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The Expression Of Connexin-43 By CD11c+ Dendritic Cells Is Required to Maintain CD4+ Foxp3+ Regulatory T Cell Population in Peripheral Lymphoid Organs

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THE EXPRESSION OF CONNEXIN-43 BY CD11c⁺ DENDRITIC CELLS IS REQUIRED TO
MAINTAIN CD4⁺ Foxp3⁺ REGULATORY T CELL POPULATION
IN PERIPHERAL LYMPHOID ORGANS

by

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and Sciences, Allahabad, India

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Old Dominion University in Partial Fulfillment of the
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MASTER OF SCIENCE
BIOLOGY

OLD DOMINION UNIVERSITY
August 2019

Approved by:
Piotr Kraj (Director)
Emilia Oleszak (Member)
Robert Ratzlaff (Member)
ABSTRACT

THE EXPRESSION OF CONNEXIN-43 BY CD11c⁺ DENDRITIC CELLS IS REQUIRED TO MAINTAIN CD4⁺ Foxp3⁺ REGULATORY T CELL POPULATION IN PERIPHERAL LYMPHOID ORGANS

Caroline Titus Miller
Old Dominion University, 2019
Director: Dr. Piotr J. Kraj

Foxp3⁺ regulatory T cells (T_R) are an immunosuppressive subset of CD4⁺ T cells that maintain homeostasis of the immune system. They are sustained by the interaction between the Major Histocompatibility Complex (MHC) molecules present on antigen presenting dendritic cells and the T Cell Receptor (TCR) expressed on T_R cells that is specific for the MHC loaded with an antigenic peptide. Here, we show that in addition to MHC/TCR interaction, Connexin-43 (Cx43) expression by dendritic cells is required to maintain the T_R cell population. CD11c⁺ dendritic cells represent a major subset of antigen presenting cells. Using flow cytometry, we have observed that mice which lack Cx43 expression in CD11c⁺ dendritic cells (Cx43⁻ DC⁻), have a lower percentage of T_R cells which express lower levels of Foxp3. These mice showed increased incidence of dermatitis as they age, even though we show that their dendritic cells can efficiently present antigen to naive T cells using proliferation inhibition assay. The decrease in the proportion of T_R cells were associated with an altered phenotype of these cells, demonstrated by lower expression of CD39 and higher expression of CD73, ectonucleotidases mediating T_R cell immunosuppressive function. We propose that the presence of Cx43 on the surface of dendritic cells is required for effective communication between T_R cells and dendritic cells so as to sustain T_R cell homeostatic expansion and Foxp3 expression.
This thesis is dedicated to the loving memory of my parents, John Titus Joseph and Lilly Titus Joseph, who I wish could have seen this moment.
ACKNOWLEDGMENTS

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I would like to thank my academic advisor, Dr. Piotr Kraj, for offering guidance and instruction in the most gracious way, and for inspiring me to never stop learning. I thank him for appreciating my efforts and setting a fast-paced work environment that I have really enjoyed. I would like to thank my friend and colleague, Dr. Lauren Browning for believing in me, sometimes even more than I did myself. I thank her for her guidance throughout the research project, her friendship during emotional times and her austerity in agreeing to confiscate my phone when I had deadlines to meet. I will greatly miss being a part of this laboratory. I extend gratitude to the members of the thesis committee, Dr. Emilia Oleszak and Dr. Robert Ratzlaff, for being available and providing prompt feedback on all the submissions over the past few months. I thank them also for their encouraging conversations and for going above and beyond to make sure this process was completed in the right way.

All of this would not have taken place the way it had, if it hadn’t been for the initial encouragement to pursue a master’s degree from the love of my life, Vaughn Miller. I am very thankful for being able to become a mother in the midst of the program to our sweetest little baby boy whose squeals and smiles I can come home to everyday.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>$^3$H</td>
<td>Tritium</td>
</tr>
<tr>
<td>AMP</td>
<td>Adenosine MonoPhosphate</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine DiPhosphate</td>
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<td>ATP</td>
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<td>APC</td>
<td>Antigen Presenting Cell</td>
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<td>cAMP</td>
<td>cyclic Adenosine MonoPhosphate</td>
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<tr>
<td>CFA</td>
<td>Complete Freund’s Adjuvant</td>
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<tr>
<td>CTLA-4</td>
<td>Cytotoxic T lymphocyte Antigen-4</td>
</tr>
<tr>
<td>CTM</td>
<td>Complete Tumor Medium</td>
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<tr>
<td>Cx</td>
<td>Connexin</td>
</tr>
<tr>
<td>CXCL8</td>
<td>C-X-C motif Ligand 8</td>
</tr>
<tr>
<td>DC</td>
<td>Dendritic Cell</td>
</tr>
<tr>
<td>DNA</td>
<td>DeoxyriboNucleic Acid</td>
</tr>
<tr>
<td>FBS</td>
<td>Fetal Bovine Serum</td>
</tr>
<tr>
<td>FGL2</td>
<td>Fibrinogen-like protein</td>
</tr>
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<td>Foxp3</td>
<td>Forkhead-box P3</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>Klrg1</td>
<td>Killer cell Lectin like Receptor G1</td>
</tr>
<tr>
<td>LAG-3</td>
<td>Lymphocyte-Activation Gene-3</td>
</tr>
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<td>MHCI</td>
<td>Major Histocompatibility Complex Class I</td>
</tr>
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<td>--------------</td>
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<tr>
<td>MHCII</td>
<td>Major Histocompatibility Complex Class II</td>
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<tr>
<td>min</td>
<td>Minute (s)</td>
</tr>
<tr>
<td>NAD</td>
<td>Nicotinamide Adenine Dinucleotide</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Solution</td>
</tr>
<tr>
<td>PD1</td>
<td>Programmed cell Death protein 1</td>
</tr>
<tr>
<td>PE</td>
<td>Phycoerythrin</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>TCR</td>
<td>T-Cell Receptor</td>
</tr>
<tr>
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<td>Tumor Growth Factor β</td>
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<td>T&lt;sub&gt;H&lt;/sub&gt;1</td>
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CHAPTER 1
INTRODUCTION

Most multi-cellular organisms, if not all, face the constant threat of infection by a variety of microbial pathogens. The probability that the host organism will survive depends on its ability to overpower and eliminate the invading foreign material, by inducing defensive responses that are termed broadly as ‘immune responses’. Ideally, these responses target the pathogenic material and not the host cells and tissues. In this introduction, we will review antigen presentation by innate immune cells and the consequent activation of a few specific types of T cell subsets that play an important role in adaptive immunity and homeostasis. In addition, we will discuss the current understanding of the role of Connexin-43 in the immunological synapse, and how it relates to current research.

