



# Dendritic Cells in Hematopoietic Cell Transplantation

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## 11.1 Human Dendritic Cells

### 11.1.1 Dendritic Cells: Critical Regulators of Immunity

Dendritic cells (DCs) are specialized bone marrow-derived leukocytes that orchestrate both innate and adaptive immunity. DCs reside in all tissues where they continuously survey the local environment and inform cells of the immune system to modulate their responses (Banchereau and Steinman 1998; Steinman and Banchereau 2007; Steinman 2012). Under physiologic steady-state conditions, DCs are predominantly immature or semi-mature and efficiently process self-antigens to induce and maintain self-tolerance (Hawiger et al. 2001; Bonifaz et al. 2002; Lutz and Schuler 2002). Under inflammatory conditions, DCs undergo terminal maturation and activation to become fully immunogenic. DC heterogeneity and differential activation states ultimately determine the type and quality of immune responses.

DCs initiate an immune response by capturing and presenting antigen in the form of peptide–major histocompatibility complex (MHC) molecule complexes to naive T cells in lymphoid tissues (Banchereau and Steinman 1998; Steinman and Banchereau 2007; Steinman 2012). DCs share most features of antigen-processing cells and class I and II MHC-restricted presentation with other antigen-presenting cells (APCs). DCs, however, are endowed with the capacity to cross-present exogenous antigens on their own class I MHC molecules to autologous T cells regardless of the MHC alleles expressed by the antigen source (Albert et al. 1998; Guermonprez et al. 2003; Ackerman et al. 2005). When compared with other APCs, like macrophages, DCs are much more efficient and can elicit responses from very low numbers of T cells (Steinman 2012).

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In peripheral tissues, DCs capture antigens by different complementary mechanisms (Trombetta and Mellman 2005) and then migrate through the afferent lymphatics to draining lymph nodes. DCs process proteins into peptides that bind to both MHC class I and class II molecules. Lipid antigens are processed differently through nonclassical MHC molecules of the CD1 family (Banchereau et al. 2000). Antigens also reach lymph node-resident DCs directly through the lymph (Itano and Jenkins 2003). On interaction with DCs, naive CD4<sup>+</sup> and CD8<sup>+</sup> T cells differentiate into antigen-specific effector cells with diverse functions. CD4<sup>+</sup> T cells can become T helper 1 (T<sub>H1</sub>) cells, T<sub>H2</sub> cells, T<sub>H17</sub> cells, or T follicular helper (T<sub>FH</sub>) cells, as well as regulatory T (T<sub>Reg</sub>) cells that suppress the function of other lymphocytes. Naive CD8<sup>+</sup> T cells can give rise to effector cytotoxic T lymphocytes (CTLs). The type of CD4<sup>+</sup> or CD8<sup>+</sup> T-cell response is at least in part dependent on the DC subset presenting the antigen (Banchereau and Steinman 1998). DCs also interact with cells of the innate immune system, including natural killer (NK) cells and mast cells (Banchereau and Steinman 1998; Steinman and Banchereau 2007; Steinman 2012). DCs control humoral immunity, directly by interacting with B cells and indirectly by inducing CD4<sup>+</sup> helper T-cell expansion and differentiation (Jego et al. 2005; Qi et al. 2006).

### 11.1.2 DC Maturation and Activation

DC maturation and activation is pivotal to the control of immune responses. Immature or non-activated DCs in peripheral tissues induce immune tolerance either through T-cell deletion or T<sub>Reg</sub> expansion (Steinman et al. 2003). Immature DCs efficiently capture antigens, accumulate MHC class II molecules in the late endosome–lysosomal compartment, express low levels of co-stimulatory molecules, and have a limited capacity for cytokine secretion (Trombetta and Mellman 2005). In response to environmental signals, immature DCs differentiate into mature forms that efficiently induce immune responses. Maturation is associated with decreased antigen-capture activity, increased expression of surface MHC class II and co-stimulatory molecules, increased cytokine secretion (Trombetta and Mellman 2005), increased CCR7 expression to enable migration to draining lymph nodes (Trombetta and Mellman 2005), and upregulation of CD83, the prototypical marker of DC maturation (Zhou and Tedder 1995). DCs also upregulate the immunomodulatory enzyme, indoleamine 2,3-dioxygenase, with maturation to induce T<sub>Regs</sub>, thus providing an intrinsic brake to counter otherwise unrestrained immune responses (Munn et al. 2002; Chung et al. 2009).

