T.C.

BIRUNI UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

MOLECULAR BIOLOGY AND GENETICS PROGRAMME

MOLECULAR AND MEDICAL GENETICS MASTER'S PROGRAMME

THE IMPACT OF THALIDOMIDE ON THE EXPRESSION OF IMMUNE CHECKPOINTS IN DENDRITIC CELLS

Nastaran AZAMI

ADVISOR

Asst. Prof. Dr. Esra AYDEMIR

July, 2024

T.C.

BIRUNI UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

MOLECULAR BIOLOGY AND GENETICS PROGRAMME

MOLECULAR AND MEDICAL GENETICS MASTER'S PROGRAMME

THE IMPACT OF THALIDOMIDE ON THE EXPRESSION OF IMMUNE CHECKPOINTS IN DENDRITIC CELLS

Nastaran AZAMI

ADVISOR

Asst. Prof. Dr. Esra AYDEMIR

July, 2024

Thesis Approval Page

Declaration

I declare that I have acquired all the information and documents for this study in compliance with academic regulations. I have presented all visual, audio, and written information and findings in adherence to scientific ethics. I have not altered any data that I have utilized and have appropriately referenced the sources in accordance with scientific standards. Furthermore, I assert that my thesis is original, except for the cases explicitly referenced.

Nastaran AZAMI

Acknowledgement

I am profoundly thankful to my supervisor, Asst. Prof. Dr. Esra AYDEMIR, for her exceptional guidance and steadfast support throughout my thesis. Her deep expertise and thoughtful insights have been pivotal in shaping both the direction and substance of my research. Her mentorship has greatly expanded my academic knowledge and significantly enriched my learning journey.

I would also like to sincerely thank the Immunology Research Center of Tabriz, Iran, for their indispensable support and resources. Their contributions were essential in enabling the thorough research that underpins this thesis.

My heartfelt gratitude goes to my family, particularly Neda and Nasim, for their unwavering support and constant encouragement during my years of study and throughout the process of researching and writing this thesis.

Finally, I am deeply appreciative of my partner, Alireza, whose unwavering support, belief in me, patience, and love have been my constant source of motivation throughout this journey.

Table of Contents

Page No

Symbols / Abbreviatıon

APCs	Antigen-presenting cells
BTLA	B and T-lymphocyte attenuator
cDCs	conventional dendritic cells
cDNA	complementary DNA
CTLA-4	Cytotoxic T lymphocyte antigen-4
CTLs	Cytotoxic T lymphocytes
DAMPs	Damage-associated molecular patterns
DCs	Dendritic cells
DMSO	Dimethyl Sulfoxide
FBS	Fetal Bovine Serum
FITC	Fluorescein Isothiocyanate
GM-CSF	Granulocyte/macrophage colony-stimulating factor
HLA-DR	Human leukocyte antigen-DR isotype
IFN-γ	Interferon-γ
LAG-3	Lymphocyte activation gene-3.
LPS	Lipopolysaccharide
MFI	Median fluorescence intensity
MHC-I	Major Histocompatibility Complex Class I
Mo-DC	Monocyte-derived dendritic cell
PAMPs	Pathogen-associated molecular patterns
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate-buffered saline
pDC	plasmacytoid dendritic cell
PD-L1	Programmed cell death proteins 1 ligand 1
qRT-PCR	Quantitive Real-Time polymerase chain reaction
TGF-β	Tumor growth factor-β
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor-α
Tregs	Regulatory T-cells
VISTA	V-domain Ig suppressor of T-cell activation

List of Tables

List of Figures

Özet ve Anahtar Kelimeler

Azami, N. (2024). The Impact of Thalidomide on the Expression of Immune Checkpoints in Dendritic Cells. Yüksek Lisans Tezi, Biruni Üniversitesi Lisansüstü Eğitim Enstitüsü, İstanbul.

Uzmanlaşmış dendritik hücreler (DC'ler) antijenleri etkili bir şekilde parçalayarak bağışıklık yanıtını tetiklemek ve kontrol etmek amacıyla aktarır. İlk olarak sedatif olarak kullanılan bir farmasötik bileşik olan talidomid, anti-enflamatuar ve anti-anjiyogenik özelliklerinden dolayı bağışıklık sistemini güçlü bir şekilde baskılama yeteneği ile tanınmaktadır. Bu çalışmanın amacı, monositlerden türetilen dendritik hücrelerde talidomid tedavisinin immünolojik kontrol noktası genlerinin ekspresyonu üzerindeki etkisini incelemektir. Talidomid, çeşitli hücresel sinyal yollarını bozma yeteneği ve bağışıklık sistemi üzerinde güçlü düzenleyici etkisi ile bilinir. PBMC ayrılması için Ficoll prosedürü kullanıldıktan sonra, PBMC'lerden monositler MACS yöntemiyle izole edilmiştir. Monositler, 6 kuyucuklu plaklarda kültüre edilip GM-CSF ve IL-4 sitokinleri ile muamele edilmiştir. Plaklar, 37°C'de bir inkübatöre yerleştirilmiş ve günlük olarak herhangi bir kontaminasyon belirtisi açısından izlenmiştir. Üçüncü günde, kuyucuklardaki ortamın yarısı, sitokin içeren taze ortam ile kademeli olarak değiştirilmiştir. Mo-DC farklılaşmasının akış sitometrisi analizini takiben, talidomid mo-DC'lere uygulanmıştır. Mo-DC'lerin fenotipik özellikleri, talidomid tedavisinin etkisini değerlendirmek amacıyla akış sitometrisi kullanılarak analiz edilmiştir. Gerçek zamanlı PCR tekniği, tedavi uygulanan ve uygulanmayan mo-DC gruplarında çeşitli kontrol noktaları ile ilişkili bağışıklık genlerinin ekspresyonunu incelemek için kullanılmıştır. Çalışma, talidomidin dendritik hücrelere uygulanmasının bu hücrelerdeki CD86 ve HLA-DR ekspresyon seviyelerinde bir artışa yol açtığını göstermiştir. Ayrıca, talidomid enjeksiyonu sonrasında dendritik LAG3 ve VISTA kontrol noktalarının seviyeleri azalmıştır. Buna karşılık, bu hücrelerde BTLA seviyeleri artmıştır. Bu deneyin sonuçları, talidomidin DC hücrelerini spesifik olarak hedef alarak bu hücrelerin kendine özgü özelliklerini değiştirebileceğini göstermektedir.

Anahtar Kelimeler: Talidomid, Bağışıklık Terapisi, Dendritik Hücreler, Bağışıklık Kontrol Noktaları

Abstract and Keywords

Azami, N. (2024). The Impact of Thalidomide on the Expression of Immune Checkpoints ın Dendritic Cells. Master Thesis, Biruni University Institute of Graduate Studies, İstanbul.

Specialized dendritic cells efficiently break down and transfer antigens to trigger and control the immune response. The interplay between dendritic cells and antigen-specific T cells may elicit either activation or suppression of antigen-specific T-cell responses. Thalidomide is a pharmaceutical compound first used as a sedative but promptly discontinued owing to its ability to cause congenital abnormalities. This medication is recognized for its strong ability to inhibit the immune system because of its anti-inflammatory and anti-angiogenic characteristics. This study aims to examine the impact of thalidomide treatment on the expression of immunological checkpoint genes in dendritic cells generated from monocytes. Thalidomide is recognized for its ability to disrupt several cellular signaling pathways and its strong regulatory impact on the immune system. The Ficoll procedure separated PBMCs, and monocytes were isolated using the MACS method. Cultured in 6-well plates, treated with GM-CSF and IL-4, the plates were incubated at 37°C, and half of the medium was replaced with fresh cytokines. After flow cytometry analysis, thalidomide was administered. The phenotypic properties of mo-DCs were analyzed using flow cytometry to assess the impact of thalidomide treatment. The real-time PCR technique was used to examine the expression of immune genes associated with various checkpoints in the mo-DC groups that received treatment and those that did not. The study demonstrated that administering thalidomide to dendritic cells led to an augmentation in the levels of CD86 and HLA-DR expression inside these cells. As a result, it may impact the expression of markers associated with the development and functioning of dendritic cells (DCs). Furthermore, the levels of immunological checkpoints LAG3 and VISTA were reduced in dendritic cells after the injection of thalidomide. Conversely, the levels of BTLA were increased in these cells. The results of this experiment suggest that thalidomide can specifically target DC cells and alter their distinct characteristics.

Keywords: Thalidomide, Immune Therapy, Dendritic Cells, Immune Checkpoints

1. INTRODUCTION AND PURPOSE

Dendritic cells (DCs) are highly efficient antigen-presenting cells and play a vital role as intermediaries between the innate and specific immune systems(van Gulijk, Dammeijer, Aerts, & Vroman, 2018). In conditions of stability, dendritic cells (DCs) are predominantly acknowledged as young antigen-presenting cells (APCs) that possess a robust ability to capture antigens. The expression of co-stimulatory molecules, including CD80, CD86, CD40, and MHC, is reduced in these cells, leading to a restricted supply of antigens. These direct current (DC) sources lead to the formation of tolerance (Steinman, Hawiger, & Nussenzweig, 2003). DCs are activated and mature when they are exposed to environmental stimuli, such as damage-related molecular patterns (DAMPs) and pathogen-related molecular patterns (PAMPs). This exposure leads to enhanced expression of co-stimulatory molecules and the generation of cytokines. Furthermore, the capacity of these cells to engulf antigens through phagocytosis diminishes. The presence of mature dendritic cells (DCs) is essential for initiating T-cell responses and plays a critical role in promoting anti-tumor immunity (Veglia & Gabrilovich, 2017). Thus, dendritic cells exhibit two separate phenotypes: immunogenic and tolerogenic. Immunogenic dendritic cells (DCs) have certain distinguishing characteristics. They can display antigens with co-stimulatory molecules (CD80 and CD86 molecules; signal 2) and other cytokines (signal 3) to CD4+ and CD8+ T cells. This elicits a targeted T-cell response against the antigen (Alvarez-Dominguez et al., 2017).

On the other hand, dendritic cells (DCs) play a vital role in beginning and maintaining self-tolerance. This is accomplished by inhibiting the functions of effector T cells, producing and stimulating regulatory T cells, and promoting the removal of T cells by clonal deletion and induction of anergy (Domogalla, Rostan, Raker, & Steinbrink, 2017). These dendritic cells (DCs) have increased amounts of many immunological checkpoints, including CTLA-4, PD-L1, BTLA4, B7H7, VISTA, and LAG3 (Ghorbaninezhad et al., 2022). Here are a few noteworthy characteristics of tolerogenic dendritic cells. Immune checkpoints serve as a regulatory mechanism to prevent the immune system from mistakenly targeting the body's cells (Ghorbaninezhad et al., 2022). The activation or inhibition of certain signaling

pathways in dendritic cells (DCs) is linked to the development of two different phenotypes in these cells (Adorini, 2004; Švajger & Rožman, 2013).

Thalidomide is a pharmacological substance that can modify the immune system and reduce inflammation by affecting various immune cells (Bamias & Dimopoulos, 2005). This medicine is indicated for the treatment of persons diagnosed with multiple myeloma as well as several forms of cancer, such as pancreatic, prostate, and lung cancer. Additionally, it is used in the management of autoimmune illnesses such as graft versus host disease (GVHD), Waldenström's macroglobulinemia, and experimental autoimmune encephalomyelitis. This medication has the potential to be beneficial for individuals with multiple sclerosis (Bamias & Dimopoulos, 2005). The objective of this study was to investigate the impact of thalidomide on the differentiation, maturation, and expression of immunological checkpoints in DC cells. Thalidomide is known for its potent immunomodulatory effects across several cell types. Our discoveries have the potential to lead to new therapeutic approaches for the treatment of immunological diseases.

2. GENERAL INFORMATION

2.1. Immune system

The immune system consists of a complex network of anatomical barriers, chemical defense barriers, and cellular components. These components may be divided into two categories: innate immunity and acquired immunity (CHAPLIN, 2006). Innate immunity encompasses the body's earliest defensive system, which includes physical barriers such as the skin and mucosal epithelium, chemical signals like cytokines, and cells with a restricted capacity to detect conserved molecular patterns or danger signals (Abul K. Abbas & Andrew H. Lichtman & Shiv Pillai, 2021). The innate immune system consists of many kinds of cells, including monocytes, macrophages, neutrophils, dendritic cells (DCs), mast cells, natural killer cells (NK cells), and innate lymphoid cells (ILCs). These cells possess germ-line-encoded pattern recognition receptors (PRRs) that are recognized by pathogen-associated molecular patterns (PAMPs), such as viral RNA or bacterial endotoxin, as well as damage-associated molecular patterns (DAMPs) from cells. These compounds are discharged when an individual dies as a result of tissue injury, as shown by their excretion. External forces initiate this process (Murphy, 2016). Macrophages and neutrophils, which are part of the innate immune system, can phagocytose, meaning they may engulf and kill infections and dead host cells (Abul K. Abbas & Andrew H. Lichtman & Shiv Pillai, 2021). Conversely, immune cells that are obtained by acquisition, such as B and T cells, have a diverse array of receptors that are capable of recognizing and attaching to certain antigens (Murphy, 2016). Specific immunity may be divided into two components: humoral immunity and cellular immunity. Humoral immunity is established via the synthesis of antibodies by plasma cells. The primary mechanism for protecting against extracellular bacteria and toxins is the synthesis of antibodies that selectively bind to these pathogens and facilitate their elimination (Abul K. Abbas & Andrew H. Lichtman & Shiv Pillai, 2021). T cells play a crucial role in the formation of cellular immunity. T cells detect antigens by recognizing major histocompatibility complex (MHC) molecules. These cells particularly recognize peptide antigens that are components of MHC-peptide complexes and are displayed by antigen-presenting cells (APCs). TCD4+ cells recognize peptide antigens shown by

MHC-II molecules, whereas TCD8+ tissues recognize peptide antigens displayed by MHC-I molecules (Abul K. Abbas & Andrew H. Lichtman & Shiv Pillai, 2021). T cells may be modulated by many strategies, such as stimulating macrophages to eradicate pathogens, directly eliminating infected cells, or secreting cytokines and modifying their immediate environment (Abul K. Abbas & Andrew H. Lichtman & Shiv Pillai, 2021). TCD4+ cells undergo cellular development into diverse subtypes of helper T cells, which subsequently release specific cytokines.

In addition, these cells could differentiate into regulatory T cells (Treg) that have anti-inflammatory properties(Abul K. Abbas & Andrew H. Lichtman & Shiv Pillai, 2021). CD8+ T cells undergo a process of differentiation into cytotoxic T lymphocytes (CTLs), which can kill target cells (Murphy, 2016). Helper T cells play a crucial role in promoting the activation of B cells to generate antibodies, stimulating macrophages to remove ingested pathogens, and initiating CTLs to kill infected target cells (Murphy, 2016).

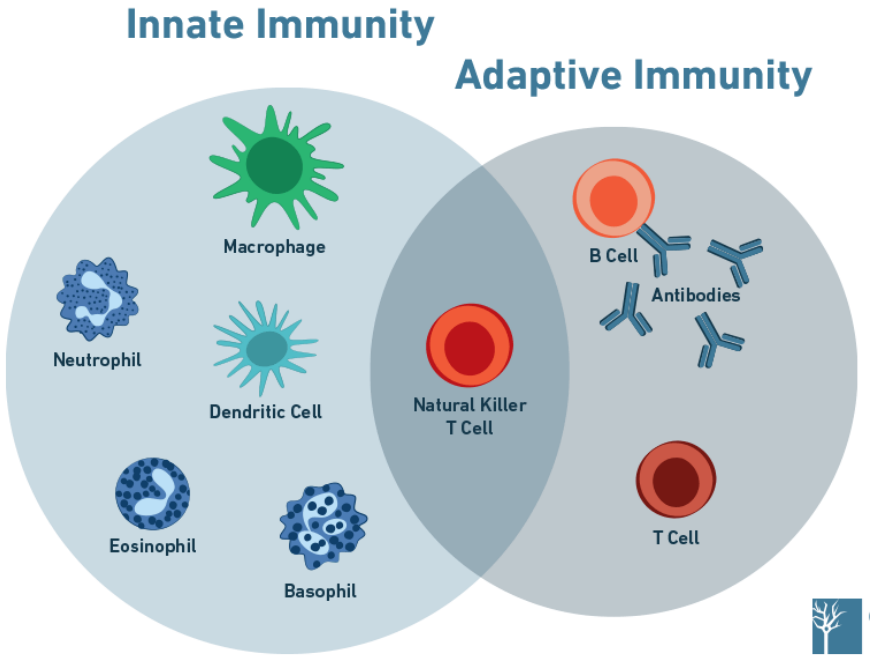


Figure 2. 1. Innate immune system versus acquired immune system

2.2. Types of DCs and their role in the immune system

Dendritic cells (DCs) are specialized cells that deliver antigens and mediate communication between the innate and adaptive immune systems. They play a crucial role in the development and regulation of immune responses that are specific to

antigens (Collin & Bigley, 2018). Moreover, dendritic cells (DCs) play a critical role in the establishment and maintenance of self-tolerance (Collin & Bigley, 2018). The cells demonstrate a significant level of variation in their attributes and roles (Steinman, 2012). During the process of DC development, these cells originate from CMP precursors located in the bone marrow. CMPs have two potential outputs. The transcription factor Nur77 promotes the development of CMPs into monocytes, which may then further develop into molecular DCs in inflammation (Domínguez & Ardavín, 2010). Nevertheless, when Nur77 expression is not present, both plasmacytoid dendritic cells (pDCs) and conventional dendritic cells (cDCs) differentiate from common myeloid progenitors (CMPs) (Domínguez & Ardavín, 2010). FLT3, a growth factor, often has a significant impact on the process of developing dendritic cells (DCs) from bone marrow progenitor cells (Balan, Saxena, & Bhardwaj, 2019).

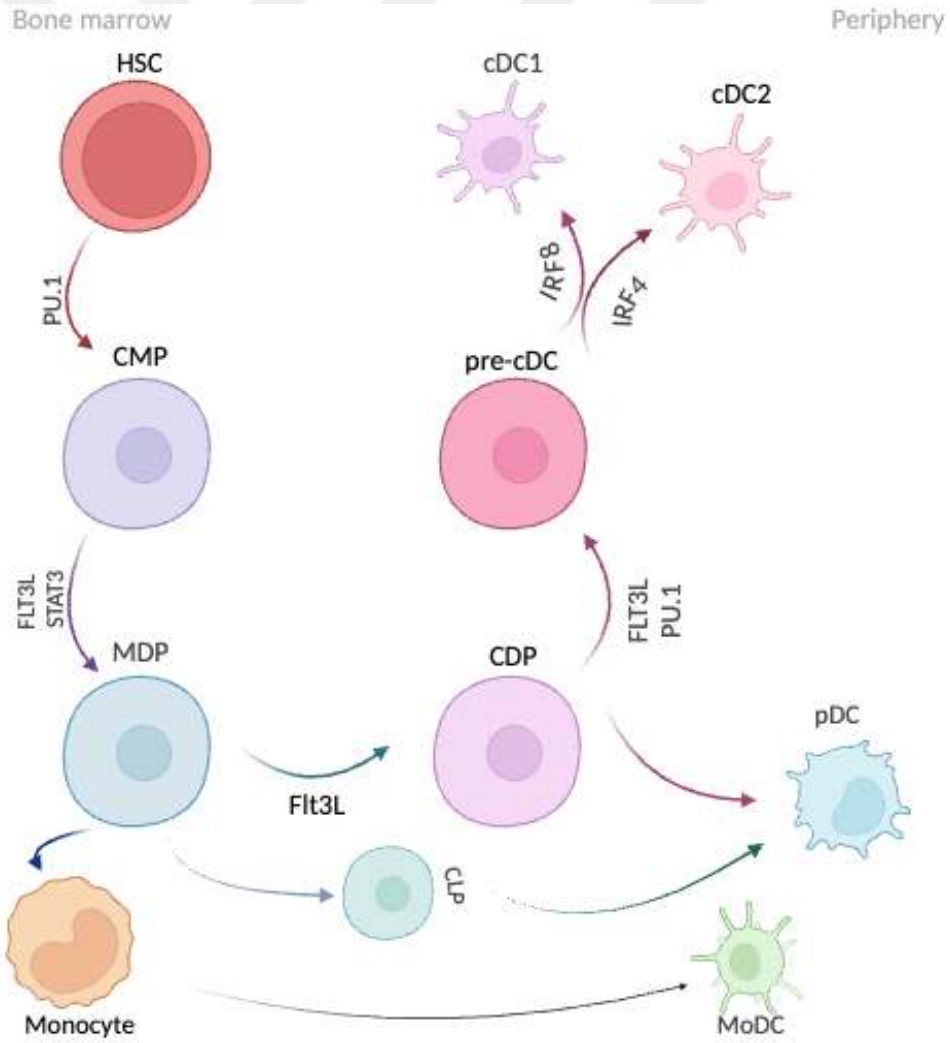


Figure 2. 2. Differentiation of DC types from their precursors in bone marrow

Dendritic cells (DCs) are not stationary entities but rather active participants in the immune system. They continuously examine the nearby microenvironment and are capable of conveying antigens, co-stimulatory signals, and cytokines to cells of the adaptive immune system (Shortman & Naik, 2007). During the steady state, dendritic cells (DCs) are often in an immature condition and have limited ability to present antigens as compared to other types of antigen-presenting cells (APCs). They possess a significant ability to capture antigens. Nevertheless, their ability to produce cytokines is restricted, and they only exhibit moderate quantities of co-stimulatory molecules, which demonstrates their highly regulated nature(Shortman & Naik, 2007). The interplay between DC receptors and PAMPs, DAMPs, and other cytokines is essential for the development and stimulation of DCs, emphasizing their capacity to adjust and react (Ghorbaninezhad et al., 2022). After reaching maturity and becoming active, dendritic cells (DCs) increase the production of major histocompatibility complex (MHC) molecules, co-stimulatory molecules (CD80/86), cytokines, chemokines, and C-C chemokine receptor type 7 (CCR7), evolving into powerful antigen-presenting cells (Patente et al., 2019).