1.1. Antigen presentation

The first line of defense against foreign material is the physical and chemical barriers of the body like skin and antimicrobial enzymes secreted at mucosal surfaces, respectively. If foreign material or a pathogen were to successfully overcome the barriers and enter the body, the innate immune system responds to it in a multi-faceted way. Neutrophils, basophils, macrophages, monocytes, dendritic cells, natural killer cells and granulocytes bind foreign molecules through Pattern Recognition Receptors (PRRs), get activated and directly or indirectly facilitate the elimination of the pathogen (1-3). In most cases, the pathogen or what is referred to as antigen, is digested and destroyed by resident phagocytes. All phagocytic cells like macrophages/monocytes, granulocytes and dendritic cells have the ability to internalize antigen, through a process called phagocytosis,
or take up large amounts of extracellular material through a process called pinocytosis and degrade the antigens that were endocytosed. Activated phagocytes secrete pro-inflammatory cytokines like Tumor Necrosis Factor-α (TNF-α), Interleukin-1 (IL-1), IL-6 and IL-18 and chemokines like CXCL-8 that recruit and activate cells of the adaptive immune system at the site of pathogen entry (4, 5).

One of the main roles of phagocytes is to present antigenic peptides on the cell's surface in order to activate cytotoxic and helper T cells of the adaptive immune system. T cells are not able to recognize native antigens and conformational epitopes; therefore, they depend on recognition of processed antigen for activation. Antigen processing relies on proteolytic digestion of ingested proteins/antigens and binding of resulting peptides to the Major Histocompatibility Complex (MHC) molecules. MHCs are polymorphic transmembrane glycoproteins found on the cell's surface having a characteristic peptide-binding groove. MHC structure is stabilized by binding peptides derived from self or exogenous proteins. There are two types of MHC molecules. MHC Class I (MHCI) molecules are expressed by all nucleated cells and present antigenic peptides to cytotoxic CD8+ T cells (6). It increases the targeting of abnormal cells by CD8+ cytotoxic T cells that recognizes the mutated peptides, like those resulting from DNA damage or cancer associated mutations (7). MHC Class II molecules are only present on phagocytic cells, of which macrophages and dendritic cells are referred to as "professional antigen presenting cells" (8). When the endocytosed material is broken down in acidified endosomes, it is loaded onto the MHCII molecule and transported to the cell membrane for antigen presentation. The helper CD4+ T cells require this presentation of antigenic peptide in the context of MHCII molecules in order to be activated (9). CD11c is a type I transmembrane protein that is constitutively present on all dendritic cells and is used in conjunction with other markers to identify various subsets. CD11c is, therefore,
used in this study to identify the dendritic cell population, which are known to be efficient activators of regulatory T cell population. This is further discussed in 1.3.

1.2. Development and activation of CD4$^+$ T cells

The adaptive immune responses are carried out by B and T lymphocytes, two types of white blood cells that are differentiated from hematopoietic stem cells in the bone marrow. B cell differentiation occurs in the bone marrow, and T cell maturation takes place in the thymus. B cells and T cells have a large repertoire of B cell receptors (BCRs) or T cell receptors (TCRs) to enable these cell types to detect and eliminate a variety of pathogens \( (10) \). A TCR is a dimer and consists of one α and one β chain, each having a variable and a constant region. The variable regions of these chains have a unique sequence that can bind to a peptide in complex with an MHC molecule. In this way, T cells are MHC-restricted and cannot be activated by antigenic peptide alone. The development of T cells or thymocytes takes place in the thymus and is a very systematic process that ensures a low probability of self-reactivity. After the successful rearrangements of the V, D and J gene segments of the TCR’s β-chain takes place, there is a rapid clonal expansion of the cells whose β-chain was successfully rearranged, accompanied by the expression of co-receptor proteins CD4 and CD8 that have the role of binding to a portion of the MHC Class II and MHC Class I molecule, respectively, to infer stability of the eventual TCR/MHC interaction \( (11, 12) \). The cell proliferation is followed by α-chain rearrangement in each individual cell \( (13) \). The next step in ensuring that these cells will bind to an MHC molecule is called positive selection where thymic cortical epithelial cells express MHC molecules for thymocytes to bind, and during this step, the T cell will commit to expressing either CD4 or CD8 depending on the strength of the affinity by which the TCR binds to the MHCII or MHCI \( (14) \). In addition, the T cells that do not bind in this
step undergo apoptosis. During negative selection, any thymocyte that binds with high affinity to an MHC molecule with a self-antigen peptide is eliminated by apoptosis (15). This significantly reduces the chance of auto-reactive T cell clones. Elimination of self-reactive T cells is complemented by a dominant tolerance mechanism relying on the presence of suppressor CD4+ T cells. These cells, called natural regulatory T cells (T\textsubscript{R}), expressing transcription factor Forkhead box P3 (Foxp3) are generated in the thymus and recognize MHCII + self-peptides. Current data support the conclusion that T\textsubscript{R} cells are generated in an instructive process when interactions between TCR and MHCII/peptide complex, stronger than that of conventional CD4+ thymocytes, induce Foxp3 expression and launch a genetic program of T\textsubscript{R} cell differentiation (16).

Immunosuppressive role of T\textsubscript{R} cells relies on various mechanisms and depends on recognition of a self-peptide on MHCII molecule, which is further discussed in 1.3. The CD4+ Foxp3+ T\textsubscript{R} thymocytes migrate out of the thymus and circulate to the lymphoid tissues through the bloodstream. Professional antigen presenting cells like dendritic cells migrate to the lymphoid tissues to interact with circulating naïve CD4+ conventional and T\textsubscript{R} cells (17). These could be CD11c+ migratory Langerhans cells or dermal dendritic cells that reside in the skin and sample antigens (18). When the naïve CD4+ T helper cell recognizes the specific antigen presented by a dendritic cell and co-stimulatory molecules, the T cell remains in the lymphoid tissue and undergoes clonal expansion with the production of cytokines that gives rise to effector T cells and memory T cells. The naïve and effector T cells migrate out of the lymph node and move toward the site of infection by chemotaxis. The CD4+ T cells recognize antigen presented by APC at the site of infection, get activated and undergo clonal expansion and cytokine production (19). Depending on the cytokines in the environment, the effector T cells differentiate into subsets like T helper 1 (T\textsubscript{H1}) cells that secrete IFN-\gamma (20), T helper 2 (T\textsubscript{H2}) cells that secrete IL-4 (21), T helper 17 (T\textsubscript{H17}) cells that
secrete IL-17 (22) or peripheral regulatory T (T<sub>R</sub>) cells that secrete IL-10, as depicted in Fig. 1 (23), and upregulate the expression of characteristic transcription factors and cytokines. T<sub>H1</sub>, T<sub>H2</sub> and T<sub>H17</sub> cells release pro-inflammatory cytokines, whereas T<sub>R</sub> performs an immunosuppressive role and controls the immune response (22).