Microbial products provide a physiologic activation stimulus to DCs via pathogen-associated molecular patterns (PAMPs), including toll-like receptors. Although most microbes activate DCs, a few can block DC maturation (Steinman and Banchereau 2007; Pulendran et al. 2001; Palucka and Banchereau 2002). Tissue-localized DCs can also be polarized into distinct phenotypes by products released from neighboring immune cells responding to injury, including interferon (IFN)- $\gamma$  from  $\gamma\delta$ -T cells and NK cells, preformed interleukin (IL)-4 and TNF from mast cells, IFN $\alpha$  from pDCs, and IL-15 and thymic stromal lymphopoietin (TSLP)

from stromal cells (Ueno et al. 2010; Cheng et al. 2010). A combination of pro-inflammatory cytokines that include IL-1- $\beta$ , IL-6, prostaglandin E2, and tumor necrosis factor (TNF)- $\alpha$  (Jonuleit et al. 1997) simulates physiologic stimulation and is often used to mature DCs for study in vitro and use in clinical vaccine trials. In addition, adjuvant components of many vaccines trigger DC activation through distinct molecular pathways, resulting in varied T-cell responses (Maldonado-Lopez et al. 1999; Pulendran et al. 1999).

### 11.1.3 Human DC Subsets

Comparative phenotypic and functional studies have identified distinct DC subsets present in all mammals. Human DCs express MHC class II (HLA-DR) at high levels but lack T cell (CD3), B cell (CD19/CD20), and NK cell (CD56) lineage markers. The classical descriptions of DCs as HLA-DR<sup>+</sup> lineage<sup>-</sup> cells have been refined to include additional positive DC lineage markers that categorize DCs as either “myeloid” (or “conventional”) or “plasmacytoid” (Ziegler-Heitbrock et al. 2010). Human DC subsets in the blood can be distinguished by the differential expression of the cell-surface molecules CD303 (BDCA2 and CLEC4C), CD1c (BDCA1), and CD141 (BDCA3 and thrombomodulin) (Dzionek et al. 2000).

*Myeloid DCs (mDCs)* express the myeloid antigens CD11c, CD13, CD33, and CD11b, corresponding to mouse CD11c<sup>+</sup> “classical” or “conventional” DCs. In humans, both monocytes and mDCs express CD11c, but DCs lack CD14 or CD16 and can be subclassified into two populations by the reciprocal expression of CD1c and CD141. These two subpopulations share homology with mouse classical DCs expressing either CD11b (CD1c<sup>+</sup> DCs) or CD8/CD103 (CD141<sup>+</sup> DCs). Human CD141<sup>+</sup> mDCs are adept at taking up dead or necrotic cells via CLEC9A, sensing viral nucleic acids via TLR3 and TLR8, and cross-presenting antigen to CD8<sup>+</sup> T cells in vitro (Collin et al. 2013). Thus, human CD141<sup>+</sup> DCs are well equipped to stimulate CD8<sup>+</sup> T-cell-mediated immune responses. It is important to note that other human DCs, such as epidermal Langerhans cells (Klechevsky et al. 2008), also cross-present antigens effectively. Whether CD141<sup>+</sup> blood mDCs are related to DC subsets in peripheral tissues is unknown. CD1c<sup>+</sup> mDCs are the major population of human mDCs in blood, tissues, and lymphoid organs, and defining their unique function(s) remains an area of active research. Overall, the division of labor between CD141<sup>+</sup> DCs and other myeloid DCs in humans appears less sharply demarcated than in the mouse, underscoring the imprecise nature of cross-species comparisons.

*Langerhans cells (LCs) and dermal interstitial DCs (dermal DCs)* are the two primary subsets of mDCs present in the skin. LCs express high levels of langerin, a C-type lectin, and CD1a, a non-polymorphic class I MHC molecule, and are superior stimulators of cytotoxic T lymphocytes (CTLs) in vitro, at least against recall viral antigen and cross-presented tumor antigen (Ratzinger et al. 2004). Dermal DCs can be further subdivided into CD1a<sup>+</sup> DCs and CD14<sup>+</sup> DCs (Valladeau and Saeland 2005; Nestle et al. 1993). CD14<sup>+</sup> DCs appear to be specialized participants

in humoral responses (Klechevsky et al. 2008; Ueno et al. 2007), as they can directly help activated B cells and induce naive T cells to differentiate into cells with T<sub>FH</sub> cell-like properties (Klechevsky et al. 2008; Caux et al. 1997).

