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Concise Review: In Vitro T-Cell Generation from Adult, Embryonic, and Induced Pluripotent Stem Cells: Many Roads to One Destination

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Abstract

T lymphocytes are critical mediators of the adaptive immune system and have the capacity to serve as therapeutic agents in the areas of transplant and cancer immunotherapy. While T cells can be isolated and expanded from patients, T cells derived *in vitro* from both hematopoietic stem/progenitor cells (HSPCs) and human pluripotent stem cells (hPSCs) offer great potential advantages in generating a self-renewing source of T cells that can be readily genetically modified. T-cell differentiation *in vivo* is a complex process requiring tightly regulated signals; providing the correct signals *in vitro* to induce T-cell lineage commitment followed by their development into mature, functional, single positive T cells, is similarly complex. In this review, we discuss current methods for the *in vitro* derivation of T cells from murine and human HSPCs and hPSCs that use feeder-cell and feeder-cell-free systems. Furthermore, we explore their potential for adoption for use in T-cell-based therapies.

Keywords

Induced pluripotent stem cells; CD34 +; Cell culture; Cord blood; Differentiation; Embryonic stem cells; Hematopoietic stem cells; T cells

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Author Contributions

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Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

Introduction

Hematopoietic Stem/Progenitor Cell-Derived T Cells on OP9-DL Cells

The identification of Notch signaling as a crucial mediator of T-cell development led to dramatically improved methods for in vitro derivation of T cells [1, 2]. At the forefront of these has been a coculture system based on a mouse bone marrow (BM)-derived stromal cell line, called OP9, engineered to overexpress the Notch ligand, Delta-like ligand 1 (Dll-1), and hence termed OP9-DL1. Similar to thymic stromal cells, OP9 cells provide key factors that support T lymphocyte development, including interleukin 7 (IL-7), chemokine (C-X-C motif) ligand 12 (CXCL12), and stem cell factor (SCF). Furthermore, OP9 cells have a dysfunctional *csf1* gene, which would normally support myelopoiesis [3].

Notch activation on hematopoietic stem/progenitor cells (HSPCs) by OP9-DL1 cells first drives their differentiation into T-lineage cells, then stimulates the cells to survive through the different stages of T-cell ontogeny, from $CD4^{-}CD8^{-}$ double negative progenitor T cells to the $CD4^{+}CD8^{+}$ double positive (DP) stage [4]. Ultimately, differentiation achieved using the human pluripotent stem cell (hPSC)/OP9-DL1 coculture system results in a large number of phenotypically and functionally mature conventional single positive (SP) $CD8^{+}$ T cells with a diverse T-cell receptor (TCR) repertoire. In many respects these $CD8^{+}$ T cells are functionally equivalent to *ex vivo* thymus-derived $CD8^{+}$ T cells in response to activating signals while maintaining tolerance for self [5].

The OP9-DL culture system permits the generation of HSPC-derived T cells *in vitro*, serving both as a means to facilitate the study of T-cell differentiation, as well as the potential to produce large numbers of cells for adoptive transfer, which is often a limiting factor [6, 7]. However, Delta-like molecules do not share equal expression, and therefore have varying potentials in terms of activation of the Notch family of proteins [8, 9]. Case in point, targeted deletion of Dll1 in thymic epithelial cells (TECs) has no effect on T-cell ontogeny [10], although its ortholog, Dll4, is the physiological, critical, nonredundant Notch1 ligand expressed in the thymus [11, 12]. Despite the functional differences of Dll1 and Dll4 *in vivo*, OP9-DL1 and OP9-DL4 cells yield similar results when driving T-cell differentiation *in vitro* [13]. The difference between the signaling capacities of Dll1 and Dll4 becomes apparent at limiting levels, where Dll4 appears to be more effective than Dll1 at activating Notch and inducing a T-lineage phenotype [14]. It has a higher capacity to bind Notch1, although unlike Dll1, Dll4 is unable to signal through Notch2 [13]. Hence, while Dll4 may be the preferred Dll to use for early stages of T-cell development, this may change depending on the expression of Notch molecules in the target hematopoietic cells.