**Fig. 1.** Naïve T cells differentiate on activation based on the cytokines in its environment. Animated drawing depicting TCR/MHC interaction between an APC and CD4<sup>+</sup> T cell through MHC-II; and interaction between the APC and CD8<sup>+</sup> T cell through MHC-I. Based on the cytokines in the environment, the effector T cells differentiate into effector cells with pro-inflammatory, anti-inflammatory or cytotoxic roles specified within the orange boxes. The yellow boxes note the genes that are upregulated in the T cell subset. (23).
1.3. Immunosuppressive CD4+ Foxp3+ regulatory T cells

Maintenance of immune tolerance is largely carried out by CD4+ Foxp3+ T<sub>R</sub> cells. Gene mapping and functional studies established that Foxp3 is an X-chromosome-encoded gene and a master regulator of CD4+ T<sub>R</sub> cell development in both humans and mice. Foxp3-deficient mice do not have functional T<sub>R</sub> cell population and develop fatal lymphoproliferative autoimmune syndrome at 3-4 weeks of age (24). T<sub>R</sub> cells constitute up to 10-20% of the CD4+ T cell compartment. Like other CD4+ T cells, CD4+ Foxp3+ T<sub>R</sub> cell population’s development and activation are strictly dependent on the TCR/MHC interactions (25).

All APCs, expressing class II MHC molecules, have the ability to induce antigen specific CD4+ Foxp3+ T<sub>R</sub> cells in the presence of IL-2 and TGF-β. However, there is evidence that dendritic cells (DCs) are the most efficient activators of T<sub>R</sub> cells (26). In the presence of high concentrations of IL-2, the apoptotic signaling in T<sub>R</sub> cells is inhibited. On recognition of the MHC/peptide complexes through their TCR, T<sub>R</sub> cells undergo clonal expansion and make direct contact or produce effector molecules to enforce immune suppression. Some of the molecules by which these T<sub>R</sub> cells maintain homeostasis have been outlined in Table 1 (27). Foxp3+ Regulatory T cells can be immunosuppressive in an antigen non-specific manner in multiple ways. Through the secretion of cytokines like TGF-β and IL-35 by T<sub>R</sub> cells, CD4+ effector cells are led to differentiate into immunosuppressive Foxp3+ cells as discussed in 1.2 (28, 29). Other cytokines like IL-10 have been shown to be required for effective immunosuppression at interfaces like the colon and lungs (30). Regulatory T cells are also able to transfer cyclic-AMP (cAMP) into conventional cells in a contact-dependent manner through gap junctions to result in the induction of the expression of the transcription factor ICER (Induced Cyclic-AMP Early Repressor) in conventional CD4+ T cells. ICER represses at the IL-2 and IL-4 gene loci and prevents the secretion of cytokines by the
activated CD4+ T cells (31). CD39 is an ectonucleotidase present on the surface of Foxp3+ T<sub>R</sub> cells, that converts adenosine diphosphate (ADP) or adenosine triphosphate (ATP) to adenosine monophosphate (AMP), where ATP is a known mediator of inflammation. CD73 is also an ectonucleotidase on the T<sub>R</sub> cell whose function complements CD39 and converts AMP to adenosine which has been shown to have an immunosuppressive role (32). T<sub>R</sub> cells can also maintain homeostasis by secreting cytolytic molecules like granzymes and perforin to induce apoptosis in conventional CD4+ T cells (33). Certain dendritic cells express indoleamine 2,3-dioxygenase (IDO) that catalyzes the degradation of tryptophan to kynurenine which starves the effector T cells and puts them in cell cycle arrest. Via CTLA-4-induced signaling, T<sub>R</sub> cells can increase IDO expression in dendritic cells and also direct T<sub>R</sub> differentiation of naïve CD4+ T cells (34).

Table 1. **Immunosuppressive effector molecules of T<sub>R</sub> cells.** The known effector molecules of T<sub>R</sub> cells can be classified into secreted immunosuppressive cytokine molecules, molecules that inhibit metabolic signaling, secreted cytolytic molecules and membrane associated molecules that inhibit T effector cell activation.

<table>
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<th>Metabolic signaling</th>
<th>Cytolytic molecules</th>
<th>Membrane-associated molecules</th>
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<td>TGF-β</td>
<td>cAMP</td>
<td>Granzyme A/B</td>
<td>CTLA-4</td>
</tr>
<tr>
<td>IL-10</td>
<td>CD39</td>
<td>Perforin</td>
<td>LAG-3</td>
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<tr>
<td>IL-35</td>
<td>CD73</td>
<td>TRAIL-DR5</td>
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<tr>
<td>FGL2</td>
<td>NAD</td>
<td>Galectin-1</td>
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</table>
As a result of the effects of these molecules and direct interactions, activated T cells and antigen-presenting cells are not able to carry out their immunological response effectively. T<sub>R</sub> cell population consumes a large portion of IL-2 produced by the activated CD4<sup>+</sup>T cells and in doing so, carries out IL-2 deprivation and induces apoptosis of both cell populations (35). For this reason, T<sub>R</sub> cell populations were initially identified by the expression of CD25, which is the α-chain of the IL-2 receptor. However, the upregulation of CD25 on all activated CD4<sup>+</sup> cells and the existence of non-immunosuppressive CD4<sup>+</sup>CD25<sup>+</sup> cells were observed, and Foxp3 expression was found to be a more precise marker for the immunosuppressive cells (24). Immunoregulation is an essential process in order to avoid potentially damaging autoimmune responses by non-regulatory T cells and to maintain tolerance to self-antigens. CD4<sup>+</sup> Foxp3<sup>+</sup> T<sub>R</sub> cells have a high turnover rate and respond to a range of signals from the environment that may make them anergic or persist as memory cells (36). Therefore, maintenance of a stable population size of functional T<sub>R</sub> is required for effective immune suppression.

