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REVIEW

From pluripotent stem cells to T cells

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The generation of T cells from human pluripotent stem cells (PSCs) opens a valuable experimental window into developmental hematopoiesis and raises the possibility of a new therapeutic approach for T-cell immunotherapy. After directing PSCs through mesoderm and early hematopoietic developmental stages, commitment to the T-cell lineage has been achieved by several groups using coculture with stromal cells that express a notch ligand, recapitulating the critical signals that initiate the first stages of normal T-cell differentiation in the thymus. However, positive selection and the production of mature T cells from human PSCs have been limited to date. Nonetheless, T-lineage cells have been generated from PSCs with tumor antigen specificity either through a prearranged clonal T-cell receptor (TCR) or lentiviral-mediated expression of chimeric antigen receptors. The recent development of a 3D artificial organoid model has demonstrated that PSCs can generate mature conventional T cells that are fully functional and express a diverse TCR repertoire. Introduction of a transgenic TCR at the PSC stage allows for the production of tumor-antigen-specific, mature conventional T cells. The tools of gene editing in PSCs are ideally suited to produce off-the-shelf universal products for T-cell immunotherapy. In this review, we describe the studies that have led to this exciting moment in PSC biology and discuss translation to clinical applications.

Since the seminal paper of Kaufman and Thomson showed in 2001 that hematopoietic progenitors (HPCs) could be generated from human embryonic stem cells (hESCs) [1], the exploration of the lineage potential of these HPCs has been of great interest to understand both the biology of PSC-derived hematopoiesis and the potential of these cells for clinical applications. The ability to differentiate T cells from hPSCs was first shown in vivo by Galic et al. [2] and in vitro by Timmermans et al. [3]. Several groups have since confirmed the ability of the in vitro systems to induce early T-cell commitment from PSCs (note: "commitment" is defined in this review as the generation of cells with a specified lineage potential, with loss of potential of all other lineages). However further T-cell maturation has tended to be inconsistent and inefficient, so studies in this area have remained in the experimental biology realm without a clear pathway toward a translational T-cell product. Now, with T-cell immunotherapy bursting into clinical prominence, interest has turned to the possibility of using PSCs to provide an inexhaustible and potentially universal source of T cells engineered for tumor-specific killing capability. This review discusses the major basic research performed over the past 10 years to generate T cells from PSCs and the latest advances that are moving the field toward the exciting goal of PSC-derived T-cell immunotherapy.

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Normal postnatal human T-cell development

As with all hematopoietic lineages, T cells originate from self-renewing hematopoietic stem cells that reside in the bone marrow during steady-state postnatal life. However, unlike other major lineages, commitment to a specific T-cell program does not occur in the marrow, but rather begins only after seeding of marrow-derived progenitors into the thymus from the circulation. Commitment to the lymphoid lineage clearly can occur in the bone marrow with the generation of lymphoid-primed multipotent progenitors (LMPPs) [4,5] and common lymphoid progenitors (CLPs) [6], both of which demonstrate T-cell potential after transplantation or in vitro differentiation; therefore, it has previously been assumed that some level of lymphoid commitment must occur prior to seeding of the thymus. However, the thymus not only contains lymphoid-restricted progenitors, but also cells with multilineage potential, such as erythroid, myeloid, dendritic, and full lymphoid (B, T, natural killer [NK] lymphoid) cells, which are transcriptionally very similar to bone marrow HSCs and LMPPs [7]. Therefore, it seems likely that more than one type of cell, including HSCs, can home to the thymus and respond to signals in the thymic microenvironment that launch the T-cell lineage. Which of these hematopoietic stem or progenitor cells, if any, are the precursors of T cells in PSC cultures is unclear.