While the OP9-DL system was originally created to support T-cell development in the mouse system, it was successfully adapted for use with human umbilical cord blood (UCB)-derived progenitor cells (UCB-HSPCs) [15]. In mice, fetal liver-derived hPSCs possess a higher capacity for in vitro T-cell development than BM HSPCs; similarly, UCB-derived HSPCs ($CD34^{+}CD38^{lo/-}$) cells are also found to generate greater numbers committing to the T-cell lineage, reaching developmental milestones in less time than BM HSPCs [16]. UCB-HSPCs undergo the expected program of human T-cell differentiation and give rise to $CD34^{+}CD7^{+}$ progenitor T cells (pro-T). When allowed to continue differentiating in vitro on

OP9-DL cells, mature SP cells with a strong CD8 bias are generated, with the majority being $CD3^+TCR\alpha\beta^+CD27^+CD1a^-$. These $CD8^+$ cells respond to CD3/CD28 stimulation in a manner similar to ex vivo CD8 SP cells as measured by surface marker modulation, proliferation, and production of proinflammatory cytokines [17].

Both human and allogeneic murine pro-T cells (murine pro-Ts are defined as $CD4^-CD8^-CD25^+$) were able to engraft within the thymus of immune-deficient mice without instigating graft versus host disease (GVHD). While human pro-Ts mature *in vivo* at least through the DP stage, expressing high levels of CD3 and TCRαβ [18, 19], their murine counterparts undergo positive, and more importantly, negative selection. Thus, the host thymus selects T cells that can respond to antigen in the context of the host major histocompatibility complex (MHC), eliminating T cells that could mediate GVHD. Engrafted cells mature with a varied TCR-Vβ repertoire that can respond to stimulation, and do not require cytokine administration to persist in vivo. In preclinical studies, the descendants of the adoptively transferred pro-T cells have been shown to be present 60 days post-transfer, at which point they are not only tolerant but offer protection against infection and tumors [20]. Pro-T cells have the additional advantage of enhancing immune system reconstitution after total body irradiation [21, 22], lessening the duration and intensity of the resulting immunodeficiency. If this also proves true for human pro-T cells, it would be monumental for patients undergoing chemo/radio-therapy, after which the lost T cells could be replenished from an *ex vivo* source.

Alternative Methods to OP9-DL Cells in HSPC-to-T-Cell Differentiation

Murine HSPC Differentiation into T Cells—Apart from OP9 cells, other murine cells have been shown to have varying degrees of success (but not as much as OP9 cells) in inducing T-cell development when forced to express Dll molecules (Table 1). Murine primary stromal cells have also demonstrated a robust ability to support T-cell development, including fetal thymic stromal cells, either in a three-dimensional matrix or in a monolayer. Exposure of human BM-derived HSPC with irradiated murine fetal thymic stromal cells in a three-dimensional matrix in the presence of IL-12 and FMS-like tyrosine kinase 3 ligand (Flt3L) resulted in the generation of mature SP CD4 and CD8 cells [23]. When cultured as a monolayer, thymic stromal cells lose their ability to support T-cell development, as expression of Dll4 is rapidly downregulated. However, ectopic expression of Dll1 or Dll4 on these same primary thymic cultures is sufficient to restore their ability to support T-cell development [24]. Nevertheless, the required reagent, namely the fetal thymic stromal cells, is the limiting factor to broadly applying this technique.

Conversely, primary fibroblasts (pFib) are highly abundant, and when modified to express Dll they can direct Notch-regulated T-cell differentiation [25, 26], despite being unable to produce endogenous cytokines to mediate lymphoid development. Interestingly, unlike OP9-DL cells, pFib-DL/HSPC cocultures do give rise to myeloid cells as well as T-lineage cells. In fact, the system that most closely approaches the efficiency of OP9-DLs appears to be pFib derived from a mouse model with a triple deletion of the myelogenic M-, G-, and GM-CSF genes, but with the addition of cytokines Flt3-L, IL-7, and SCF [26].

Generating such a system using human resources would have great clinical impact. Primary fibroblasts are accessible, and could be used to create an autologous system where both the hematopoietic and the feeder cells expressing Dll could be derived from the same individual, eliminating the possibility of rejection upon transplant. Moreover, they are able to satisfy two distinct interests in generating T cells: (a) they support the generation of pro-Ts that can give rise to mature T cells in vivo when adoptively transferred [25, 26]; and (b) the primary fibroblasts generate mature $CD3^+$ SP CD8 and CD4 T cells in the context of self-MHC. While selection in the context of MHC for specific peptides does occur on OP9-DL/HSPC cocultures [27, 28], creating an individualized feeder system could potentially allow for selection of mature T cells that would specifically recognize and react against particular tumors.