*Plasmacytoid DCs (pDCs)* secrete copious amounts of type I interferon (IFN- $\alpha/\beta$ ) in response to foreign nucleic acids and thereby mediate antiviral immunity (Siegal et al. 1999). pDCs are distinguished by the absence of myeloid antigens and expression of CD123 (IL-3R), CD303, and CD304. Freshly isolated plasmacytoid DCs express much lower levels of MHC and co-stimulatory molecules than their conventional DC counterparts (Grouard et al. 1997). Non-activated pDCs capture, process, and load antigens onto MHC molecules less effectively and are therefore relatively poor stimulators of T cells. In their resting state, pDCs participate in immune tolerance, including oral tolerance (Reizis et al. 2011). IL-3, in combination with CD40L or microbial products, leads to full pDC activation, abundant type I IFN secretion, and more potent lymphocyte stimulation (Siegal et al. 1999; Cella et al. 1999; Fonteneau et al. 2003). Activated pDCs also induce the maturation of activated B cells into plasma cells through cytokines and surface signaling (Jego et al. 2003; Shaw et al. 2010).

### 11.1.4 DC Receptors

DCs sense the environment with a diverse repertoire of surface and intracellular receptors, including toll-like receptors (TLRs), C-type lectins (CLRs), and helicases. TLRs recognize specific components conserved among microorganisms, and ligand binding to TLRs on DCs initiates the entire range of innate and acquired immunity (Takeda et al. 2003). TLR ligands include peptidoglycan (TLR2), viral dsRNA (TLR3), LPS (TLR4), viral ssRNA (TLR7), and unmethylated bacterial CpG DNA motif (TLR9). Myeloid DCs express various combinations of TLR1–TLR6 and TLR8, depending on the subset and activation state (Kadowaki et al. 2001; Jarrossay et al. 2001). pDCs are the only human DC subtype with TLR7 and TLR9 expression (Kadowaki et al. 2001; Jarrossay et al. 2001). CLRs bind carbohydrate moieties of glycoprotein self-antigens and pathogens, as well as many non-carbohydrate ligands such as lipids and proteins, by mechanisms that are not yet fully understood, to variably trigger pro-inflammatory or anti-inflammatory reactions (Reis e Sousa 2006). CLRs can synergize with, antagonize, or regulate signals from other receptors, thereby fine-tuning responses to infection or damage. CLR expression by DCs varies with activation status (Valladeau et al. 2000; Ebner et al. 2004; Bonifaz et al. 2004) and includes DEC-205 (CD205), DC-SIGN (CD209), BDCA-2, Dectin-1, Langerin (CD207), and CLEC9A. Helicases are members of a large family of molecules, including retinoic acid-inducible gene I (RIGI), which recognize nucleic acids. Activation of helicases can differentially affect DC function to yield distinct immune responses (Takeuchi and Akira 2010; Zhang et al. 2011). DCs also express a combination of activating (CD16, CD32a, and CD64) and inhibitory (CD32b) Fc- $\gamma$  receptors that influence processing and presentation of antigens in the steady state and during inflammation (Guilliams et al. 2014).

## 11.2 Human DCs in Clinical Hematopoietic Cell Transplantation

### 11.2.1 DC Antigen Presentation and Chimerism After Allogeneic Hematopoietic Cell Transplantation

DCs initiate T-cell responses to MHC and minor histocompatibility antigens (miHAs) and are both initiators and targets of graft–host interactions in hematopoietic cell transplantation (HCT). Specifically, DCs participate in the induction of graft-versus-tumor (GVT) activity and graft-versus-host disease (GvHD), two distinct but overlapping syndromes. The cytokine storm associated with pretransplantation conditioning and the early peri-transplant period can activate DCs to present MHC and miHAs through two separate pathways in an immunogenic manner (Hill and Ferrara 2000). Persistent host DCs present antigen by direct ligation of the donor T-cell receptor (TCR) by MHC molecules on recipient DCs. Engrafting donor DCs use an indirect pathway to cross-present host antigens. In both cases, antigens are presented to engrafting donor T cells. Polymorphic residues in the MHC binding groove, which themselves are not accessible to TCRs, affect binding of peptides recognized by allogeneic T cells. This intensifies the antigenic effect of MHC polymorphisms and explains the much higher frequency of T cells (1–10%) reactive with allogeneic MHC compared with those that react with miHAs presented by MHC-identical individuals (Sherman and Chattopadhyay 1993). Donor T cells use an indirect pathway to recognize miHAs, which are peptides derived from polymorphic genes unique to the host but recognized because they are presented by shared MHC molecules in matched allogeneic HCT (allo-HCT) (Bleakley and Riddell 2004). The ultimate goal in clinical transplantation is to stimulate T cells against miHAs unique to a malignancy that are absent from normal tissues, thus achieving GVT activity without GvHD.