1.4. Connexin-43

Connexins are a family of small transmembrane proteins that assemble into membrane complexes to create small channels called gap junctions in order to allow communication between cells or carry out paracrine functions as hemi-channels (37, 38). Connexin-43 (Cx43) has a relatively long cytoplasmic domain with a number of phosphorylation sites that have been proposed to interact with cytoskeletal elements and signaling molecules like protein kinase C, mitogen-activated protein kinase and β-catenin (39). Recent evidence has shown that Cx43 may have the ability to act as a transcription factor (40). The expression of Cx43 is widespread among immunological
cells like dendritic cells, monocytes, B cells, T cells and Natural Killer cells (41). On antigen activation, expression of Cx43 is upregulated in T cells (42). Hemi-channel function of Cx43 is pro-inflammatory as it involves the release of ATP, a mediator of inflammation (43). In contrast, channels can form at gap junctions between effector and T\(_R\) cells to facilitate the transfer of cAMP from T\(_R\) cells to effector cells (31). In summary, modulating connexin-43 expression or gap junction formation may alter the course of adaptive immune response mediated by T cells. When the hemi-channel activity of Cx43 was reduced while simultaneously promoting gap junction plaque formation using a mimetic peptide-\(\alpha\)CT1 in a non-obese diabetic mouse model, T\(_R\) suppressor ability was partially restored (44). Phase III clinical trials of the use of \(\alpha\)CT1 for wound healing during treatment of diabetic foot ulcers is currently underway (45). Additionally, higher expression of Cx43 has been observed in human and murine thymic epithelial cells (42). Although the mechanism of its function has not been elucidated, several reports conclude that Cx43 is a component of the immunological synapse.

1.5. Purpose of research and experimental design

To examine the role of gap junctions and communication between T\(_R\) cells and DCs, we used a mouse model in which \(Gja1\) gene that codes for Cx43 was knocked out in CD11c\(^+\) dendritic cells alone. Several of these mice spontaneously developed dermatitis and a preliminary experiment showed a reduction in the number of CD4\(^+\)Foxp3\(^+\) T\(_R\) cells in both the lymph nodes and spleens of the Cx43\(^{DC-}\) mice. This provided the initial motivation for the study of the role of connexin in maintaining the regulatory T cell population. This research contributes to the literature on the involvement of Cx43 in the maintenance of regulatory T cell population, with the hope of shedding light on novel targets for therapeutic treatments of auto-immune diseases. We look into the changes
caused in mice due to the lack of expression of Cx43 in dendritic cells. For this reason, phenotypic and functional analyses of peripheral populations of DC and CD4$^+$ T cells using flow cytometry were carried out. Flow cytometry data on the dendritic cell population in the skin of wild-type (WT) control mice and Cx43$^{DC-}$ mice were also analyzed. The ability of the CD11c$^+$ dendritic cells with no expression of Cx43 to activate CD4$^+$ naïve T cells and the ability of their T$_R$ cells to suppress proliferation of activated CD4$^+$ T cells were measured through radioactive thymidine incorporation assays described in 2.6. and 2.7.
CHAPTER 2
METHODS

2.1. Generation of Cx43DC-mice

Conditional knockout of \textit{Gja1} gene that codes for Connexin-43 (Cx43\textsuperscript{loxP}) were crossed with CD11\textsuperscript{cre} and Foxp3\textsuperscript{GFP} reporter mice (39, 46). These mice were compared to wild-type control mice during analyses. Mice were housed and sacrificed according to the guidelines of the Institutional Animal Care and Use Committee of Old Dominion University under IACUC protocol number 17-017. Subsequent cell work was carried out with the approval of the Institutional Biosafety Committee of Old Dominion University under IBC protocol number 19-007.

2.2. Isolation of dendritic cells from the skin

Dorsal skin from the mice were cut and incubated in 0.5 mL of 0.2 mg/mL Dispase I solution in a 24-well plate overnight at 4\degree C. Hair was removed and incubated at 37\degree C in 1 mL Collagenase IV-DNase solution (RPMI, 10\% FBS, 1.5 mg/mL Collagenase IV and 0.5 mg/mL DNase) followed by addition of 1mL of Collagenase IV-DNase solution every 30 minutes for 2 hours. The solution was then filtered and centrifuged at 1200 rpm for 6 minutes. The pellet was resuspended in PBS + 2\% FBS for antibody staining and subsequent flow cytometry analysis.

2.3. Cell purification, flow cytometry and cell sorting

Lymph nodes and spleens of Cx43\textsuperscript{DC-} and WT mice were isolated and passed through nylon mesh to form cell suspensions in PBS + 2\% FBS. Spleen suspensions were centrifuged at 1200 rpm for 6 minutes and resuspended with 0.16 M sterile ammonium chloride to lyse red blood cells in the
sample. This sample was incubated at room temperature for 6 minutes and centrifuged at 1200 rpm and resuspended in PBS + 2% FBS. Aliquots of the cell suspension were stained with eight color antibody sets with antibodies specific for CD4, CD8, CD44, CD62L, 4-1BB, Tim-3, PD-1, IL-7R,Klr-1, BTLA, Lag3, CD25, CD39, CD73, Sirp1α, CD80, 4-1BBL, MHCII, CD24, CD86, PDL1, F4/80, Ox40L and Gr1 (BD Bioscience, BioLegend, eBioscience) for 30 minutes at 4°C with subsequent washes with PBS + 2% FBS, and analyzed using a flow cytometer (BDFACSCanto). CD4⁺ cells were negatively selected using streptavidin beads (mentioned in 2.4), while CD11c⁺ cells were positively selected using PE magnetic beads (mentioned in 2.5). In addition to this, these cells were sorted from the cell suspensions using a flow cytometer (BD Influx) for increased purity of 98-99%.

2.4. Negative selection of CD4⁺ cells using streptavidin beads.

Cell pellets were resuspended in 150 µL of PBS + 2% FBS and stained with biotinylated antibodies against CD8, Ter119, B220, CD49b (BD Bioscience, BioLegend, eBioscience) for 30 minutes at 4°C. After a wash with PBS+ 2%FBS, the cells were incubated with streptavidin magnetic beads (Appendix) for 20 minutes at 4°C, and the tube was placed in a magnet for 6 minutes. The clear solution was carefully pipetted out, centrifuged at 1200 rpm for 6 minutes and resuspended in PBS + 2% FBS as a CD4⁺ sample. Purity of the sample was checked using flow cytometry.