Human HSPC Differentiation into T Cells In Vitro—*In vitro* differentiation of murine T cells has been a valuable tool for investigating T-cell development and has allowed preclinical testing of T-cell-based cellular therapies. Despite its many advantages, OP9-DL cells have not yet been used for clinical purposes because of their mouse origin. To circumvent the potential issue of clinical applicability, two approaches have been taken to develop an effective xenogeneic-free technique for *in vitro* T-cell differentiation. One is to create feeder cells of human origin that can support T-cell development similar to OP9-DL cells. The second is to create a feeder-cell-free culture system where all the molecular requirements to induce T-cell development are provided through addition of defined media, cytokines, and proteins with minimal animal or human products (Table 2).

While the results of murine pFib-DL/HSPC cocultures were very encouraging, it is not clear why the human pFib-DL failed to accommodate T-cell development, despite the addition of macrophage colony stimulating factor inhibitors [25]. In another strategy for the development of a human feeder-cell-based system, primary fibroblasts and human keratinocytes, both primary [28] and immortalized lines [29], were combined and seeded with UCB $CD34^+$ progenitors. When in a three-dimensional matrix, keratinocytes and fibroblasts upregulate Dll4 and IL-7, and with additional IL-7, IL-15, and Flt3L, support T-cell development. However, the difficulty and the inefficiency of this method to generate pro-Ts or functional SPs have resulted in controversy regarding the authenticity and potential of this method [30]. A more consistent method has been the coculture of UCB $CD34^+$ cells on a human TEC line overexpressing murine Dll1 or Dll4 in the presence of IL-7, SCF, and Flt3L. The TEC line supports T-cell expansion and differentiation up to the DP stage [31]. However, there is a decrease in viability after 2–3 weeks in culture, and T-lineage cells do not progress to $CD4^+$ or $CD8^+$ $CD3^+$ SPs, necessitating a different approach to their generation.

Feeder-Free Systems of T-Cell Development—So far, attempts at creating human-derived feeder cells for T-cell development have been met with disappointing results and more research is required to discover the right source of cells. In contrast, there has been a steady improvement of the outcome of the feeder-free system, increasing the clinical translational potential of this method. The essential component of this system has been the Dll1 or Dll4 recombinant protein fused to the Fc portion of human IgG, and hence named

Dll-Fc. When it was first attempted, Dll-Fc was bound to a matrix or plate supplemented with combinations of Flt3-L, IL-7, and SCF. The outcome was inefficient, with the generation of $CD90^+$ cells from BM HSPC [4, 32]. It has since been determined that the IL-7 concentration must be adjusted throughout the hPSC differentiation to T-lineage cells, with high levels at the start of the culture diminishing to lower levels, to allow the DPs to emerge [33]. Other factors that appear to improve the outcome of T-cell differentiation in this system, either with murine or human HSPCs, are the addition of CXCL12 [34], ascorbic acid [35] or Wnt3a [36].

In order to expedite clinical use, one of the goals of the feeder-cell-free method is to use defined media that contain no animal products. Remarkably, this was achieved with the culture of $CD34^+CD38^-$ UCB cells on plates coated with immobilized Dll-Fc fragments in serum-free Stemspan media with the addition of SCF, Flt3L, IL-6, IL-3, thrombopoietin, and low-density lipoprotein [37]. This led to a many-fold expansion of $CD34^+$ cells within 2 weeks of culture and significant improvement in preclinical engraftment into immunodeficient mice. Based on its success, this methodology has been used to clinically investigate the efficacy in repopulating the hematopoietic cells in patients undergoing cord blood transplantation following chemo- and/or radiation therapy. However, clinical trials showed that using $CD34^+$ cells preincubated with Dll1-Fc resulted in a more rapid *myeloid* reconstitution after adoptive transplant, as opposed to T-lineage [37]. This suggested that further improvements are required to enhance T-cell expansion. Nonetheless, the approval of this methodology for clinical trials reinforces the use of Dll-Fc as a viable path to expand and differentiate T cells for clinical applications. In addition, following this method of expansion, human $CD8^+$ T cells were generated *in vitro* with a diverse TCR-Vβ repertoire and selected on antigen-specific peptide loaded tetramers, although only in small numbers [38].