DCs are terminally differentiated and, due to their nonproliferating state, are resistant to myeloablative regimens that target dividing cells, including total body irradiation. This results in the persistence of host DCs that coexist with new donor-derived DCs after allo-HCT. Most chimerism studies have evaluated conventional or myeloid DCs, identifying rapid conversion to donor type even though small numbers of residual host DCs may persist for extended periods, especially after reduced-intensity conditioning. In one study, approximately 80% of peripheral blood DCs were of donor origin by day +14 after allo-HCT, increasing to >95% by day +56 (Auffermann-Gretzinger et al. 2002). The kinetics of DC chimerism in peripheral tissues varies by conditioning regimen. A study of epidermal LC chimerism after allo-HCT found an average 97% donor-derived LCs with full-intensity conditioning, but only 36.5% donor-derived LCs with reduced-intensity conditioning 40 days after allo-HCT; at least 90% of LCs were donor-derived by day +100 (Collin et al. 2006). Donor chimerism is delayed in the presence of acute GvHD (Collin et al. 2006; Auffermann-Gretzinger et al. 2006), but the presence of residual host DCs is also seen in the absence of acute GvHD (Andani et al. 2014).

### 11.2.2 DCs and GvHD

GvHD is a frequent complication of allo-HCT and causes significant morbidity and mortality. At its root, GvHD is an inflammatory process mediated by both the innate and adaptive arms of the immune system (Ball and Egeler 2008; Ferrara et al. 2009). Residual host- and donor-derived DCs participate in GvHD pathogenesis. In murine models, host-derived DCs are essential for the induction of acute GvHD, whereas donor-derived DCs amplify acute GvHD and may be involved in the development of chronic GvHD (Shlomchik et al. 1999; Matte et al. 2004). During the effector phase of GvHD, tissue-resident, host-derived macrophages and DCs control the migration of alloreactive donor T cells into the tissues and subsequent local development of GvHD in mice (Zhang et al. 2002).

Mouse models of GvHD demonstrate that DC homeostasis after transplant influences GvHD outcome. LCs can self-renew in the skin of parabiotic mice from local precursors and remain of host origin for a prolonged period (Merad et al. 2004). LCs and dermal DCs can survive myeloablative radiation and persist for months after transplantation of purified stem cells or T-cell-depleted bone marrow in the absence of GvHD (Merad et al. 2004; Bogunovic et al. 2006). The presence or absence of GvHD is crucial to DC composition. In the absence of GvHD, trace populations of low-level cycling precursors in the skin can replace LCs or dermal DCs that exit to secondary lymphoid tissues, thus maintaining DCs of host origin. In contrast, in the setting of GvHD, the loss of DCs exceeds the capacity of local precursors to replenish host populations, allowing for circulating donor marrow-derived DC precursors to fill the resulting void. Elimination of host LCs and replacement by donor DCs prevent cutaneous GvHD in MHC-mismatched allo-HCT (Merad et al. 2004). In addition, residual allogeneic T cells from donor marrow, once primed against host MHC or miHA, eliminate host DCs from GvHD target organs, with subsequent replacement by donor marrow-derived DCs (Merad et al. 2004). Previous acute GvHD of the skin in humans correlates with complete donor LC chimerism, again supporting a role of allogeneic T cells in promoting donor LC engraftment (Collin et al. 2006).

Resident populations of DCs in peripheral tissues may be more relevant to acute GvHD, as addressed in the murine studies cited above. In humans, host LCs decreased and then recovered with donor LCs more rapidly after myeloablative conditioning than with reduced-intensity conditioning, although the nadirs were comparable between days 14 and 21 (Collin et al. 2006). Donor LC recovery to pretransplant levels was more brisk in the absence of acute GvHD but more complete in the presence of acute GvHD, indicating a role for donor T cells in promoting LC engraftment as in mice (Merad et al. 2002, 2004). Dermal DC reconstitution can exhibit similarly rapid turnover by about day +100 (Auffermann-Gretzinger et al. 2006), although some host dermal DCs persist, especially after reduced-intensity conditioning (Bogunovic et al. 2006). Co-expression of the activation marker, CMRF-44, by conventional or myeloid CD11c<sup>+</sup> DCs in peripheral blood precedes the onset of clinically significant acute GvHD (Lau et al. 2007), suggesting the predictive value of monitoring such subsets in the blood.