2.5. Positive selection of CD11c⁺ cells using PE beads.

Cell pellets were resuspended in 150 µL of PBS + 2% FBS and stained with CD11c-PE antibody for 30 minutes at 4°C, followed by a wash with PBS + 2% FBS. The cells were incubated with PE beads for 20 minutes at 4°C. Then the cells were washed and resuspended in 750 µL of PBS + 2
% FBS and loaded into the column attached on a magnetic plane. The flow through was disposed, and the column was detached from the magnet. PBS + 2% FBS was used to flush the CD11c⁺ cells into a collection tube. Purity of the sample was checked by flow cytometry.

2.6. Proliferation assay
In a 96-well round bottomed plate, 3x10⁴ sorted CD11c⁺ DCs and 2x10⁴ CD4⁺ cells from a transgenic mouse whose CD4⁺ T cells are specific for a peptide PCC50V54A were stimulated with soluble peptide (5 μM) in CTM (Appendix) and incubated at 37°C with 5% CO₂. Three days after stimulation, 1 μCi/well of ³H-thymidine was supplied. The radioactivity of the resulting cells after washing were measured on Tri-Carb 3110TR Liquid Scintillation Analyzer.

2.7. Proliferation inhibition assay
In a 96-well round bottomed plate, 5x10⁴ sorted naïve (CD44lo CD62Lhi) CD4⁺ T cells and 5x10⁴ WT DCs from lymphopenic mice (TCRα⁻) were stimulated using antibody against CD3 (BD Bioscience- 3 μg/mL) in CTM (Appendix) and incubated at 37°C with 5% CO₂. In different wells, T₉R were added in increasing concentrations of 0%, 5%, 10% 20% and 40% of total naïve CD4⁺ T cells. After three days of stimulation, 1 μCi/well of ³H-thymidine was added, and radioactivity of the sample after washing was measured on Tri-Carb 3110TR Liquid Scintillation Analyzer.

2.8. Data analysis
Flow Cytometry data was analyzed in dot plots and histograms using FlowJo v10. The results of the proliferation assay and the proliferation inhibition assay were graphed using Exel.
CHAPTER 3

RESULTS

3.1. Development of dermatitis in Cx43<sup>DC</sup>- mouse

The Cx43<sup>DC</sup>- mice, having a lack of expression of Connexin-43 in CD11c<sup>+</sup> dendritic cells, seem to visually develop normally apart from the dermatitis observed. About 25% of mice aged 1 to 4 months begin to develop a form of dermatitis on their dorsal skin (Fig. 2), which is first seen as barbering. Moreover, 60% of the mice older than 5 months had dermatitis progressing from barbering to open skin lesions. This skin disease is likely caused by an autoimmune response that has been sustained due to lack of immune suppression. During an auto-immune response, the T<sub>R</sub> population is usually lower than in healthy mice. Lymph nodes and spleens were isolated from these mice and T cell populations were analyzed to further investigate the cell populations affected by the lack Cx43 in DCs that may have caused the disease.

![Fig. 2. Cx43<sup>DC</sup>- mice spontaneously develop dermatitis. Image of barbering seen on the dorsal skin of two-month old Cx43<sup>DC</sup>- mice](image)
3.2. Dendritic cell population density in the skin of Cx43\textsuperscript{DC} mice varies compared to that of wild-type control mice

To investigate the changes in skin’s dendritic cell population due to the lack of Cx43 on CD11c\textsuperscript{+} DC cells, skin was digested, and the cells were isolated to be stained. There was a presence of CD11c\textsuperscript{+} MHCII\textsuperscript{+} CD24\textsuperscript{-} CD103\textsuperscript{-} dermal DCs in young 2-month old Cx43\textsuperscript{DC} mice, which was barely detectable in control mice (Fig. 3). These cells are usually only detectable at 3 months of age as the immune system matures. It is possible that the existence of larger proportion of dermal dendritic cells that are migrating to the skin, is the reason why Cx43\textsuperscript{DC} mice are predisposed to developing skin disease. The CD11c\textsuperscript{+} MHCII\textsuperscript{+} CD24\textsuperscript{+} CD11b\textsuperscript{+} Langerhans cell population was increased in Cx43\textsuperscript{DC} mice with respect to age, whereas CD11c\textsuperscript{+} MHCII\textsuperscript{+} CD24\textsuperscript{+} CD11b\textsuperscript{-} dermal dendritic cell population was decreased, also with respect to age compared with age-matched WT mice older than 3 months.
Fig. 3. Increased presence of dermal dendritic cells in the dorsal skin of 2 month old Cx43\textsuperscript{DC-} mice. Mice lacking Cx43 expression by CD11c\textsuperscript{+} dendritic cells have a larger proportion of CD24\textsuperscript{-}CD103\textsuperscript{-} dermal dendritic cells in the dorsal skin of 2 month old mice. Dermal dendritic cells usually are present in low numbers in the skin till 3 months of age when the immune system is mature.

3.3. Increased activation is seen in older Cx43\textsuperscript{DC-} mice with dermatitis.

The flow cytometry data analysis of cells from lymph nodes showed that in Cx43\textsuperscript{DC-} mice with dermatitis, more than 70% of the CD4\textsuperscript{+} cells in the lymph nodes were activated, depicted as CD44\textsuperscript{hi}CD62\textsuperscript{lo} cell population in Fig. 4. These activated cells from Cx43\textsuperscript{DC-} mice had a decrease in activation markers 4-1BB and Klrg1 expression, accompanied by an increase in inhibitory molecule PD-1 in old mice, compared to age-matched WT (Fig. 5). The changes in population of activated cells and expression of activation markers shown in Fig. 4 and 5 were not seen in Cx43\textsuperscript{DC-} mice without dermatitis or in the WT mice. Therefore, the increased activated population could be
attributed to the response to the dermatitis seen on the dorsal skin, while the changes in expression of 4-1BB and Klr g1 may be due to the way Cx43\textsuperscript{DC-} cell populations interact after the onset dermatitis.

Fig. 4. Increased activation in Cx43\textsuperscript{DC-} mice with dermatitis. Total CD4\textsuperscript{+} cells can be separated into activated and naïve cells based on the expression of CD44 and CD62L. The plots show that old Cx43\textsuperscript{DC-} mice with dermatitis had almost 80\% of CD4\textsuperscript{+} cells activated in the lymph nodes.
3.4. Decrease of CD4+ Foxp3+ T<sub>R</sub> population in Cx43<sup>DC</sup>− mice with relation to age.