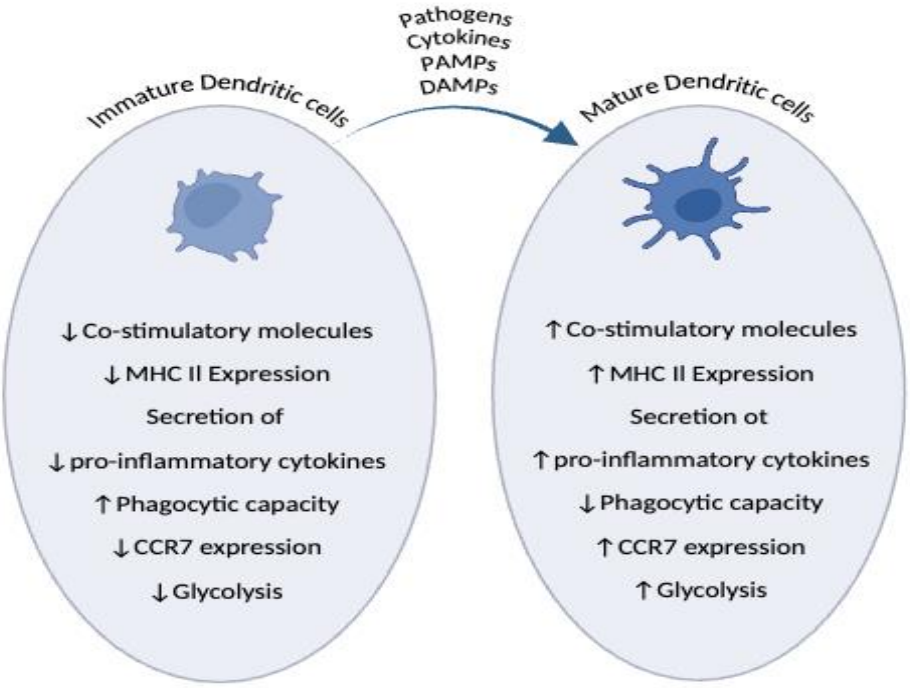
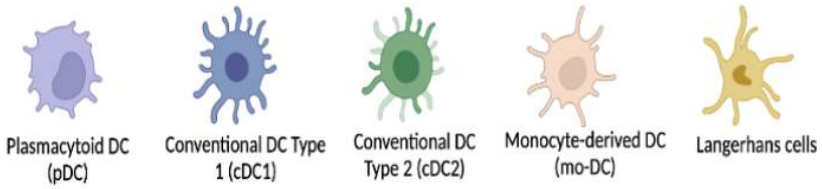


Figure 2. 3. Differences between immature and mature DCs (Patente et al., 2019).

Undoubtedly, the pivotal role of dendritic cells (DCs) in peripheral tissues, migrating to draining lymph nodes after capturing antigens, is widely acknowledged. These lymph nodes serve as conduits, transmitting intracellular antigens to naive CD8+ T cells and extracellular antigens to naive CD4+ T cells. This process triggers a myriad of reactions from effector T cells (Colbert, Cruz, & Rock, 2020). Furthermore, the activation of T lymphocytes, which express the CD40 ligand (CD40L), is promoted by dendritic cells (Mildner & Jung, 2014). The ability of DCs to display different antigens activates T helper (Th) 1 cells, Th2 cells, Th17 cells, T follicular helper (TFH) cells, regulatory T cells, and CD8+ CTLs (Guermonprez, Valladeau, Zitvogel, Théry, & Amigorena, 2002). Hence, dendritic cells (DCs) have rightfully earned recognition as the primary cells responsible for producing unique immunity to a specific antigen (Gerhard, Bill, Messemaker, Klein, & Pittet, 2021). The main subsets of dendritic cells (DCs) encompass cDCs, pDCs, mo-DCs, and Langerhans cells (LCs) (Gerhard et al., 2021). CDCs are further classified into two subtypes, namely cDC1 and cDC2 (Gerhard et al., 2021).



Transcription Factors	IRF4, IRF7, IRF8	BATF3, ID2, IRF8, ZBTB46	ID2, IRF4, KLF4, ZBTB46	IRF4, KLF4, ZBTB46	
Description	Plasma cell-like morphology. Rapid production of type l and type Ill interferons for viral infections.	Notable for cross-presen tation, effective in priming CD8+ T cells against extracellular antigens.	Dominant DC subset in blood, tissues, and lymphoid organs. Robust repertoire of pattern recognition receptors.	Differentiates from monocytes during inflammation . Has dendritic cell morphology.	Macrophages in origin, but with dendritic cell-like phenotype. Resides in squamous epithelium.
Cell Markers	CCR7, CD45RA, CD209, CLEC4C, LILRB4, NRP1, B220, SiglecH, IFN-a	BTLA, CADM1, CD8A, CLECIA, ITGAE, ITGAX, LY75, THBD, XCR1	CD14, CD163, CLEC10A, NOTCH2, ITGAM, SIRPA, CX3C1, CD1C, CD2	CD14, CD1A, CD1C, CD209, FCER1, ITGAM, MRC1, SIRPA	CD1A, CD207, ID2

Figure 2. 4. pDCs, markers, and their functions

2.2.1. cDCs

Conventional dendritic cells (CDCs) are antigen-presenting cells that are known for their exceptional efficiency in the innate immune system (Merad, Sathe, Helft, Miller, & Mortha, 2013). Despite having a relatively brief lifespan of around 3-6 days, cDCs undergo ongoing differentiation from their precursor cells in the bone marrow via the FLT-3-dependent pathway (McKenna et al., 2000). Human conventional dendritic cells (cDCs) may be distinguished by the expression of certain markers, such as CD123-HLA DR+CD11c+, CD14low, CD16low, and the lack of lineage markers (CD3, CD19, CD20, and CD56) (O'Keeffe, Mok, & Radford, 2015). The CD26 antigen may be used to characterize the conventional dendritic cells (cDCs) in the human population (Guilliams et al., 2016). Intestinal cDCs have been shown to possess CD56, and CD56 may also be present in cDCs found in other tissues(Guilliams et al., 2016).

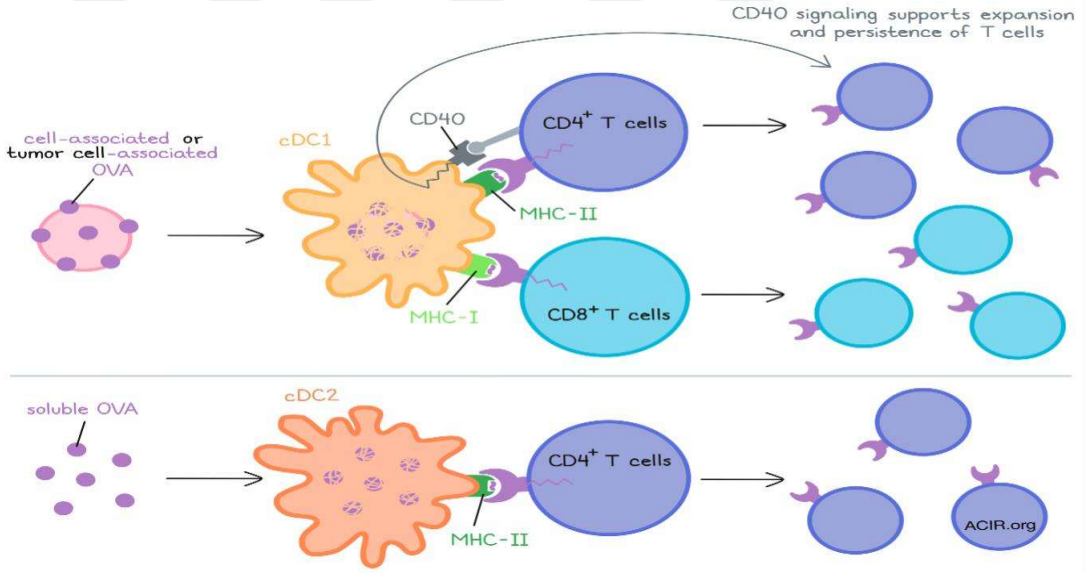


Figure 2. 5. Comparison of CDC1s versus CDC2s population performance (Lauren Hitchings, 2020)

2.2.1.1. cDC1s. Blood, lymphoid, and non-lymphoid tissues are among the human body's several places where studies have shown the existence of cDC1 cells (Haniffa et al., 2012). Human conventional dendritic cell type 1 (cDC1s) may be distinguished by the expression of DEC-205, CLEC9A, CADM1, and XCR1 markers (Patente et al., 2019). BDCA-3 (CD141) is an identifying characteristic that differentiates the cDC1 subgroup in humans (Jongbloed et al., 2010). CD103 is

expressed by conventional type 1 dendritic cells (cDC1s) and migratory cDC1s in non-lymphoid tissue, while cDC1s express CD8α in lymphoid tissue (Mildner & Jung, 2014).

In addition, cDC1 endosomes contain Toll-like receptors 3 and 9, which selectively recognize double-stranded RNA and double-stranded DNA, respectively (Villani et al., 2017). Furthermore, cDC1s demonstrate the presence of TLR-11, which is essential for detecting intracellular parasites (Mashayekhi et al., 2011). Several transcription factors, including GATA2, PU.1, GFI1, ID2, IRF-8, and BATF3, play a part in the process of differentiating myeloid cDC1s (Collin, Dickinson, & Bigley, 2015; Grajales-Reyes et al., 2015). Studies have shown that cDC1s can transport foreign antigens to CD8+ T cells via MHC-I. Furthermore, CD8+ T cells are indirectly activated by cDC1s via Th1 responses.

Studies have shown that cDC1s have a vital function in promoting the support of CD4+ T cells in CD8+ T lymphocytes. The absence of cDC1s leads to insufficient development of memory CD8+ T cells in viral infections (Eickhoff et al., 2015). Human conventional dendritic cells type 1 (cDC1s) can stimulate both CD4+ T cells and CD8+ T lymphocytes. In addition, they contribute to the eradication of bacterial and fungal skin diseases by attracting neutrophils (del Fresno et al., 2018).

2.2.1.2. cDC2s. Circulating dendritic cells type 2 (cDC2s) are present in the blood, lymphoid, and non-lymphoid tissues (Haniffa et al., 2012). Human cDC2 cells are BDCA-1+ DCs or CD1c+ DCs due to CD1c, CD11c, and signal regulatory protein alpha (SIRPα) (Heger et al., 2018). They can exhibit modest levels of CD14, CD123, and CD26 in blood or tissue while typically not expressing CD209 and BTLA (J.-S. Shin & Greer, 2015).

In line with their distribution, cDC2s express additional markers such as CD1a in the dermis and CD103 in the gut (Watchmaker et al., 2014). IRF-4 is the main transcription factor in differentiating cDC2 cells, although other transcription factors are also necessary for this process (Schlitzer et al., 2013). IRF-8 is another significant transcription factor involved in human cDC2 cell development (Hambleton et al., 2011).

DCs primarily utilize MHC class II molecules to deliver antigens to naïve CD4+ T cells and activate the effector responses of these cells (Matsuo et al., 2021). CDC2s can activate many CD4+ T cells, including Th1 cells, Th2 cells, Th17 cells,

regulatory T cells, and TFH cells (Nizzoli et al., 2016; Tussiwand et al., 2015). Furthermore, cDC2s can activate CD8+ T cells. Nevertheless, cDC2s can reduce the activation of CD8+ T cells (Laoui et al., 2016). Furthermore, cDC2s demonstrate the presence of several Toll-like receptors (TLRs), including TLR-2, TLR-4, TLR-5, TLR-6, TLR-8, and TLR-9 (Collin & Bigley, 2018). Conventional dendritic cell type 2 (cDC2s) activates Toll-like receptors (TLRs) to release inflammatory cytokines such as tumor necrosis factor-alpha (TNF-), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-12 (IL-12), and interleukin-18 (IL-18). Additionally, these cells are responsible for stimulating regulatory T cells in the gut (Watchmaker et al., 2014)and preserving tolerance in the liver (Bamboat et al., 2009).

2.2.2. pDCs

Plasmacytoid dendritic cells (pDCs) are frequently present in lymphoid organs and can also be found in peripheral blood. CCR7, ChemR23, CXCR3, L-selectin, and E-selectin regulate the movement of pDCs from the bloodstream to the lymph nodes via high endothelial venules (Diacovo, Blasius, Mak, Cella, & Colonna, 2005; Seth et al., 2011). pDCs, or plasmacytoid dendritic cells, are a distinct subgroup of dendritic cells that play a critical role in the immune response against viral infections these cells' defining features are the absence of CD11c expression and the presence of CD123, CD303, and CD45RA, and CD304 in human pDCs (Reynolds & Haniffa, 2015) These cells also display CD4, BDCA-2, HLA-DR, ILT-3, and ILT-7 markers on their surface and TLR-7 and TLR-9 indicators in their endosomes (Cao, 2009). A significant study reveals that the transcription factor E2.2 and its ligand FLT-3 influence the development of human plasmacytoid dendritic cells (pDCs) (Reizis, 2019; Swiecki & Colonna, 2015). These cells' antiviral properties are characterized by their ability to release large quantities of IFN-α/β and other inflammatory cytokines, including IL-6 and TNF-α, in response to TLR-7/9 stimulation (Reizis, Bunin, Ghosh, Lewis, & Sisirak, 2011). Although pDCs express co-stimulatory molecules and MHC-II, their ability to deliver antigens is limited (Villadangos & Young, 2008). Compared to conventional dendritic cells (cDCs), plasmacytoid dendritic cells (pDCs) can potentially present antigens to CD8+ T lymphocytes. However, pDCs have a lesser capacity for antigen cross-presentation (Dudziak et al., 2007). These cells play a conflicting role in cancer. On the one hand, they can activate the body's defenses against cancer cells. On the other hand, they can hinder the immune system's

responses, allowing tumor cells to evade immunity and facilitating the growth and spread of tumors (Brewitz et al., 2017; Gerlini et al., 2007). In the realm of autoimmune illnesses, they serve a dual purpose. Excessive plasmacytoid dendritic cells (pDCs) activation due to high levels of type I interferon (IFN-I) leads to the development of autoimmune diseases such as systemic lupus erythematosus and psoriasis (Minaga et al., 2021). Conversely, these cells can prevent disease by promoting tolerance. It is important to take steps to prevent the occurrence of autoimmune disorders (Hasegawa & Matsumoto, 2018).

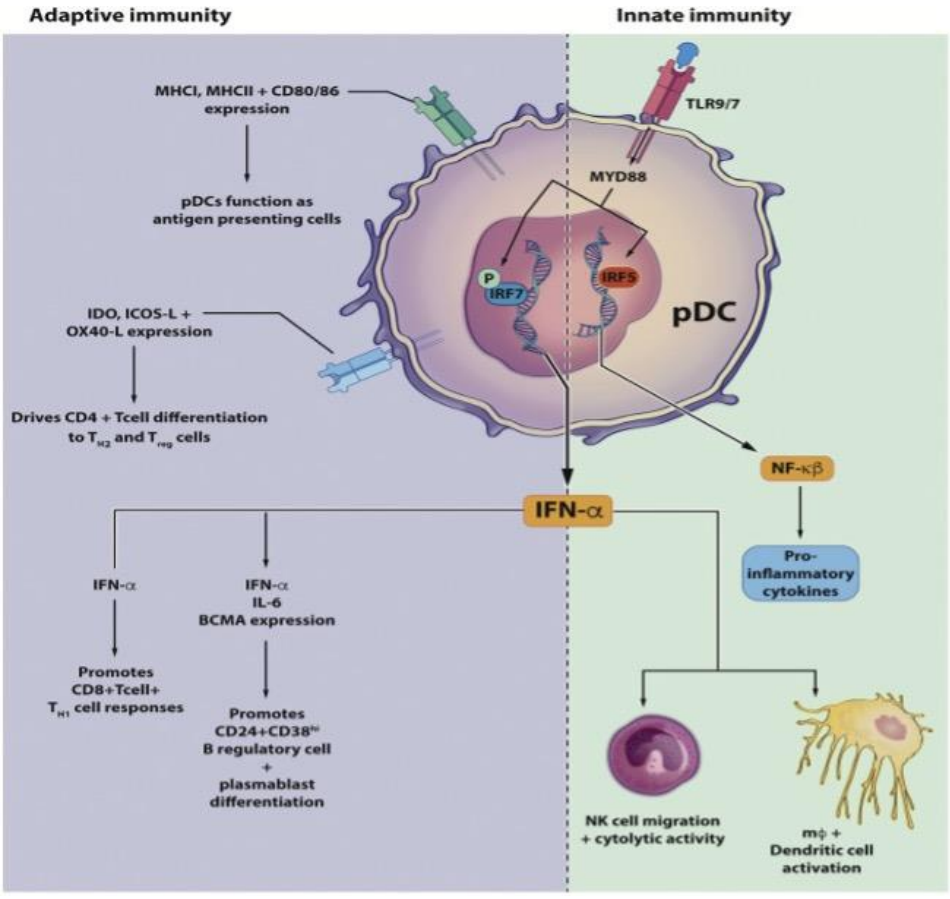


Figure 2. 6. pDCs, markers, and their functions

(Mitchell et al., 2018)

2.2.3. LCs

Langerhans cells (LCs) are a specific subset of dendritic cells (DCs) that are in the outermost layer of the skin, known as the epidermis (Merad, Ginhoux, & Collin, 2008). In the 19th century, Paul Langerhans first documented these cells, and today, we recognize them as a subset of the DC class (Romani, Clausen, & Stoitzner, 2010).

Longrin, CD1a, and epithelial cell adhesion molecules (EpCAM) are high in these cells, while CD11c, CD11b, and CD13 expression levels are low (Bigley et al., 2015; De Monte et al., 2016). They are predominantly located in the outermost layer of the skin, known as the epidermis, and have a close relationship with keratinocytes. Moreover, researchers have discovered LCs in stratified epithelia (Kashem, Haniffa, & Kaplan, 2017). TGF-β controls the differentiation process of LCs, which is entirely unrelated to the FLT-3 ligand (Merad et al., 2002)(Merad et al., 2002). These DCs exhibit resistance to radiation and have a half-life of two months, which is longer than average DCs (Merad et al., 2002). TNF-α and IL-1β are created in the skin when there is inflammation. This production causes LCs to detach from the epithelium and move through the basement membrane toward the afferent lymphatic capillaries. In humans, they can transform into highly effective cross-reactive antigen-presentation dendritic cells (DCs). These cells can produce significant quantities of IL-15, present mycobacterial glycolipid antigens, and activate CD8+ T cells (Banchereau et al., 2012; Romano et al., 2012).

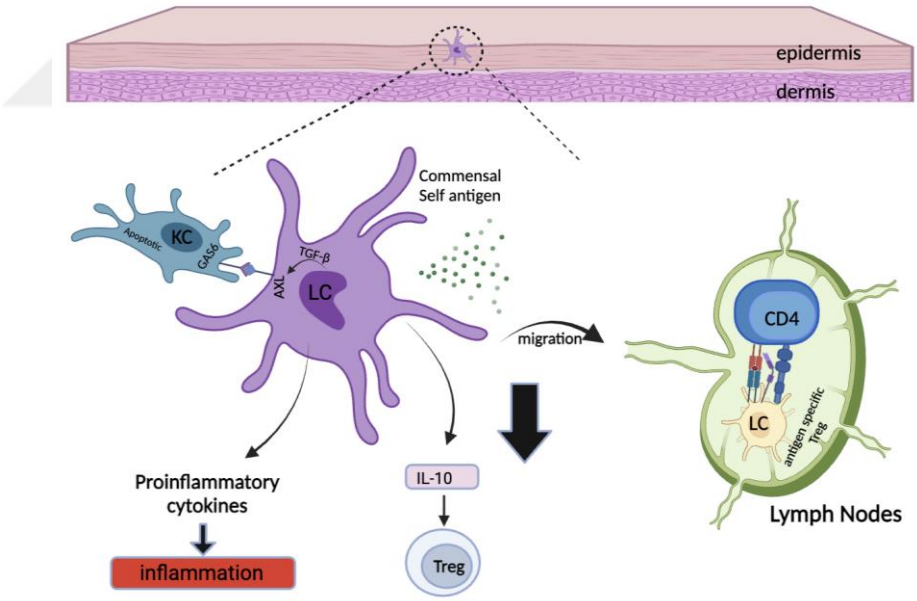


Figure 2. 7. LCs and their related functions (Zhou et al., 2022)

2.2.4. mo-DCs

Mo-DC, a subgroup of dendritic cells (DC), is formed from monocytes that undergo a process of differentiation into DC when they penetrate the tissue in an inflammatory environment (Guttman-Yassky et al., 2007). Monocytes can transform into mo-DCs, both in controlled laboratory environments (in vitro) and inside live

creatures (in vivo). Several studies (Lavin et al., 2017; Michea et al., 2018)have shown the presence of Mo-DCs in the microenvironment of lung, breast, and colorectal cancers. Furthermore, studies have demonstrated the presence of these cells in the gastrointestinal tract and healthy lungs, even in the absence of any signs of inflammation (Patel et al., 2017; Richter et al., 2018). Like cDC2s, mo-DCs depend on the transcription factor IRF-4 for their development in the bone marrow (Inaba et al., 1992). Monocyte-derived dendritic cells (mo-DCs) in humans are formed from monocytes that have elevated levels of CD14 expression (Domínguez & Ardavín, 2010; León & Ardavín, 2008)((Domínguez & Ardavín, 2010; León & Ardavín, 2008). Human monocyte-derived dendritic cells (mo-DCs) express many surface markers, including BDCA-1, CD1a, CD11c, CD14, and CD172a (14. These cells have several functional characteristics, such as co-stimulatory molecules, the production of various cytokines, and the capacity to present antigens to CD4+ and CD8+ T lymphocytes (Sallusto & Lanzavecchia, 1994; Serbina, Salazar-Mather, Biron, Kuziel, & Pamer, 2003). Consequently, mo-DCs facilitate the differentiation of naïve CD4+ T cells into Th1 cells (León, López-Bravo, & Ardavín, 2007), Th2 cells (Plantinga et al., 2013), and Th17 cells (Segura et al., 2013), as well as the differentiation of naïve CD8+ T cells into CTLs (K.-S. Shin et al., 2019). Under controlled laboratory circumstances, GM-CSF and IL-4 facilitate the transformation of monocytes into mo-DC via an IRF4-dependent mechanism (Helft et al., 2015). Several mo-DC vaccines have been developed and evaluated utilizing these cell culture techniques. Mo-DC vaccines have shown varying effectiveness in treating acute myeloid leukemia (AML) in clinical studies (Anguille, Willemen, Lion, Smits, & Berneman, 2012). For example, the vaccination procedure included the use of mo-DCs that were exposed to leukemic lysates from AML patients who relapsed after receiving autologous hematopoietic stem cell transplantation. As a result, immunological responses were triggered. Moreover, this increased the ability of the autologous T cell to activate dendritic cells (Yamazaki et al., 2003).

