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REVIEW ARTICLE

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In vitro Production of Dendritic Cells as Cancer Immunotherapy: Highlights on Sample Source, Culture Period, Differentiation and Maturation Cytokines

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Dendritic cells (DC) are antigen-presenting cells between innate and adaptive immune cells and commonly used as immunotherapy. Despite this promising potential, protocols detailing the specifics of the DC production are varied, affecting the potency of dendritic as immunotherapy. There are various factors affecting the production and DC potency, such as sample source, culture period, differentiation and maturation cytokines. Due to the limited number and quality of DC in humans, the monocyte could be isolated and differentiated to mature DC. The purity and viability monocytes shall be maintained to produce a high yield of DC. Negative sorting maintains the potency of DC as a therapeutic agent. Monocytes from umbilical cord blood (UCB) are naïve and can be differentiated to DC easily. Meanwhile, the tumor microenvironment (TME) may inhibit DC maturation from monocyte-derived peripheral blood. Without pro-inflammatory cytokines and a short maturation period, DC remain immature and fails to activate T cells. Long-period culture correlates with decreased DC viability and function. This review outlines several factors which can produce higher cytotoxic T cells and pro-inflammatory cytokines that might help each facility in developing its protocol to ensure the best procedure in DC production. Increasing purity and yield through close and automatic system under GMP production are mandatory to decrease risk of contamination during DC production.

Keywords: *differentiation cytokines, maturation cytokines, culture period, sample source, isolation technique*

Introduction

Cancer is one of the leading causes of death worldwide, with 49.3% of incidence occurring in Asia.[1] The immune system fails to defend against cancer cells because of high-concentration of pro-inflammatory cytokines and activation of T regulatory cells in tumor microenvironment (TME).[2] In cancer patients, the immune system fails to defend against viral infection because of high-concentration pro-inflammatory cytokines. Several pro-inflammatory cytokines such as chemokine (C-C motif) ligand 18 (CCL18), interleukin (IL)-6/8/10/18, tumor growth factor (TGF)-β, vascular endothelial growth factor (VEGF), interferon (IFN)-γ, and IL-1β accumulate in the environment of

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TME.[2] TGF-β and IL-10 cytokines play a role in activating regulatory T cells and tumor-associated macrophage (TAM), resulting in immunosuppressive TME and causing tumor cells to evade the immune system.[2] Furthermore, there was a change in the expression of surface markers from tumor cells that inhibit the dendritic cells (DC) maturation through the Fas/FasL mechanism and activate apoptosis signaling in DC.[3]

Despite it is commonly used as a treatment, chemoradiation may cause side effects such as fatigue, vomiting, nausea, dermatitis, stomatitis, diarrhea, weight loss, leukopenia, neutropenia, thrombocytopenia, anemia, and ototoxic.[4-8] The current goal of cancer treatment is to provide a target-specific immune system with high efficacy and safety, diminishing the chemoradiation effect.[9] DC have the potential to present antigens and mediating crosstalk between innate and adaptive immune responses through major histocompatibility complex (MHC).[10] The antigen-presenting MHC-I complex activates cytotoxic T cells (CD8), while MHC-II activates helper T cells (CD4) as memory immune cells to fight future similar viral infections.[11] Currently, there are about 343 clinical trials on DC for cancer-based on the website ClinicalTrials.gov (as of May 2023), indicating the trending of DC as immunotherapy. Most studies directly compared the efficacy of DC as immunotherapy in clinical application. However, the results vary within similar cancer cases, indicating there are unnoticeable factors affecting the stimulation of powerful DC. Moreover, the response rates of DC as anti-tumor immunity are not fully elaborated. Several factors that may affect the *in vitro* production of those DC including sample sources, isolation techniques, differentiation and maturation cytokines, and culture time. This review briefly describes several factors affecting the production and potency of dendritic cells.