Notch signaling from ligands in the thymic stroma (Delta-like ligand 1, DLL-1, and the more potent DLL-4) is essential for commitment of progenitors to the T lineage, a fate decision that is made at the expense of B-cell development [8]. The earliest cells in the human thymus express CD34, as do their HSC and progenitor counterparts in the bone marrow; expression of the cell surface marker CD7 in conjunction with either CD1a or CD5 further divides the $CD34^+$ thymocytes into three distinct stages that mark the progressive loss of non-T-cell potential: $CD34^+CD7^-CD1a^-CD5^-$ (early thymic progenitors, ETP aka "Thy1"), $CD34^+CD7^+CD1a^-/CD5^-$ (Thy2), and finally T-committed $CD34^+CD7^+/CD5^+$ (Thy3) [7,9,10]. Each of the thymic $CD34^+$ populations lack lineage-specific cell surface marker and correspond approximately to the $CD4^-CD8^-$ ("double-negative" DN) fractions in the murine thymus. Although the transcriptional control of these early stages of T-cell development is mostly conserved between mice and humans, careful analyses of endogenous human thymocytes and the use of in vitro systems of T-cell development from human HSCs are beginning to reveal subtle but important differences [11–13].

The remarkable ability of the adaptive immune system to respond in an antigen-specific manner to an unlimited number of unknown pathogens encountered throughout life, resides in the process of random DNA rearrangements that produce a diverse repertoire of T-cell receptors (TCRs) and B-cell-derived immunoglobulins [14]. Although DNA rearrangements are randomly produced, they occur through a carefully orchestrated process at very specific stages after lymphoid commitment. During development of the conventional T-cell pathway, rearrangement of the Vβ locus of the TCR ("beta selection") occurs as cells move from the final $CD34^+$ progenitor stage (Thy3) into the $CD3^-CD8^-CD4^+$ (immature single-positive CD4, ISP4) stage. Although, in mice, notch signaling is clearly required for beta selection [8], data from human studies suggest notch is required for proliferation but not for beta selection [15,16]. CD8 (in the form of the CD8$\alpha\beta$ heterodimer) is then expressed to form the $CD4^+CD8^+$ (double-positive, DP) stage. During the latter part of the DP stage, CD3 and TCR$\alpha\beta$ move to the cell surface to create a TCR complex capable of signaling. Further differentiation of DP generates either $CD3^+CD4^+CD8^-$ (single-positive, SP4) or $CD3^+CD4^-SP8^+$ (SP8). This process is known as positive selection and results in survival only of those cells that express a TCR complex able to engage through either class II (for CD4 selection) or class I (for CD8 selection) major histocompatibility complex (MHC) molecules expressed on other hematopoietic or stromal cells in the thymus. The majority of DPs will die during positive selection because of suboptimal TCR expression. In addition to intercellular signals, certain cytokines are also key for thymopoiesis, most notably interleukin-7 (IL-7), which drives proliferation and survival at various stages.

In summary, for the ultimate production of conventional naïve SP8 or SP4 T cells, the cells that seed the thymus from the circulation must signal through notch, rearrange the Vβ and Vα loci, express the TCR$\alpha\beta$ complex on the surface, and undergo positive selection. Further shaping of the TCR repertoire then occurs through the process of negative selection in which T cells that express high-affinity TCRs that recognize self-antigens undergo apoptosis, thus producing central tolerance through deletion of potentially autoreactive T cells.

In vivo evidence for T-cell potential of PSC-derived hematopoiesis

The first evidence that hPSCs possessed T-cell potential was shown in vivo using the chimeric SCID-hu (Thy/Liv) model, in which fragments of human fetal liver and thymus were implanted under the renal capsule of sublethally irradiated immune-deficient (SCID) mice to establish a humanized microenvironment for T-cell development [2]. In this report, hESCs (H1 cells) were initially cultured on OP9 stroma for 7–14 days and then progenitors (either $CD34^+$ or $CD133^+$ cells) were isolated and transferred directly into the Thy/Liv graft. PSC-derived T cells were distinguished from the fetal thymocytes in the implants based on expression of the green fluorescent protein (GFP) gene introduced into hPSCs via lentiviral

transduction ± differential expression of HLA-class I. Thy/Liv grafts implanted into irradiated Rag1/2 knockout (Rag-hu) mice also showed T-cell differentiation. GFP^+ T cells harvested after 5 weeks from the chimeric mice were activated by CD3/CD28 costimulation as shown by upregulation of the activation marker CD25. Although these studies were the first to demonstrate the T-cell potential of PSCs, the complexity of the in vivo model did not allow further characterization of the T cells or manipulation of the differentiation process, so this is not a system that can be used to generate T cells for therapeutic purposes.