Prophylactic and therapeutic immunosuppressive agents for GvHD affect DC numbers. During clinically significant acute GvHD, circulating DC levels decline, reflecting the effects of therapy (especially steroids), more rapid turnover and migration into tissues, or both (Reddy et al. 2004). Alemtuzumab rapidly depletes circulating host DCs but does not alter donor DC engraftment or deplete other DCs that lack the CD52 target epitope (LCs or dermal DCs) (Ratzinger et al. 2003; Collin et al. 2005; Klanginsirikul et al. 2002). Cyclosporin A and tacrolimus can impair antigen processing by DCs (Lee et al. 2005) but, like steroids, are nonselective and also exert broad effects on T cells by calcineurin inhibition. Sirolimus (rapamycin), which blocks the signal transduction resulting from ligation of the IL-2, IL-4, and IL-6 receptors in T cells, also suppresses DC immunogenicity (Hackstein et al. 2003). Thus, drugs that block DC function should modulate immune interactions in allo-HCT. More targeted reagents are still required, however, especially if the goal is to maintain viral immunity and GVT effects while eliminating GvHD and avoiding overly global immune suppression and its attendant complications. The use of tolerogenic recipient DCs to pretreat donor stem cell sources and minimize allogeneic T-cell responses is an alternative consideration.

### 11.2.3 DCs and GVT Responses

Host DCs presenting tumor antigen(s) either directly or by cross-presentation should induce at least a portion of the GVT response. The precise role of the different human DC subtypes in GVT responses after allo-HCT, however, remains poorly understood. Mouse studies have shown that host DCs may play an important role in GVT effects (Mapara et al. 2002), especially those that are able to cross-present tumor-specific antigen(s) to donor T cells (Toubai et al. 2013). The participation of host DCs in GVT in humans was supported by a study where the combination of donor T cells and mixed chimerism in DC subsets stimulated a potent GVL effect in association with GvHD, whereas donor lymphocyte infusions in patients with donor chimerism in both T cells and DC subsets resulted in GVL reactivity without GvHD (Levenga et al. 2007). Whether DC subtypes separately direct GVH or GVT reactions at the level of antigen presentation to responding T cells is incompletely understood. In the absence of concomitant tissue damage, persistent host LCs migrating from the skin to draining lymph nodes can stimulate potent graft responses against host antigens, thus supporting GVT without GvHD in an MHC-matched murine allo-HCT model (Durakovic et al. 2006). This finding is also relevant to the immunologic effects mediated by donor lymphocyte infusions to treat relapsed/recurrent disease. The ultimate goal is to preferentially target miHAs that are only expressed by tumor cells and not shared with normal tissue to avoid the overlapping development of GvHD (Bleakley and Riddell 2004). Maintaining DCs in an immature or semi-mature state to preserve graft–host tolerance while promoting GVT is an area of ongoing study.



### 11.2.4 DC Vaccines: General Considerations

Methods for the large-scale generation of human DCs have enabled their clinical evaluation as vaccines. Although early phase I and II trials of DC-based vaccines showed limited success, many studies used immature DCs that were insufficiently immunogenic, suboptimal routes and schedules of vaccination, and patients with advanced disease in whom there was inadequate time to respond. Importantly, numerous other studies have demonstrated the feasibility of DC-based immunization to induce both immune and objective clinical responses against tumors (Palucka and Banchereau 2012), although primarily in the non-transplant setting.

DC precursors can be obtained from several sources, including nondividing peripheral blood monocytes or cycling CD34<sup>+</sup> progenitors in cord blood, granulocyte colony-stimulating factor-elicited peripheral blood, or bone marrow. Regardless of source, precursor cells require recombinant cytokine support *in vitro* to generate DCs, with subsequent terminal maturation to ensure optimal stimulation of T-cell immunity. A potential advantage of using CD34<sup>+</sup>-derived DCs, especially in the setting of cord blood transplantation, is their capacity for expansion prior to DC differentiation to generate larger numbers of DCs from a limited pool of precursor cells. In addition to cytokine-supported methods, preformed circulating DCs can also be isolated from blood by density gradient (Hsu et al. 1996; Timmerman et al. 2002) or direct immunoselection (Dzionek et al. 2000; Lopez et al. 2003).

Almost all previous DC vaccine trials have used monocyte-derived DCs (moDCs), in large part because monocyte precursors are easier to obtain and culture *in vitro* than CD34<sup>+</sup>-derived subsets, including LCs. LCs, however, are superior to moDCs and other conventional DC subsets at inducing antigen-specific CTLs against viral and tumor antigens *in vitro* (Klechevsky et al. 2008; Ratzinger et al. 2004). When compared with moDCs, LCs secrete more IL-15 (Klechevsky et al. 2008; Ratzinger et al. 2004; Munz et al. 2005), which in turn reduces IL-2-induced T-cell apoptosis and decreases T<sub>Reg</sub> expansion during LC-mediated CTL generation (Romano et al. 2012). LCs can overcome tolerance against tumor-associated antigens by an IL-15R $\alpha$ /IL-15/pSTAT5-dependent mechanism (Romano et al. 2012). Clinical trial data have shown greater efficacy of DC vaccines that contain LCs (Banchereau et al. 2001), as well as greater tetramer reactivity stimulated by LCs when compared with moDCs (Romano et al. 2011). Thus, selection of DC subtype for use in vaccine formulations is an important consideration.