In vitro production of dendritic cells

Due to limited potency of patient's immune cells, another alternative shall be proceeded to increase the efficacy of immunotherapy. Dendritic cells can be differentiated from its source such as monocyte that can be isolated from umbilical cord blood (UCB) and peripheral blood (PB) with different concentration. By adding certain pro-inflammatory cytokines to the culture for certain period, monocyte will differentiate to potato-shaped structure with ability to attach on the plastic surface. Additional maturation cytokine and antigen specific cancer from tumor lysate or personalized nucleic acid pool promote maturation of immature dendritic cells for up to 48 hours (Figure 1). Several tests shall be conducted before mature DC injection to ensure safety and efficacy of product to patient. The absence of contamination can be tested using sterility, endotoxin, and mycoplasma. Cell count, viability, and phenotype of positive mature DC provide additional information about the quality of DC product.[12]

The most well-known maturation markers on the surface of mature DC are CD83, CD80, and CD86 which act as costimulatory molecules for complete T-cell activation. When they were monocytes, positive surface markers were CD14 and CD54, while negative surface markers included CD80, CD83, CD86, HLA-DR, and CD40. During the differentiation process into immature dendritic cells, there was a decrease in the CD14 marker, while there was an increase in the CD83, CD80, HLA-DR, CD40, and CD86 markers.[13] There is no official regulatory standard in the world for the minimal targets of surface markers and the resulting cytokines. However, some dendritic cell manufacturers for cancer use a minimum specification of positive surface markers of 60-70% for CD80, CD83, CD86, HLA-DR, while a maximum of 20% for negative surface markers such as CD14 obtained from internal validation data.[12,14]

The long culture process and processing in a non-standardized environment can be the cause of failure in DC production. Washing and changing medium can cause contamination in open system culture.[15] High lymphocytes contamination can be introduced during adherent culture, which decreases after 2 hours incubation and two times washing.[15] The use of a closed system equipment at the time of separation, GMP-grade reagents, and cell culture carried out at a Good Manufacturing Practice (GMP) facility can provide high and pure DC concentration, high turn-over time, and low risk of contamination.[12,14,15] The use of single-use disposable system such as collection bag and tubing also limit the risk of contamination during DC production.[15] In addition, the success of DC production is influenced by the selection of samples, cell types, isolation technique, and specific antigens for differentiation and maturation.

Monocytes sources

DC can be isolated directly from UCB and PB. However, only <1.0% of DC can be isolated from healthy adult PB,

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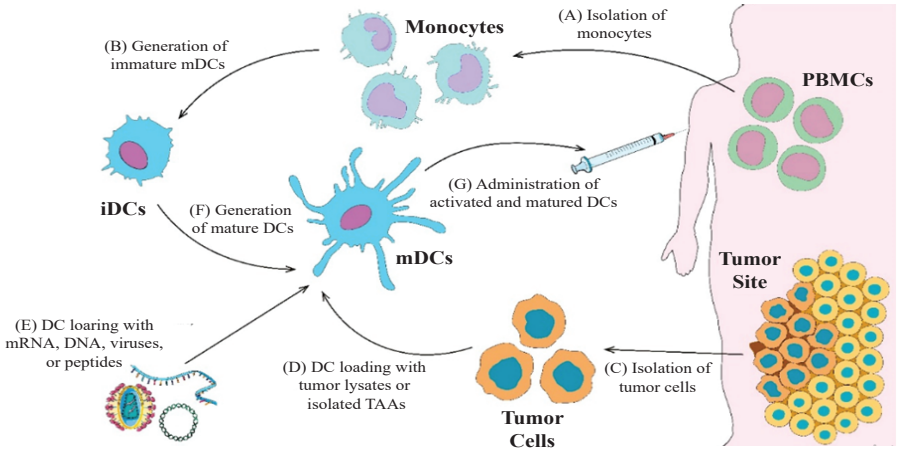

Figure 1. Monocytes (A) can be isolated from peripheral blood or umbilical cord blood and matured by addition of patient tumor lysate (B-D) or personalized mRNA, DNA, viruses or peptides (E); the mature DCs (F) will be injected to patient after pass quality control testing (G).(44) (Adapted with permission from MDPI).

but lower in cancer patients.[16] Therefore, DC precursor, such as monocytes, should be isolated and differentiated *in vitro* before being administered to patients.