In vitro assays of T-cell potential using primary HSPCs

Various in vitro systems have been developed to try to mimic the thymic microenvironment's ability to induce T-cell differentiation from multipotent primary HSPCs. The earliest assays used the healthy microenvironment of the fetal thymus to assess the T-cell potential of a test population. In these fetal thymic organoid cultures (FTOCs), the experimental populations, usually congenic or marked syngeneic murine bone marrow HSPCs, were injected into cultured whole thymus harvested from murine embryos. A more recent report used decellularized murine thymus as a 3D scaffold for in vitro seeding of thymic epithelial cells, which, when transplanted into nude mice, could generate T cells from endogenous murine HSPCs [17]. Although providing qualitative information, T-cell potential in FTOC cultures is difficult to quantitate and these have rarely been used for the study of human HSPCs.

In 2002, Schmitt and Zuniga-Pflucker [18] reported a more user-friendly monolayer coculture system in which T-cell differentiation from murine HSPCs was accomplished by coculture on an adherent layer of the murine stromal cell line OP9 that was retrovirally engineered to express the notch ligand DLL1. The OP9-DLL1 system has since then become the de facto gold standard for T-cell differentiation; it uses a fetal calf serum-containing medium with IL-7 ± other cytokines, and cells are analyzed and replated weekly onto fresh stromal layers over the next 6 or more weeks.

In 2005, the use of the OP9-DLL1 system for human T-cell cultures was first reported [19]. When applied to primary human HSPCs (typically $CD34^+$ cells from umbilical cord blood), the OP9-DLL1 (or DLL4) system is very efficient at inducing commitment to the T-cell lineage as shown by vigorous production of $CD7^+CD5^{+/-}$ prothymocytes [20]. Within the $CD7^+$ population, DN, ISP4, and, to a lesser extent, DP cells are generated; however, cell surface production of CD3 and TCR is low, leading to limited positive selection and inefficient production of mature SP4 and SP8 T cells. A similar monolayer culture system with the MS5 stromal line engineered to express DLL1 has been used to assess multilineage (including T-cell potential) of human HSPCs [21].

Our group recently reported a new in vitro system for T-cell differentiation from human HSPCs that combines elements of the FTOC (3D culture in an air–fluid interface) and the notch ligand expressing stromal systems [22]. The artificial thymic organoid (ATO) model uses a serum-free medium (avoiding the variability of the serum-containing cultures) and expresses human DLL1 or DLL4 in the murine stromal line MS5, which can tolerate serum-free culture. MS5-DLL1 or DLL4 cells are centrifuged with human HSPCs to form small 3D aggregates, which are then plated onto micropore filters and cultured undisturbed for several weeks until harvesting for analysis. ATOs induce T-cell differentiation that is remarkably faithful to normal human thymopoiesis with production of all three $CD34^+$ subsets (Thy1, Thy2, and Thy3), as well as ISP4 and DP. Efficient positive selection in ATO cultures leads to spontaneous production of SP8 and SP4 cells [22].

In vitro T-cell differentiation from human PSCs using monolayer systems

The generation of T cells from PSC is a more complex process than from HSPCs, initially requiring the induction of mesoderm specification followed by hematopoietic commitment before the process of T-cell differentiation can begin. The reports of in vitro T-cell differentiation from hPSCs have typically induced these initial stages of specification by applying similar approaches to those used to generate other hematopoietic lineages (Figure 1A) [1,23–31]. The pathway from PSCs to mesoderm is initiated either by generating embryoid bodies or cocultivating PSCs on murine stromal lines (usually OP9), including morphogens that favor mesoderm specification (e.g., BMP4, VEGF, FGF2 ± activin) for the first few days of culture. Cultures are then switched to cytokine conditions that favor hematoendothelial specification; the $CD34^+$ hematopoietic cells produced are then isolated and replated onto OP9-DLL1 or DLL4 stromal monolayers for T-cell differentiation.