2.3. Different phenotypes of DC cells

Dendritic cells (DCs) maintain the equilibrium between the body's immune response and its tolerance for foreign substances. They fulfill this role by acting as an intermediary between the innate immune system and the adaptive immune system (Domogalla et al., 2017). Dendritic cells (DCs) may be categorized into two primary

phenotypes: immunogenic and tolerogenic. Immunogenic dendritic cells (DCs) possess essential features such as antigen uptake and display in CD4+ and CD8+ T lymphocytes. The presentation is accompanied by co-stimulatory molecules, including CD80 and CD86 molecules, which serve as signal 2. Furthermore, other cytokines, known as signal 3, are involved in this process. Ultimately, these molecules and cytokines work together to promote T-cell responses. They initiate an immunological response to the antigen (Alvarez-Dominguez et al., 2017). Immunogenic dendritic cells (DCs) can present antigens to T cells, making them useful in the treatment of several types of cancer, including mesothelioma (Aerts et al., 2018), blood malignancies (Van den Bergh et al., 2017), pancreatic cancer (Schnurr et al., 2001), and others. Conversely, dendritic cells (DCs) have a crucial function in initiating and sustaining self-tolerance. To achieve this, they suppress the activity of effector T cells, stimulate the proliferation and activation of regulatory T cells, and enable the elimination of T cells by clonal deletion and anergy induction (Domogalla et al., 2017). The traits described above are unique characteristics of tolerogenic dendritic cells. Presently, researchers are actively investigating the potential therapeutic applications of tolerogenic dendritic cells (DCs) in many diseases that need immunosuppression. These illnesses include autoimmune conditions such as multiple sclerosis (Xiao, Zhu, & Lu, 2007), primary Sjogren's syndrome (Volchenkov, Brun, Jonsson, & Appel, 2013), and autoimmune diabetes (ADORINI, 2003).

2.3.1. Tolerogenic Dcs and Their Therapeutic Uses in Autoimmune Diseases

Tolerogenic dendritic cells use several immunosuppressive mechanisms to promote the development of tolerance. Tolerogenic dendritic cells (DCs) often have an immature or partially mature nature. This is supported by a reduction in the expression of co-stimulatory molecules, the major histocompatibility complex (MHC), and changes in cytokine production. When antigens are provided without simultaneous co-stimulatory signals, small amounts of antigens cause T cell inactivation and promote the growth of regulatory T cells. Both laboratory settings (in vitro) and real beings (in vivo) have observed this effect (Kretschmer et al., 2005; Steinbrink, Graulich, Kubsch, Knop, & Enk, 2002). When there are no co-stimulatory signals present, a certain set of genes becomes activated, resulting in the production of regulatory T cells. Disruption of the CD28-mediated Ras/MAPK pathway hinders this process, leading to a shortage of overall cellular activation. It transforms and becomes

AP-1. In the absence of AP-1, NFAT proteins can start a process of transcribing genetic information, maybe by forming dimers or working along with other transcription factors. This program can cause T cell exhaustion and promote the formation of regulatory T cells (Baine, Abe, & Macian, 2009).

However, studies have shown that dendritic cells (DCs) that have reached a mature state may also contribute to the development of regulatory T cells. This suggests that the cell phenotype of DCs does not solely control their immunogenic or regulatory role. In addition, dendritic cells (DCs) release anti-inflammatory cytokines, including IL-10 and TGF-β, while suppressing the production of pro-inflammatory cytokines. These acts are essential for promoting the development of tolerance (Hsu et al., 2015). Tolerogenic dendritic cells (DCs) secrete interleukin-10 (IL-10), which is essential for their capacity to control diverse circumstances. Furthermore, DCs secrete TGF-β, which is critical for inducing tolerance(Hsu et al., 2015; Travis et al., 2007). Tolerogenic dendritic cells (DCs) secrete TGF-β, which has a vital function in controlling the activation of Th17 cells in neuroinflammation, as shown in CD11cDNR mice (Speck et al., 2014). Tolerogenic dendritic cells promote the demise of effector T cells by activating the interactions between Fas ligand/Fas and TRAIL/TRAIL receptors (Izawa et al., 2012; Kurts, Heath, Kosaka, Miller, & Carbone, 1998)Tolerogenic dendritic cells (DCs) may produce many inhibitory receptors, including PDL-1, PDL-2, ILT, and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), that work to dampen the immune response. They undergo cellular differentiation to transform into T lymphocytes. Tolerogenic dendritic cells (DCs) control the concentrations of tryptophan and carbon monoxide via the production of HO-1, which activates IDO, and by modifying metabolic factors to affect T cell activity. IDO catalyzes the conversion of tryptophan into N-formyl kynurenine, resulting in a reduction in T-cell proliferation (Bonanno et al., 2012). HO-1 suppresses hemoglobin activity, resulting in carbon monoxide formation and a decrease in DC cell responsiveness (Chora et al., 2007). Furthermore, tolerogenic dendritic cells facilitate the proliferation of regulatory T cells through retinoic acid secretion (Hill et al., 2008). A recent study suggests that the process of dendritic cells (DCs) losing CD25 results in the termination of IL-2 absorption. This might serve as an alternate approach to suppressing the expansion of effector T cells (Kryczanowsky, Raker, Graulich, Domogalla, & Steinbrink, 2016).

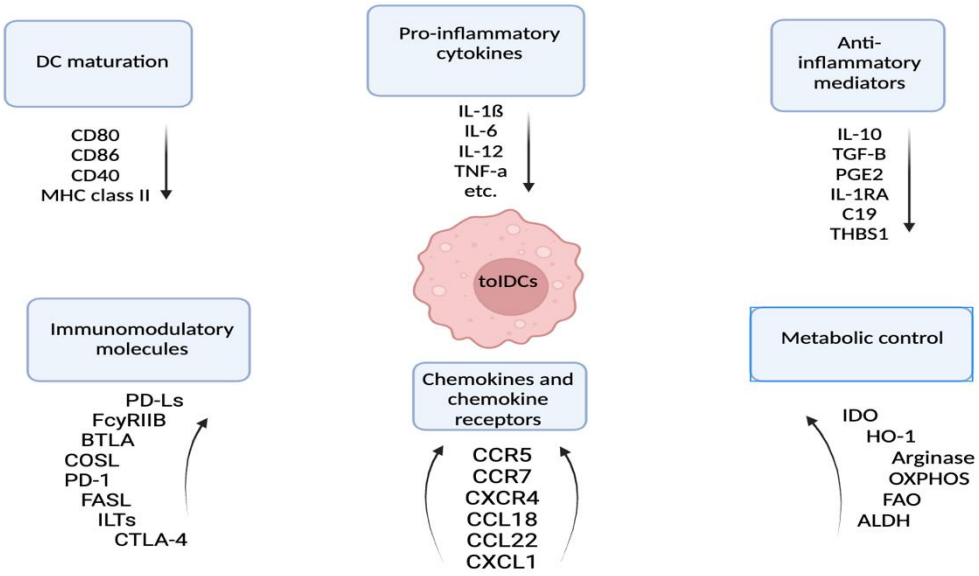


Figure 2. 8. Characteristics of tolerogenic DCs (Ritprajak et al., 2019)

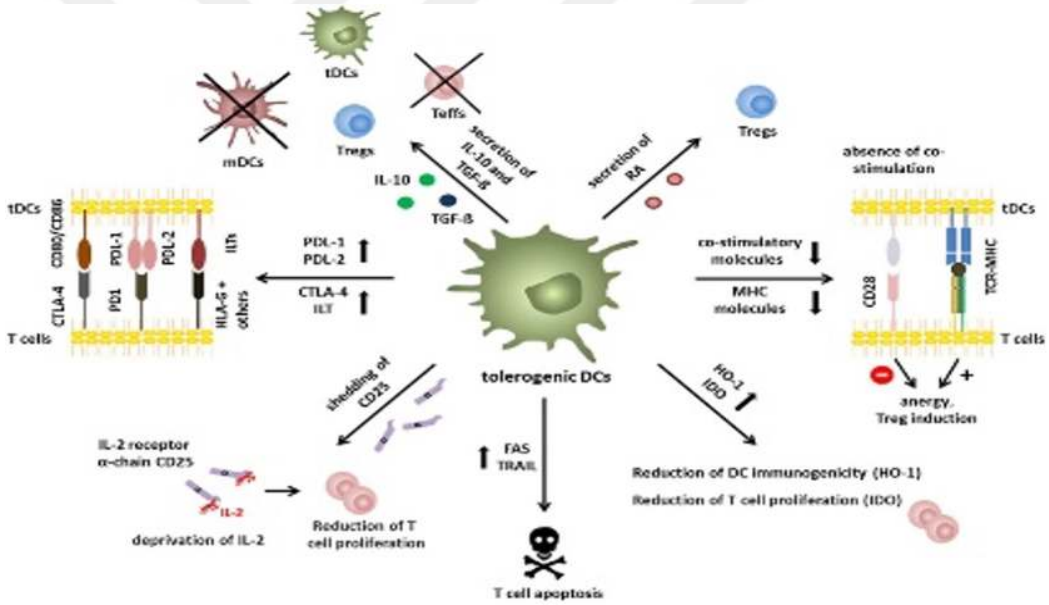


Figure 2. 9. Regulatory functions of tolerogenic DCs (Domogalla et al., 2017)

Tolerogenic dendritic cells (DCs) have been used in many immunotherapies approaches to treat inflammatory, autoimmune, and allergic diseases, as well as transplant rejection. In contrast to conventional immunosuppressive therapies, which often do not directly target the underlying cause of the illness and are associated with notable negative consequences, ex-vivo-generated tolerogenic DCs may be a feasible therapeutic approach to stimulate, augment, or restore tolerance to antigens. Strong data from several studies using rodent models has clearly shown that tolerogenic DCs are very effective in treating inflammatory diseases, autoimmune disorders, allergy

conditions, and transplant rejection (García-González, Ubilla-Olguín, Catalán, Schinnerling, & Aguillón, 2016). To apply the results of these experiments to the human body, several ex-vivo studies have shown that human tolerogenic dendritic cells (DCs) effectively inhibit immune responses related to diseases by stimulating regulatory T-cell responses or inducing T-cell anergy and death. He does. Extracorporeal experiments have shown that tolerogenic DCs, a kind of immune cell, may mitigate some immune responses related to TH2 cells in allergic illnesses by producing a molecule called IL-10. Studies have shown that these tolerogenic dendritic cells (DCs) may stimulate the generation of regulatory T cells that express a specific protein known as FOXP3. In addition, dexamethasone-treated tolerogenic DCs may effectively inhibit T-cell responses. They enhance the production of IL-10 by using regulators that are specifically targeted to Hevb 5 latex antigens (Escobar et al., 2014; Li et al., 2010). Several ex-vivo studies have been carried out employing tolerogenic dendritic cells (DCs) derived from people with autoimmune disorders. These dendritic cells (DCs) can reinstate tolerance for certain self-antigens that are established inside tissues. Administering vitamin D3-treated mo-DCs (which have immunosuppressive properties) carrying myelin peptides to people with multiple sclerosis reduced the sustained activation of autologous T lymphocytes that specifically target this antigen. This phenomenon was discovered to be contingent upon TGF-β (Anderson et al., 2016; Raïch-Regué et al., 2012). Moreover, a study conducted on individuals with type 1 diabetes showed that the creation of tolerogenic dendritic cells (DCs) in the presence of vitamin D or IL-10/TGF-β, followed by exposure to pancreatic islet antigen glutamic acid decarboxylase 65, led to a decrease in T cell activity. It primarily focuses on this antigen (Segovia-Gamboa et al., 2014)Moreover, in patients with systemic lupus erythematosus, the administration of autologous mo-DCs treated with dexamethasone or rosiglitazone and loaded with self-antigens led to a modification of CD4+ T cell activation, making it a suitable approach for immunotherapy of this disease (Obreque et al., 2017).

2.3.2. Immunogenic DCs and their therapeutic uses in cancer

Dendritic cells (DCs) are potent professional antigen-presenting cells (APCs) that possess an exceptional ability to coordinate immune responses (Steinman, 2012). Activated dendritic cells (DCs) in cancer can generate significant quantities of pro-inflammatory cytokines and eliminate tumor cells (Chung, Ysebaert, Berneman, &

Cools, 2013; O'Keeffe et al., 2015). Furthermore, they can enhance the cytotoxicity of natural killer (NK) cells and T lymphocytes, hence facilitating the eradication of cancerous cells (Chung et al., 2013; O'Keeffe et al., 2015). While the presentation of tumor antigens with MHC-I molecules by tumor cells is essential for the activation and function of cytotoxic T lymphocytes (CTLs), dendritic cells (DCs) are necessary to sustain the immune responses that target and destroy tumors (Fu & Jiang, 2018). Dendritic cells (DCs) capture antigens and then degrade them. Next, MHC-I or MHC-II molecules are introduced to initiate the CD8+ T and CD4+ T cell responses (Gardner & Ruffell, 2016). Multiple studies have demonstrated the involvement of CD8+ T cells in the production of immunological responses against tumors. Effector-killer T cells utilize their receptors to locate antigens associated with malignancies shown by MHC-I molecules (Fu & Jiang, 2018). The infiltration and extended presence of CD8+ T cells in the tumor microenvironment are correlated with a favorable prognosis for cancer(Dumauthioz et al., 2021; Rosenberg & Restifo, 2015). Upon activation, cytotoxic T lymphocytes (CTLs) can trigger programmed cell death (apoptosis) in tumor cells by utilizing either the Fas-FasL pathway or granule exocytosis. These cells also exhibit the secretion of interferon-gamma (IFN-γ) and TNFα, which can potentially induce toxicity in malignant cells (Lei et al., 2020). Research on mice indicates that CD4+ helper T cells are necessary to initiate immune responses by CD8+ T cells against tumors (Melssen & Slingluff, 2017). CD4+ T cells assist antitumor cytotoxic T lymphocytes (CTLs) through two mechanisms (Borst, Ahrends, Bąbała, Melief, & Kastenmüller, 2018). Their primary function in the immune system is to combat cancer cells. CD4+ T cells that have been activated release interleukin 2, which stimulates the development and proliferation of CD8+ cytotoxic T lymphocytes (CTLs) with a high degree of CD25 expression. CD4+ T cells can enhance the production of co-stimulatory molecules or cytokines from antigen-presenting dendritic cells (DCs) connected to cytotoxic T lymphocytes (CTLs). This indirect action helps to strengthen the immune response against tumors (Ahrends & Borst, 2018; Tay, Richardson, & Toh, 2021).

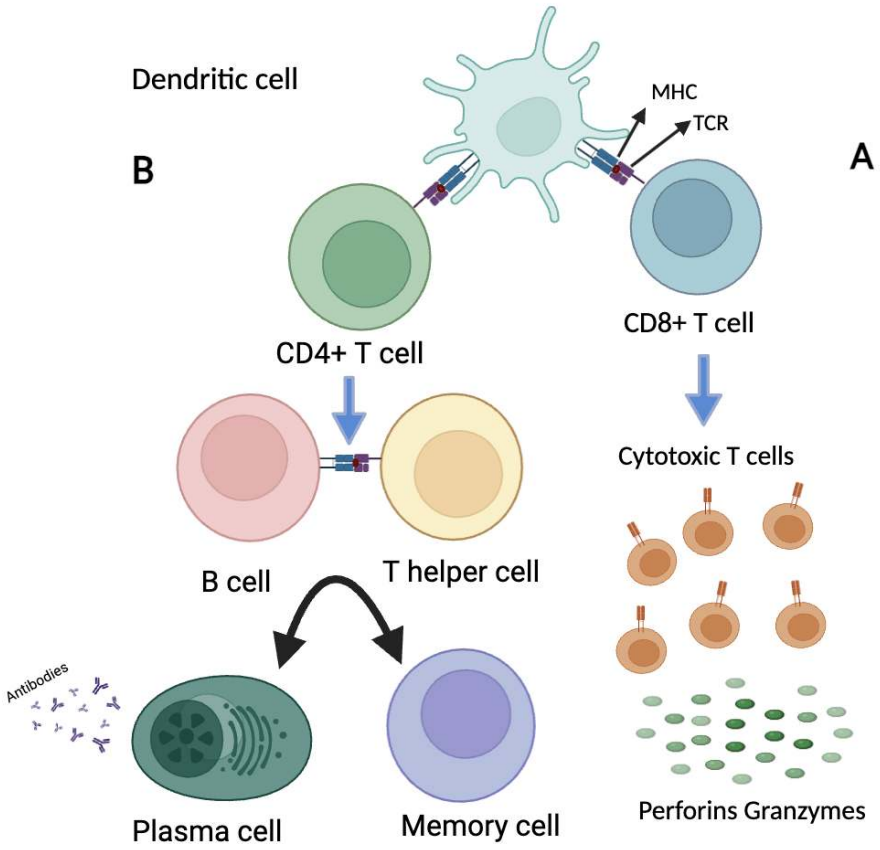


Figure 2. 10. The role of DCs in inducing the response of TCD4+ and TCD8+ cells and antitumor responses (Salah et al., 2021)

2.4. Thalidomide

In 1954, scientists synthesized thalidomide by chemical means and then released it to the market in 1967 as a treatment for respiratory infections. Pregnant women later advocated its use as a remedy for morning sickness. However, investigations from several nations have indicated the substance's ability to cause birth defects, resulting in its cessation. Thalidomide has developed into a medicine with immunomodulatory, anti-inflammatory, anti-angiogenic, and anti-proliferative properties. It has the potential to be used in the treatment of several types of cancer and autoimmune illnesses (Sherbet, 2015). This medicine is indicated for the treatment of persons diagnosed with multiple myeloma as well as several forms of cancer, such as pancreatic, prostate, and lung cancer. Additionally, it is used for the management of autoimmune illnesses such as graft versus host disease (GVHD), Waldenström's macroglobulinemia, and experimental autoimmune encephalomyelitis. There are

potential benefits to using it for multiple sclerosis treatment (Bamias & Dimopoulos, 2005). The intriguing feature of this immunomodulating drug is its capacity to influence angiogenesis, cell proliferation, and tumor advancement. Studies have shown that thalidomide impedes the effects of vascular endothelial growth factor (VEGF) and bFGF-induced angiogenesis in ocular angiogenesis tests (D'Amato, Loughnan, Flynn, & Folkman, 1994; KENYON, BROWNE, & D'AMATO, 1997). A further investigation has shown that lenalidomide, a derivative of thalidomide, hampers the functioning of VEGF, the P13K/protein kinase-B (AKT) pathway, and the formation of angiogenesis-related impacts in primary endothelial cells under controlled laboratory conditions. A distinct investigation examined the impact of the NOTCH/WNT pathway and the regulatory gene Kremen 2 on signaling. These genes control the inhibition of WNT signaling through Dickkopf 2 (DKK2) and Deltex 4 (DTX4). After being treated with pomalidomide and lenalidomide, which are drugs similar to thalidomide, specific changes were seen in colorectal cancer cells (Liu et al., 2010). In a phase I clinical study including patients with multiple myeloma, thalidomide showed the capacity to suppress the NF-kB, TRAF1, and IAP2 signaling pathways. When lenalidomide is coupled with everolimus, an inhibitor of the mTOR pathway, it leads to a reduction in the expression of mTOR, resulting in the development of treatment resistance in cancer (Yee et al., 2014).

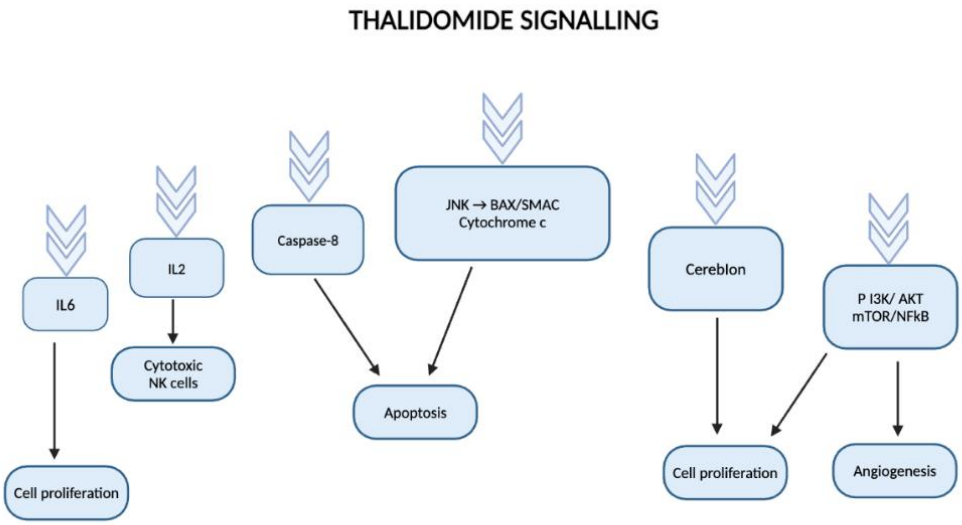


Figure 2. 11. Thalidomide-related signaling pathways (Sherbet, 2015)

Research has shown that thalidomide enhances the growth of CD4+ and CD8+ human T lymphocytes and boosts the production of IL-2 and IFN-Γ (MARRIOTT et al., 2002; Shannon & Sandoval, 2002). This medication can also decrease the synthesis of IL-6, which is a factor that promotes the development of tumors (Franks, Macpherson, & Figg, 2004). Studies have shown that thalidomide may effectively suppress the angiogenic effects caused by COX-2 in human tumor models (Fujita J, 2001).