Monocyte cells are the most commonly used source for DC differentiation compared to another source such as CD34. The isolation procedure is simpler, does not require a long culture time, and has fewer adjuvants than $CD34^+$ cells. For maturation, $CD34^+$ cells take 7-12 days to differentiate using granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-4, and stronger pro-inflammatory cytokines such as stem cell factor (SCF), FMS-like tyrosine kinase 3 (Flt3L), thrombopoietin (TPO), TNF-α, IL-3, IL-4, and IL-6.[10,13,17,18] Meanwhile, monocytes only take 5-6 days for maturation.[19-23] There are approximately 5.28% and 7.91% monocytes that can be isolated from adult PB and UCB, respectively.[16] Current trend still uses autologous peripheral blood as DC immunotherapy as summarized in Table 1. There are no differences in surface markers between mature DC differentiated from UCB and PB.[19,20,24-26] Adult PB contained significantly higher monocytes and CD40 surface marker than UCB.[21] However, another researcher found that CD40 marker is higher in UCB than PB.[26] This co-stimulation has important role as one of the early signals required for the eagerness of DC to making contact with T cells.[22] The presence of CD40 can upregulate the expression of other costimulatory molecules such as CD80 and CD86.

In the midst of debate about higher surface markers between UCB and PB, the quality of DCs shall be prioritized in activating cytotoxic T cells. Detection of DC potency is measured by cytotoxic T cells number or released cytokine. DC-derived from UCB had lower T cells activation capability than DC-derived from PB, detected by lower T cell number at the end of co-culture.[20,24] However, another research states there is no significant differences in T cell number.[21,26] Besides cytotoxic T cell number, the potency of DC can be measured from released cytokine after DC-T cells activation. UCB can produce higher IL-12p70 and IL-1β compared to PB, but levels of IL-6, IL-8 and IL-10 are similar.[20,27,28] A higher concentration of IFN-γ is released in UCB-derived DC than PB-derived DC.[19,26] Both UCB and PB can produce mature DC, but it is necessary to investigate the potency of DC and not solely depend on the surface markers detection. More effort shall be implemented for UCB-derived DCs, since UCD-derived DCs have not been introduced to 'danger signal', unlike PB-derived DCs.

Monocytes isolation technique

Gradient Centrifugation

Isolation technique may affect the amount and potency of DC. Monocyte from UCB and PB can be isolated efficiently using two common techniques such as gradient centrifugation and magnetic sorting. Gradient centrifugation separates mononuclear cells (MNCs) from other blood components based on the density using specific media such as Ficoll® Paque Plus.[13] Erythrocytes and polymorphonuclear cells are deposited at the bottom of the tube, followed by Ficoll, buffy coat, and platelet. The buffy coat layer includes B and T lymphocytes, monocytes, NK cells, and dendritic cells. This buffy coat is taken and cultured into culture dish for 2 hours. Monocytes attach to the culture flask while other components remain in supernatant such as B and T lymphocytes, NK cells, and DC.[13,23,29] This method is inexpensive compared to magnetic sorting. Monocytes isolated with gradient centrifugation produce higher CD80 and CD83 compared to positively isolated monocytes.[27]

Table 1. Different protocol in dendritic cells production.