In the first report of in vitro T-lineage differentiation from hPSCs, Timmermans et al. [3] elegantly showed that T-cell potential resided in the $CD34^+$ cells isolated from the "hematopoietic zones" seen in OP9 cultures; these zones appeared to be similar to the in vivo blood islands of the embryonic yolk sac. By replating different fractions of the $CD34^+$ isolated from OP9 into T-cell conditions (OP9-DLL1), the investigators found that only the $CD34^{hi}CD43^{lo}$ subset possessed T-cell potential. A subset of the initial $CD34^+CD43^+$ cells harvested from OP9 expressed CD7 but, interestingly, it was only the $CD34^+CD7^-$ fraction that possessed T-cell potential; however, CD7 was rapidly upregulated

when the $CD34^+CD43^+$ cells were replated onto OP9-DLL1 stroma. The same report demonstrated that over the next few weeks, most of the expected early stages of T-cell differentiation could be generated from the PSC-derived $CD34^+$ cells on OP9-DLL1, including ISP4 and DP [3]. Although cytoplasmic expression of CD3 (CyCD3) was readily detected in a majority of cells, cell surface expression of CD3 and TCR$\alpha\beta$ was uncommon, and it was unclear to what extent mature ($CD3^+$) SP8 and SP4 cells were generated.

In a similar approach, Kennedy et al. [32] used embryoid bodies (EBs) from the H1 cell line to initially generate $CD34^+$ cells, which were then replated onto OP9-DLL4 for T-cell differentiation. This group found that at day 9 of EB culture, T-cell potential was restricted to $CD34^+CD43^-$ cells, but by day 11 of EB culture, the T-cell potential was present both in $CD34^+CD43^-$ and $CD34^+CD43^+$ progenitors. Similar to Timmermans et al., the $CD34^+$ cells from EBs produced ISP4 and DP on OP9-DLL4 and, although CD3 expression was detected, evidence of positive selection and production of conventional mature T cells (i.e., SP8 [$CD8ab^+CD3^+$] and SP4) appeared to be limited.

Kennedy et al. also found that inhibition of activin/nodal signaling in EBs using SB 431542 prevented specification of the primitive erythroid lineage, but T-cell differentiation was not affected [32]. These findings led to the conclusion that T-cell potential marked the emergence of definitive hematopoiesis early in EB formation independently of activin/nodal signaling and that the pathway for T-cell differentiation was separate from that of primitive hematopoiesis, which required activin/nodal signaling.

Other groups have used similar approaches to generate T cells from hPSCs. Chang et al. tested the H1 ESC line and iPSCs (from skin fibroblast reprogramming) using both OP9 stroma and EBs to induce hematopoiesis and replated $CD34^+$ onto OP9-DLL4 [33]. Again, efficient production of $CD7^+$ cells and TCR rearrangement was seen, but further positive selection and maturation were not clearly demonstrated.

During normal mammalian fetal development, conventional T cells first appear after the development of the thymus organ, a time point that follows the establishment of definitive hematopoiesis. It has therefore been assumed that production of T cells from PSCs in vitro is evidence that definitive hematopoietic stem cells have been achieved in culture [32]. However, it is likely that lympho–myeloid or lymphoid-restricted progenitors produced in culture harbor T-cell potential, revealed only when the appropriate microenvironmental signals such as notch ligand are provided. Therefore, the nature of the PSC-derived hematopoietic derivatives that can generate T cells when they engage with notch ligand is not clear at this time. A discussion of the notch pathway during hematoendothelial development and arterial specification is provided elsewhere in this special edition [34].

Reprogramming of T cells into iPSCs

The limitations of producing T cells from PSCs in vitro led to several early studies in which iPSCs were reprogrammed from primary T cells to produce so-called T-iPSCs [35–38]. In 2013, Nishimura and Vizcardo [39,40] each took the T-iPSC approach further by reprogramming from a clone of T cells, which possess a single TCR. Nishimura reprogrammed to iPSCs a T-cell clone with a TCR specific to an HIV epitope and then cultured these iPSCs in OP9-DLL1 culture to produce T cells that were functional and showed antigen-specific cytotoxicity. As would be expected, CD3 was coexpressed with the clonal TCR expression; however, because CD8$\alpha\beta$ expression was not reported, it remains unclear whether conventional T-cell differentiation was achieved. Vizcardo et al. [39] generated T-iPSCs reprogrammed from the JFK6 cell line, a clonally derived T-cell line that expresses a class I-restricted TCR specific for the melanoma antigen MART-1. Differentiation of the T-iPSCs first on OP9 and then on OP9-DLL1 led to the production of DP cells, most of which expressed the MART-1 TCR; further differentiation of TCR^+ DP to $CD8^+$ cells was accomplished using stimulation with CD3 antibody. $CD3^+TCR^+CD8^+$ cells were expanded further with CD3/CD28 stimulation [39]; however, although antigen-specific cytokine release was seen, no in vitro or in vivo data on cytotoxicity mediated by the T cells were reported and, again, CD8$\alpha\beta$ data were not reported.