2.5. Checkpoint immune molecules

Immune checkpoint molecules are pathways that regulate the immune system's response to infections and prevent excessive tissue damage. They also maintain immune tolerance to themselves and restrict the breadth of immunological responses in peripheral tissues (Kong et al., 2019). The expression of these inhibitory substances on immune cells hinders their activity. Immunological checkpoints are crucial in maintaining immunological homeostasis by preventing excessive immune responses. Nevertheless, these molecules are present in antigen-presenting cells (APCs) and lymphocytes that infiltrate the tumor microenvironment. As a result, T-lymphocyte-associated responses are suppressed, allowing the tumor to evade the immune system (Zhang et al., 2018)Tumor cells increase the expression of immunological checkpoints, which inhibit the function of DC cells. This suppresses the activity of T lymphocytes that are specifically primed to combat malignancies (Ghorbaninezhad et al., 2022). Upregulating immunological checkpoints like CTLA-4, PD-L1, BTLA4, B7H7, VISTA, and LAG3 leads to tolerating DC cells, enhancing their suppressive characteristics. The immune system utilizes this method as a means for tumors to evade immune surveillance (Ghorbaninezhad et al., 2022).

3. MATERIALS AND METHODS

Table 3. 1. Instruments Used

	Instrument	Manufacturing Company	Manufacturing Country
1	Invert Microscope	Optika	Italy
2	Nanodrop	Thermo Scientific	USA
3	Flow Cytometry	Miltenyi Biotec	Germany
4	Real-Time PCR	StepOne Plus	USA
5	Thermal Cycler PCR	Bio-Rad	USA
6	Incubator	Memert	Germany
7	Class 2 Hoods	Jal Tajhiz	Iran
8	Centrifuge	Sigma	Germany

In the study, a variety of sophisticated instruments were employed to ensure precise and accurate results.

3.1. Chemicals and Solutions

The study used various chemicals and solutions to achieve its goals, including BSA solution, DMSO solvent, PBS, RPMI-1640 culture media, cDNA Synthesis Kit, TRIzol for RNA extraction, TRIzol for RNA precipitation, primer synthesis, Ficoll for cell separation, 2-mercaptoethanol for cell culture, glutamine for cell culture media, and Fetal Bovine Serum for cell proliferation. Immunoglobulins targeting specific genes were used for flow cytometry.

3.2. Computer Software

Microsoft Excel and ImageJ were utilized for data processing in a study, enabling comprehensive investigation of cell populations, while FlowJo V10 supported image analysis, and GraphPad Prism 8 provided sophisticated statistical analysis and graphical representation.

3.3. Method

3.3.1. Cell Counting Protocol

A precise understanding of the cell count is essential for several cell culture operations, such as cell passage and freezing. The neobar slide, an apparatus built explicitly for this purpose, is commonly used to measure the number of cells per unit volume. To do the counting, the sediment of the cell was dissolved in 1 ml of culture fluid. Afterward, the solution with a volume of 20 ml was combined with an equivalent amount of trypan blue. Ultimately, a volume of ten microliters from the resulting combination was relocated to a fresh bar slide. The precise quantity of cells was determined using the below formula. Neobar's lam has four compartments for white blood cells (W); the average number of these compartments is used in the following formula.

$$N = a \times b \times c \times 10^4$$

(N: the total cell count; a: the average count of 4 cells; b: the photograph showing the dilution factor with trypan blue; c: the amount of the sample in milliliters)

The viability test in the cell suspension is conducted using Trypan blue dye to detect the proportion of living cells. Trypan blue solution can permeate and colorize deceased cells, but it cannot permeate living cells. Dead cells are blue, making them distinguishable from living cells that are translucent and colorless. This attribute calculates the proportion of viable cells using the following mathematical equation.

$$Viability\% = \frac{a}{T} \times 100$$

(a: number of living cells; T: total number of cells)

3.3.2. Distinction of DCs

3.3.2.1. Extraction of peripheral blood PBMCs. Healthy individuals were used to acquire peripheral blood, which was then collected in a sterile falcon tube containing heparin. Ficoll solution was utilized to separate peripheral blood mononuclear cells (PBMCs). Ficoll is a polysaccharide that is attracted to water and has several branches. Ficoll comes in various densities. However, Ficoll, with a 1.077 g/ml density, is specifically employed to isolate PBMCs. To achieve this objective, utilizing Ficoll and centrifugation generates a density gradient that allows for the distinct visualization of individual blood components. Following centrifugation, erythrocytes and neutrophils traverse the Ficoll and accumulate beneath it on the Falcon floor. PBMC cells are situated at the boundary between platelet-rich plasma and Ficoll.

The Falcon tube was used to obtain peripheral blood treated with heparin. Subsequently, the blood was centrifugated at room temperature with zero braking and one acceleration for 15 minutes at a force of 400 g. Following centrifugation, the upper layer containing a high concentration of PBMCs, and erythrocyte sediment was meticulously removed and transferred to a Falcon tube. Subsequently, an equivalent amount of PBS was added to the Falcon tube to dilute the cells collected from the center part. An equal volume of Ficoll was introduced to a separate falcon, matching the volume of the undiluted cell suspension. Subsequently, the cell suspension was delicately transferred to the Ficoll overlay after being diluted with PBS. Subsequently, a second centrifugation was carried out at room temperature at 800 g for 20 minutes, with the rotor abruptly stopped and started at a rate of one. The PBMC layer was carefully positioned between the Ficoll using a sterile Pasteur pipette, while the serum was collected in a separate falcon tube. PBMCs were washed twice with PBS at 200 g and 120 g for 10 minutes at room temperature to remove platelets. Subsequently, the cells were disrupted under specific conditions, with a brake setting of 5 and an acceleration of 5. After collecting the peripheral blood mononuclear cells (PBMCs), their quantity was determined using neobar slides, and their viability was assessed using a trypan blue solution.

3.3.2.2. Isolation of monocytes from PBMCs. The MACS technique was employed to isolate the autologous monocytes from PBMCs, as per the instructions included in the kit. The cell suspension was centrifuged at 300 g for 10 minutes

immediately following the isolation of PBMCs and subsequently frozen at a low temperature. A volume of one milliliter of MACS buffer and 100 L of biotin-bound anti-CD14 antibody (Ab-CD14-Biotin) were introduced for every 108 cells after the liquid containing dispersed particles was removed. 100 µl of streptavidin nanobeads were introduced for every 108 cells after 15 minutes of incubation at a low temperature. After a 15-minute incubation period on ice, 2 ml of buffer solution was introduced and centrifuged at 300 g for 10 minutes. The liquid portion was discarded, and one milliliter of buffer solution was reintroduced. The MACS column must be prepared before being inserted into the magnetic field of the MACS separator. We introduced approximately 600 microliters of buffer into the column, permitted the buffer to pass through, and subsequently saturated the column. Ultimately, the Falcon's contents were disposed of after the buffer was transferred into it after traversing the column. The cell suspension was incorporated after the column was prepared. The non-labile cells were initially transversed through the column. Monocyte cells that express CD14 surface markers become immobilized and ensnared within the column. We separated the column from the magnetic field and added a buffer after collecting non-tagged cells. The labeled cells, specifically monocytes, were subsequently collected using a piston. The monocytes were isolated and subsequently centrifuged at 300 g for 10 minutes. The vitality of monocytes was evaluated using a trypan blue solution, and their quantity was determined using a neobar slide. The MACS technique was employed to isolate autologous monocytes from PBMCs, and the kit's instructions were adhered to. After the isolation of PBMCs, the cell suspension was centrifuged at 300 g for 10 minutes and subsequently frozen at a low temperature. A volume of one milliliter of MACS buffer and 100 L of biotin-bound anti-CD14 antibody (Ab-CD14-Biotin) were introduced for every 108 cells after the liquid containing dispersed particles was removed. 100 µl of streptavidin nanobeads were introduced for every 10^8 cells after 15 minutes of incubation at a low temperature. After a 15-minute incubation period on ice, 2 ml of buffer solution was introduced and centrifuged at 300 g for 10 minutes. The liquid portion was discarded, and one milliliter of buffer solution was reintroduced. The MACS column must be prepared before being inserted into the magnetic field of the MACS separator. The column was lubricated after approximately 600 microliters of buffer were introduced. The buffer was then permitted to pass through the column. Ultimately, the Falcon's contents were disposed of after the buffer was transferred into it after traversing the column. The cell suspension was

incorporated after the column was prepared. The non-labile cells were initially transversed through the column. Monocyte cells that express CD14 surface markers become immobilized and ensnared within the column. The column was separated from the magnetic field, and a buffer was applied after the collection of non-tagged cells. The labeled cells, specifically monocytes, were subsequently collected using a piston. The monocytes were isolated and subsequently centrifuged at 300 g for 10 minutes. The vitality of monocytes was evaluated using a trypan blue solution, and their quantity was determined using a neobar slide.

3.3.2.3. Monocyte cultivation and their subsequent differentiation into dendritic cells. After performing cell counting, monocytes with an initial concentration of 1.5 x 106 per mL were placed on a 6-well plate along with a full medium for culture. The medium was comprised of RPMI-1640 supplemented with 15% fetal bovine serum, 100 IU/mL penicillin cyclin,100 µg/mL streptomycin, and two mM L-glutamine. In addition, the cytokines GM-CSF, a 50 µM solution of 2-mercaptoethanol (2ME) and IL-4, with concentrations of 40 and 25 ng/mL, respectively, were introduced into the culture. Subsequently, the cells were placed in an incubation environment. The cells changed the culture medium on the third day. The new medium included a comprehensive blend of cultures, consisting of GM-CSF, two mM of L-glutamine, and IL-4 at doses of 40 and 25 ng/mL, respectively. The cells were subsequently treated again. Dendritic cells (DC cells) at an immature stage were gathered on the fifth day.

3.3.3. Treatment of DCs with Thalidomide

On the fifth day, 1 mL of the liquid remaining on top of each well was extracted, and 1.5 mL of fresh RPMI-1640 solution containing 15% FBS was introduced. For the subsequent stage, it is recommended that an optimal dosage of thalidomide be administered to the DCs. The effective dose of thalidomide was determined using flow cytometry and the Annexin V-FITC/PI method. Once the optimal dosage of the thalidomide drug was determined, it was administered to the treatment group of cells. The treated and the control cells were not exposed to the drug and were then incubated at 37°C for 2 hours. Throughout the incubation period, 100 ng/mL of LPS was introduced to both the treatment and control group's cells. The cells were then

subjected to another round of incubation under the same conditions as before. After 24 hours, we collected thalidomide-treated mDCs and mDCs from the control group.

3.3.3.1. Annexin V-FITC/PI Staining. Phosphatidylserine, a naturally occurring molecule in the inner layer of the membrane, is translocated to the outer surface of the membrane. This displacement is caused by either the dysfunction of the ATP-dependent translocase enzyme or the initiation of another activity. Scramblase 2 is a supplementary enzyme. The positioning of phosphatidylserine on the external surface of the cell membrane serves as a signal for macrophages and surrounding cells to identify and ingest apoptotic cells. Annexin V, a protein with anticoagulant properties and a molecular weight of 18 kilodaltons, has been discovered to firmly attach to phosphatidylserine in the presence of calcium. This protein is commonly combined with FITC fluorescence dye to study apoptosis using flow cytometry. Annexin V necessitates a particular calcium-enriched buffer to attach to phosphatidylserines on the surface of the membrane. Otherwise, it will not adhere. During the late phases of apoptosis, the nuclear membrane gradually loses structural integrity, increasing its permeability. Here, the fluorescent dye PI, which specifically binds to DNA, attaches to the nucleic acid, resulting in the cells being PI-positive. This experiment consists of two groups: the control group, which does not get any treatment with the drug, and the treatment group, which is subjected to various doses of the drug.

To conduct this test, the medication was added, and the DCs were incubated for 24 hours. Following the designated incubation period, the cells undergoing treatment were gathered to examine apoptosis. Once the cells were assembled in 1.5 ml microtubes, the microtubes underwent centrifugation at 300g and 4°C for 10 minutes. Next, the liquid above the sediment was removed, and 200 µL of a buffer designed explicitly for inducing programmed cell death was added to each little tube. Next, 3 µL of annexin-labeled fluorescein isothiocyanate (FITC) solution was introduced to the cell suspension. The microtubes were then incubated in darkness and on ice for 10 minutes. Subsequently, 0.8 µL PI was introduced and subjected to another incubation period of 5 minutes under conditions of darkness and low temperature. Next, we subjected the microtubes to centrifugation at a force of 300 g at a temperature of 4°C for 10 minutes. Following this, The upper liquid fraction was pipetted off and 200 ml of PBS was added. Finally, we analyzed the samples using a flow cytometry instrument.

3.3.4. Phenotypic examination of DCs

The study investigated the presence of numerous surface markers in dendritic cells (DCs). Specifically, the concentrations of markers such as HLA-DR, CD11c, and CD86 were determined using anti-HLA-DR-APC, anti-CD86-PE, and anti-CD11c-FITC antibodies, respectively. Additionally, we evaluated the expression of the monocyte marker CD14 on both dendritic cells (DCs) and monocytes using anti-CD14-FITC. Fully developed dendritic cells (mDCs) and mDCs that were treated with thalidomide were represented in the experimental groups. The cells from these groups were transferred from six-well plates to microtubes. After the liquid portion was removed, cell enumeration was conducted on neobar slides after centrifugation at 300 ×g for 10 minutes. The microtubes were subsequently subjected to an additional round of centrifugation under the same conditions. The cell clusters from each group were subjected to FACS reagents and appropriate antibodies at a low temperature in the absence of light for 25 minutes after the liquid part was separated. PBS was administered to each microtube, and the tubes were subsequently centrifuged at 300 rpm for 10 minutes. The cell clusters were re-suspended in 200 µL of PBS and analyzed by flow cytometry after the liquid above the particulate residue was decanted.

3.3.5. Assessing the expression of several immune checkpoint genes using the real-time PCR technique.

The test groups consisted of two types of cells: mDCs and mDCs treated with thalidomide. The test aimed to examine the immunological expression of different checkpoints, including PD-L1, BTLA, B7H7 CTLA-4, VISTA, and LAG3.

3.3.5.1. RNA extraction. The RNA extraction process involved the utilization of Trizol solution. RNA extraction was performed using one million cells from each group. All tools used for this phase must be free of RNase contamination. To accomplish this objective, the cells were initially transplanted to 1.5-ml microtubes. Following a 10-minute centrifugation at 1300 rpm, the supernatant liquid was discarded, and Trizol solution was introduced to the remaining 800 µL solution. Trizol is a hazardous substance and should be carefully introduced into the microtubes within a chemical hood. Trizol facilitates the release of internal contents, such as RNA and DNA, by lysing cells. Subsequently, a 200 ml aliquot of chloroform solution was

introduced, and the solution was gently agitated for 15 seconds using swirling motions, avoiding vortexing, in each microtube.

Next, the microtubes underwent centrifugation in a chilled centrifuge at a speed of 13,000 revolutions per minute for 15 minutes at a temperature of 40 °C. Consequently, this process divides the cell contents according to the concentration gradient and molecular weight. Following centrifugation, the solution within the microtube was separated into three distinct phases. The top phase, which is colorless and transparent, is the chloroform phase and contains RNA. The intermediate layer, which has a ring-like shape and is white, contains DNA. The lowermost layer is crimson and comprises triazole and several other chemicals. Chloroform is used to extract RNA from biological components and proteins. To extract the RNA from the cells, we specifically collected the clear and colorless supernatant using a nuclease-free sampler head. This supernatant was transferred into another nuclease-free 1.5 ml microtube, and 1 ml of isopropanol was added. Subsequently, the solution was rapidly mixed by gently agitating the upper and lower parts of the microtube; then, the microtubes were transferred to a freezer set at a temperature of -20°C for 24 hours. Following this period, the microtubes underwent centrifugation in a chilled centrifuge at a speed of 13000 revolutions per minute for 15 minutes at a temperature of 40 °C. Following the centrifugation process, the liquid portion of the microtubes, known as the supernatant, was extracted. The centrifuged white precipitate was dissolved in a 1ml solution of 75% ethanol. The microtube underwent centrifugation in a chilled centrifuge at a speed of 8000 revolutions per minute for 10 minutes while maintaining a temperature of 40 °C. Using ethanol for washing negates the efficacy of isopropanol. Following the second cleaning, the microtubes were positioned in the laboratory with the door left open to facilitate the evaporation and complete removal of the alcohol contained within them. Ultimately, the sediment contained within the microtubes was dissolved using 20 µL DEPC water. Subsequently, the microtubes were subjected to a temperature of 580 °C for 10 minutes in a dry bath machine. Subsequently, the isolated RNA's optical density (OD) and purity were assessed using a nanodrop device. The extracted RNAs were preserved at a temperature of -80°C until the synthesis of cDNA.

3.3.5.2. cDNA synthesis. The cDNA synthesis was performed using a BIOFACT kit manufactured in Korea. This kit utilizes the Moloney murine cancer virus reverse transcriptase enzyme, which possesses modest RNase characteristics. This kit can synthesize complementary DNA (cDNA) from templates of considerable

size, including those as large as 13 kilobases. The contents of this kit include the following, Random Hexamer, oligo dt, RNase-free water, and 2X RT pre-mix.

A random hexamer primer generated a cDNA strand from the RNA strand. This primer binds indiscriminately to several locations on the RNA molecule, allowing it to serve as a template for producing all forms of RNA, such as tRNA, rRNA, and mRNA. Before cDNA synthesis, it is crucial to normalize the concentration of samples. To achieve this objective, the samples were measured using a nanodrop device to determine their optical density (OD). Subsequently, an RNA concentration of 500 ng/µL was generated. An alternative method exists to complete this task. In this approach, the nanodrop device is used to measure the concentration of RNA. Based on this measurement, the necessary quantity of each sample may be determined to achieve a final concentration of 500 ng/µL. This amount is extracted from the original sample and combined with other substances. This work added RNase-free water to the residual solution to achieve a final volume of 20 µL. CDNA synthesis was performed to evaluate the expression of the relevant genes. The substances were initially introduced into 0.2 ml microtubes for PCR.

Afterward, the microtubes containing the specified ingredients were placed in a thermocycler and kept at 60°C for 5 minutes. The temperature-induced alteration of the RNA from the clamp state facilitates the uniform attachment of the primer to all sites. Subsequently, the samples were subjected to thermal cycling in a thermocycler, with a temperature of 50°C maintained for 30 minutes and a temperature of 95°C for 5 minutes. After the cycle, the microtubes containing the samples were transferred to a freezer set at a temperature of -20 °C to preserve their integrity.

3.3.5.3. Real-time PCR test. Real-time PCR equipment is utilized to assess the degree of gene expression in living organisms.The drawback of the primary PCR technology is its incapacity to assess the gene's expression level quantitatively. This study utilized the ABI StepOne Plus brand real-time PCR instrument. This technique employs intercalating dyes that selectively attach to two DNA strands.There is a direct correlation between the quantity of fluorescence in the device and the quantity of the ultimate product produced. These dyes cannot bind without double-stranded DNA, decreasing their fluorescence intensity. One of the most crucial colors is cyber green. Simultaneously, Syber Green is affixed to the items during the production process. Hence, the rise in fluorescence of this substance during the elongation phase of each

cycle is directly proportional to the quantity of PCR product generated in that cycle. The quantification of gene expression or presence involves normalizing the PCR product of the gene with a reference gene, which serves as a control. The reference gene must possess identical copies within the given cell line, and the treatment should not influence its expression. The often-used reference genes comprise beta-actin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), beta-globin, 18S ribosomal RNA, U6, and SNORD. The benefits of utilizing the real-time polymerase chain reaction (PCR) technique include Reproduction is observable at every moment. This system allows for real-time response monitoring and examining the multiplication process in each cycle. It is feasible to halt the reaction at the intended time since the PCR process is observable. The response can be halted through non-proliferation or entering a resting phase, thus preventing the unnecessary expenditure of time and energy.

3.3.5.4. Quantitative PCR. The primary template can be accurately measured using absolute and relative quantification approaches to determine its precise and proportional quantities. Reactions typically occur more rapidly, necessitating less time, and this method's detection range surpasses traditional PCR's.

3.3.5.5. Melting Curve Analysis. High-resolution melt curve analysis is an affordable and uncomplicated technique used after PCR to detect genetic alterations and determine genotypes. This technique relies on the melting temperature of PCR products, corresponding to the temperature at which double-stranded DNA splits into single strands. The melting temperature of DNA is an important characteristic that is influenced by its structure, as well as elements such as nucleotide length and quantity, probe concentration, salt concentration in the environment, and the percentage of cytosine and guanine nucleotides. An advantage of real-time PCR with varied nucleotide sequences is the ability to determine the melting curve (High-Resolution et al.). This curve is generated after the PCR is completed to validate the results. When there is a variation in the arrangement of nucleotides between two samples, it results in a distinct variance in the form of the curve. Distinguishing cases is accomplished by comparing the curves of the samples either among themselves or with standardized samples (sequenced). Each peak in this curve corresponds to the melting point of a PCR result, determined by analyzing fluorescence variations at different temperatures.

Another distinctive benefit of this test is its exceptional speed, precision, and affordability.

3.3.5.6. Gene expression ratio analysis. The PCR signal indicates the corresponding transcription level of the target gene in comparison to other genes, including the calibrator gene. The -ΔΔCt2 technique determines gene expression changes in Real-time PCR. The last step in Real-time PCR involves conducting a threshold analysis of cycles, also known as CT. This is done by graphing the logarithm of the PCR signal versus the number of cycles. The study involved using the earlier methods to conduct qRT-PCR tests on the cDNA generated from each group's samples. The CT results were verified using Microsoft Excel Spreadsheet software. The expression of the relevant genes was calculated through the following equation:

$$\text{Relative gene expression} = 2^{-\Delta\Delta Ct}$$

$$\Delta CT = CT_{Target} - CT_{Refrence}$$

$$\Delta\Delta CT = \Delta CT_{Sample} - \Delta CT_{Calibrator}$$

3.3.5.7. Real-time PCR protocol. The mRNA expression level of immunological checkpoint genes was assessed using real-time PCR and Syber Green. The primers were selected by analyzing the primers mentioned in the publications associated with the target genes through the website http://www.primerdesigntools.com/.