Sample Source	Isolation Technique	Cytokine Differentiation	Cytokine Maturation	Culture period	Markers Detection	References
Peripheral Blood	Gradient Centrifugation	- GM-CSF (50 ng/mL) - IL-4 (1000 U/mL)	- TNF-α (10 ng/mL) - CD-40L (Not mentioned)	6 days differentiation + 1 day maturation	CD1a, CD1c, CD1b, CD14, HLA-ABC, CD11c, CD40	66
Peripheral Blood	Gradient Centrifugation	- GM-CSF (600 U/mL) - IL-7 (6 U/mL)	Not mentioned	7 days culture in total	HLA-DR, CD1a, CD11c, CD23, CD40, CD54, CD58, CD80, CD86, CD95, CD23, CD21	39
Peripheral Blood	Gradient Centrifugation + $CD14^-$ Beads	- GM-CSF (500 U/mL) - IFN-α (1000 U/mL)	LPS (1 μg/mL)	3 days differentiation + 2 days maturation	CD14, CD11c, CD54, CD80, HLA-DR, CD95, CD1a, CD40, CD86, Fas-L (NOK-1), CD83	35
Peripheral Blood	Gradient Centrifugation + $CD14^+$ Beads	- GM-CSF (100 ng/mL) - IFN-α (500 IU/mL)	CD40L (Not mentioned)	5 days differentiation + 2 days maturation	CD40, CD54, CD1a, CD14, CD58, CD80, CD83, HLA-DR, HLA-ABC	54
Peripheral Blood	Gradient Centrifugation + $CD14^+$ Beads	- GM-CSF (50 ng/mL) - IL-4 (10 ng/mL) and IL-2 (100 U/mL)	LPS (200 ng/mL)	5 days differentiation + 2 days maturation	CD1a, CD25. HLA-ABC, CD80, CD86, CD83, HLA-DR, CD14, CD11c	46
Peripheral Blood	Gradient Centrifugation	- GM-CSF (800 U/mL) - IL-4 (500 U/mL)	- IFN-γ (5000 U/mL) - IL-1β (10 ng/mL) and Poly I:C (20 ng/mL) - TNF-α (10 ng/mL)	5 days differentiation + 2 days maturation	CD83, CD80, CD86, HLA-DR	19
Peripheral Blood	$CD14^+$ Beads	- GM-CSF (800 U/mL) - IL-4 (1000 U/mL)	LPS (20 ng/mL)	6 days differentiation + 2 days maturation	CD1c, CD14, HLA-DR, HLA-ABC, CD1a, CCR7, CD11b, CD11c, CD40, CD80, CD83, CD86	60
Peripheral Blood	Gradient Centrifugation + $CD14^+$ Beads	- GM-CSF (400 U/mL) - IL-4 (250 U/mL)	- TNF-α (50 ng/mL) - IL-1β (25 ng/mL) - poly I:C (20 ng/mL) - IFN-γ (100 ng/mL)	6 days differentiated (Not mentioned maturation days)	CD1a, CD14, CD11c, CD1c, CD86, CD83, CD80, CD40, HLA-DR, HLA-ABC, CCR1, CCR2, CCR5, CCR7, CXCR4	67
Peripheral Blood	Gradient Centrifugation + $CD34^+$ Beads	- SCF (20 ng/mL) and GM-CSF (50 ng/mL) - IL-4 (50 ng/mL) and Flt-3L (50 ng/mL)	TNF-α (25 ng/mL)	4 weeks culture in total	CD1a, CD86, CD80, HLA-DR, CD14, CD83, CD40	68

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Table 1. Different protocol in dendritic cells production (cont.).

Sample Source	Isolation Technique	Cytokine Differentiation	Cytokine Maturation	Culture period	Markers Detection	References
Peripheral Blood	Gradient Centrifugation	- GM-CSF (50 ng/mL) - IL-4 (100 ng/mL)	LPS (100 µg/mL) or IFN-γ (10 ng/mL)	6 days differentiation + 1 day maturation	CD14, CD11c, CD80, HLA-DR	20
Peripheral Blood	Gradient Centrifugation	- GM-CSF (50 ng/mL) - IL-4 (10 ng/mL)	Not mentioned	6 days culture in total	CD83, HLA-DR	45
Peripheral Blood	Gradient Centrifugation + $CD14^+$ Beads	- GM-CSF (200 ng/mL) - IL-4 (1000 U/mL)	- TNF-α (100 ng/mL) - IFN-γ (500 U/mL) - IL-1β (5 ng/mL) and IFN-α (3000 U/mL) - Poly I:C (1µM)	24-36 hours differentiation + 18 hours maturation	HLA-DR, CD14, CD11b, CCR7	37
Peripheral Blood	Gradient Centrifugation + $CD14^+$ Beads	- GM-CSF (1000 IU/mL) - IL-4 (500 IU/mL)	- TNF-α (1000 IU/mL) - IL-6 (1000 IU/mL) - IL-1β (1000 IU/mL) - PGE2 (1 µg/mL)	5 days differentiation + 1 day maturation	CD14, CD11c, CD80, CD86, CD83, HLA-DR, CD40	48
Peripheral Blood	Gradient Centrifugation + $CD14^-$ Beads	- GM-CSF (100 ng/mL) - IL-4 (20 ng/mL)	- IL-1β (10 ng/mL) - TNF-α (10 ng/mL) - IL-6 (1000 U/mL)	2 days differentiation + 1 day maturation	CD1a, CD11b, CD11c, CD18, CD14, HLA-ABC, CD38, CD83, CD40, CD86, CD80, HLA-DR, CD197, CD274, CD273	42
Peripheral Blood	Gradient Centrifugation + $CD14^+$ Beads	- GM-CSF (1000 U/mL) - IL-4 (500 U/mL)	- IL-1β (10 ng/mL) - TNF-α (50 ng/mL) - IFN-γ (100 ng/mL) - LPS (100 ng/mL)	5 days differentiation + 3 days maturation	CD40, CD80, CD86	69
Peripheral Blood	Gradient Centrifugation	- GM-CSF (1000 U/mL) - IL-4 (20 ng/mL)	- TNF-α (120 ng/mL) - IL-1β (120 ng/mL) - PGE2 (20 ng/mL)	7 days differentiation + 1 day maturation	HLA-DR, CD14, CD1a, CD25, CD83, CD80, CD86	70
Peripheral Blood & Umbilical Cord Blood	Gradient Centrifugation	- GM-CSF (100 ng/mL) - IL-4 (5 ng/mL)	- TGF-β1 (0.5 ng/mL) - Flt3 (25 ng/mL)	7 days of culture in total	CD1a, CD80, CD14, CD83, CD40	24
Peripheral Blood	Gradient Centrifugation + $CD14^+$ Beads	- GM-CSF (800 U/mL) - IL-4 (1000 U/mL)	- Poly I:C (20 µg/mL) - TNF-α (1000 U/mL) - IFN-α (3000 U/mL)	6 days differentiation + 1 day maturation	CD1c, CD14, HLA-ABC, CD1a, CCR7, CD11b, CD11c, CD40, CD80, CD83, CD86, DC-SIGN, HLA-DR	47