ATO system for conventional mature T-cell production from PSCs

Our group has recently demonstrated that the ATO system can efficiently generate conventional, mature naïve T cells from both ESCs and fibroblast-derived iPSCs (Figure 1B) [41]. Mesoderm specification into $CD326^-CD56^+$ embryonic mesodermal progenitors (EMPs) was induced over 3 days using a modification of the Evseenko protocol [42]. Cells were then aggregated with MS5-DLL4 (or DLL1) and cultured as PSC-ATOs. Sequential changes to the medium induced commitment of EMPs to the hematoendothelial ($CD34^+$VE-cadherin$^{+/-}$) lineage, production of hematopoietic-restricted $CD34^+CD43^+$ and $CD34^+CD45^+$ cells, and finally T-cell commitment and differentiation, all without disruption of the initial ATOs. Surprisingly, T-cell commitment and differentiation in the PSC-ATOs appeared more rapidly than in ATOs derived from primary HSPCs, with production of DP by the first week in T-cell conditions. By week 5, 90% of the cells were $CD3^+TCR\alpha\beta^+$ and, of these, ~80% were CD8SP. Although innate-like CD8$\alpha\alpha$ $CD3^-$ cells were seen transiently, the CD8SP cells that took over the culture were conventional CD8$\alpha\beta$