Optimizing antigen loading is another key parameter of DC vaccine preparation. The simplest and most often used approach is “peptide pulsing,” which is the incubation of DCs with synthetic peptides of limited length and defined HLA restrictions, most commonly HLA-A\*0201. DNA (Yuan et al. 2006)- or RNA (Gilboa and Vieweg 2004)-based methods of antigen delivery offer the potential advantage of facilitating the processing and presentation of a broad repertoire of multiple class I and II MHC-restricted epitopes from the translated protein (Romano et al. 2011), together with more sustained antigen expression than peptide pulsing. Other approaches have used tumor lysates for uptake and cross-presentation (Ratzinger et al. 2004; Berard et al. 2000; Palucka et al. 2006), DC receptor targeting for



antigen delivery *in vivo* (Bonifaz et al. 2004; Birkholz et al. 2010), and systemic delivery of antigen-encoding RNA lipoplexes to DCs *in vivo* (Kranz et al. 2016). The majority of DC vaccine studies have been limited to single antigen and restricted epitope targets. Simultaneously targeting more than one antigen, however, offers the potential to improve the breadth of immune responses and clinical response rates (Karan et al. 2011; Walter et al. 2012).

Different routes of immunization have been tested, with subcutaneous administration by far the most common method. Other approaches include intradermal, intravenous, intranodal, and intratumoral injections. Although direct comparisons are generally lacking, intradermal vaccination may be more effective than subcutaneous vaccination due to the rich lymphatics at the epidermal–dermal junction. Intravenous administration does not compare favorably with the intradermal route in animal and limited clinical comparisons. Intranodal vaccination removes considerable uncertainty but cannot be widely implemented. The ideal frequency and duration for vaccination is unknown, but maintaining an ongoing vaccination schedule in responding patients may provide benefit (Palucka et al. 2006).

Early-phase trials often rely on proxy measurements *in vitro* of responses to vaccines. These include antigen-driven assays for measurement of IFN- $\gamma$  secretion, intracellular cytokine secretion assays, T-cell reactivity with tetramers/pentamers of defined peptides with known MHC restrictions, CTL assays against antigen-expressing targets, and, more recently, next-generation deep sequencing of the TCR-V-beta CDR3 region to assess changes in T-cell clonal diversity. Clinical responses remain mostly anecdotal in the relatively small numbers of patients among the many treated in the presence of persistent systemic disease.

### 11.2.5 DC Vaccination After Autologous Stem Cell Transplantation

DC-based vaccination to induce or restore antitumor immunity offers a promising approach to target residual malignancy and to improve clinical outcomes after autologous HCT (auto-HCT). The minimal residual disease state and lymphopenia after auto-HCT afford a unique platform to induce antitumor immune responses by limiting tumor-driven immunosuppression (Kim et al. 2006), eliminating cytokine sinks (Gattinoni et al. 2005), and transiently depleting T<sub>Regs</sub> (Zhang et al. 2005; Chung et al. 2016). Importantly, CD8<sup>+</sup> T cells can respond to autologous DCs presenting tumor antigen *in vitro* as early as day +12 posttransplant, becoming antigen-specific cytolytic T-lymphocyte effectors and thereby demonstrating preservation of cellular reactivity after transplant (Chung et al. 2016). DC-based vaccination in this setting therefore offers one approach to redirect recovering T cells toward specific MHC-restricted antigen(s).

The feasibility of DC vaccines in the setting of auto-HCT has been demonstrated in multiple myeloma, the most common indication for auto-HCT (Table 11.1). DCs from patients with multiple myeloma are functionally intact, comparable to those from healthy donors, and induce autologous antigen-specific T cells with lytic

**Table 11.1** DC vaccine trials after auto-HCT

DC subtype	Cell source	Target	Antigen(s)	Antigen form	Vaccine route	Immune response	Clinical response	SAE	Reference
CD14+	PBMC	MM	Id KLH	Protein	IV/SC	Id 2/12 KLH 11/12	N/A	NR	Reichardt et al. (1999)
CD14+	PBMC	MM	Id	Protein	IV	Not reported	OS benefit	NR	Lacy et al. (2009)
CD14+	PBMC	MM	Autologous tumor	DC-tumor fusion	SC	Expansion of MM-specific T cells 36/36	Increased CR at 1 year	NR	Rosenblatt et al. (2013)
CD14+	PBMC	MM	Autologous tumor	DC-tumor fusion	SC	N/A	N/A	N/A	<sup>a</sup> NCT 02728102
LC	CD34+ HPC	MM	CT7 MAGE-A3 WT1	mRNA	ID	N/A	N/A	N/A	<sup>a</sup> NCT 01995708