Embryonic Stem Cell and Induced Pluripotent Stem Cell-Derived T Cells

Despite the advantage of using human UCB over BM in its robustness to expand and differentiate *in vitro*, the relatively paucity of UCB and the difficulty of its maintenance in an undifferentiated form in culture are great obstacles to overcome. In contrast, embryonic stem cell (ESC) or induced pluripotent stem cell (iPSC), which are renewable sources of cells, can in theory be perpetually propagated and hence provide an unlimited source for generating hematopoietic cells. Investigations into using ESC or iPSC as an alternative source of stem cells for the *in vitro* generation of T cells have been ongoing in the mouse system with some recent successes in the human system (Figure 1).

Shortly after its advent, the OP9-DL1 coculture system was adapted to generate T cells from mouse ESCs *in vitro*. With modification of ESC passaging protocols and the timed addition of first Flt3L and then IL-7, cells cultured in this manner completed a normal course of T-cell differentiation, eventually resulting in mature, functional $CD8^+$ SP cells by day 22. When implanted into immunodeficient hosts, pro-T cells were able to complete differentiation into mature CD4 and CD8 SP cells and provide protection against viral challenge [39]. Addition of tetramers to the coculture was able to induce generation of

antigen-specific mature $CD8^+$ T cells, which—despite being a small percentage of the total hematopoietic cells— were able to induce activated caspase 3 expression in target cells [40].

In vitro differentiation of human ESC to T cells could not be readily established in either OP9-DL1 coculture or fetal thymic organ cultures, despite the successes of these protocols when used with human UCB- or BM-derived HSPC and mouse ESCs. However, a combination of initial culture on OP9 cells followed by in vivo differentiation in human thymic tissue in an immunodeficient mouse model allowed ESCs to fully differentiate into mature T cells [41]. Comparison of human ESC with $CD34^+$ UCB suggested that the human ESC gene expression program strongly favored the NK lineage at the expense of the T lineage [42]. Further investigation of human ESC cultured on OP9 cells led to the discovery of T-lineage potential in human ESC, specifically the $CD34^+CD43lo$ fraction, when allowed to form hematopoietic zones. When transferred to OP9-DL1 cultures, these cells gave rise to $CD3^+TCR\alpha\beta^+$ or $TCR\gamma\delta^+$ T cells that were functionally mature [43].

With the discovery of induced pluripotency came renewed efforts in the development of methods for *in vitro* generation of T cells from iPSC. Mouse iPSCs were successfully differentiated to mature T cells *in vitro*, beginning with an embryonic fibroblast-derived iPSC line cultured on OP9-DL1 cells in the presence of IL-7 and Flt3L [44]. In contrast with other adult or ESCs, iPSCs derived from either fibroblast or lymphoid cells demonstrate a bias for the T lineage over the B lineage in this culture system [45]. This was followed by relatively well-defined and successful differentiation strategies that directed human iPSCs to generate definitive-like HSPC and subsequently T-cell differentiation on OP9-DL4 cells. Inhibition of the Activin/Nodal pathway and stimulation of the Wnt pathway during mesoderm specification greatly enhanced T-cell potential of this method as defined by $CD34^+$ cells having the capacity to produce $CD45^+CD43^+CD7^+CD5^+$ pro-T cells [46].

The success of these methods to differentiate iPSCs into T cells provided the opportunity to exploit these techniques to potentially achieve two very important goals of immunotherapy: (a) the genetic manipulation of iPSCs to correct mutations that result in immunodeficiencies, as exemplified by the correction of a SCID-X1 mutation in a human iPSC line [32]; and (b) the ability to create iPSCs from T cells with Ag-specificity that could be clinically effective, virtually guaranteeing an unlimited supply of antigen-specific T cells. Several groups have reported deriving human iPSCs from mature peripheral blood T cells [47–49] with unique TCRs. iPSC-derived T cells displayed a broad T-cell repertoire [50] and retained antigen specificity at the DNA level, although this specificity was sometimes lost during differentiation as a result of RAG-mediated recombination. Prevention of undesired recombination events required stimulation of TCR signaling using anti-CD3/CD28 mAb-coated beads in the presence of exogenous cytokines [51]. Furthermore, these cells expanded up to 1,000-fold in response to cytokine signaling, maintained a memory T-cell surface antigen expression profile, and produced cytolytic molecules to lyse target cells in an antigen-specific manner [52].