Using flow cytometry, we analyzed the different T cell populations in the lymph nodes of the knockout mice alongside age-matched controls. We found that Cx43<sup>DC</sup>− mice had a smaller population of CD4<sup>+</sup> Foxp3<sup>+</sup> T<sub>R</sub> cells compared to WT mice (Fig. 6, 7). Additionally, these T<sub>R</sub> cells expressed Foxp3 with a lesser intensity (Fig. 8) as indicated by the decrease in mean fluorescence intensity measurement of the fluorophore by flow cytometry. The lower amounts of immunosuppressive regulatory T cells may have led to the development of dermatitis seen among Cx43<sup>DC</sup>− mice, due to the consequent failure to control immune response at the dorsal skin.

**Fig. 5.** Changes in expression of molecular markers for activation and inhibition in activated cells of Cx43<sup>DC</sup>−. Histograms showing the expression of 4-1BB, PD-1, IL-7R, Klrq1, Lag3 and BTLA by activated CD4<sup>+</sup> CD44<sup>hi</sup>CD62<sup>lo</sup> cells of WT and Cx43DC− mice.
Fig. 6. Lower CD4⁺ Foxp3⁺ T<sub>R</sub> cell population in Cx43<sup>DC⁻</sup> mice. Flow cytometry analysis of Foxp3 expression by gated CD4⁺ Foxp3⁺ cells showing that Cx43<sup>DC⁻</sup> mice had lower proportion of Foxp3⁺ T<sub>R</sub> cells compared to age-matched WT at 2 months, 9 months and 12 months.

Fig. 7. Decrease in CD4⁺ Foxp3⁺ T<sub>R</sub> population in Cx43<sup>DC⁻</sup> mice with respect to age. The percentage of T<sub>R</sub> cells in lymph nodes of Cx43<sup>DC⁻</sup> mice decrease with respect to age categorized as 1-3 months, 4-7 months and greater than 8 months, based on flow cytometry data.
Fig. 8. Decrease in Foxp3 expression of CD4$^+$ Foxp3$^+$ T$_R$ cells in Cx43$^{DC-}$ mice with respect to age. Mean fluorescence Intensity of Foxp3 expression of the T$_R$ cells of Cx43$^{DC-}$ mice is also reduced, portraying a reduced expression of Foxp3 at 1-3 months of age and greater than 8 months of age based on flow cytometry data.

3.5. An altered phenotype of CD4$^+$ Foxp3$^+$ T$_R$ is observed in Cx43$^{DC-}$ mice.

As mentioned earlier, CD39 and CD73 are ectonucleotidases on the surface of Foxp3$^+$ regulatory T cells. Flow cytometric data analysis of Foxp3$^+$ T$_R$ cells of Cx43$^{DC-}$ mice showed that a large majority of the cells took on a CD39$^+$ CD73$^+$ phenotype (Fig. 9), unlike the populations of different level of expressions seen in WT mice. This population also had a decrease in CD39 expression and an increase in CD73 expression compared to the same population of WT mice (Fig. 10).
Fig. 9. Majority of CD4^+ Foxp3^+ cells of Cx43^{DC-} mice have acquired CD39^+ CD73^+ phenotype. Flow cytometry data that shows that more than 90% of CD4^+ Foxp3^+ T_R cells from Cx43^{DC-} mice have acquired CD39^+ CD73^+ phenotype at young (2 months) and old (12 months) age compared to age-matched WT.
Fig. 10. CD4+ Foxp3+ T<sub>R</sub> cells of Cx43<sup>DC</sup> mice have altered expression of CD39 and CD73. The CD4+ Foxp3+ CD39+ CD73+ T<sub>R</sub> population gated in Fig. 7 displays varied expressions of CD39 and CD73 among Cx43<sup>DC</sup>- and WT. There is reduced expression of CD39 and increased expression of CD73, measured by flow cytometry in both young and old mice.

3.6. CD4+ Foxp3+ T<sub>R</sub> cells from Cx43<sup>DC</sup>- mice suppress the proliferation of CD4+ cells.

It was proposed that presence of CD4+ Foxp3+ CD39+ CD73+ T<sub>R</sub> cells with altered phenotype might offset the conversion of pro-inflammatory ATP to immunosuppressive adenosine and be responsible for a possible lack of immune suppression leading to the dermatitis seen on the mice. To check this, we set up a proliferation inhibition assay with APCs and CD4+ naïve T cells along with increasing proportions of CD4+ Foxp3+ T<sub>R</sub> cells. The splenocytes were taken from TCR<sub>α</sub>KO mice which did not have functioning T cells, and used as APCs in this assay. Soluble CD3 antibody was supplied to ensure activation of the CD4+ naïve T cells. The amount of tritium labelled thymidine (³H-thymidine) incorporated was used to measure proliferation of the cells. The results from this experiment show that the CD4+ Foxp3+ cells from the Cx43<sup>DC</sup>- mice suppressed the proliferation of CD4+ naïve WT cells at a comparable level as CD4+ Foxp3+ cells from WT mice, with only a
slight decrease, as the readings were within one standard deviation of the WT and considered not significant (Fig. 11). Although the phenotype of the regulatory cells developed in Cx43DC- mice had altered levels of CD39 and CD73 expression, perhaps they were able to suppress using mechanisms of immunosuppression.

**Fig. 11.** Proliferation inhibition assay results. Data from the scintillation counter showing the proliferation of CD4+ T cells on activation decreases when CD4+ Foxp3+ T_R cells are added to the well.

3.7. Prominent CD4+ Foxp3- CD25+ cell population is seen in Cx43DC- mice

Flow cytometry analysis showed that WT mice have less than one percent of Foxp3- CD25+ cells at 2 months and at 12 months, whereas Cx43DC- have about 4% at 2 months and about 10% when they are 12 months or older (Fig. 12). CD25 is the IL-2 receptor required for differentiation into CD4+ Foxp3+ T_R cells in the presence of IL-2. There is a possibility that Foxp3+ cells lose their Foxp3 expression while still expressing CD25 but are not immunosuppressive. Increased population of CD25+Foxp3- cells is present in mice where T_R cell ontogeny is disrupted by deletion
of connexin gene in double positive thymocytes. Increased proportion of this population in Cx43<sup>DC<sup>-</sup> mice indicates that there is impaired communication between T<sub>R</sub> and DC cells that affects T<sub>R</sub> cell stability in the peripheral tissues. Thus, transition of Foxp3<sup>+</sup> cells to Foxp3<sup>-</sup> CD25<sup>+</sup> cells could be the cause for decreased proportion of CD4<sup>+</sup> Foxp3<sup>+</sup> T<sub>R</sub> population in Cx43<sup>DC<sup>-</sup> mice. Cx43 expression by dendritic cells may be involved in triggering CD4<sup>+</sup> Foxp3<sup>+</sup> T cells that do not lose their Foxp3 expression.