*ALL* acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *CMV* cytomegalovirus, *CR* complete response, *CT7* cancer-testis antigen 7, *DC* dendritic cell, *DTH* delayed-type hypersensitivity, *HPC* hematopoietic progenitor cell, *Id* idotype, *ID* intradermal, *IV* intravenous, *KLH* keyhole limpet hemocyanin, *LC* Langerhans-type dendritic cell, *MAGE-A3* melanoma-associated antigen 3, *MiHA* minor histocompatibility antigen, *MM* multiple myeloma, *N/A* not applicable, *NHL* non-Hodgkin lymphoma, *NR* none reported, *OS* overall survival, *PBMC* peripheral blood mononuclear cell, *PBSC* peripheral blood stem cell, *RCC* renal cell carcinoma, *SAE* severe adverse events, *SC* subcutaneous, *WT1* Wilms' tumor 1

<sup>a</sup>Trial ongoing

activity *in vitro* (Chung et al. 2016). Clinical validation of this approach was shown with an idiotype-pulsed autologous DC vaccine for multiple myeloma after auto-HCT that induced idiotype-specific T-cell responses in a subset of patients (Reichardt et al. 1999). Subsequently, posttransplant vaccination with an idiotype-pulsed cellular product containing DCs was associated with improved progression-free survival compared with a historical control cohort (Lacy et al. 2009). More recently, vaccination with a DC–myeloma fusion vaccine following autologous transplant was associated with an increase in myeloma-specific T cells and conversion from partial response to complete response in a subset of patients (Rosenblatt et al. 2013). Vaccination in these three studies was well tolerated without evidence of significant autoimmunity or adverse effect on posttransplant engraftment.

Posttransplant maintenance therapy with lenalidomide improves progression-free and overall survival after auto-HCT (McCarthy et al. 2012; Attal et al. 2012; Palumbo et al. 2014). In addition, lenalidomide has immunostimulatory properties (Benson Jr et al. 2011; Luptakova et al. 2013; Noonan et al. 2012) that could further augment vaccine-induced immunity. This is being assessed in two DC vaccine studies that include lenalidomide maintenance in the treatment regimen. A phase I study is examining early posttransplant vaccination using autologous LCs electroporated with mRNA encoding three MM-associated antigens followed by lenalidomide maintenance (NCT01995708). A phase II, multicenter trial will study posttransplant lenalidomide maintenance alone or in conjunction with serial vaccination with DC–myeloma fusions (NCT02728102).

Immune checkpoint inhibitors enhance vaccine-induced antitumor immune responses in various preclinical models. Both CD4<sup>+</sup> and CD8<sup>+</sup> T cells from multiple myeloma patients express the negative regulatory molecules, CTLA-4, PD-1, LAG-3, and TIM-3, before and after auto-HCT (Chung et al. 2016). In addition, a subpopulation of hyporesponsive, exhausted/senescent PD-1-expressing CD8<sup>+</sup> T cells that characterize immune impairment and relapse after auto-HCT can be revived with PD-1 blockade *in vitro* (Chung et al. 2016). The combination of DC vaccines with checkpoint blockade to “prime and boost” antitumor immune responses warrants investigation.

### 11.2.6 DC Vaccination After Allogeneic Stem Cell Transplantation

As in the auto-HCT setting, DC-based vaccination after allo-HCT offers similar advantages in exploiting a minimal residual disease state and the autoreactive potential of recovering T-cell populations, relatively devoid of T<sub>Regs</sub>. In contrast to auto-HCT, however, allo-HCT regimens often include immunosuppressant agents for GvHD prophylaxis, which could impede responses to vaccines. Nonetheless, DC vaccination after allo-HCT has been well tolerated with evidence of both clinical and immunological antiviral and antitumor responses without increases in adverse events, albeit in a limited number of patients (Table 11.2).

Virus-specific immunity against human cytomegalovirus (CMV) can be induced by DC vaccination after allo-HCT. Patients at high risk for developing CMV