Table 1. Different protocol in dendritic cells production (cont.).

Sample Source	Isolation Technique	Cytokine Differentiation	Cytokine Maturation	Culture period	Markers Detection	References
Peripheral Blood & Umbilical Cord Blood	Gradient Centrifugation	- GM-CSF (50 ng/mL) - IL-4 (30 ng/mL) - TNF-α (50 ng/mL)	- TNF-α (100 ng/mL) - LPS (100 ng/mL) - CD40L (100 ng/mL)	3 days differentiation (GM-CSF + IL-4) + 4 days differentiation (GM-CSF + IL-4 + TNF-α) + 2 days maturation	CD1a, CD11c, CD40, CD58, CD54, CD80, CD83, CD86, HLA-DR, HLA-ABC	26
Peripheral Blood	Gradient Centrifugation + $CD14^+$ Beads	- GM-CSF (500 UI/mL) - IL-4 (500 UI/mL)	LPS (0.5 µg/mL)	4 days differentiation + 1 day maturation	CD80, CD86, CD209, CD14	18
Umbilical Cord Blood	Gradient Centrifugation + $CD14^+$ Beads	- GM-CSF (800 IU/mL) - IL-4 (1000 IU/mL)	IFN-γ (1000 IU/mL)	7 days differentiation + 12 hours maturation	CD1a, CD14, CD83, CD40	71
Umbilical Cord Blood	$CD14^+$ Beads	- GM-CSF (800 U/mL) - IL-4 (500 U/mL)	- IFN-γ (5000 U/mL) - IL-1β (10 ng/mL) and Poly I:C (20 ng/mL) - TNF-α (10 ng/mL)	5 days differentiation + 2 days maturation	CD83, CD80, CD86, HLA-DR	19
Umbilical Cord Blood	Gradient Centrifugation + $CD34^+$ Beads	- Flt-3L (25 ng/mL) - TPO (10 ng/mL), SCF (20 ng/mL), IL-4 (20 ng/mL), and GM-CSF (50 ng/mL)	- TNF-α (30 ng/mL) - CD40L (100 ng/mL) - CCL-19 (500 ng/mL)	3 weeks for $CD34^+$ expansion + 7 days differentiation + 2 days maturation	CD1a, CD11c, CD14, CD20, CD40, CD54, CD58, CD80, CD83, CD86, HLA-DR, HLA ABC, DC-LAMP. CCR-7	72
Umbilical Cord Blood	Gradient Centrifugation + $CD34^+$ Beads	- Flt-3L (25 ng/mL) - IL-3 (10 ng/mL), SCF (10 ng/mL), IL-6 (10 ng/mL), TPO (10 ng/mL), IL-4 (20 ng/mL), GM-CSF (50 ng/mL)	Poly I:C (10 µg/mL)	4 weeks $CD34^+$ expansion + 5-6 days differentiation + 1 day maturation	CD1a, CD14, HLA- DR, CD40, CD80, CD86	52
Umbilical Cord Blood	Gradient Centrifugation + $CD34^+$ Beads	- GM-CSF (1000 U/mL) - IL-4 (1000 U/mL)	TNF-α (Not Mentioned)	5 days differentiation + 2 days maturation	CD80, HLA-DR, CD11c	23
Umbilical Cord Blood	CD34+ beads	- GM-CSF (50 ng/mL) - IL-4 (50 ng/mL) and SCF (50 ng/mL)	LPS (100 µg/mL) or IFN-γ (10 ng/mL)	16 days differentiation + 1 day maturation	CD14, CD11c, CD80, HLA-DR	20