At this point, the most viable autologous anti-cancer T-cell-based therapies are chimeric antigen receptor (CAR) and TCR gene transfer technology into mature, functional $CD8^+$ T cells. Most often, mature T cells are harvested from peripheral blood or tumor infiltrating

lymphocytes, activated and transduced with a CAR or tumor-specific TCR *in vitro* to confer antigen specificity, and adoptively transferred back to the patient. The autologous nature of this procedure ensures that there is no GVHD unless the antigen is present at extratumor sites. This attractive feature is offset by obstacles to the successful clinical implementation of this method, which necessitates long-term T-cell survival and function *in vivo* and potential need for additional autologous T-cell products over time for repetitive T-cell infusions, if needed to control tumor burden [53].

Clinical Possibilities for In Vitro-Derived T-Cell Precursors and Conclusion

Continued advancement in *in vitro* production of human T cells will have a great impact in ameliorating the prospects of patients with immunodeficiencies, congenital or otherwise, or for those undergoing chemo-/radiotherapy. It is conceivable that such systems could lend themselves to the production of T cells for the reversal of autoimmunity, but these possibilities have yet to be investigated. Much success has been achieved using progenitors purified from human UCB, which are already routinely used in hematopoietic stem cell transplantation [54], but represent a much more finite source of starting material. If a feeder-cell-free process of pro-T-cell generation from iPSCs can be actuated, refined, and scaled up to provide large quantities, the hope is that the advent of a curative method for T-cell adoptive transfer could be realized. Unlike CAR-based immunotherapy, pro-T cells do not require an MHC match between the donor and recipient or the disruption of the endogenous TCR, permitting universal clinical application. Upon maturation, an appropriate subset can become central memory T cells, which show the greatest potential for long-term *in vivo* persistence [55]. For immunotherapy, the expectation is that hPSCs derived from iPSC culture systems can lend themselves well to genetic manipulation, either to correct deficiencies, or to express exogenous designer TCRs to counter cancer. Creative approaches have been taken to improve the timing of CAR expression; for example, the restricted expression of a CAR reactive against hCD19 for anti-B cell-leukemia response until after negative selection, enabled complete and long-term protection against a hCD19-expressing syngeneic tumor [56]. The incorporation of CTLA-4 or PD-1-based inhibitory CARs, promoting self-regulation of cytokine secretion, proliferation, and cytotoxicity, are strategies that have been taken to address complications or inadequate specificity of engineered T cells [57]. However, iPSC-derived cells are just now entering the clinic, and their long-term safety is unknown. Methods to permit high levels of proliferation and robust differentiation to T-lineage cells are imperative since these immunotherapies must undergo rigorous clinical testing to reveal their safety and benefit. Although first-in-human trials of pro-Ts using OP9-DL1 cells are being considered for obtaining proof-of-principle that pro-Ts can speed immune recovery in patients, approval of clinical translation would be facilitated by the development of effective differentiation techniques independent of feeder cells or animal products. While such techniques are in their beginning stages [58, 59] and cannot yet support robust and efficient T-cell differentiation, they are being improved and will hopefully unlock the immense potential of immunotherapy.

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Significance

This concise review covers recent advances in the in vitro generation of T cells from adult, embryonic, and induced pluripotent stem cells. Today's leading techniques are described and evaluated on the basis of cell yield, cell function, and ease of translation to the clinic. The potential use of in vitro-derived T cells as therapeutic agents is discussed. This review is an unbiased survey of current advances and obstacles in the field, and as such is a valuable resource for those conducting related research.

