THE INVESTIGATION OF THERAPEUTIC POTENTIAL OF BORON DERIVATIVES AND MESENCHYMAL STEM CELL LYSATE ON HOUSE DUST MITE-INDUCED ASTHMA IN MICE

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ABSTRACT

THE INVESTIGATION OF THERAPEUTIC POTENTIAL OF BORON DERIVATIVES AND MESENCHYMAL STEM CELL LYSATE ON HOUSE DUST MITE-INDUCED ASTHMA IN MICE

Asthma is a respiratory disease and affects more than 350 million people worldwide, especially in developed countries. It is characterized with airway hyperresponsiveness (AHR), airway inflammation and remodeling. Asthma brings a serious economic burden on countries because of the high treatment costs and loss of labor. Approximately 50-70 percent of asthma patients have a T helper 2 (Th2) immunity which is characterized by type 2 inflammation. Allergic asthma is associated with sensitization to allergen such as pollen, house dust mite (HDM), and pets. Different treatment methods are used to reduce or control the symptoms, but no definitive treatment for asthma has been found, yet. Some of these treatments are corticosteroids, beta-2 agonist drugs, omalizumab which is anti-IgE based therapy, anti-IL-5 based therapy with mepolizuman, reslizumab and benralizumab, and mesenchymal stem cell (MSC)-based immune modulation. However, definitive treatment is needed. In this thesis, the therapeutic effects of boron derivatives such as sodium pentaborate pentahydrate (SPP), sodium perborate tetrahydrate (SPT), and MSC lysate on HDM-induced allergic asthma model were investigated. To determine the therapeutic effects of these agents on 6-8 weeks female BALB/c mice, these agents were delivered on day 12 and 14 intranasally ensuing initiation and sensitization phase using 50 μg/day HDM during the first four days. A challenge phase was initiated by the re-administration of HDM between days 15-18 days. Lung and the mediastinal lymph node (mLN) were harvested and blood samples were collected from all experimental groups on day 19. Lung histopathology, serum IgE levels and immune cell numbers and percentages of total dendritic cells (DC), $CD11b^+$ DCs, $CD103^+$ DCs, alveolar macrophages, T lymphocytes, and neutrophils were determined using hematoxylin eosin staining, ELISA, flow cytometry, respectively. We found that SPP has a therapeutic potential for asthma treatment. It decreased the number of lung infiltrating immune cells and serum IgE levels. However, neither SPT nor MSC lysate introduced any changes on the pathology or immunology of asthma in a therapeutical sense. In a conclusion, we showed for first time in the literature, intranasal delivery of SPP has a therapeutic potential of asthma.

ÖZET

BOR TÜREVLERİ VE MEZENKİMAL KÖK HÜCRE LİZATININ EV TOZU AKARI-ASTIM MODELİ ÜZERİNDEKİ TERAPÖTİK POTANSİYELİNİN İNCELENMESİ