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Moreover, the production of IL-12, IFN-γ, and cytotoxic T cells is 10-fold higher using density gradient than positive beads sorting.[27] However, the tricky part is to ensure that only buffy coat is taken. Untargeted cells can attach and disturb the differentiation of DC. Importantly, monocytes may be unintendedly disposed during medium replacement after 2-hours incubation. Thus, using magnetic beads sorting provide simpler technique and higher purity without interference from NK, B, and T lymphocytes.

Magnetic Sorting

There are two types of sorting, negative and positive sorting. The monocytes concentration is higher in positive selection, approximately 95% of the MNC can be isolated from adult peripheral blood.[13,30,31] Monocytes are the only cells in blood that exhibit high expression of CD14 on their membranes, which can be positively interacted with antibodies coupled with magnetic beads (positive sorting). The magnetic bead flows through magnetic field and trapped. Meanwhile, other cells that are not bound with the antibody will be eluted.[18] In contrast, negative sorting beads only bind with cells other than monocytes, such as macrophage, B cells, and T cells.[18] Still, any falsely unlabeled cell is present in the cell suspension.

There is higher proportion of monocytes isolated using positive sorting beads compared to negatively sorted monocytes, approximately 92.2% and 70.3%, respectively.[17] Thus, most of researches use positive sorting as summarized in Table 1. However, the potency to attach, migrate, and releasing cytokines are lower than negatively selected monocytes.[17,28] This may be because of interaction between antibody and CD14 indicating "danger signal", causing early maturation. In addition, by flowing through the magnetic field, the monocytes can shrink and lead to cell death.[17,28] Due to early maturation and dead cells, the monocyte had a lower adherence which is associated with a reduced migratory and metabolic potency compared to negatively sorted monocytes.[17,28] In addition, the beads from positive sorting still persist in patient, may cause hypersensitivity reaction. Even though there is no proof if the beads have also been injected to patient, but the beads still present after 6 days culture in laboratory.[28] This method can improve the monocyte purity during culture and eliminate 2-hours incubation as mentioned with density gradient centrifugation method, but culture time for differentiation and maturation is still similar between monocyte derived UCB and PB.

Differentiation cytokines for immature dendritic cells

GM-CSF

After ensuring there are enough monocytes as the source for DC, the next step is to ensure all the monocytes can be differentiated and matured efficiently. GM-CSF and IL-4 are commonly used to produce immature dendritic cells from monocytes.[16] GM-CSF is a double-edged sword that can lead to tolerogenic or immunogenic DCs depending on the concentration. High doses of GM-CSF correlate with cell proliferation, but low concentrations do not promote cell proliferation.[32] The optimum GM-CSF concentration to induce an immunogenic effect is 40-80 µg, while a concentration around 100-500 µg can suppress the immune system.[33] The GM-CSF appears to downregulate macrophage colony-stimulating factor (M-CSF) receptor expression on monocytes, inhibiting differentiation from monocytes to macrophages.[34] This mechanism is important to ensure monocyte only differentiate into dendritic cells and not macrophages.

IL-4

Completing the GM-CSF function, the IL-4 cytokine helps monocytes differentiation from DC by inhibiting the formation of macrophage colonies. Without IL-4, the DC detaches easily, leading to a lower concentration of mature DC.[35] The use of GM-CSF should not be in very high amounts, but IL-4 can be increased so that it does not cause the conversion of monocyte cells into macrophages.[36] The concentration of IL-4 that is commonly used is 5-100 ng/mL and GM-CSF is around 50-100 ng/mL (Table 1). There is no fixed concentration for dendritic cell activation. GM-CSF and IL-4 cannot be cultured with another cytokine during differentiation. This step is pivotal for the differentiation of monocyte to become immunogenic DC. A research found that differentiation with combined cytokines (GM-CSF, IL-4, and TNF-α) can reduce the potency of DC to activate cytotoxic T cells by decreasing IL-12p70 secretion and enhancing IL-10 release.[34] In contrast, another research found that GM-CSF, IL-4, and TNF-α can increase surface markers of mature DC and phagocytosis capability.[34,37]