**Table 11.2** DC vaccine trials after allo-HCT

DC subtype	Cell source	Target	Antigen(s)	Antigen form	Vaccine route	Immune response	Clinical response	SAE	Reference
CD14+	Donor PBMC	CMV	pp65 pp150	Peptide	SC	Tetramer 7/17	15/17	NR	Grigoletti et al. (2007)
CD14+	Donor PBMC	CMV	pp65	Protein	SC	Protein recall 1/1	1/1	NR	Feuchtinger et al. (2010)
CD14+	Donor PBSC	AML, ALL, NHL	Autologous tumor	Irradiated tumor cells	IV	DTH 3/4	3/4	NR	Fujii et al. (2001)
CD14+	Donor PBSC	RCC	Autologous tumor	Lysate	ID	DTH 0/1	0/1	NR	Tatsugami et al. (2004)
CD14+	Donor PBMC	AML	WT1 KLH	Peptide	ID	WT1 no KLH yes	0/1	NR	Kitawaki et al. (2008)
CD14+	Host PBMC	MM	MiHA	N/A	IV/ID	Protein recall 6/6	0/6	NR	Levenga et al. (2010)

ALL acute lymphoblastic leukemia, AML acute myeloid leukemia, CMV cytomegalovirus, CR complete response, CT7 cancer-testis antigen 7, DC dendritic cell, DTH delayed-type hypersensitivity, HPC hematopoietic progenitor cell, Id idiotype, ID intradermal, IV intravenous, KLH keyhole limpet hemocyanin, LC Langerhans-type dendritic cell, MAGE-A3 melanoma-associated antigen 3, MiHA minor histocompatibility antigen, MM multiple myeloma, N/A not applicable, NHL non-Hodgkin lymphoma, NR none reported, OS overall survival, PBMC peripheral blood mononuclear cell, PBSC peripheral blood stem cell, RCC renal cell carcinoma, SAE severe adverse events, SC subcutaneous, WT1 Wilms' tumor 1

disease, defined as a CMV-seropositive patient and a CMV-seronegative donor and/or receipt of a T-cell-depleted graft, received donor CD14-derived peptide-loaded DCs after allo-HCT, with induction of measurable CMV-specific T-cell responses and evidence of clinical benefit, without the stimulation or expansion of allo-reactive T cells (Grigoleit et al. 2007). Vaccination with CMV pp65-pulsed DCs induced antigen-specific CD4 and CD8 cells and sustained CMV viral clearance in a patient with recurrent CMV viremia resistant to standard antiviral therapies (Feuchtinger et al. 2010).

Tumor-specific immunity elicited by donor-derived DCs loaded with irradiated tumor cells was observed in three of four patients with hematologic malignancies relapsed after allo-HCT (Fujii et al. 2001). Additional reports have demonstrated antigen-specific immune responses in patients immunized with DC-based vaccines after allo-HCT (Tatsugami et al. 2004; Kitawaki et al. 2008; Levenega et al. 2010).

Because allo-HCT patients may not be sufficiently immune reconstituted to respond to direct immunization, alternative approaches merit consideration. Donors could be vaccinated with DCs bearing their recipients' tumor antigen(s) before stem cell collection and transplantation. Donor DCs could also be used to stimulate donor lymphocytes *ex vivo* against specific tumor antigens for adoptive immunotherapy, with less off-target effects that could trigger GvHD.

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### 11.3 Expert Point of View

DCs comprise a complex system of bone marrow-derived leukocytes that are critical to the onset and modulation of immunity. The divisions of labor among distinct human DC subsets maintain an equilibrium between steady-state tolerance and stimulation of antigen-specific immunity against pathogens, tumors, and other insults. Maintenance of tolerance in the steady state is an active process mediated by resting or semi-mature DCs. Under inflammatory conditions, this homeostasis is disrupted, leading to the maturation and activation of DCs and triggering a cascade of events leading to an immune response. In the setting of HCT, the mechanisms that regulate DC homeostasis offer potential targets to fine-tune graft–host interactions. It is not yet known whether a particular subtype of DC is more or less responsible for initiating or being targeted (or both) by GvHD reactions. The precise role of the different DC subtypes in GVT responses after HCT also remains poorly understood. Animal models are providing important data about distinct DC precursors, homeostasis of tissue-resident DCs, and turnover of DCs in response to inflammatory stimuli and pathological conditions like GvHD. Ultimately, therapeutic interventions that use or specifically target defined DC subtypes to selectively induce both the innate and adaptive arms of immunity, either in combination or in a prime-boost sequence, may provide optimal clinical utility by harnessing both effector cell compartments.

## 11.4 Future Directions

Advancing our knowledge of how different DC subsets are related, their roles in graft–host interactions and disease pathogenesis, and their most favorable therapeutic implementation are among the key issues for future studies. Progress in systems immunology is expected to lend insights into the molecular pathways that determine DC-guided immunity. Thus, an integrated approach combining transcriptional profiling, genetic and small-molecule screening, and proteomics will further our understanding of DC biology and thereby enable the discovery of novel adjuvants and strategies to induce protective immune responses while minimizing the risk of autoimmunity or GvHD. In turn, this will yield more rational and refined clinical applications of DC-based therapies in HCT.

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