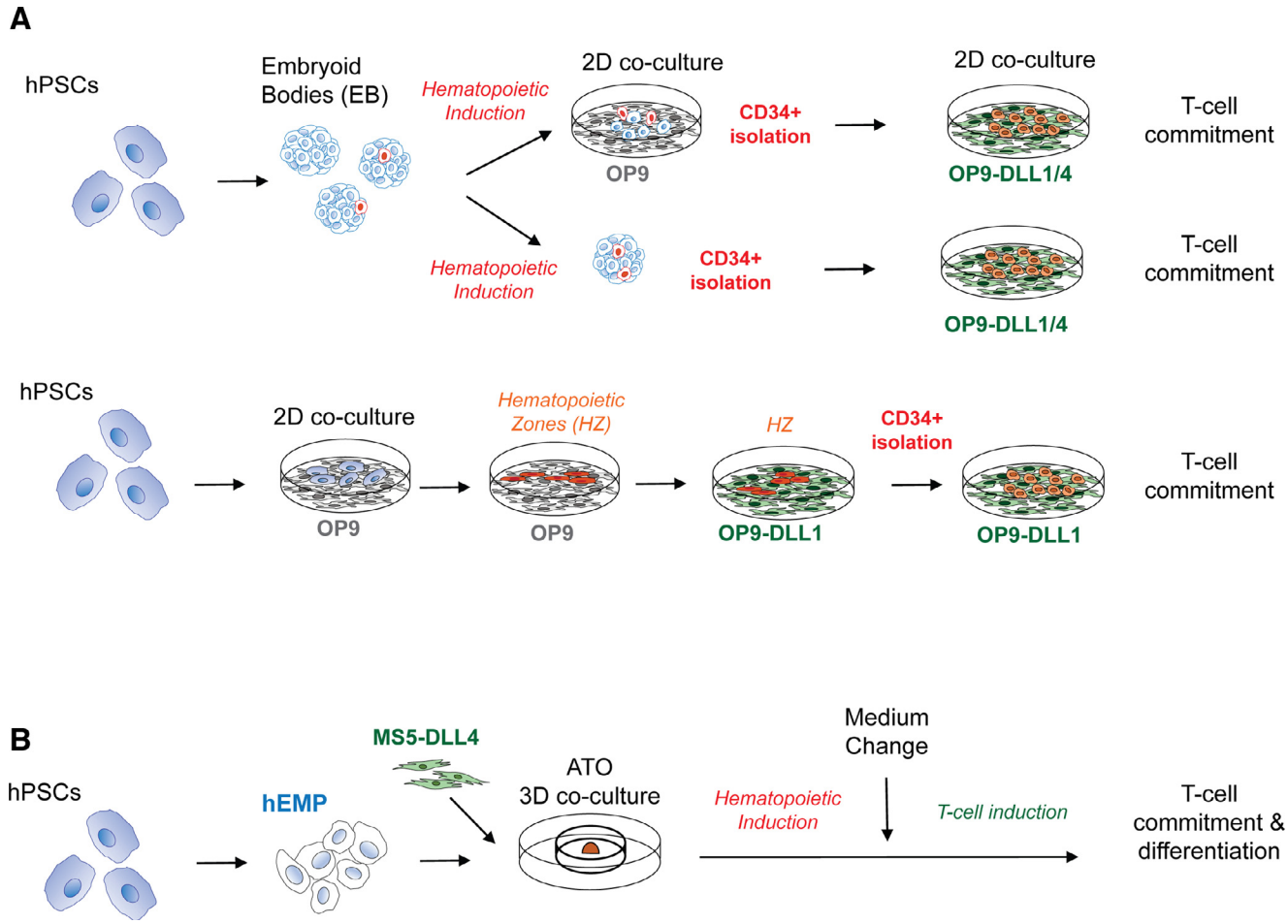

Figure 1. Schema of current approaches for T-cell differentiation from hPSCs. **(A)** Common models of T-cell differentiation from hPSCs consist of a multistep process with the specification of mesoderm by either generating EBs or cocultivating PSCs on OP9 stromal lines in the presence of morphogens. Culture conditions are then changed to favor hematoendothelial induction. The $CD34^+$ hematopoietic cells generated from OP9 cocultures, EBs, or hematopoietic zones are then isolated and replated onto OP9-DLL1 or DLL4 stromal monolayers for T-cell differentiation. **(B)** In the ATO model, mesoderm specification is initiated from PSCs to produce EMPs that are then aggregated with MS5-DLL4 in 3D organoids placed at a liquid–air interface. Hematopoietic specification and T-cell commitment and differentiation into mature naïve $CD3^+$ T cells (either $CD8(ab)^+$ or $CD4^+$) are then induced by simple medium changes.

T cells. The CD8SPs displayed a diverse TCR repertoire and were functional, responding to PMA and ionomycin stimulation by secreting tumor necrosis factor-alpha, IL-2, and interferon-gamma/proliferation becoming activated in response to CD3/CD28 and IL-2 stimulation. Functional, highly diverse $CD3^+CD4$SPs were also produced, although at lower frequencies than in normal thymus. Studies are under way to explore the potential of scaling up the ATO platform to produce a clinically relevant number of T cells under GMP conditions.

RNA sequencing of PSCs, EMPs, and the CD8SPs derived from EMPs showed the orderly transcriptional progression from pluripotency through epithelial–mesenchymal transition and finally to lymphoid-specific gene expression [41]. Genes differentially expressed in CD4 and CD8 cells showed an identical pattern in cells isolated from the thymus to those derived in PSC-ATOs. CD8SP cells harvested from hESC-ATOs and iPSC-ATOs possessed transcriptional profiles that were closely aligned with those of naïve mature $CD8^+$ primary T cells from thymus and peripheral blood.

Interestingly, in recent murine studies, Vizcardo et al. [43] showed that the OP9-DLL1 monolayer system produced DP cells from murine iPSCs and T-iPSCs, but that the CD8 cells produced in monolayer cultures represented an alternative innate-like differentiation pattern based on expression of nonclassical MHC class I and NK associated genes. However, when immature T cells derived from the OP9-DLL1 monolayers were inserted to a modified 3D FTOC, further maturation into conventional SP8s was demonstrated phenotypically and transcriptionally. These studies suggest that the 3D nature of the FTOC was necessary to complete normal positive selection,

although undefined factors supplied by the primary thymus tissue may have also been required for conventional maturation.

Engineered PSC-derived T cells for tumor immunotherapy

The ability to generate T-lineage cells in vitro from PSCs has led logically to attempts to generate cells with antigen-specific cytotoxicity; to date, this has consisted of endowing PSC-derived T cells with receptors to specific tumor antigens using either known TCR$\alpha\beta$ or chimeric antigen receptors (CARs) [38,44,45].