Table 3. 2. Real-Time PCR Primer Sequences

Gene	Forward/Reverse	Sequence
CTLA-4	F	CATGATGGGGAATGAGTTGACC
	R	TCAGTCCTTGGATAGTGAGGTTC
BTLA	F	GCACCAGGCAAAATTCCCAAG
	R	TTCGGTCCAATGACAGAATGG
PD-L1	F	TGCCGACTACAAGCGAATTACTG
	R	CTGCTTGTCCAGATGACTTCGG
B7H7	F	TGAAGCAAACATGGACAGGG
	R	CAGATAGTAAGCCAGGACAGAG
VISTA	F	GCGGATGGACAGCAACATT
	R	TTGGAGAGTCAGGGACAGGG
LAG3	F	CTGGGACCTACACCTGCCAT
	R	TACTGGAGTCACCTCACAAAGCA
18S	F	ACCCGTTGAACCCCATTCGTGA
	R	GCCTCACTAAACCATCCAATCGG

To prevent damage to the cDNAs, they were placed on ice immediately after removal from the freezer at -20 °C. Then, according to the number of samples, RNase-free strip tubes for PCR were prepared, and in each of them, the following materials were added.

Table 3. 3. Real-Time PCR Materials

Volume in microliters	Material
0/5	cDNA
5	Master Mix 1X
0/5	Mixed forward and reverse primers
4	Deps Water
10	the final Volume

After preparing the samples according to Table 7-4 protocol, the samples were transferred to the Real-time PCR machine. The considered conditions for PCR include 10 minutes at 95°C, three steps of 13 seconds at 95°C, 30 seconds at 60-62°C, 20 seconds at 72°C, and 45 cycles.

Table 3. 4. Protocol of cycles used in Real-Time PCR

Cycle		Time and Temprature
Hold Step		95^0C, 10 min
Cycling (45 Repeats)	Denaturation	95^0C, Hold 13 secs
	Annealing	60-62^0C, Hold 30 secs
	Extension	72^0C, Hold 20 secs

3.3.5.8. Internal standard. To compare the expression of a particular gene in two different samples, a gene is chosen as a control or internal standard. This gene is present in the sample and considers the varied amount of RNA retrieved from the cell and the contribution of one to two percent of mRNA from the total cellular RNA. Various genes exhibit comparable levels of expression (House et al.), and the expression of additional genes is quantified relative to this baseline. The 18S gene-specific primers were designed to amplify the target genes as an internal control, contributing to the relative presentation of the data, to achieve this objective.

3.3.6. Statistical analysis

The experiments in this study were done three times. The data were provided as the mean value (± SD). The statistical analysis used the student's t-test to determine the differences in variables between the groups being studied. The statistical analyses

were performed using GraphPad Prism software version 8.0.2. A significance threshold of $P < 0.05$ was used.

4. RESULTS

4.1. Determining the effective dose of the thalidomide drug

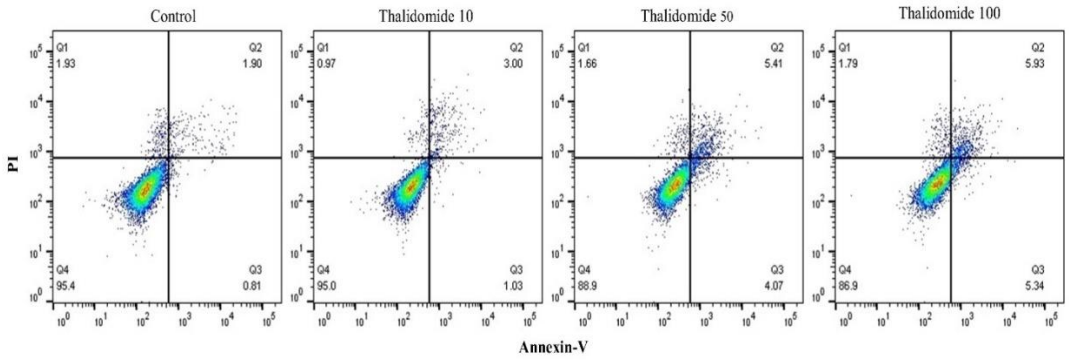


Figure 4. 1. Determining the optimal dose of thalidomide. Cells treated with a dose of 10 μM of this drug showed the highest percentage of being healthy (95%).

The effective dosage of thalidomide was determined using flow cytometry and the Annexin V-FITC/PI technique. Dendritic cells (DCs) were subjected to various concentrations of thalidomide (10, 50, and 100 μM). The highest dose that caused the least apoptosis in cells was selected as the effective dose. The number of live cells in the population of cells treated with 10, 50, and 100 μM drug doses was 95%, 88.9%, and 86.9%, respectively (cell population located in the Q4 region, which is PI- and Annexin-v-). The cell populations located in the Q3 region are Annexin-v+ and PI- cells. This population of cells is in the early stage of apoptosis, and the number of cells in this stage of apoptosis was 1.03%, 4.07%, and 5.34% for the cells treated with 10, 50, and 100 μM doses of the drug, respectively. The population of cells located in the Q2 region is Annexin-v+ and PI+, the cells located in the late stage of apoptosis, and the number of cells in this stage of apoptosis for cells treated with 10, 50, and 100 μM doses of the drug; the order was 3%, 5.41%, and 5.93%. Therefore, according to the data obtained, a dose of 10 micromolar drugs was considered the optimal dose for treating cells (Figure 4.1).

4.2. Differentiation and maturation of DCs

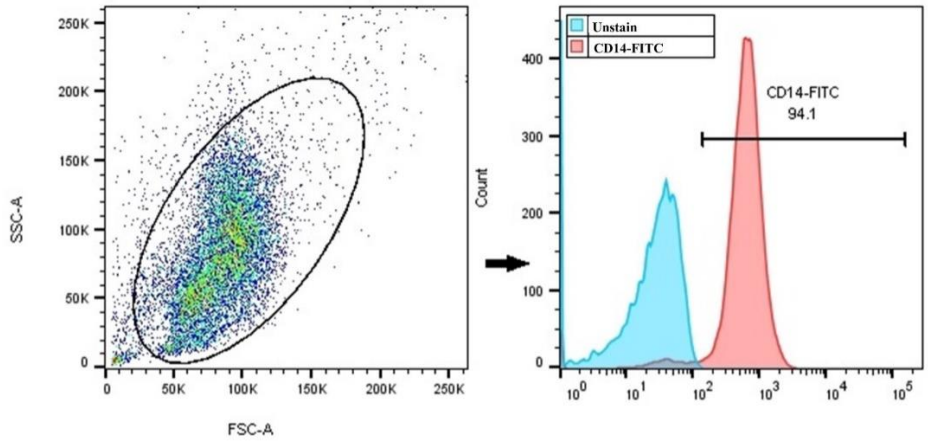


Figure 4. 2. The purity of monocytes derived from peripheral blood mononuclear cells (PBMCs)

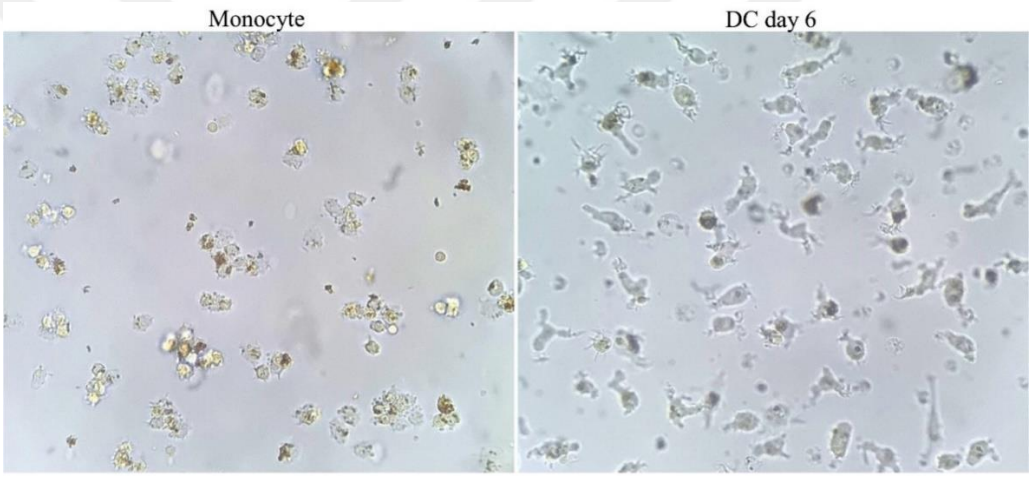


Figure 4. 3. Morphological changes during in vitro culture of adherent monocytes and differentiated DCs.

The monocytes produced from PBMCs utilizing the MACS approach exhibited a purity of around 94% (Figure 4.2). Monocytes attached to the surface of the plate and transformed into thalidomide-mDCs over six days. This transformation occurred when the cells were being cultured in the presence of IL-4 and GM-CSF cytokines, along with thalidomide treatment (Figure 4.3). The evaluation of the physical characteristics of fully developed dendritic cells (DCs) was performed by analyzing the presence of certain markers related to the process of maturation and the presentation of antigens, including CD11c, HLA-DR, and CD86, on the cell surface. Furthermore, the presence of monocyte markers (CD14) on the surface of dendritic cells (DCs) was assessed. The evaluation was conducted on the mDC group, and the

group received the correct amount of medicine. The presence of the CD14 monocyte marker was detected in a small subset of differentiated DC cells, suggesting that the differentiation of DC cells from monocytes was effective (Figure 4.2). The results demonstrated that CD11c, HLA-DR, and CD86 markers were present in both the mDC groups and those treated with the optimal medication dosage (Figure 4.2). Afterward, the difference in the way these markers appear on the surface of the two groups of dendritic cells (DCs) was evaluated using the mean fluorescence intensity (MFI). The flow cytometry findings showed a notable rise in HLA-DR ($P < 0.01$) and CD86 ($P \leq 0.01$) levels in the thalidomide-treated group (Figure 4.2). The expression of the CD11c marker showed a little increase in the treated group, but this change did not reach statistical significance (Figure 4.2).

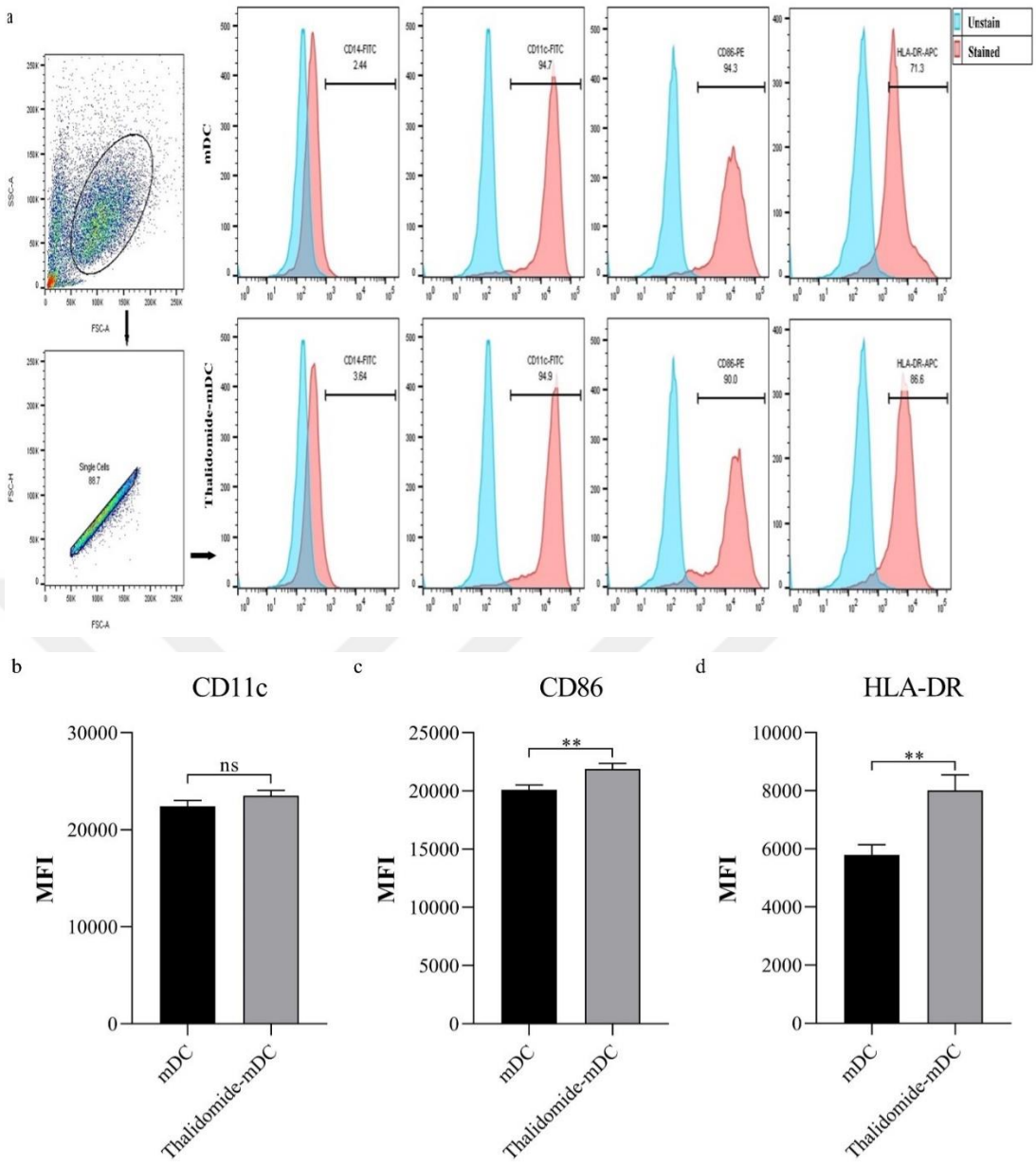


Figure 4. 4. Phenotypic assessment of mDCs and Thalidomide-mDCs

The surface marker expression profiles of both subsets of dendritic cells (DCs) were assessed using flow cytometry, which included CD11c, HLA-DR, and CD86. The results of category a are represented as the percentage of cells that express these markers. The levels of CD11c, HLA-DR, and CD86 expression across various DC types are represented by the mean fluorescence intensity (MFI) in categories b, c, and d. Statistical significance is indicated by the symbol (**: P≤ 0.01, ns: not significant).

4.3. Determining the expression of B7H7, PD-L1, CTLA-4, BTLA, VISTA and LAG3 genes in mDCs and Thalidomide-mDCs

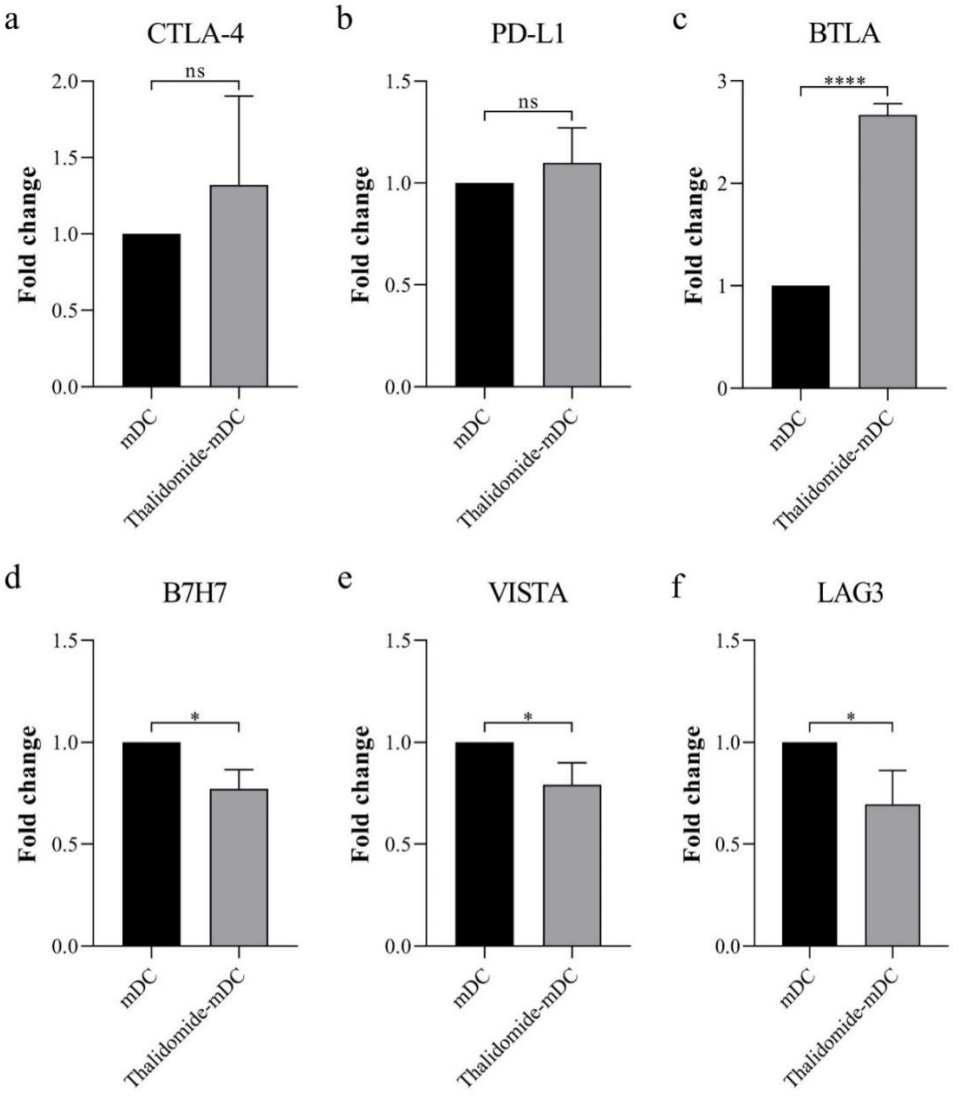


Figure 4. 5. Expression profile of immune checkpoints in mDCs and Thalidomide-mDCs

RNA was extracted from mDC and thalidomide-mDC group cells after the cells were treated with the thalidomide drug. Subsequently, cDNA was synthesized from the extracted RNA. The real-time PCR technique was employed to investigate the expression levels of immune checkpoint genes, such as B7H7, PD-L1, CTLA-4, BTLA, VISTA, and LAG3. The results suggest that the thalidomide treatment of mDCs resulted in a substantial decrease in the expression levels of immunological checkpoints B7H7 ($P \leq 0.05$), VISTA ($P \leq 0.05$), and LAG3 ($P \leq 0.05$) in the thalidomide-mDC group compared to mDC (Figure 4.5). However, the treated group demonstrated a substantial increase in BTLA gene expression in comparison to the

control group (P≤0.0001). The thalidomide-treated group exhibited higher gene expression levels of immunological checkpoints PD-L1 and CTLA-4 than the mDC group (Figure 4.5). Nevertheless, this increase was not statistically significant.

The level of immune gene expression of various checkpoints, including BTLA, CTLA-4, PD-L1, VISTA, B7H7, and LAG3, was assessed using the qRT-PCR technique (ns: not significant, *: P≤ 0.05, ****: P≤ 0.0001).

5. DISCUSSION

The equilibrium and consistency of immunological reactions that rely on T cells are regulated and maintained by dendritic cells (DCs), which are immune cells (Hubo et al., 2013). The activation of the antigen-specific T-cell immune response and the maintenance of tissue-specific immunological tolerance are the two functions of dendritic cells (DCs), which are derived from distinct phases of DC cell development, specifically the immunogenic and tolerogenic stages (Hubo et al., 2013). Immunogenic dendritic cells (DCs) facilitate the expansion of antigen-specific T lymphocytes by transmitting signals that promote their clonal proliferation. In the interim, tolerogenic dendritic cells (DCs) maintain tolerance by either eliminating antigen-specific T cells or promoting the clonal expansion of regulatory T (Treg) cells (Sim, Malinarich, Fairhurst, & Connolly, 2016). DC cells have garnered significant interest as potential therapeutic agents for autoimmune disorders and cancer as a result of this distinctive characteristic (Sim et al., 2016). Immature tissue-resident dendritic cells (DCs) differentiate into either tolerogenic or immunogenic DCs, contingent upon the encompassing environment and inflammatory stimuli. These distinct dendritic cells exhibit consistent characteristics and can adjust to their functions (Hubo et al., 2013). The specific microenvironment of dendritic cells (DCs) significantly influences their activation state, which is the primary factor determining their ability to establish immune tolerance or induce an immunological response. Dendritic cells (DCs) possess a variety of receptors on their surface and within their cells, which allow them to identify signals associated with infection, threat, and inhibition. The activation of DCs can be influenced by these signals (Iberg, Jones, & Hawiger, 2017). Immature dendritic cells (DCs) are typically located on the body's surfaces. The primary function of T cells is to collect and assimilate antigens to present them to T cells that are specifically targeted to those antigens. Immature dendritic cells (DCs) produce limited or no proinflammatory cytokines and exhibit reduced expression of inflammatory markers. Dendritic cells (DCs) transition to their contrasting activation state, which is described as mature DCs, when pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) are present. These fully developed dendritic cells subsequently migrate to adjacent lymph nodes and demonstrate a strong

ability to activate effector T cells (Dudek, Martin, Garg, & Agostinis, 2013). A study was conducted to cultivate fully developed dendritic cells (DCs) that possess a strong ability to stimulate immune cells. Various methods were employed, including LPS, CD40-L, TNF-α, prostaglandin E2, IL-6, and IL-1β. The results indicated that the maturation procedures that employed TNF-α and CD40L transformed DCs into a Th2 immune response. In contrast, interventions that rely on IFNs have a greater ability to activate Th1 cells (Castiello et al., 2011). Numerous immunosuppressive factors, such as IL-10 and TGF-β, as well as endogenous immunosuppressants like glucocorticoids and specific immunosuppressive medications like rapamycin, induce the production of tolerogenic dendritic cells (DCs) (Adorini, 2004; Švajger & Rožman, 2013). Consequently, the development of two distinctive phenotypes in DCs—immunogenic and tolerogenic—is associated with the activation or inhibition of specific signaling pathways. Numerous investigations have demonstrated the consequences of administering various medications to dendritic cells (DCs), including anti-cancer treatments (Joo, 2003), medroxyprogesterone acetate (Quispe Calla, Ghonime, Cherpes, & Vicetti Miguel, 2015), anti-inflammatory pharmaceuticals (Toebak et al., 2007), chloroquine (Thomé et al., 2014), and others. Each of these medications activates a distinct signaling pathway. Furthermore, Inhibit has provided information on the biological characteristics of DC cells and the process for promoting their diverse phenotypes for immunotherapy applications. Thalidomide is a pharmaceutical agent that has the potential to reduce inflammation and modify the immune system. It affects the functionality of numerous immune cells (Bamias & Dimopoulos, 2005). The objective of this investigation is to examine the impact of thalidomide on the maturity, differentiation, and expression of immunological checkpoints in DC cells. Thalidomide is recognized for its extensive modulatory effects on numerous cells. The optimal dosage of thalidomide for treating DCs was ten μM, as per our research. We assessed the impact of thalidomide treatment on the biology of DC cells after determining the optimal dosage for treating cells. The analysis involved the examination of the maturation process, capacity for presenting antigens, and distinguishing features of DCs. To evaluate these specific aspects, we implemented an exhaustive examination of the expression patterns of surface molecules. The results of our study indicate that the transformation of monocytes into dendritic cells is not impeded by thalidomide when GM-CSF and IL-4 cytokines are employed. The medication substantially increased the expression of HLA-DR and CD86 indicators,

while the CD11c marker in dendritic cells (DCs) treated with thalidomide showed only a minor change. This implies that the cells that have been treated possess distinctive characteristics and capabilities. They exhibit a substantial degree of antigen presentation. Furthermore, the investigation examined the impact of thalidomide on the expression of immune checkpoint genes in DC cells. As previously mentioned, tolerogenic DC cells exhibit elevated expression of numerous inhibitory checkpoints, including CTLA-4, PD-L1, BTLA4, B7H7, VISTA, and LAG3 (Ghorbaninezhad et al., 2022). Our findings indicate that the expression of genes associated with B7H7, VISTA, and LAG3 is substantially diminished in DCs treated with thalidomide in comparison to the mDC group. Nevertheless, the BTLA gene expression was significantly elevated in the treatment group. Furthermore, the expression of PD-L1 and CTLA-4 exhibits a modest increase following thalidomide treatment; however, this increase is not statistically significant. Additional research has been conducted in this discipline. According to a study, the process of monocyte differentiation into dendritic cells, initiated by the cytokines GM-CSF and IL-4, was not impeded by thalidomide. Our observations were corroborated by the discovery (Mohamad Mohty 1, 2002). The results of our investigation suggest that the expression of CD86 and HLA-DR markers is substantially increased by the combination of thalidomide treatment and DC cell maturation with CD40-L (Mohamad Mohty 1, 2002). The investigation conducted by Henry et al. has demonstrated that the expression of MHC-II and CD86 is increased in DC cells when they are treated with thalidomide analogs, as per our observations. As a result, this improves the capacity of dendritic cells (DCs) to use cross-presentation to present antigens to TCD8+ (Henry et al., 2013). Another study found that dendritic cells (DCs) produced from monocytes obtained from multiple myeloma patients treated with thalidomide exhibited a decrease in CD86 and HLA-DR expression, which contrasts with our findings (Schütt et al., 2005). You et al. discovered that the expression of CD80/CD86 was not influenced by the administration of thalidomide to DC cells, irrespective of the duration of the stimulation, as reported in reference (You, Xu, Zhang, Chen, & Gao, 2018). Research has demonstrated that thalidomide can reduce the ability of LC cells to transport antigens while maintaining the levels of CD80 and CD86 molecules (Deng, Ding, & Granstein, 2003).A significant increase in CD86 expression was observed in splenic dendritic cells (DCs) from C57BL/6 mice that were treated with thalidomide, as demonstrated by Kim et al (Kim et al., 2021). However, there were no alterations in