**Fig. 12.** Prominent presence of CD4<sup>+</sup> Foxp3<sup>-</sup> CD25<sup>+</sup> cells in Cx43<sup>DC<sup>-</sup> mice. Age-matched WT and Cx43<sup>DC<sup>-</sup> have differing proportions of CD4<sup>+</sup> Foxp3<sup>-</sup> CD25<sup>+</sup> cells shown by flow cytometry at young (2 months) and old (12 months) age.
3.8. CD11c+ dendritic cells from Cx43\textsuperscript{DC-} mice can present antigen effectively

CD11c+ dendritic cells sorted from WT or Cx43\textsuperscript{DC-} mice were added to a well with CD4+ naïve T cells sorted from a mouse with transgenic TCR for a peptide PCC50V54A in a proliferation assay. After four days of incubation with the peptide, \textsuperscript{3}H-Thymidine was added. The incorporation of \textsuperscript{3}H-Thymidine was used to measure proliferation of CD4+ that were successfully activated by CD11c+ dendritic cells. Fig. 13 shows that CD4+ T cells activated by DCs from Cx43\textsuperscript{DC-} mice proliferate more vigorously than the same cells activated by wild type dendritic cells. This data shows that inhibition of communication between TR cells and DCs also affect DCs by making them more effective antigen presenting cells. This further suggests that TR cells modulate antigen presenting cells. Enhanced function of antigen presenting cells in Cx43\textsuperscript{DC-} mice likely affects organs which are exposed to contact with environment like skin and gut airways. Enhanced responses to antigen could be a factor that promotes the skin inflammation observed in Cx43\textsuperscript{DC-} mice. In the current assay, this could result in the enhanced proliferation of effector CD4+ T cells.

![Fig. 13. Proliferation assay results. Data from the proliferation assay with CD11c+ APCs from WT and Cx43\textsuperscript{DC-} mice with CD4+ T cells from transgenic mice specific for peptide PCC50V54A. \textsuperscript{3}H-Thymidine measurements show that CD11c+ DC from Cx43\textsuperscript{DC-} triggered greater proliferation.](image-url)
4.1. Cx43 expression by CD11c+ dendritic cells maintains CD4+ Foxp3+ cell population

Lack of Cx43 expression in CD11c+ dendritic cells alone resulted in decrease in population of CD4+ Foxp3+ T_R, and a possible formation of dermatitis on the dorsal skin of the mouse. These findings support that of Yamazaki et al. (2003) in that it confirms that CD11c+ dendritic cells maintain the T_R population (26). However, our data shows that in addition to the interactions between TCR on T_R cells and MHC on DCs are important for both cell populations and that the communications through gap junctions is most likely required for sustaining functions of both cell subsets.

CD25 is the IL-2 receptor required for differentiation into T_R cells. A prominent population of CD4+ Foxp3+ CD25+ cells were seen in the lymph nodes of Cx43^{DC-} mice. An explanation could be that the T_R cells that were formed in the periphery during activation lose their Foxp3 expression and may assume features of exT_R cells (47, 48). These cells are known to produce pro-inflammatory cytokines and promote autoimmune diseases. It is currently not known what promotes loss of Foxp3 and generation of exT_R cells. It is possible that the loss of Foxp3 by T_R cells, and thereby its immunosuppressive function, is the reason for the increase in proliferation seen in the proliferation assay where CD11c+ DC from Cx43^{DC-} mice presented PCC50V54A antigen to CD4+ naïve T cells. In case of T_R cells that interacted with the peptide + MHCII on CD11c+ DC that did not have Cx43 for proper signaling, T_R cells may not receive some, at present unknown, signal dependent on gap junction formation, leading to loss of Foxp3
expression. At the same time, Cx43 deficient DCs also loose T<sub>R</sub> cell mediated tonic signal which conditions these cells to be over-stimulatory. Overtime, lack of interactions between DCs and T<sub>R</sub> cells which involve Cx43 affects both populations altering their response to homeostatic interactions and antigenic stimulation.

We hypothesized that the CD4<sup>+</sup> Foxp3<sup>+</sup> T<sub>R</sub> cell from Cx43<sup>DC-</sup> mice were not able to suppress proliferation due to the changes in CD39 and CD73 expression which may offset the conversion of pro-inflammatory ATP to immunosuppressive adenosine. However, the results of the proliferation inhibition assay show that these T<sub>R</sub> cells suppressed the proliferation of activated CD4<sup>+</sup> T cells with only a slight decrease in immunosuppressive function. However, the proliferation inhibition assay used to examine the suppressor function of T<sub>R</sub> cells from Cx43<sup>DC-</sup> mice may not be the most suitable assay. This was the first assay used to probe T<sub>R</sub> cell function <i>in vitro</i> and there has been multiple examples when the short term suppressor function of T<sub>R</sub> cells is preserved <i>in vitro</i> but <i>in vivo</i> functions are impaired.

Taken together, our results show that lack of Cx43 expression by dendritic cells does not affect the ability of these cells to present antigen to CD4<sup>+</sup> naïve T cells and activate them. However, the CD4<sup>+</sup> Foxp3<sup>+</sup> population was significantly reduced. Knowing that dendritic cells maintain the T<sub>R</sub> cell population (26) and that Cx43 signaling enhances the generation of Foxp3<sup>+</sup> T<sub>R</sub> cells (39), we propose that Cx43 expression by CD11c<sup>+</sup> dendritic cells is required for maintaining CD4<sup>+</sup> Foxp3<sup>+</sup> T<sub>R</sub> cell population.
4.2. Future Research

It is possible that CD11c+ DC interact with an intermediary component that directly or indirectly maintain the TR cell population, but there is no evidence of such a component as of now. We were not able to test the antigen-specific activation of regulatory T cells by antigen presenting cells because TR cells with transgenic TCR do not make it through thymic selection. Thus, we were unable to run an in vitro test of aspects of the interaction between APC and antigen specific TR cells. The findings of a study like this would be of great importance to our proposal of the role of Cx43.