Several attempts to replace GM-CSF and IL-4 were reported by exploring the important signal during DC differentiation and maturation. Besides TNF-α, other cytokines have been selectively studied such as IFN-β, IL-3, and IL-7. The DC-derived from IFN-β and IL-3 maturation

produces higher levels of HLA-ABC, HLA-DR, IFN-α, IL-6, IL-8, IL-5, IFN-γ, and TNF-α, but low IL-12 compared to DC-derived from GM-CSF and IL-4.[38] DC maturation cannot be asses only from IFN-β and IL-3, but should be completed with LPS or CD40L stimulation. Similar to GM-CSF and IL-4 combination, IFN-β cannot be used alone. In the absence of IL-3, the cells detach and die quickly.[38] Similarly, IL-7 stimulate higher T cell potency compared to DC-derived from GM-CSF and IL-4.[39]

Maturation cytokines for mature dendritic cells

In the state of immature DC, all maturation cytokines and tumor-associated antigens should be introduced. In this last process, more types of cytokine adjuvants are used compared to monocyte cell differentiation into immature DC. Several choices of cytokine adjuvants that can be used, including: (a) Lipopolysaccharide (LPS) with either monophosphorylate lipid A (MPLA), resiquimod (R848), IL-2, or CD40L; (b) IFNs (IFN-α/IFN-γ), TNF-α, IL-6, IL-1β with either Prostaglandin E2 (PGE2) or poly (I:C) ; (c) TNF-α alone or combines with LPS, CD40L, and IFN-γ.

The maturation cocktails (a) produce high concentration of IL-12p70 either with or without CD40L. Higher concentration of IL-8, IL-12p70, and IL-6 is released in response to CD40L.[38,40] CD40 ligand (CD40L) is expressed primarily by activated T cells and B cells, and binds to its CD40 receptor on DC.[41] The IL-12p70 modulates Th1 and Th2 responses and contributes in IFN-γ-production. In addition, LPS can increase production of IL-15 that is important in differentiation of NK cells, effector $CD8^+$ T cells and memory $CD8^+$ T cells.[42-45] IL-2 can be used in combination with LPS and promoted an increase of IL-1β, TNF-α, and IL-12 production.[46] The use of MPLA with IFN-γ efficiently induced mature DC with ≥60.0% positive markers ($HLA\text{-}DR^+$ $CD86^+$ cells) and ≥50 pg/mL IL12p70.[12,47]

The golden standard for maturation cytokine that is most widely used is TNF-α, IL-6, IL-1β, PGE2.[16,37,48] However, some researchers have different opinion about this cocktail because of low IL-12p70 secretion, leading to Th2-type immune responses. This is may be because of PGE2 that can impair the secretion of IL-12p70 and induce $CD4^+$ T cells both using GM-CSF and IL-4 or IL-1β and TNFα or LPS alone.[37,45] In addition, PGE2 can increase tumor metastasis because of IL-10 secretion that is blocking DC to release IL-12p70 which plays a role in cytotoxic T cell activation.[24,49,50] Nowadays, the use of LPS and PGE2 are limited because of its toxicity and tolerogenic response, respectively.[13,51] The use of LPS and PGE2 can be replaced by Poly I:C with similar potency in producing immunogenic DC. Poly I:C can increase IL-12p70 production compared to LPS.[40,47,52,53]

IFN-α has direct interaction with cancer cells by downregulating oncogene and upregulating tumor suppressor genes. In early infection, IFN-α increases MHC class I and II expression in monocytes, stimulates NK and DC cell development and activation, activates Th1 cells, and induces B cell differentiation.[12] The addition of IFN-α to monocytes in the presence of GM-CSF and IL-4 significantly impairs their ability to differentiate into IL-12 secretion.[25] However, another report suggested the large production of inflammatory cytokines such as IL-1β, IL-6, IL-10, TNF-α, and IL-18.[54] IFN-α induced high levels of CD40, CD54, CD80, CD86, and HLA-DR molecules after 3 days culture.[35]