Themeli et al. [44] (Figure 2A) demonstrated that T-iPSCs, when transduced with a lentiviral vector expressing a CAR against the CD19 antigen, could be differentiated (via EB and transfer of CD34$^+$ cells onto OP9-DLL1) into innate-like cells that were transcriptionally similar to gamma delta T cells. Following expansion, the CAR-expressing T cells upregulated CD56 and NKG2D and mediated in vitro killing of CD19$^+$ cells. CD19$^+$ Raji tumor cells were also killed in vivo after intraperitoneal injection of both tumor and CAR T cells. The CAR approach has also been applied to PSC-derived NK cells [46] and is discussed more fully elsewhere in this special issue [47].

Maeda et al. cloned CTLs that expressed a TCR to the WT1 antigen and reprogrammed them into T-iPSCs (Figure 2B) [45]. Culturing of T-iPSCs with OP9-DLL1 induced differentiation to the DP stage; further differentiation to CD8$\alpha\beta$ cells was accomplished by isolating DPs and stimulating them with anti-CD3 antibody using a similar approach to Vizcardo et al. [39]. The WT1-TCR$^+$ CD8$^+$ cells showed antigen-specific killing in vitro, and the survival of immune-deficient mice injected intraperitoneally with WT1$^+$ tumor was

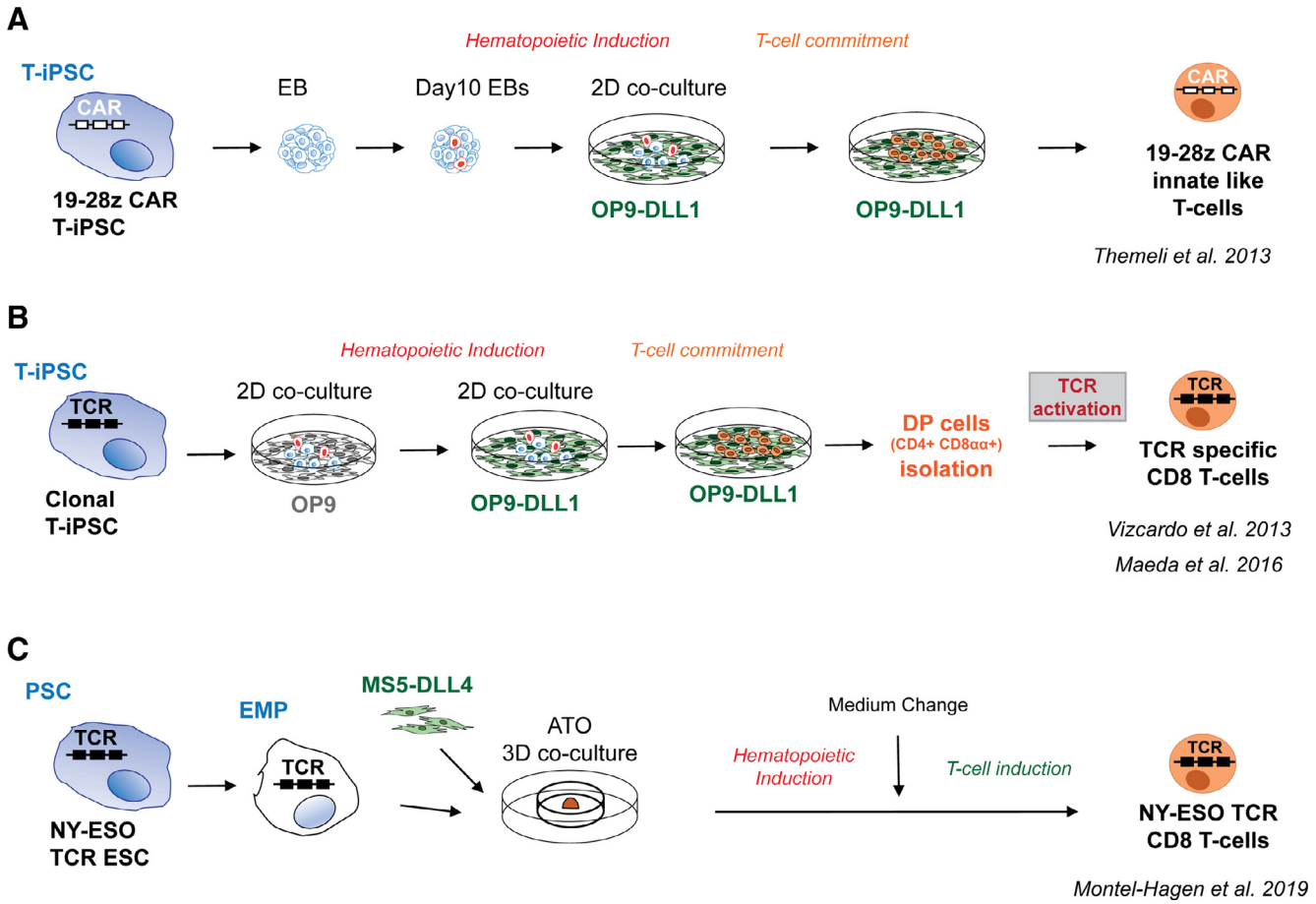

Figure 2. Schemas for the generation of antigen-specific T-lineage cells from PSCs for immunotherapy. **(A)** Lentiviral expression of CAR in PSCs, followed by the differentiation model using EB generation and coculture on OP9-DLL1 monolayers, produces innate-like CAR T cells. This model can be applied to any PSCs including T-iPSCs. **(B)** iPSCs derived from a clone of T cells (T-iPSCs) expressing a specific TCR and differentiated using multistep monolayer coculture on OP9 and OP9-DL1 stromal cells produce CD4$^+$CD8aa DP cells, which, when isolated and activated with anti-CD3, produce CD8ab T cells that express the original TCR of the T-cell clone. **(C)** Lentiviral expression of a class I-restricted TCR (in this case specific for the tumor antigen NYESO-1) TCR in PSCs, followed by the production of EMPs and the generation of ATOs as shown in Figure 1, leads directly to the production of conventional mature NY-ESO TCR$^+$ CD8ab T cells that possess antigen-specific cytotoxicity.

prolonged by intraperitoneal injection of T-iPSC-derived CD8 T cells. Interestingly, the Maeda group showed that sorting of the DPs prior to stimulation was important for success because the DN population that predominated in these mixed cultures killed DPs after stimulation, suggesting the presence of NK or innate-like cells.