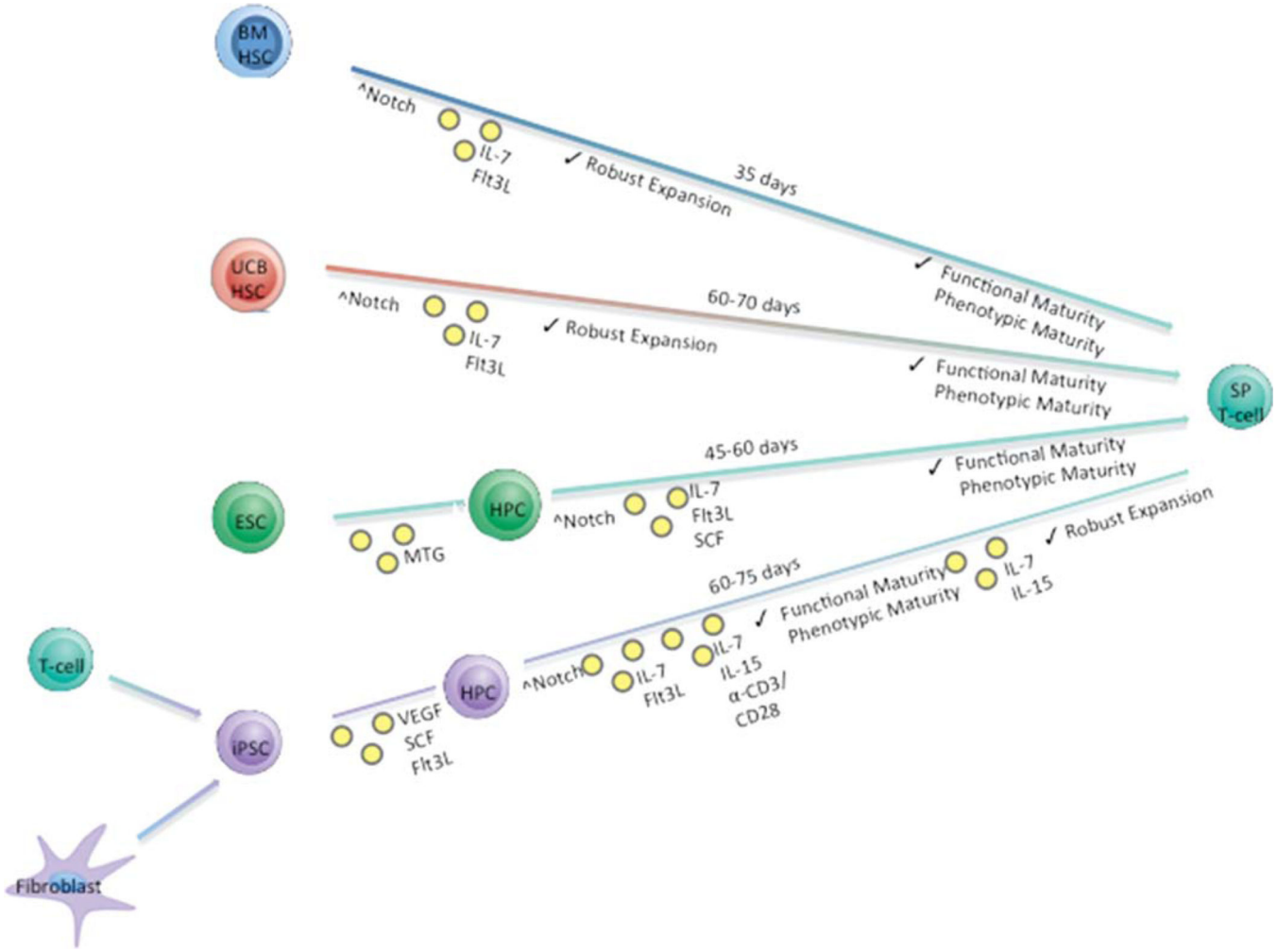

Figure 1.

Simplified *in vitro* differentiation schema describing the approximate time needed to generate mature SP T cells from four starting progenitor cell populations. The relative timing of Notch signaling induction and the addition of various cytokines are also depicted, along with the proliferative potential induced by each method and general characterizations of the resulting SP T cells obtained from each culture condition. Abbreviations: BM, bone marrow; ESC, embryonic stem cell; Flt3L, FMS-like tyrosine kinase 3 ligand; IL, interleukin; iPSC, induced pluripotent stem cell; MTG, monothioglycerol; SCF, stem cell factor; SP, single positive; UCB, umbilical cord blood; VEGF, vascular endothelial growth factor.