Another cytokine that may have an important role in DC maturation is IL-6, a pleiotropic cytokine produced in response to tissue damage and infection produced by almost all immune cell types. After targeting its specific receptors, IL-6 initiates a series of signaling events primarily associated with the Janus kinase/Signal Transducer and Activator of Transcription protein 3 (JAK/STAT3) activation pathway. IL-6 can induce CD4 T cells to secrete IL-4 and drive a Th2 response and also influence IFN-γ secretion by CD4 T cells to promote Th1 polarization.[55]

In addition to the options above, there are many more modifications made by researchers so that the resulting DC varies for each laboratory. TNF-α used alone (maturation cocktail (c)) can induce high expression of MHC class II, but not enough for IL-12 production, CCR7 expression, and DC migration.[34] TNF-α shall not be used in early differentiation, but has potential in maturation of DC. The combination of TNF-α with IFN-γ also provide new trend for DC maturation (Table 1). IFN-γ is produced by DC-T cell crosstalk.[41,56] IFN-γ can promote macrophage activation, mediate antiviral and antibacterial immunity, enhance antigen presentation, regulate innate immune system activation, coordinate lymphocyte-endothelial interactions, regulate Th1/Th2, and control cell proliferation and apoptosis.[12] IFN-γ and its receptor interaction results in the activation of the receptor-associated protein tyrosine kinases JAK1 and JAK2 and subsequent tyrosine phosphorylation and activation of STAT1, which translocate to the nucleus and activate the

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Interferon-Stimulated Gene (ISG) for antigen-presenting molecules (including MHC molecules), phagocytic receptors, and various proteins that play antiviral and antibacterial roles.[57] Most of differentiation and maturation cytokines are using JAK/STAT and Nuclear factor kappa B (NF-kB) pathways to activate dendritic cells.[58,59]

Culture period

By adding pro-inflammatory cytokines to the culture for a certain period, the monocyte differentiates into a potato-shaped structure and attach to the plastic surface. Several studies have shown that in vitro development of mature DC takes up to 48 hours.[19,23,35,54,60] The viability of mature DC is reduced when cultured for more than 24 hours.[48] Therefore, most researchers only take 24 hours for DC maturation (Table 1). However, T cell stimulatory capacity in allogeneic mixed lymphocyte reaction and cytokine production, including IL-12p40, IL-12p70 and IL-10, are similar between 24 and 48 h maturation periods.[48] Replacing the cytokine with the highly pro-inflammatory cytokines or increasing the number of cytokines used in culture may decrease the incubation period.

Proposed *in vitro* production of dendritic cells

Nowadays, DC has potency for long lasting and safe immunotherapy for cancer. UCB could be a potential source due to its high monocyte concentration and high capacity in differentiation. TNF-α, IL-1β, and IFN-γ as maturation cytokines could be introduced up to 48 hours (Table 1). Long-period culture decreases the viability of DC through apoptosis because of the dual-role of persistent maturation cytokines. Culture under 24 hours could be proposed by applying higher concentration of cytokines. Thus, it is necessary to learn more about different cytokine concentration related with monocytes amount during culture. Viable DCs with expression of co-stimulatory molecules (CD83, CD86/CD80, HLA-DR) and induction of IL-12p70 cytokine, could activate T cells. Due to inconsistent results in laboratories, the standardized protocol of closed and automatic systems under GMP production such as centrifugal force-based instrument, should be applied. In addition, allogeneic DC from different donors could provide better T cell activation by providing high HLA compatibility with patient.[61-63] Exosome from DC showed similar co-stimulatory molecules and cytokines as owned by their parent cells.[64] This exosome could provide off-the-shelf treatment with similar potency through direct or indirect interaction with immune cells.[65] Further studies in epigenetics provide better understanding for important pathway in DC differentiation, maturation, and function.

Conclusion

DCs are the most potent antigen presenting cells for naive T cells and are essentially master regulators of the entire immune system both as immunogenic and immunotolerance depends on presented antigen. Donor variability, isolation technique, culture time, and cytokines for differentiation and maturation should be considered in developing standardized protocol. Thorough investigation in the development of standardized protocol should be implemented before moving towards the clinical application. Viability, surface markers, and cell potency should be used as quality parameters in DC productions as immunotherapy.

Authors Contribution

GF, CRS, and TR were involved in concepting the topic and drafted the manuscript. GF revised the manuscript and reviewed by CRS and TR. All authors took parts in giving critical revision of the manuscript.

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