Cx43 is undeniably a part of the immunological synapse and this has been proposed by multiple research studies. The results of application of αCT1, a mimetic peptide of Cx43, to increase wound healing mentioned earlier is a big example of this association. Our results implicate gap junction protein, Cx43 in the communication between CD11c+ dendritic cells and CD4+ Foxp3+ TR cells. It is possible that Cx43 is used by CD4+ effector T cells or CD4+ Foxp3+ T cells to execute their immunological roles and this could be a topic of interest for future research. In addition, it is very likely that Cx43 is not the only connexin involved in maintaining regulatory T cells. Furthermore, looking into the Cx43 signaling pathway may shed some light onto the precise changes taking place at a molecular level that leads to maintenance of regulatory T cells.

Undeniably, understanding the cells that use or require Cx43 to directly or indirectly control auto-immune responses will open up pathways for targeted therapies that may correct one of the underlying factors of autoimmune diseases which is the low amount of CD4+ Foxp3+ TR at the site of immune response.
In the Cx43\textsuperscript{DC} mouse model, Connexin-43 molecule is not expressed by CD11c\textsuperscript{+} dendritic cells and a higher probability of developing dermatitis was seen in these mice. The low population of T\textsubscript{R} in these mice, support the notion that the skin disease may be autoimmune in nature. The ability of CD11c\textsuperscript{+} dendritic cells to present antigen to naïve T cells were not affected by the loss of Cx43. However, the drastic decrease in T\textsubscript{R} cell population and Foxp3 expression, combined with the altered CD39\textsuperscript{+} CD73\textsuperscript{+} phenotype, indicate that Cx43 is an important player in the communication between antigen presenting cells and the T\textsubscript{R} cell subset. These results also suggest that TCR/MHC interaction along with defined co-stimulatory processes are not enough in itself, but require Cx43 expression by CD11c dendritic cells, to maintain immunosuppressive CD4\textsuperscript{+} Foxp3\textsuperscript{+} T\textsubscript{R} cell population in the peripheral lymph nodes.


46. E. Mazzini, L. Massimiliano, G. Penna, M. Rescigno, Oral tolerance can be established via gap junction transfer of fed antigens from CX3CR1⁺ macrophages to CD103⁺ dendritic cells. *Immunity* 40, 248-261 (2014).


APPENDIX

REAGENTS

0.16M Ammonium Chloride
\( \text{NH}_4\text{Cl}: 8.3\text{g, KH}_3\text{CO}: 1\text{g, MiliQ added up to 1L with pH at 7.2.} \)

Antibody Stains (eBioscience, BD Bioscience, BioLegend)

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<td>CD73</td>
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<td>4-1BB</td>
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<td>KlrG1</td>
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<td>Ter119</td>
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<td>200mM</td>
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BSS
\( \text{NaCl 8g, KCl 400mg, CaCl}_2 140mg, \text{ MgSO}_4.7\text{H}_2\text{O 100mg, MgCl}_2.6\text{H}_2\text{O 100mg, Na}_2\text{HPO}_4.2\text{H}_2\text{O 60mg, KH}_2\text{PO}_4 60mg, Glucose 1g, NaHCO}_3 350mg, MiliQ added up to 1L. \)

Collagenase IV (Worthington)

Complete Freund’s Adjuvant (CFA) (Sigma-Aldrich)
Mixed with 1x PBS at 1:1 before administration

Complete Tumor Medium (CTM)
\( \alpha\text{MEM 500mL, “tumor cocktail” 50mL, FBS 50mL, L-glutamine (200mM) 5mL.} \)
Tumor cocktail- \( \alpha\text{MEM 630mL, dextrose 7.5g, essential amino acids 50x (Cellgro) 200mL, endogenic amino acids 100x (Cellgro) 100mL, Sodium pyruvate 100x (Cellgro) 100mL, adjust pH to 7.0, NaHCO}_3 8.5g, Gentamycin (Gemini Bioproducts) 0.5g, Penicillin G 0.6g, Streptomycin 1g, }\beta-\text{mercaptoethanol 34uL.} \)

Dispase I (Sigma-Aldrich)
DNase (Sigma-Aldrich)
FBS (Atlanta Biologicals)
Fetal Bovine Serum

1X PBS (Cellgro)
Dulbecco’s Phosphate Buffered Saline without calcium and magnesium

PE Magnetic beads (Miltenyi Biotec)

RPMI (HyClone)
Roswell Park Memorial Institute growth medium 1640 with L-Glutamine

Streptavidin Magnetic beads (BD Bioscience)
VITA

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EDUCATION
M.S. Biology  
Old Dominion University  
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2019

B. Tech. Biotechnology (Molecular and Cellular Engineering)  
Sam Higginbottom University of Agriculture, Technology and Sciences  
Allahabad, India  
2012

Certified Training Modules (2 month programs)
Online: Introduction to Genomic Technologies  
Johns Hopkins University, MA, USA  
2016

Construction of Reporter Vectors- Wnt5a-EGFP and WNT5a-d2EGFP  
Rajiv Gandhi Centre for Biotechnology, Kerala India  
2012

Plant Tissue Culture  
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Sangenomics Research Labs, Bengaluru, Karnataka, India  
DNA Forensics  
2010

Bioaxis DNA Research Centre, Secunderabad, Andhra Pradesh, India

WORK EXPERIENCE
Graduate Teaching Assistant- Instructor (General Biology Lab I & II)  
Old Dominion University, Virginia, USA  
2017-2019

Laboratory Supervisor  
Al Hekma International School, Kingdom of Bahrain  
2014-2015

Laboratory Demonstrator/ Research Assistant (FY Medical)  
Royal College of Surgeons, Ireland- Medical University of Bahrain (RCSI-MUB)  
Kingdom of Bahrain  
2012-2013

PUBLICATIONS

PRESENTATIONS
“Expression of Connexin-43 by CD11c+ Dendritic Cells is Required for Maintaining CD4+ Foxp3+ Regulatory T Cell Population.” (Poster)  
Old Dominion University’s Graduate Research Achievement Day 2019

“Connexin-43 expression by CD11c+ dendritic cells is required for maintaining CD4+ Foxp3+ regulatory T cell population” (Oral)  
Virginia Academy of Sciences 2019