Table 1

Summary of culture conditions using mouse cells as a starting source

	Feeder cells	Supplements		Media	Duration	Functional SP?	Reference
0P9-DL							
BM-HSPC	0P9-DL	IL-7	Flt3L	α-MEM + 20% FBS	5 weeks	Yes	Schmitt [6]
FL-HSPC	OP9-DL	IL-7	Flt3L	α-MEM + 20% FBS	5 weeks	Yes	Schmitt [6]
ESC	OP9 0P9-DL	IL7 IL-15	Flt3L	α-MEM + 20% FBS	3 weeks	Yes	Schmitt [39]
iPSC	OP9-DL	IL-7	Flt3L	α-MEM + 20% FBS	3 weeks	Yes	Lei [44]
pFib-DL							
FL-HSPC	Primary fibroblast-DL	IL-7 SCF	Flt3L GW2580	α-MEM + 15% FBS	2 weeks	Yes	Mohtashami [25]
Dll-Fc							
FL-HSPC	–	IL-7 SCF	Flt3L	RPMI + 10% FBS	2 weeks	Yes	Ikawa [33]

Abbreviations: BM-HSPC, bone marrow-derived hematopoietic stem progenitor cells; DL/Dll, delta-like ligand; ESC, embryonic stem cell; FBS, fetal bovine serum; FL-HSPC, fetal liver-derived hematopoietic stem progenitor cells; Flt3L, FMS-like tyrosine kinase 3 ligand; IL, interleukin; iPSC, induced pluripotent stem cell; α-MEM, minimum essential medium α; pFib, primary fibroblast; RPMI, Roswell Park Memorial Institute medium; SCF, stem cell factor; SP, single positive.

Table 2

Summary of culture conditions using human cells as a starting source

	Feeder cells	Supplements		Media	Duration	Functional SP?	Reference
0P9-DL							
UCB-HSPC	OP9-DL	IL-7	FIt3L	α-MEM + 20% FBS	9–10 Weeks	Yes	Awong [17]
ESC	OP9 0P9-DL	Ascorbic acid MTG transferrin BMP4 bFGF Activin A SB VEGF	Dkk IL-6 IGF-1 IL-11 SCF EPO TPO IL-3 Flt3L	Stem Pro 34	4 weeks	Yes	Kennedy [46]
iPSC	0P9 OP9-DL	Ascorbic acid MTG transferrin BMP4 bFGF Activin A SB VEGF	Dkk IL-6 IRF-1 IL-11 SCF EPO TPO IL-3 Flt3L	Stem Pro 34	4 weeks	No	Kennedy [46]
FTSC							
BM-HSPC	Fetal thymic stromal cells	IL-2 IL-7 IL-12	Flt3L SCF	IMDM + 20% FBS	5 weeks	Yes	Freedman [23]
pFib-DL							
UCB-HSPC	Primary fibroblast-DL	IL-7 SCF	FIt3L	α-MEM1 20% FBS	5 weeks	Yes	Mohtashami [25]
pFib							
UCB-HSPC primary fibroblast	Keratinocytes	IL-7 IL-15	FIt3L	DMEM + 10% FBS	3–4 weeks	No	LaPenna [29]

	Feeder cells	Supplements		Media	Duration	Functional SP?	Reference
DII-Fc							
UCB-HSPC	-	IL-7 SCF TPO	Flt3L ascoribic acid	α-MEM + 20% FBS	3–4 weeks	No	Huijskens [35]

Abbreviations: bFGF, basic fibroblast growth factor; BM-HSPC, bone marrow-derived hematopoietic stem progenitor cells; BMP, bone morphogenic protein; Dkk, Dickkopf protein; DL/Dll, delta-like ligand; DMEM, Dulbecco's modified Eagle's medium; EPO, erythropoietin; ESC, embryonic stem cell; FBS, fetal bovine serum; Flt3L, FMS-like tyrosine kinase 3 ligand; FTSC, fetal thymic stromal cells; IGF, insulin-like growth factor; IL, interleukin; IMDM, Iscove's Modified Dulbecco's Medium; iPSC, induced pluripotent stem cell; α-MEM, minimum essential medium α; MTG, monothioglycerol; pFib, primary fibroblast; RPMI, Roswell Park Memorial Institute medium; SB, SB-431542; SCF, stem cell factor; SP, single positive; TPO, thrombopoietin; UCB-HSPC, umbilical cord blood-derived hematopoietic stem progenitor cells; VEGF, vascular endothelial growth factor.