We have recently shown that the efficient differentiation of conventional T cells in the ATO system can be used to generate functional conventional CD8$\alpha\beta$ T cells with antigen-specific cytotoxicity (Figure 2C) [41]. hPSCs were stably transduced with a lentivirus encoding a class I (A0201)-restricted TCR specific for an epitope of the NY-ESO-1 antigen, and T cells were generated from PSCs in ATOs using the identical protocol described previously and in Figure 1B [38]. T-cell differentiation from TCR-transduced hPSCs was rapid and efficient; 95% of CD45$^+$ cells expressed TCR$^+$CD3$^+$ from week 1 and CD8$\alpha\beta$ dominated the ATOs by week 5, passing first through the DP stage. CD8SP cells demonstrated antigen-specific cytokine release, proliferation, and activation and expanded more than 100-fold after isolation from the ATOs. After NY-ESO-TCR$^+$CD8SP cells were isolated from ATOs and stimulated in the presence of artificial antigen-presenting cells, they converted from a CD45RA mature naïve to a CD45RO effector memory phenotype. Expanded cells showed vigorous antigen-specific cytotoxicity in vitro and, when injected intravenously into immune-deficient mice, controlled the growth of NYESO-expressing K562 cells in vivo. Forty-eight hours after intravenous injection, NYESO TCR$^+$ CD8$^+$ cells were detectable in the blood and spleen of immune-deficient mice, demonstrating that these PSC-derived T cells were able to circulate and home to distal tissue [41].

Summary

Several studies have now shown that functional tumor antigen-specific T-lineage cells can be generated from PSCs. The monolayer culture systems allow T-cell commitment, but further differentiation occurs predominantly through the innate pathway. Efficient spontaneous production of conventional CD8$^+$ T cells and, to a lesser extent, CD4$^+$ T cells has recently been accomplished using the 3D ATO platform. Scale-up and relative cost will of course be important considerations for whether PSC-derived T cells will ultimately have a role to play clinically in the field of T-cell immunotherapy. However, their unique capacity for unlimited self-renewal makes PSCs particularly appealing for gene modification because optimally engineered clones can be identified, expanded, and fully characterized ahead of use. These characteristics offer a unique opportunity to create an off-the-shelf product suitable for an unlimited number of patients.

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