CD11c expression. Furthermore, they demonstrated that the expression of PD-L1 in these dendritic cells (DCs) did not exhibit a significant increase (Kim et al., 2021). Thalidomide administration to C57BL/6 mice increased the expression of PD-1 in splenic effector T cells (Kim et al., 2021).

6. CONCLUSION

This investigation delves into the complex relationship between thalidomide and dendritic cells (DCs), offering new perspectives on its impact on these essential immune system components. A network of interconnected responses that substantially influence the function, maturation, and expression of crucial immunological checkpoints in monocyte-derived DCs has been identified through the administration of thalidomide. Our results indicate that thalidomide has significant and concurrent effects on DCs, boosting the expression of markers that are implicated in antigen presentation. The potential of thalidomide to improve the immune system's capacity to detect and combat infections or anomalous cells is suggested by the improvement in DCs' ability to present antigens and initiate immune responses effectively.

Furthermore, our findings indicate that thalidomide has the potential to depress the expression of immunological checkpoints, including VISTA and LAG3, in DCs. To preserve immune homeostasis, these checkpoints are essential for regulating the duration and intensity of immune responses. Consequently, this drug affects the DC phenotype, likely shifting it towards a more immunogenic phenotype. Thalidomide's capacity to decrease the expression of VISTA and LAG3 indicates a potential mechanism by which the drug enhances immune activation and influences immunological tolerance.

Thalidomide treatment boosts markers involved in antigen presentation and lowers the levels of inhibitory checkpoints like VISTA and LAG3. This likely changes the DC phenotype to a more immunogenic state.

Additionally, immune regulation and therapeutic strategies are significantly affected by the profound changes in DC attributes that thalidomide induces. Thalidomide can treat immune-related disorders, such as cancer and autoimmune diseases, by reorienting DCs to a more immunogenic state. This entails the reduction of immune checkpoint molecule expression while simultaneously improving antigen presentation. However, the precise molecular mechanisms that underlie the effects of thalidomide on DCs remain uncertain, despite these compelling findings. To completely realize the therapeutic potential of thalidomide and to elucidate the complex biochemical pathways through which it exerts its immunomodulatory effects, additional research is necessary. These insights suggest that DCs are prospective

targets for thalidomide-induced immunomodulation, necessitating further research into specific biochemical pathways to develop more precise and effective therapies for immune-mediated diseases.

I. REFERENCES

Abul K. Abbas & Andrew H. Lichtman & Shiv Pillai. (2021). Cellular And Molecular Immunology, 10th Edition.

ADORINI, L. (2003). Tolerogenic Dendritic Cells Induced By Vitamin D Receptor Ligands Enhance Regulatory T Cells Inhibiting Autoimmune Diabetes. Annals Of The New York Academy Of Sciences, 987(1), 258–261. Https://Doi.Org/10.1111/J.1749-6632.2003.Tb06057.X

Adorini, L. (2004). Pharmacological Induction Of Tolerogenic Dendritic Cells And Regulatory T Cells. Seminars In Immunology, 16(2), 127–134. Https://Doi.Org/10.1016/J.Smim.2003.12.008

Aerts, J. G. J. V., De Goeje, P. L., Cornelissen, R., Kaijen-Lambers, M. E. H., Bezemer, K., Van Der Leest, C. H., Mahaweni, N. M., Kunert, A., Eskens, F. A. L. M., Waasdorp, C., Braakman, E., Van Der Holt, B., Vulto, A. G., Hendriks, R. W., Hegmans, J. P. J. J. & Hoogsteden, H. C. (2018). Autologous Dendritic Cells Pulsed With Allogeneic Tumor Cell Lysate In Mesothelioma: From Mouse To Human. Clinical Cancer Research, 24(4), 766–776. Https://Doi.Org/10.1158/1078-0432.CCR-17-2522

Ahrends, T. & Borst, J. (2018). The Opposing Roles Of <Scp>CD</Scp> 4 + T Cells In Anti-Tumour Immunity. Immunology, 154(4), 582–592. Https://Doi.Org/10.1111/Imm.12941

Alvarez-Dominguez, C., Calderón-Gonzalez, R., Terán-Navarro, H., Salcines-Cuevas, D., Garcia-Castaño, A., Freire, J., Gomez-Roman, J. & Rivera, F. (2017). Dendritic Cell Therapy In Melanoma. Annals Of Translational Medicine, 5(19), 386–386. Https://Doi.Org/10.21037/Atm.2017.06.13

Anderson, A. E., Swan, D. J., Wong, O. Y., Buck, M., Eltherington, O., Harry, R. A., Patterson, A. M., Pratt, A. G., Reynolds, G., Doran, J.-P., Kirby, J. A., Isaacs, J. D. & Hilkens, C. M. U. (2016). Tolerogenic Dendritic Cells Generated With Dexamethasone And Vitamin D3 Regulate Rheumatoid Arthritis CD4+ T Cells Partly Via Transforming Growth Factor- B 1. Clinical And Experimental Immunology, 187(1), 113–123. Https://Doi.Org/10.1111/Cei.12870

Anguille, S., Willemen, Y., Lion, E., Smits, E. L. & Berneman, Z. N. (2012). Dendritic Cell Vaccination In Acute Myeloid Leukemia. Cytotherapy, 14(6), 647–656. Https://Doi.Org/10.3109/14653249.2012.693744

Baine, I., Abe, B. T. & Macian, F. (2009). Regulation Of T-Cell Tolerance By Calcium/NFAT Signaling. Immunological Reviews, 231(1), 225–240. Https://Doi.Org/10.1111/J.1600-065X.2009.00817.X

Balan, S., Saxena, M. & Bhardwaj, N. (2019). Dendritic Cell Subsets And Locations (Pp. 1–68). Https://Doi.Org/10.1016/Bs.Ircmb.2019.07.004

Bamboat, Z. M., Stableford, J. A., Plitas, G., Burt, B. M., Nguyen, H. M., Welles, A. P., Gonen, M., Young, J. W. & Dematteo, R. P. (2009). Human Liver Dendritic Cells Promote T Cell Hyporesponsiveness. The Journal Of Immunology, 182(4), 1901–1911. Https://Doi.Org/10.4049/Jimmunol.0803404

Bamias, A. & Dimopoulos, M. A. (2005). Thalidomide And Immunomodulatory Drugs In The Treatment Of Cancer. Expert Opinion On Investigational Drugs, 14(1), 45–55. Https://Doi.Org/10.1517/13543784.14.1.45

Banchereau, J., Thompson-Snipes, L., Zurawski, S., Blanck, J.-P., Cao, Y., Clayton, S., Gorvel, J.-P., Zurawski, G. & Klechevsky, E. (2012). The Differential Production Of Cytokines By Human Langerhans Cells And Dermal CD14+ Dcs Controls CTL Priming. Blood, 119(24), 5742–5749. Https://Doi.Org/10.1182/Blood-2011-08-371245

Bigley, V., Mcgovern, N., Milne, P., Dickinson, R., Pagan, S., Cookson, S., Haniffa, M. & Collin, M. (2015). Langerin-Expressing Dendritic Cells In Human Tissues Are Related To CD1c+ Dendritic Cells And Distinct From Langerhans Cells And CD141high XCR1+ Dendritic Cells. Journal Of Leukocyte Biology, 97(4), 627–634. Https://Doi.Org/10.1189/Jlb.1HI0714-351R

Bonanno, G., Mariotti, A., Procoli, A., Folgiero, V., Natale, D., De Rosa, L., Majolino, I., Novarese, L., Rocci, A., Gambella, M., Ciciarello, M., Scambia, G., Palumbo, A., Locatelli, F., De Cristofaro, R. & Rutella, S. (2012). Indoleamine 2,3-Dioxygenase 1 (IDO1) Activity Correlates With Immune System Abnormalities In Multiple Myeloma. Journal Of Translational Medicine, 10(1), 247. Https://Doi.Org/10.1186/1479-5876-10-247

Borst, J., Ahrends, T., Bąbała, N., Melief, C. J. M. & Kastenmüller, W. (2018). CD4+ T Cell Help In Cancer Immunology And Immunotherapy. Nature Reviews Immunology, 18(10), 635–647. Https://Doi.Org/10.1038/S41577-018-0044-0

Brewitz, A., Eickhoff, S., Dähling, S., Quast, T., Bedoui, S., Kroczek, R. A., Kurts, C., Garbi, N., Barchet, W., Iannacone, M., Klauschen, F., Kolanus, W., Kaisho, T., Colonna, M., Germain, R. N. & Kastenmüller, W. (2017). CD8+ T Cells Orchestrate Pdc-XCR1+ Dendritic Cell Spatial And Functional Cooperativity To Optimize Priming. Immunity, 46(2), 205–219. Https://Doi.Org/10.1016/J.Immuni.2017.01.003

Cao, W. (2009). Molecular Characterization Of Human Plasmacytoid Dendritic Cells. Journal Of Clinical Immunology, 29(3), 257–264. Https://Doi.Org/10.1007/S10875-009-9284-X

Castiello, L., Sabatino, M., Jin, P., Clayberger, C., Marincola, F. M., Krensky, A. M. & Stroncek, D. F. (2011). Monocyte-Derived DC Maturation Strategies And Related Pathways: A Transcriptional View. Cancer Immunology, Immunotherapy, 60(4), 457–466. Https://Doi.Org/10.1007/S00262-010-0954-6

CHAPLIN, D. (2006). 1. Overview Of The Human Immune Response. Journal Of Allergy And Clinical Immunology, 117(2), S430–S435. Https://Doi.Org/10.1016/J.Jaci.2005.09.034

Chora, Â. A., Fontoura, P., Cunha, A., Pais, T. F., Cardoso, S., Ho, P. P., Lee, L. Y., Sobel, R. A., Steinman, L. & Soares, M. P. (2007). Heme Oxygenase–1 And Carbon Monoxide Suppress Autoimmune Neuroinflammation. Journal Of Clinical Investigation, 117(2), 438–447. Https://Doi.Org/10.1172/JCI28844

Chung, C. Y. J., Ysebaert, D., Berneman, Z. N. & Cools, N. (2013). Dendritic Cells: Cellular Mediators For Immunological Tolerance. Clinical And Developmental Immunology, 2013, 1–8. Https://Doi.Org/10.1155/2013/972865

Colbert, J. D., Cruz, F. M. & Rock, K. L. (2020). Cross-Presentation Of Exogenous Antigens On MHC I Molecules. Current Opinion In Immunology, 64, 1–8. Https://Doi.Org/10.1016/J.Coi.2019.12.005

Collin, M. & Bigley, V. (2018). Human Dendritic Cell Subsets: An Update. Immunology, 154(1), 3–20. Https://Doi.Org/10.1111/Imm.12888

Collin, M., Dickinson, R. & Bigley, V. (2015). Haematopoietic And Immune Defects Associated With GATA2 Mutation. British Journal Of Haematology, 169(2), 173–187. Https://Doi.Org/10.1111/Bjh.13317

D'Amato, R. J., Loughnan, M. S., Flynn, E. & Folkman, J. (1994). Thalidomide Is An Inhibitor Of Angiogenesis. Proceedings Of The National Academy Of Sciences, 91(9), 4082–4085. Https://Doi.Org/10.1073/Pnas.91.9.4082

De Monte, A., Olivieri, C.-V., Vitale, S., Bailleux, S., Castillo, L., Giordanengo, V., Maryanski, J. L., Segura, E. & Doglio, A. (2016). CD1c-Related Dcs That Express CD207/Langerin, But Are Distinguishable From Langerhans Cells, Are Consistently Present In Human Tonsils. Frontiers In Immunology, 7. Https://Doi.Org/10.3389/Fimmu.2016.00197

Del Fresno, C., Saz-Leal, P., Enamorado, M., Wculek, S. K., Martínez-Cano, S., Blanco-Menéndez, N., Schulz, O., Gallizioli, M., Miró-Mur, F., Cano, E., Planas, A. & Sancho, D. (2018). DNGR-1 In Dendritic Cells Limits Tissue Damage By Dampening Neutrophil Recruitment. Science, 362(6412), 351–356. Https://Doi.Org/10.1126/Science.Aan8423

Deng, L., Ding, W. & Granstein, R. D. (2003). Thalidomide Inhibits Tumor Necrosis Factor-A Production And Antigen Presentation By Langerhans Cells. Journal Of Investigative Dermatology, 121(5), 1060–1065. Https://Doi.Org/10.1046/J.1523-1747.2003.12565.X

Diacovo, T. G., Blasius, A. L., Mak, T. W., Cella, M. & Colonna, M. (2005). Adhesive Mechanisms Governing Interferon-Producing Cell Recruitment Into Lymph Nodes. The Journal Of Experimental Medicine, 202(5), 687–696. Https://Doi.Org/10.1084/Jem.20051035

Domínguez, P. M. & Ardavín, C. (2010). Differentiation And Function Of Mouse Monocyte-Derived Dendritic Cells In Steady State And Inflammation. Immunological Reviews, 234(1), 90–104. Https://Doi.Org/10.1111/J.0105-2896.2009.00876.X

Domogalla, M. P., Rostan, P. V, Raker, V. K. & Steinbrink, K. (2017). Tolerance Through Education: How Tolerogenic Dendritic Cells Shape Immunity. Frontiers In Immunology, 8, 1764. Https://Doi.Org/10.3389/Fimmu.2017.01764

Dudek, A. M., Martin, S., Garg, A. D. & Agostinis, P. (2013). Immature, Semi-Mature, And Fully Mature Dendritic Cells: Toward A DC-Cancer Cells Interface That Augments Anticancer Immunity. Frontiers In Immunology, 4. Https://Doi.Org/10.3389/Fimmu.2013.00438

Dudziak, D., Kamphorst, A. O., Heidkamp, G. F., Buchholz, V. R., Trumpfheller, C., Yamazaki, S., Cheong, C., Liu, K., Lee, H.-W., Park, C. G., Steinman, R. M. & Nussenzweig, M. C. (2007). Differential Antigen Processing By Dendritic Cell Subsets In Vivo. Science, 315(5808), 107–111. Https://Doi.Org/10.1126/Science.1136080

Dumauthioz, N., Tschumi, B., Wenes, M., Marti, B., Wang, H., Franco, F., Li, W., Lopez-Mejia, I. C., Fajas, L., Ho, P.-C., Donda, A., Romero, P. & Zhang, L. (2021). Enforced PGC-1α Expression Promotes CD8 T Cell Fitness, Memory Formation And Antitumor Immunity. Cellular & Molecular Immunology, 18(7), 1761–1771. Https://Doi.Org/10.1038/S41423-020-0365-3

Eickhoff, S., Brewitz, A., Gerner, M. Y., Klauschen, F., Komander, K., Hemmi, H., Garbi, N., Kaisho, T., Germain, R. N. & Kastenmüller, W. (2015). Robust Anti-Viral Immunity Requires Multiple Distinct T Cell-Dendritic Cell Interactions. Cell, 162(6), 1322–1337. Https://Doi.Org/10.1016/J.Cell.2015.08.004

Escobar, A., Aguirre, A., Guzmán, M. A., González, R., Catalán, D., Acuña-Castillo, C., Larrondo, M., López, M., Pesce, B., Rolland, J., O'Hehir, R. & Aguillón, J. C. (2014). Tolerogenic Dendritic Cells Derived From Donors With Natural Rubber Latex Allergy Modulate Allergen-Specific T-Cell Responses And Ige Production. Plos ONE, 9(1), E85930. Https://Doi.Org/10.1371/Journal.Pone.0085930

Franks, M. E., Macpherson, G. R. & Figg, W. D. (2004). Thalidomide. The Lancet, 363(9423), 1802–1811. Https://Doi.Org/10.1016/S0140-6736(04)16308-3

Fu, C. & Jiang, A. (2018). Dendritic Cells And CD8 T Cell Immunity In Tumor Microenvironment. Frontiers In Immunology, 9. Https://Doi.Org/10.3389/Fimmu.2018.03059

Fujita J, M. J. Z. J. S. K. D. A. (2001). Thalidomide And Its Analogues Inhibit Lipopolysaccharide-Mediated Iinduction Of Cyclooxygenase-2.

