cells. This would explain our recent observation of the appearance of high numbers of activated DCs mainly in lymph nodes after injection of poly(I:C) at the peak of DC expansion even in the absence of vaccination [27,115]. It would also explain the enhanced ex vivo DC-based vaccination in combination with CTX treatment without adjuvant [44,72] or with poly(I:C) [37].

- It is also possible that some of the antigen loaded in the exogenous DCs might be transferred into the endogenous DCs when both arrive to lymph nodes, resulting in antigen presentation by both of these DCs and better antigen display to T cells. This would explain the enhanced anti-tumor responses after the administration of DCs with no antigen [20,124,127].

Advantages of the sequential activation of DC at lymphopenic and recovery phases

Recent studies have reported the capability of CD8⁺ T cells to induce regression of established B16 tumor. The anti-tumor effects in these studies, however, required aggressive treatment protocols consisting of: (1) TBI-induced lymphodepletion or myelodepletion followed by hematopoietic stem cell transplant; (2) adoptive transfer of in vitro cytokine-conditioned antigen-stimulated T cells; (3) vaccination with 2×10^7 plaque forming units of a recombinant fowlpox virus encoding gp100₂₅₋₃₃ or with repeated ex vivo vaccination with peptide-pulsed DCs; and (4) exogenous administration of high doses of IL-2 [61,132,134]. The efficacy of these anti-tumor treatment protocols could be explained by the hierarchy of activation of DCs during the lymphopenic phase and their expansion during the restoration phase (Fig. 2). This hierarchy of DC responses would suggest a harmonized multiple mechanisms involved in the beneficial effect of lymphodepletion to adoptive T cell therapy and warrants the reconsideration of the timing between adoptive T cell transfer into a lymphodepleted host and subsequent vaccinations. As shown in Fig. 2, antigen priming along with a TLR agonist could be delivered at the lymphopenic phase, when T cells could benefit from the activated DCs, and then at the recovery phase again with a TLR agonist when T cells can benefit from the DC surge. This prime-boost vaccination regimen at these certain time points post-induction of lymphodepletion would obviate the need for more complicated and potentially toxic treatment regimens such as in vivo IL-2 therapy. In line with this notion, a complete regression of a large B16 melanoma was achieved when adoptive T cell transfer and vaccination with adenoviral vector expressing gp100 antigen post-CTX preconditioning was boosted by persistent stimulation of innate immunity through adjuvant peritumoral injections of the TLR9 agonist CpG and the TLR3 agonist poly(I:C) [66]. Interestingly, the administration of CpG and poly(I:C) in this study was performed about 12 days after CTX treatment, the time point when DC rebounding is maximal [104,114,115], indicating that the requirement of CPG/poly(I:C) injection to the success of this protocol is due to the activation of the rebounding DCs. Given the robust anti-tumor effect of our prime-boost vaccination with gp100 peptide in combination with poly(I:C), we suggest that not only IL-2 can be replaced by TLR agonist but also vaccination with adenoviral expressing the target antigen can be replaced with antigenic peptide mixed with a TLR agonist. This strategy would lead to a significant improvement in the application of chemo-immunotherapy, opening a new avenue for a simple but effective anti-cancer therapy.

Conclusion and prospective

There is growing evidence that DCs are altered in numbers and phenotypes at precise time points after the induction of lymphodepletion, resulting in a biphasic effect on DCs: a rapid induction of DC activation during the lymphopenic phase, and increasing their frequency without affecting their activation during the recovery phase. Exogenous administration of inflammatory adjuvants such as TLR agonists can induce stimulation and maturation of this expanded DCs and their migration to LNs. These fine-tuned responses of DCs are of a paramount significance since a precise prime-boost vaccination along with a TLR-mediated targeting of DCs at both the lymphopenic and recovery phases post-lymphodepletion could profoundly improve the anti-tumor responses of adoptive T cell therapy. These roles of DCs

would provide a useful foundation for the design of immunotherapy regimens combining tumor vaccines, primed T cells, and lymphodepletion. Future studies are required to focus on maximizing the role of DCs during both the lymphopenic and recovery phases post-lymphodepletion. For instance, DC rebounding can be augmented by agents such as Flt3L, and DC survival and trafficking to lymph nodes can be enhanced by chemokines such as CCL21 (CCR7 ligand), and DC activation can be augmented by combination of multiple TLR agonists. Further studies are also required to unveil the molecular mechanisms underlying the expansion of DCs post-induction of lymphodepletion. Progress in the research in this area can advance our understanding of the application of lymphodepletion for the maximal benefit of immunotherapy in the different clinical settings.

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Fig. 1.

Suggested phases post-CTX therapy and proposed approaches for their manipulation in vivo to benefit adoptive T cell therapy (ACT). CTX treatment induces a rapid lymphopenia for about 5 days followed by a gradual cellular recovery with a full recovery after 18–20 days of treatment. The cellular recovery is characterized by an expansion of cells with immature myeloid-derived suppressor cell (MDSC) phenotype ($\text{Gr-1}^+\text{CD11b}^+$) followed by an expansion of DCs with $\text{CD11c}^+\text{CD11b}^+$ phenotype in the peripheral blood, spleen, and liver. The expansion of DCs occurs when the expanded MDSC start to contract. Vaccination with a tumor antigen (Ag) and a potent adjuvant, such as a TLR ligand (TLRL) can activate the expanded DCs and MDSC, resulting in beneficial host microenvironment to T cell responses

Fig. 2.

Proposed paradigm for DC activation post-lymphodepletion and its impact on antigen-specific responses of adoptive T cell therapy. Treatment with a lymphodepleting dose of CTX or TBI induces a rapid lymphopenia likely associated with microbial translocation due to the damage of the intestinal tract. Lymphopenia can result in elimination or an imbalance of regulatory elements, including T_{reg} cells and myeloid-derived suppressor cells. Microbial translocation can also result in the release of microbial products such as LPS, which leads to activation of antigen presenting cells, in particular DCs. The intensity of these events, however, decreases gradually during the recovery phase from lymphopenia, in which DCs are expanded in circulation due to mobilization of DC precursors (*solid line*). Adoptively transferred antigen-specific T cells (*dotted line*) during the lymphopenic phase can benefit from the space “niche” and the more available survival cytokines and thus show homeostatic proliferation. The proliferation of these cells is further increased if the host is also primed with a specific antigen, particularly in the presence of endogenous activated DCs. The inflammatory microenvironment created during the peak of the antigen-specific responses of donor T cells to antigen priming might result in activation of the expanded DCs during the recovery phase, and thus could slightly augment the T cell responses when the antigen boosting occurs at this time point. Addition of an inflammatory adjuvant (e.g. TLR agonist) at the time of DC surge, however, would accentuate the inflammatory microenvironment that can induce the full activation and maturation of the expanded DCs and their migration to LNs, resulting in robust antigen-specific responses of donor cells (*dotted line*)