García-González, P., Ubilla-Olguín, G., Catalán, D., Schinnerling, K. & Aguillón, J. C. (2016). Tolerogenic Dendritic Cells For Reprogramming Of Lymphocyte Responses In Autoimmune Diseases. Autoimmunity Reviews, 15(11), 1071–1080. Https://Doi.Org/10.1016/J.Autrev.2016.07.032

Gardner, A. & Ruffell, B. (2016). Dendritic Cells And Cancer Immunity. Trends In Immunology, 37(12), 855–865. Https://Doi.Org/10.1016/J.It.2016.09.006

Gerhard, G. M., Bill, R., Messemaker, M., Klein, A. M. & Pittet, M. J. (2021). Tumor-Infiltrating Dendritic Cell States Are Conserved Across Solid Human Cancers. Journal Of Experimental Medicine, 218(1). Https://Doi.Org/10.1084/Jem.20200264

Gerlini, G., Urso, C., Mariotti, G., Di Gennaro, P., Palli, D., Brandani, P., Salvadori, A., Pimpinelli, N., Reali, U. M. & Borgognoni, L. (2007). Plasmacytoid Dendritic Cells Represent A Major Dendritic Cell Subset In Sentinel Lymph Nodes

Of Melanoma Patients And Accumulate In Metastatic Nodes. Clinical Immunology, 125(2), 184–193. Https://Doi.Org/10.1016/J.Clim.2007.07.018

Ghorbaninezhad, F., Asadzadeh, Z., Masoumi, J., Mokhtarzadeh, A., Kazemi, T., Aghebati-Maleki, L., Shotorbani, S. S., Shadbad, M. A., Baghbanzadeh, A., Hemmat, N., Bakhshivand, M. & Baradaran, B. (2022). Dendritic Cell-Based Cancer Immunotherapy In The Era Of Immune Checkpoint Inhibitors: From Bench To Bedside. Life Sciences, 297, 120466. Https://Doi.Org/10.1016/J.Lfs.2022.120466

Grajales-Reyes, G. E., Iwata, A., Albring, J., Wu, X., Tussiwand, R., KC, W., Kretzer, N. M., Briseño, C. G., Durai, V., Bagadia, P., Haldar, M., Schönheit, J., Rosenbauer, F., Murphy, T. L. & Murphy, K. M. (2015). Batf3 Maintains Autoactivation Of Irf8 For Commitment Of A CD8α+ Conventional DC Clonogenic Progenitor. Nature Immunology, 16(7), 708–717. Https://Doi.Org/10.1038/Ni.3197

Guermonprez, P., Valladeau, J., Zitvogel, L., Théry, C. & Amigorena, S. (2002). Antigen Presentation And T Cell Stimulation By Dendritic Cells. Annual Review Of Immunology, 20(1), 621–667. Https://Doi.Org/10.1146/Annurev.Immunol.20.100301.064828

Guilliams, M., Dutertre, C.-A., Scott, C. L., Mcgovern, N., Sichien, D., Chakarov, S., Van Gassen, S., Chen, J., Poidinger, M., De Prijck, S., Tavernier, S. J., Low, I., Irac, S. E., Mattar, C. N., Sumatoh, H. R., Low, G. H. L., Chung, T. J. K., Chan, D. K. H., Tan, K. K., … Ginhoux, F. (2016). Unsupervised High-Dimensional Analysis Aligns Dendritic Cells Across Tissues And Species. Immunity, 45(3), 669–684. Https://Doi.Org/10.1016/J.Immuni.2016.08.015

Guttman-Yassky, E., Lowes, M. A., Fuentes-Duculan, J., Whynot, J., Novitskaya, I., Cardinale, I., Haider, A., Khatcherian, A., Carucci, J. A., Bergman, R. & Krueger, J. G. (2007). Major Differences In Inflammatory Dendritic Cells And Their Products Distinguish Atopic Dermatitis From Psoriasis. Journal Of Allergy And Clinical Immunology, 119(5), 1210–1217. Https://Doi.Org/10.1016/J.Jaci.2007.03.006

Hambleton, S., Salem, S., Bustamante, J., Bigley, V., Boisson-Dupuis, S., Azevedo, J., Fortin, A., Haniffa, M., Ceron-Gutierrez, L., Bacon, C. M., Menon, G., Trouillet, C., Mcdonald, D., Carey, P., Ginhoux, F., Alsina, L., Zumwalt, T. J., Kong, X.-F., Kumararatne, D., … Gros, P. (2011). IRF8 Mutations And Human Dendritic-Cell Immunodeficiency. New England Journal Of Medicine, 365(2), 127–138. Https://Doi.Org/10.1056/Nejmoa1100066

Haniffa, M., Shin, A., Bigley, V., Mcgovern, N., Teo, P., See, P., Wasan, P. S., Wang, X.-N., Malinarich, F., Malleret, B., Larbi, A., Tan, P., Zhao, H., Poidinger, M., Pagan, S., Cookson, S., Dickinson, R., Dimmick, I., Jarrett, R. F., … Ginhoux, F. (2012). Human Tissues Contain CD141hi Cross-Presenting Dendritic Cells With Functional Homology To Mouse CD103+ Nonlymphoid Dendritic Cells. Immunity, 37(1), 60–73. Https://Doi.Org/10.1016/J.Immuni.2012.04.012

Hasegawa, H. & Matsumoto, T. (2018). Mechanisms Of Tolerance Induction By Dendritic Cells In Vivo. Frontiers In Immunology, 9. Https://Doi.Org/10.3389/Fimmu.2018.00350

Heger, L., Balk, S., Lühr, J. J., Heidkamp, G. F., Lehmann, C. H. K., Hatscher, L., Purbojo, A., Hartmann, A., Garcia-Martin, F., Nishimura, S.-I., Cesnjevar, R., Nimmerjahn, F. & Dudziak, D. (2018). CLEC10A Is A Specific Marker For Human CD1c+ Dendritic Cells And Enhances Their Toll-Like Receptor 7/8-Induced Cytokine Secretion. Frontiers In Immunology, 9. Https://Doi.Org/10.3389/Fimmu.2018.00744

Helft, J., Böttcher, J., Chakravarty, P., Zelenay, S., Huotari, J., Schraml, B. U., Goubau, D. & Reis E Sousa, C. (2015). GM-CSF Mouse Bone Marrow Cultures Comprise A Heterogeneous Population Of CD11c+MHCII+ Macrophages And Dendritic Cells. Immunity, 42(6), 1197–1211. Https://Doi.Org/10.1016/J.Immuni.2015.05.018

Henry, J. Y., Labarthe, M., Meyer, B., Dasgupta, P., Dalgleish, A. G. & Galustian, C. (2013). Enhanced Cross-Priming Of Naive <Scp>CD</Scp> 8 + <Scp>T</Scp> Cells By Dendritic Cells Treated By The <Scp>IM</Scp> I <Scp>D</Scp> S ® Immunomodulatory Compounds Lenalidomide And Pomalidomide. Immunology, 139(3), 377–385. Https://Doi.Org/10.1111/Imm.12087

Hill, J. A., Hall, J. A., Sun, C.-M., Cai, Q., Ghyselinck, N., Chambon, P., Belkaid, Y., Mathis, D. & Benoist, C. (2008). Retinoic Acid Enhances Foxp3 Induction Indirectly By Relieving Inhibition From CD4+CD44hi Cells. Immunity, 29(5), 758–770. Https://Doi.Org/10.1016/J.Immuni.2008.09.018

Hsu, P., Santner-Nanan, B., Hu, M., Skarratt, K., Lee, C. H., Stormon, M., Wong, M., Fuller, S. J. & Nanan, R. (2015). IL-10 Potentiates Differentiation Of Human Induced Regulatory T Cells Via STAT3 And Foxo1. The Journal Of Immunology, 195(8), 3665–3674. Https://Doi.Org/10.4049/Jimmunol.1402898

Hubo, M., Trinschek, B., Kryczanowsky, F., Tuettenberg, A., Steinbrink, K. & Jonuleit, H. (2013). Costimulatory Molecules On Immunogenic Versus Tolerogenic

Human Dendritic Cells. Frontiers In Immunology, 4. Https://Doi.Org/10.3389/Fimmu.2013.00082

Iberg, C. A., Jones, A. & Hawiger, D. (2017). Dendritic Cells As Inducers Of Peripheral Tolerance. Trends In Immunology, 38(11), 793–804. Https://Doi.Org/10.1016/J.It.2017.07.007

Inaba, K., Inaba, M., Romani, N., Aya, H., Deguchi, M., Ikehara, S., Muramatsu, S. & Steinman, R. M. (1992). Generation Of Large Numbers Of Dendritic Cells From Mouse Bone Marrow Cultures Supplemented With Granulocyte/Macrophage Colony-Stimulating Factor. The Journal Of Experimental Medicine, 176(6), 1693–1702. Https://Doi.Org/10.1084/Jem.176.6.1693

Izawa, T., Kondo, T., Kurosawa, M., Oura, R., Matsumoto, K., Tanaka, E., Yamada, A., Arakaki, R., Kudo, Y., Hayashi, Y. & Ishimaru, N. (2012). Fas-Independent T-Cell Apoptosis By Dendritic Cells Controls Autoimmune Arthritis In MRL/Lpr Mice. Plos ONE, 7(12), E48798. Https://Doi.Org/10.1371/Journal.Pone.0048798

Jongbloed, S. L., Kassianos, A. J., Mcdonald, K. J., Clark, G. J., Ju, X., Angel, C. E., Chen, C.-J. J., Dunbar, P. R., Wadley, R. B., Jeet, V., Vulink, A. J. E., Hart, D. N. J. & Radford, K. J. (2010). Human CD141+ (BDCA-3)+ Dendritic Cells (Dcs) Represent A Unique Myeloid DC Subset That Cross-Presents Necrotic Cell Antigens. Journal Of Experimental Medicine, 207(6), 1247–1260. Https://Doi.Org/10.1084/Jem.20092140

Joo, H.-G. (2003). Altered Maturation Of Dendritic Cells By Taxol, An Anticancer Drug. Journal Of Veterinary Science, 4(3), 229–234.

Kashem, S. W., Haniffa, M. & Kaplan, D. H. (2017). Antigen-Presenting Cells In The Skin. Annual Review Of Immunology, 35(1), 469–499. Https://Doi.Org/10.1146/Annurev-Immunol-051116-052215

KENYON, B. M., BROWNE, F. & D'AMATO, R. J. (1997). Effects Of Thalidomide And Related Metabolites In A Mouse Corneal Model Of Neovascularization. Experimental Eye Research, 64(6), 971–978. Https://Doi.Org/10.1006/Exer.1997.0292

Kim, E. J., Kim, J. Y., Choi, H. Y., Lee, H., Lee, J., Kim, M. S., Kim, Y. S., Huh, K. H. & Kim, B. S. (2021). Systemic Immunomodulatory Effects Of Combinatorial Treatment Of Thalidomide And Dexamethasone On T Cells And Other

Immune Cells. Yonsei Medical Journal, 62(2), 137. Https://Doi.Org/10.3349/Ymj.2021.62.2.137

Kong, B. Y., Bolton, H., Kim, J. W., Silveira, P. A., Fromm, P. D. & Clark, G. J. (2019). On The Other Side: Manipulating The Immune Checkpoint Landscape Of Dendritic Cells To Enhance Cancer Immunotherapy. Frontiers In Oncology, 9. Https://Doi.Org/10.3389/Fonc.2019.00050

Kretschmer, K., Apostolou, I., Hawiger, D., Khazaie, K., Nussenzweig, M. C. & Von Boehmer, H. (2005). Inducing And Expanding Regulatory T Cell Populations By Foreign Antigen. Nature Immunology, 6(12), 1219–1227. Https://Doi.Org/10.1038/Ni1265

Kryczanowsky, F., Raker, V., Graulich, E., Domogalla, M. P. & Steinbrink, K. (2016). IL-10–Modulated Human Dendritic Cells For Clinical Use: Identification Of A Stable And Migratory Subset With Improved Tolerogenic Activity. The Journal Of Immunology, 197(9), 3607–3617. Https://Doi.Org/10.4049/Jimmunol.1501769

Kurts, C., Heath, W. R., Kosaka, H., Miller, J. F. A. P. & Carbone, F. R. (1998). The Peripheral Deletion Of Autoreactive CD8+ T Cells Induced By Cross-Presentation Of Self-Antigens Involves Signaling Through CD95 (Fas, Apo-1). The Journal Of Experimental Medicine, 188(2), 415–420. Https://Doi.Org/10.1084/Jem.188.2.415

Laoui, D., Keirsse, J., Morias, Y., Van Overmeire, E., Geeraerts, X., Elkrim, Y., Kiss, M., Bolli, E., Lahmar, Q., Sichien, D., Serneels, J., Scott, C. L., Boon, L., De Baetselier, P., Mazzone, M., Guilliams, M. & Van Ginderachter, J. A. (2016). The Tumour Microenvironment Harbours Ontogenically Distinct Dendritic Cell Populations With Opposing Effects On Tumour Immunity. Nature Communications, 7(1), 13720. Https://Doi.Org/10.1038/Ncomms13720

Lavin, Y., Kobayashi, S., Leader, A., Amir, E. D., Elefant, N., Bigenwald, C., Remark, R., Sweeney, R., Becker, C. D., Levine, J. H., Meinhof, K., Chow, A., Kim-Shulze, S., Wolf, A., Medaglia, C., Li, H., Rytlewski, J. A., Emerson, R. O., Solovyov, A., … Merad, M. (2017). Innate Immune Landscape In Early Lung Adenocarcinoma By Paired Single-Cell Analyses. Cell, 169(4), 750-765.E17. Https://Doi.Org/10.1016/J.Cell.2017.04.014

Lei, X., Lei, Y., Li, J.-K., Du, W.-X., Li, R.-G., Yang, J., Li, J., Li, F. & Tan, H.-B. (2020). Immune Cells Within The Tumor Microenvironment: Biological

Functions And Roles In Cancer Immunotherapy. Cancer Letters, 470, 126–133. Https://Doi.Org/10.1016/J.Canlet.2019.11.009

León, B. & Ardavín, C. (2008). Monocyte-Derived Dendritic Cells In Innate And Adaptive Immunity. Immunology & Cell Biology, 86(4), 320–324. Https://Doi.Org/10.1038/Icb.2008.14

León, B., López-Bravo, M. & Ardavín, C. (2007). Monocyte-Derived Dendritic Cells Formed At The Infection Site Control The Induction Of Protective T Helper 1 Responses Against Leishmania. Immunity, 26(4), 519–531. Https://Doi.Org/10.1016/J.Immuni.2007.01.017

Li, X., Yang, A., Huang, H., Zhang, X., Town, J., Davis, B., Cockcroft, D. W. & Gordon, J. R. (2010). Induction Of Type 2 T Helper Cell Allergen Tolerance By IL-10–Differentiated Regulatory Dendritic Cells. American Journal Of Respiratory Cell And Molecular Biology, 42(2), 190–199. Https://Doi.Org/10.1165/Rcmb.2009-0023OC

Liu, W. M., Laux, H., Henry, J. Y., Bolton, T. B., Dalgleish, A. G. & Galustian, C. (2010). A Microarray Study Of Altered Gene Expression In Colorectal Cancer Cells After Treatment With Immunomodulatory Drugs: Differences In Action In Vivo And In Vitro. Molecular Biology Reports, 37(4), 1801–1814. Https://Doi.Org/10.1007/S11033-009-9614-3

MARRIOTT, J. B., CLARKE, I. A., DREDGE, K., MULLER, G., STIRLING, D. & DALGLEISH, A. G. (2002). Thalidomide And Its Analogues Have Distinct And Opposing Effects On TNF- A And TNFR2 During Co-Stimulation Of Both CD4+ And CD8+ T Cells. Clinical And Experimental Immunology, 130(1), 75–84. Https://Doi.Org/10.1046/J.1365-2249.2002.01954.X

Mashayekhi, M., Sandau, M. M., Dunay, I. R., Frickel, E. M., Khan, A., Goldszmid, R. S., Sher, A., Ploegh, H. L., Murphy, T. L., Sibley, L. D. & Murphy, K. M. (2011). CD8α+ Dendritic Cells Are The Critical Source Of Interleukin-12 That Controls Acute Infection By Toxoplasma Gondii Tachyzoites. Immunity, 35(2), 249–259. Https://Doi.Org/10.1016/J.Immuni.2011.08.008

Matsuo, K., Yoshie, O., Kitahata, K., Kamei, M., Hara, Y. & Nakayama, T. (2021). Recent Progress In Dendritic Cell-Based Cancer Immunotherapy. Cancers, 13(10), 2495. Https://Doi.Org/10.3390/Cancers13102495

Mckenna, H. J., Stocking, K. L., Miller, R. E., Brasel, K., De Smedt, T., Maraskovsky, E., Maliszewski, C. R., Lynch, D. H., Smith, J., Pulendran, B., Roux,

E. R., Teepe, M., Lyman, S. D. & Peschon, J. J. (2000). Mice Lacking Flt3 Ligand Have Deficient Hematopoiesis Affecting Hematopoietic Progenitor Cells, Dendritic Cells, And Natural Killer Cells. Blood, 95(11), 3489–3497.

Melssen, M. & Slingluff, C. L. (2017). Vaccines Targeting Helper T Cells For Cancer Immunotherapy. Current Opinion In Immunology, 47, 85–92. Https://Doi.Org/10.1016/J.Coi.2017.07.004

Merad, M., Ginhoux, F. & Collin, M. (2008). Origin, Homeostasis And Function Of Langerhans Cells And Other Langerin-Expressing Dendritic Cells. Nature Reviews Immunology, 8(12), 935–947. Https://Doi.Org/10.1038/Nri2455

Merad, M., Manz, M. G., Karsunky, H., Wagers, A., Peters, W., Charo, I., Weissman, I. L., Cyster, J. G. & Engleman, E. G. (2002). Langerhans Cells Renew In The Skin Throughout Life Under Steady-State Conditions. Nature Immunology, 3(12), 1135–1141. Https://Doi.Org/10.1038/Ni852

Merad, M., Sathe, P., Helft, J., Miller, J. & Mortha, A. (2013). The Dendritic Cell Lineage: Ontogeny And Function Of Dendritic Cells And Their Subsets In The Steady State And The Inflamed Setting. Annual Review Of Immunology, 31(1), 563–604. Https://Doi.Org/10.1146/Annurev-Immunol-020711-074950

Michea, P., Noël, F., Zakine, E., Czerwinska, U., Sirven, P., Abouzid, O., Goudot, C., Scholer-Dahirel, A., Vincent-Salomon, A., Reyal, F., Amigorena, S., Guillot-Delost, M., Segura, E. & Soumelis, V. (2018). Adjustment Of Dendritic Cells To The Breast-Cancer Microenvironment Is Subset Specific. Nature Immunology, 19(8), 885–897. Https://Doi.Org/10.1038/S41590-018-0145-8

Mildner, A. & Jung, S. (2014). Development And Function Of Dendritic Cell Subsets. Immunity, 40(5), 642–656. Https://Doi.Org/10.1016/J.Immuni.2014.04.016

Minaga, K., Watanabe, T., Hara, A., Yoshikawa, T., Kamata, K. & Kudo, M. (2021). Plasmacytoid Dendritic Cells As A New Therapeutic Target For Autoimmune Pancreatitis And Igg4-Related Disease. Frontiers In Immunology, 12. Https://Doi.Org/10.3389/Fimmu.2021.713779

Mohamad Mohty 1, A.-M. S. D. B. D. I. J.-A. G. D. O. B. G. (2002). Mohty M, Stoppa AM, Blaise D, Isnardon D, Gastaut JA, Olive D, Gaugler B. Differential Regulation Of Dendritic Cell Function By The Immunomodulatory Drug Thalidomide.

Murphy, K. And W. C. (2016). Janeway’s Immunobiology. Garland Science.

Nizzoli, G., Larghi, P., Paroni, M., Crosti, M. C., Moro, M., Neddermann, P., Caprioli, F., Pagani, M., De Francesco, R., Abrignani, S. & Geginat, J. (2016). IL-10 Promotes Homeostatic Proliferation Of Human CD8 + Memory T Cells And, When Produced By CD1c + Dcs, Shapes Naive CD8 + T-Cell Priming. European Journal Of Immunology, 46(7), 1622–1632. Https://Doi.Org/10.1002/Eji.201546136

Obreque, J., Vega, F., Torres, A., Cuitino, L., Mackern-Oberti, J. P., Viviani, P., Kalergis, A. & Llanos, C. (2017). Autologous Tolerogenic Dendritic Cells Derived From Monocytes Of Systemic Lupus Erythematosus Patients And Healthy Donors Show A Stable And Immunosuppressive Phenotype. Immunology, 152(4), 648–659. Https://Doi.Org/10.1111/Imm.12806

O'Keeffe, M., Mok, W. H. & Radford, K. J. (2015). Human Dendritic Cell Subsets And Function In Health And Disease. Cellular And Molecular Life Sciences, 72(22), 4309–4325. Https://Doi.Org/10.1007/S00018-015-2005-0

Patel, V. I., Booth, J. L., Duggan, E. S., Cate, S., White, V. L., Hutchings, D., Kovats, S., Burian, D. M., Dozmorov, M. & Metcalf, J. P. (2017). Transcriptional Classification And Functional Characterization Of Human Airway Macrophage And Dendritic Cell Subsets. The Journal Of Immunology, 198(3), 1183–1201. Https://Doi.Org/10.4049/Jimmunol.1600777

Patente, T. A., Pinho, M. P., Oliveira, A. A., Evangelista, G. C. M., Bergami-Santos, P. C. & Barbuto, J. A. M. (2019). Human Dendritic Cells: Their Heterogeneity And Clinical Application Potential In Cancer Immunotherapy. Frontiers In Immunology, 9. Https://Doi.Org/10.3389/Fimmu.2018.03176

Plantinga, M., Guilliams, M., Vanheerswynghels, M., Deswarte, K., Branco-Madeira, F., Toussaint, W., Vanhoutte, L., Neyt, K., Killeen, N., Malissen, B., Hammad, H. & Lambrecht, B. N. (2013). Conventional And Monocyte-Derived CD11b+ Dendritic Cells Initiate And Maintain T Helper 2 Cell-Mediated Immunity To House Dust Mite Allergen. Immunity, 38(2), 322–335. Https://Doi.Org/10.1016/J.Immuni.2012.10.016

Quispe Calla, N. E., Ghonime, M. G., Cherpes, T. L. & Vicetti Miguel, R. D. (2015). Medroxyprogesterone Acetate Impairs Human Dendritic Cell Activation And Function. Human Reproduction, 30(5), 1169–1177. Https://Doi.Org/10.1093/Humrep/Dev035

Raïch-Regué, D., Grau-López, L., Naranjo-Gómez, M., Ramo-Tello, C., Pujol-Borrell, R., Martínez-Cáceres, E. & Borràs, F. E. (2012). Stable Antigen-Specific T-

Cell Hyporesponsiveness Induced By Tolerogenic Dendritic Cells From Multiple Sclerosis Patients. European Journal Of Immunology, 42(3), 771–782. Https://Doi.Org/10.1002/Eji.201141835

Reizis, B. (2019). Plasmacytoid Dendritic Cells: Development, Regulation, And Function. Immunity, 50(1), 37–50. Https://Doi.Org/10.1016/J.Immuni.2018.12.027

Reizis, B., Bunin, A., Ghosh, H. S., Lewis, K. L. & Sisirak, V. (2011). Plasmacytoid Dendritic Cells: Recent Progress And Open Questions. Annual Review Of Immunology, 29(1), 163–183. Https://Doi.Org/10.1146/Annurev-Immunol-031210-101345

Reynolds, G. & Haniffa, M. (2015). Human And Mouse Mononuclear Phagocyte Networks: A Tale Of Two Species? Frontiers In Immunology, 6. Https://Doi.Org/10.3389/Fimmu.2015.00330

Richter, L., Landsverk, O. J. B., Atlasy, N., Bujko, A., Yaqub, S., Horneland, R., Øyen, O., Aandahl, E. M., Lundin, K. E. A., Stunnenberg, H. G., Bækkevold, E. S. & Jahnsen, F. L. (2018). Transcriptional Profiling Reveals Monocyte-Related Macrophages Phenotypically Resembling DC In Human Intestine. Mucosal Immunology, 11(5), 1512–1523. Https://Doi.Org/10.1038/S41385-018-0060-1

Romani, N., Clausen, B. E. & Stoitzner, P. (2010). Langerhans Cells And More: Langerin-Expressing Dendritic Cell Subsets In The Skin. Immunological Reviews, 234(1), 120–141. Https://Doi.Org/10.1111/J.0105-2896.2009.00886.X

Romano, E., Cotari, J. W., Barreira Da Silva, R., Betts, B. C., Chung, D. J., Avogadri, F., Fink, M. J., St Angelo, E. T., Mehrara, B., Heller, G., Münz, C., Altan-Bonnet, G. & Young, J. W. (2012). Human Langerhans Cells Use An IL-15R-A/IL-15/Pstat5-Dependent Mechanism To Break T-Cell Tolerance Against The Self-Differentiation Tumor Antigen WT1. Blood, 119(22), 5182–5190. Https://Doi.Org/10.1182/Blood-2011-09-382200

Rosenberg, S. A. & Restifo, N. P. (2015). Adoptive Cell Transfer As Personalized Immunotherapy For Human Cancer. Science, 348(6230), 62–68. Https://Doi.Org/10.1126/Science.Aaa4967

Sallusto, F. & Lanzavecchia, A. (1994). Efficient Presentation Of Soluble Antigen By Cultured Human Dendritic Cells Is Maintained By Granulocyte/Macrophage Colony-Stimulating Factor Plus Interleukin 4 And

Downregulated By Tumor Necrosis Factor Alpha. The Journal Of Experimental Medicine, 179(4), 1109–1118. Https://Doi.Org/10.1084/Jem.179.4.1109

Schlitzer, A., Mcgovern, N., Teo, P., Zelante, T., Atarashi, K., Low, D., Ho, A. W. S., See, P., Shin, A., Wasan, P. S., Hoeffel, G., Malleret, B., Heiseke, A., Chew, S., Jardine, L., Purvis, H. A., Hilkens, C. M. U., Tam, J., Poidinger, M., … Ginhoux, F. (2013). IRF4 Transcription Factor-Dependent CD11b+ Dendritic Cells In Human And Mouse Control Mucosal IL-17 Cytokine Responses. Immunity, 38(5), 970–983. Https://Doi.Org/10.1016/J.Immuni.2013.04.011

Schnurr, M., Galambos, P., Scholz, C., Then, F., Dauer, M., Endres, S. & Eigler, A. (2001). Tumor Cell Lysate-Pulsed Human Dendritic Cells Induce A T-Cell Response Against Pancreatic Carcinoma Cells: An In Vitro Model For The Assessment Of Tumor Vaccines. Cancer Research, 61(17), 6445–6450.

Schütt, P., Buttkereit, U., Brandhorst, D., Lindemann, M., Schmiedl, S., Grosse-Wilde, H., Seeber, S., Nowrousian, M. R., Opalka, B. & Moritz, T. (2005). In Vitro Dendritic Cell Generation And Lymphocyte Subsets In Myeloma Patients: Influence Of Thalidomide And High-Dose Chemotherapy Treatment. Cancer Immunology, Immunotherapy, 54(5), 506–512. Https://Doi.Org/10.1007/S00262-004-0633-6

Segovia-Gamboa, N., Rodríguez-Arellano, M. E., Rangel-Cruz, R., Sánchez-Díaz, M., Ramírez-Reyes, J. C., Faradji, R., González-Domínguez, É. & Sánchez-Torres, C. (2014). Tolerogenic Dendritic Cells Induce Antigen-Specific Hyporesponsiveness In Insulin- And Glutamic Acid Decarboxylase 65-Autoreactive T Lymphocytes From Type 1 Diabetic Patients. Clinical Immunology, 154(1), 72–83. Https://Doi.Org/10.1016/J.Clim.2014.06.009

Segura, E., Touzot, M., Bohineust, A., Cappuccio, A., Chiocchia, G., Hosmalin, A., Dalod, M., Soumelis, V. & Amigorena, S. (2013). Human Inflammatory Dendritic Cells Induce Th17 Cell Differentiation. Immunity, 38(2), 336–348. Https://Doi.Org/10.1016/J.Immuni.2012.10.018

Serbina, N. V, Salazar-Mather, T. P., Biron, C. A., Kuziel, W. A. & Pamer, E. G. (2003). TNF/Inos-Producing Dendritic Cells Mediate Innate Immune Defense Against Bacterial Infection. Immunity, 19(1), 59–70. Https://Doi.Org/10.1016/S1074-7613(03)00171-7

Seth, S., Oberdörfer, L., Hyde, R., Hoff, K., Thies, V., Worbs, T., Schmitz, S. & Förster, R. (2011). CCR7 Essentially Contributes To The Homing Of Plasmacytoid

Dendritic Cells To Lymph Nodes Under Steady-State As Well As Inflammatory Conditions. The Journal Of Immunology, 186(6), 3364–3372. Https://Doi.Org/10.4049/Jimmunol.1002598

Shannon, E. J. & Sandoval, F. G. (2002). Thalidomide Can Costimulate Or Suppress CD4+ Cells' Ability To Incorporate [H3]-Thymidine—Dependence On The Primary Stimulant. International Immunopharmacology, 2(8), 1143–1153. Https://Doi.Org/10.1016/S1567-5769(02)00066-8

Sherbet, G. V. (2015). Therapeutic Potential Of Thalidomide And Its Analogues In The Treatment Of Cancer. Anticancer Research, 35(11), 5767–5772.

Shin, J.-S. & Greer, A. M. (2015). The Role Of Fcεri Expressed In Dendritic Cells And Monocytes. Cellular And Molecular Life Sciences, 72(12), 2349–2360. Https://Doi.Org/10.1007/S00018-015-1870-X

Shin, K.-S., Jeon, I., Kim, B.-S., Kim, I.-K., Park, Y.-J., Koh, C.-H., Song, B., Lee, J.-M., Lim, J., Bae, E.-A., Seo, H., Ban, Y. H., Ha, S.-J. & Kang, C.-Y. (2019). Monocyte-Derived Dendritic Cells Dictate The Memory Differentiation Of CD8+ T Cells During Acute Infection. Frontiers In Immunology, 10. Https://Doi.Org/10.3389/Fimmu.2019.01887

Shortman, K. & Naik, S. H. (2007). Steady-State And Inflammatory Dendritic-Cell Development. Nature Reviews Immunology, 7(1), 19–30. Https://Doi.Org/10.1038/Nri1996

Sim, W. J., Malinarich, F., Fairhurst, A.-M. & Connolly, J. E. (2016). Generation Of Immature, Mature And Tolerogenic Dendritic Cells With Differing Metabolic Phenotypes. Journal Of Visualized Experiments, 112. Https://Doi.Org/10.3791/54128

Speck, S., Lim, J., Shelake, S., Matka, M., Stoddard, J., Farr, A., Kuchroo, V. & Laouar, Y. (2014). TGF-B Signaling Initiated In Dendritic Cells Instructs Suppressive Effects On Th17 Differentiation At The Site Of Neuroinflammation. Plos One, 9(7), E102390. Https://Doi.Org/10.1371/Journal.Pone.0102390

Steinbrink, K., Graulich, E., Kubsch, S., Knop, J. & Enk, A. H. (2002). CD4+ And CD8+ Anergic T Cells Induced By Interleukin-10–Treated Human Dendritic Cells Display Antigen-Specific Suppressor Activity. Blood, 99(7), 2468–2476. Https://Doi.Org/10.1182/Blood.V99.7.2468

Steinman, R. M. (2012). Decisions About Dendritic Cells: Past, Present, And Future. Annual Review Of Immunology, 30(1), 1–22. Https://Doi.Org/10.1146/Annurev-Immunol-100311-102839

Steinman, R. M., Hawiger, D. & Nussenzweig, M. C. (2003). Tolerogenic Dendritic Cells. Annual Review Of Immunology, 21(1), 685–711. Https://Doi.Org/10.1146/Annurev.Immunol.21.120601.141040

Švajger, U. & Rožman, P. (2013). Tolerogenic Dendritic Cells: Molecular And Cellular Mechanisms In Transplantation. Journal Of Leukocyte Biology, 95(1), 53–69. Https://Doi.Org/10.1189/Jlb.0613336

Swiecki, M. & Colonna, M. (2015). The Multifaceted Biology Of Plasmacytoid Dendritic Cells. Nature Reviews Immunology, 15(8), 471–485. Https://Doi.Org/10.1038/Nri3865

Tay, R. E., Richardson, E. K. & Toh, H. C. (2021). Revisiting The Role Of CD4+ T Cells In Cancer Immunotherapy—New Insights Into Old Paradigms. Cancer Gene Therapy, 28(1–2), 5–17. Https://Doi.Org/10.1038/S41417-020-0183-X

Thomé, R., Issayama, L. K., Digangi, R., Bombeiro, A. L., Da Costa, T. A., Ferreira, I. T., De Oliveira, A. L. R. & Verinaud, L. (2014). Dendritic Cells Treated With Chloroquine Modulate Experimental Autoimmune Encephalomyelitis. Immunology & Cell Biology, 92(2), 124–132. Https://Doi.Org/10.1038/Icb.2013.73

Toebak, M. J., De Rooij, J., Moed, H., Stoof, T. J., Von Blomberg, B. M. E., Bruynzeel, D. P., Scheper, R. J., Gibbs, S. & Rustemeyer, T. (2007). Differential Suppression Of Dendritic Cell Cytokine Production By Anti-Inflammatory Drugs. British Journal Of Dermatology, 0(0), 071119222739002-??? Https://Doi.Org/10.1111/J.1365-2133.2007.08297.X

Travis, M. A., Reizis, B., Melton, A. C., Masteller, E., Tang, Q., Proctor, J. M., Wang, Y., Bernstein, X., Huang, X., Reichardt, L. F., Bluestone, J. A. & Sheppard, D. (2007). Loss Of Integrin Avβ8 On Dendritic Cells Causes Autoimmunity And Colitis In Mice. Nature, 449(7160), 361–365. Https://Doi.Org/10.1038/Nature06110

Tussiwand, R., Everts, B., Grajales-Reyes, G. E., Kretzer, N. M., Iwata, A., Bagaitkar, J., Wu, X., Wong, R., Anderson, D. A., Murphy, T. L., Pearce, E. J. & Murphy, K. M. (2015). Klf4 Expression In Conventional Dendritic Cells Is Required For T Helper 2 Cell Responses. Immunity, 42(5), 916–928. Https://Doi.Org/10.1016/J.Immuni.2015.04.017

Van Den Bergh, J. M. J., Smits, E. L. J. M., Berneman, Z. N., Hutten, T. J. A., De Reu, H., Van Tendeloo, V. F. I., Dolstra, H., Lion, E. & Hobo, W. (2017). Monocyte-Derived Dendritic Cells With Silenced PD-1 Ligands And Transpresenting Interleukin-15 Stimulate Strong Tumor-Reactive T-Cell Expansion. Cancer Immunology Research, 5(8), 710–715. Https://Doi.Org/10.1158/2326-6066.CIR-16-0336

Van Gulijk, M., Dammeijer, F., Aerts, J. G. J. V. & Vroman, H. (2018). Combination Strategies To Optimize Efficacy Of Dendritic Cell-Based Immunotherapy. Frontiers In Immunology, 9. Https://Doi.Org/10.3389/Fimmu.2018.02759

Veglia, F. & Gabrilovich, D. I. (2017). Dendritic Cells In Cancer: The Role Revisited. Current Opinion In Immunology, 45, 43–51. Https://Doi.Org/10.1016/J.Coi.2017.01.002

Villadangos, J. A. & Young, L. (2008). Antigen-Presentation Properties Of Plasmacytoid Dendritic Cells. Immunity, 29(3), 352–361. Https://Doi.Org/10.1016/J.Immuni.2008.09.002

Villani, A.-C., Satija, R., Reynolds, G., Sarkizova, S., Shekhar, K., Fletcher, J., Griesbeck, M., Butler, A., Zheng, S., Lazo, S., Jardine, L., Dixon, D., Stephenson, E., Nilsson, E., Grundberg, I., Mcdonald, D., Filby, A., Li, W., De Jager, P. L., … Hacohen, N. (2017). Single-Cell RNA-Seq Reveals New Types Of Human Blood Dendritic Cells, Monocytes, And Progenitors. Science, 356(6335). Https://Doi.Org/10.1126/Science.Aah4573

Volchenkov, R., Brun, J. G., Jonsson, R. & Appel, S. (2013). In Vitro Suppression Of Immune Responses Using Monocyte-Derived Tolerogenic Dendritic Cells From Patients With Primary Sjögren's Syndrome. Arthritis Research & Therapy, 15(5), R114. Https://Doi.Org/10.1186/Ar4294

Watchmaker, P. B., Lahl, K., Lee, M., Baumjohann, D., Morton, J., Kim, S. J., Zeng, R., Dent, A., Ansel, K. M., Diamond, B., Hadeiba, H. & Butcher, E. C. (2014). Comparative Transcriptional And Functional Profiling Defines Conserved Programs Of Intestinal DC Differentiation In Humans And Mice. Nature Immunology, 15(1), 98–108. Https://Doi.Org/10.1038/Ni.2768

Xiao, B.-G., Zhu, W.-H. & Lu, C.-Z. (2007). The Presence Of GM-CSF And IL-4 Interferes With Effect Of TGF-B1 On Antigen Presenting Cells In Patients With Multiple Sclerosis And In Rats With Experimental Autoimmune Encephalomyelitis.

Cellular Immunology, 249(1), 30–36. Https://Doi.Org/10.1016/J.Cellimm.2007.10.006

Yamazaki, S., Iyoda, T., Tarbell, K., Olson, K., Velinzon, K., Inaba, K. & Steinman, R. M. (2003). Direct Expansion Of Functional CD25+ CD4+ Regulatory T Cells By Antigen-Processing Dendritic Cells. The Journal Of Experimental Medicine, 198(2), 235–247. Https://Doi.Org/10.1084/Jem.20030422

Yee, A. J., Hari, P., Marcheselli, R., Mahindra, A. K., Cirstea, D. D., Scullen, T. A., Burke, J. N., Rodig, S. J., Hideshima, T., Laubach, J. P., Ghobrial, I. M., Schlossman, R. L., Munshi, N. C., Anderson, K. C., Weller, E. A., Richardson, P. G. & Raje, N. S. (2014). Outcomes In Patients With Relapsed Or Refractory Multiple Myeloma In A Phase I Study Of Everolimus In Combination With Lenalidomide. British Journal Of Haematology, 166(3), 401–409. Https://Doi.Org/10.1111/Bjh.12909

You, X., Xu, D. D., Zhang, D., Chen, J. & Gao, F. G. (2018). PYR-41 And Thalidomide Impair Dendritic Cell Cross-Presentation By Inhibiting Myddosome Formation And Attenuating The Endosomal Recruitments Of P97 And Sec61 Via NF-K B Inactivation. Journal Of Immunology Research, 2018, 1–14. Https://Doi.Org/10.1155/2018/5070573

Zhang, J., Chen, W., Alvarez, J. B., Jia, K., Shi, L., Wang, Q., Zou, N., He, K. & Zhu, H. (2018). Cancer Immune Checkpoint Blockade Therapy And Its Associated Autoimmune Cardiotoxicity. Acta Pharmacologica Sinica, 39(11), 1693–1698. Https://Doi.Org/10.1038/S41401-018-0062-2

II. ATTACHMENTS

Appendix 1. Ethics Committee Approval

Tabriz University of Medical Sciences

Research Ethics Committees Certificate

Approval ID:	IR.TBZMED.REC.1402.979	Approval Date:	2024-03-04
Evaluated by:	Research Ethics Committees of Tabriz University of Medical Sciences		
Status:	Approved		
Approval Statement:	The project was found to be in accordance to the ethical principles and the national norms and standards for conducting Medical Research in Iran. Notice: 1. Although the proposal has been approved by the Biomedical Research Ethics Committee, meeting the professional and legal requirements is the sole responsibility of the PI and other project collaborators. 2. This certificate is reliant on the proposal/documents received by this committee on 2024-03-04. The committee must be notified by the PI as soon as the proposal/documents are modified.		
Proposal Title:	investigation of the effect of treatment with TNF-alpha inhibitor on the expression of inflammatory and non-inflammatory factors in dendritic cells derived from tolerogenic monocytes		
Principal Investigator:	Name: Behzad Baradaran Email: behzad_im@yahoo.com		

Dr. Bahman Naghipour
Committee Director
Tabriz University of Medical Sciences

Dr. Parviz Shahabi
Committee Secretary
Tabriz University of Medical Sciences

III. PLAGIARISM REPORT

The Impact of Thalidomide on Immune Checkpoints in Dendritic Cells

Yazar NASTARAN AZAMI

Gönderim Tarihi: 12-Tem-2024 01:42PM (UTC+0300)
Gönderim Numarası: 2415654764
Dosya adı: Nastaran_Azami.docx (2.91M)
Kelime sayısı: 11977
Karakter sayısı: 69617

The Impact of Thalidomide on Immune Checkpoints in Dendritic Cells

ORİJİNALLİK RAPORU

%13	%9	%8	%2
BENZERLİK ENDEKSİ	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCİ ÖDEVLERİ

BİRİNCİL KAYNAKLAR

1	onlinelibrary.wiley.com İnternet Kaynağı	%2
2	Sepideh Sohrabi, Javad Masoumi, Bahar Naseri, Farid Ghorbaninezhad et al. "STATs signaling pathways in dendritic cells: As potential therapeutic targets?", International Reviews of Immunology, 2023 Yayın	%1
3	www.ncbi.nlm.nih.gov İnternet Kaynağı	%1
4	worldwidescience.org İnternet Kaynağı	%1
5	Submitted to ITESM: Instituto Tecnologico y de Estudios Superiores de Monterrey Öğrenci Ödevi	%1
6	www.science.gov İnternet Kaynağı	%1
7	www.mdpi.com İnternet Kaynağı	%1

8	mdpi-res.com İnternet Kaynağı	<%1
9	mts.intechopen.com İnternet Kaynağı	<%1
10	www.frontiersin.org İnternet Kaynağı	<%1
11	Tumor-Induced Immune Suppression, 2014. Yayın	<%1
12	Weigang Xiu, Juan Ma, Ting Lei, Man Zhang, Shangyan Zhou. "Immunosuppressive effect of bladder cancer on function of dendritic cells involving of Jak2/STAT3 pathway", Oncotarget, 2016 Yayın	<%1
13	Cancer Immunology, 2015. Yayın	<%1
14	www.nature.com İnternet Kaynağı	<%1
15	c.coek.info İnternet Kaynağı	<%1
16	journals.plos.org İnternet Kaynağı	<%1
17	Submitted to University of Westminster Öğrenci Ödevi	<%1

18	Popescu, Medeea Claudia. "Bacteriophage and Antibacterial Innate Immunity in Health and Disease", Stanford University, 2023 Yayın	<%1
19	Submitted to University of Birmingham Öğrenci Ödevi	<%1
20	Reza Dabbaghipour, Elham Ahmadi, Mona Entezam, Omid Rahbar Farzam et al. "Concise review: The heterogenous roles of BATF3 in cancer oncogenesis and dendritic cells and T cells differentiation and function considering the importance of BATF3-dependent dendritic cells", Immunogenetics, 2024 Yayın	<%1
21	munin.uit.no İnternet Kaynağı	<%1
22	Matthias P. Domogalla, Patricia V. Rostan, Verena K. Raker, Kerstin Steinbrink. "Tolerance through Education: How Tolerogenic Dendritic Cells Shape Immunity", Frontiers in Immunology, 2017 Yayın	<%1
23	vetdergi.kafkas.edu.tr İnternet Kaynağı	<%1
24	www.freshpatents.com İnternet Kaynağı	<%1

25	Francisco Ramírez-Valle, Joseph C. Maranville, Sophie Roy, Robert M. Plenge. "Sequential immunotherapy: towards cures for autoimmunity", Nature Reviews Drug Discovery, 2024 Yayın	<%1
26	da Silva, Maria Araújo Alves Martins. "Elucidating the Role of Glycans in Leucocyte Function", Universidade NOVA de Lisboa (Portugal), 2024 Yayın	<%1
27	docplayer.net İnternet Kaynağı	<%1
28	patents.google.com İnternet Kaynağı	<%1
29	www.trademarkelite.com İnternet Kaynağı	<%1
30	Xuan Zhang, Yang Liu, Yang Lu, Song Li et al. "N-3-(oxododecanoyl)-l-homoserine lactone suppresses dendritic cell maturation by upregulating the long noncoding RNA NRIR", Journal of Biosciences, 2021 Yayın	<%1
31	cdr.lib.unc.edu İnternet Kaynağı	<%1
32	d.docksci.com İnternet Kaynağı	

		<%1
33	hal-univ-tours.archives-ouvertes.fr İnternet Kaynağı	<%1
34	kipdf.com İnternet Kaynağı	<%1
35	www.archivesofrheumatology.org İnternet Kaynağı	<%1
36	Xiaohan Qin, Mengzhu Zhang, Zhipeng Zhao, Qian Du, Qian Li, Yue Jiang, Fumin Xue, Yuxia Luan. "A Carrier-Free Photodynamic Nanodrug to Enable Regulation of Dendritic Cells for Boosting Cancer Immunotherapy", Acta Biomaterialia, 2022 Yayın	<%1
37	unsworks.unsw.edu.au İnternet Kaynağı	<%1
38	encyclopedia.pub İnternet Kaynağı	<%1
39	files.ecommercedns.uk İnternet Kaynağı	<%1
40	link.springer.com İnternet Kaynağı	<%1
41	refubium.fu-berlin.de İnternet Kaynağı	<%1

42	www.texilajournal.com İnternet Kaynağı	<%1
43	Anthony Meager. "Viral Inhibitors and Immune Response Mediators: The Interferons", Encyclopedia of Molecular Cell Biology and Molecular Medicine, 09/15/2006 Yayın	<%1
44	Brain, Danielle Emma. "Investigating the Impact Of, Novel, Therapeutic Delivery Systems on Immunological Responses - Determination of Short, and Long-Term, Exposure Effects", The University of Liverpool (United Kingdom), 2023 Yayın	<%1
45	hdl.handle.net İnternet Kaynağı	<%1
46	www.biocompare.com İnternet Kaynağı	<%1
47	www.ous-research.no İnternet Kaynağı	<%1
48	www.repository.cam.ac.uk İnternet Kaynağı	<%1

Alıntıları çıkart	Kapat	Eşleşmeleri çıkar	< 10 words
Bibliyografyayı Çıkart	Kapat		

42	www.texilajournal.com İnternet Kaynağı	<%1
43	Anthony Meager. "Viral Inhibitors and Immune Response Mediators: The Interferons", Encyclopedia of Molecular Cell Biology and Molecular Medicine, 09/15/2006 Yayın	<%1
44	Brain, Danielle Emma. "Investigating the Impact Of, Novel, Therapeutic Delivery Systems on Immunological Responses - Determination of Short, and Long-Term, Exposure Effects", The University of Liverpool (United Kingdom), 2023 Yayın	<%1
45	hdl.handle.net İnternet Kaynağı	<%1
46	www.biocompare.com İnternet Kaynağı	<%1
47	www.ous-research.no İnternet Kaynağı	<%1
48	www.repository.cam.ac.uk İnternet Kaynağı	<%1

Alıntıları çıkart Kapat
Bibliyografyayı Çıkart Kapat
Eşleşmeleri çıkar < 10 words