Astım, dünya genelinde 350 milyondan fazla insanı etkileyen ve özellikle de gelişmiş ülkelerde görülen bir solunum yolu hastalığıdır. Hava yolu aşırı duyarlılığı, hava yolu iltihabı ve yeniden şekillenme ile karakterizedir. Astım tedavi maliyetlerinin yüksek olması ve iş gücü kayıplarına neden olmasından dolayı ülkelere ciddi bir ekonomik yük olmaktadır. Astım hastalarının yaklaşık yüzde 50-70'i T yardımcı tip 2 bağışıklığına sahiptir ve tip 2 inflamasyon ile karakterizedir. Alerjik astım; polen, ev tozu akarı (HDM; Dematophagoides pteroyssinus) ve evcil hayvanlar gibi alerjenlere karşı duyarlılık ile ilişkilidir. Günümüzde astımın kesin tedavisi henüz bulunamamıştır ama semptomları azaltmak veya kontrol altına almak için farklı tedavi yöntemleri uygulanmaktadır. Uygulanan tedavi yöntemlerinden bazıları kortikosteroidler, beta-2 agonist ilaçlar, anti-IgE tedavisi olan omalizumab, mepolizuman ile anti-IL-5 tedavisi, reslizumab ve benralizumab ve mezenkimal kök hücre (MKH) immünmodülasyonudur. Fakat günümüzde astım hastalığı için kesin çözümlü tedavilere ihtiyaç duyulmaktadır. Bu yüzden, bu tezde bor türevlerinden olan sodyum pentaborat pentahidrat (SPP) ve sodyum perborat tetrahidrat (SPT) ve MKH lizatının ev tozu akarı ile oluşturulmuş alerjik astım modeli üzerindeki terapötik etkileri araştırıldı. Bu ajanların 6-8 haftalık dişi BALB/c fareler üzerindeki terapötik etkilerini belirlemek için, 50 µg/gün HDM ilk 4 gün boyunca duyarlılaşma fazı olarak, terapötik ajanlar 12. ve 14. günlerde ve 15-18 günleri arasında 50 µg/gün HDM intranazal yolla verildi. Kullanılan ajanların terapötik etkilerini belirlemek için 19. günde akciğerleri ve mediastinal lenf nodları toplandı ve her gruptan kan alımı yapıldı. Akciğer histopatolojisi, serum IgE seviyesi, toplam dendritik hücre (DH), $CD11b^+$ DH, $CD103^+$ DH, alveolar makrofajlar, T lenfositler ve nötrofil immün hücreleri sırasıyla hematoksilen eozin boyama, ELISA ve akış sitometrisi ile belirlendi. Elde edilen sonuçlarla, SPP'nin astım tedavisi için terapötik bir potansiyele sahip olduğunu bulundu. SPP ile tedavi edilen grupta; akciğer infiltrasyonu, immün hücre sayısını ve serum IgE seviyesi azaldı. SPT ve MKH lizatı ile tedavi edilmiş gruplar, astımdan kaynaklı patolojik ve immünolojik parametreleri terapötik olarak değiştirmedi. Bu çalışma ile literatürde ilk kez SPP'nin intranazal verilmesinin astım için terapötik bir potansiyele sahip olduğunu gösterilmiştir.

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LIST OF SYMBOLS/ABBREVIATIONS

AAD	Allergic airway disease
AHR	Airway hyperresponsiveness
APC	Antigen presenting cell
ASM	Airway smooth muscle
BAL	Bronchoalveolar lavage
BM	Bone marrow
BSA	Bovine serum albumin
c-AMP	Cyclic adenosine monophosphat
cDC	Conventional dendritic cells
CF	Cystic fibrosis
COPD	chronic obstructive pulmonary disease
DAMP	Damage-associated molecular patterns
DC	Dendritic cell
DMEM	Dulbecco's modified eagle's medium
EAR	Early asthmatic response
EC	Epithelial cells
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbent assay
FcεRI	High affinity Fc receptor
FeNO	Fractional excretion of nitric oxide
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GC	Glucocorticolds
GM-CSF	Granulocyte macrophage colony stimulating factor
H&E	Hematoxylin and eosin
HDM	House dust mite
HSC	Hematopoietic stem cells
I.N.	Intranasal
ICS	Inhaled corticostreoids
IDO	Indoleamine 2,3-dioxygenase
IFN-α	Interferon-α

IFN-γ	Interferon-γ
IgE	Immunoglobulin E
IHC	Immunohistochemistry
IL	Interleukin
IL-1α	Interferon-α
IL-1β	Interferon- β
IL-3	Interleukin-3
IL-4	Interleukin-4
IL-5	Interleukin-5
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
IL-12	Interleukin-12
IL-13	Interleukin-13
IL-17	Interleukin-17
IL-18	Interleukin 18
IL-22	Interleukin 22
IL-25	Interleukin 25
IL-33	Interleukin 33
ILC2	Innate lymphoid cell types 2
ISAAC	International study for asthma and allergies in childhood
iPS	Induced pluripotent stem
iTreg	Induced regulatory T cells
LPS	Lipopolysaccharide
LTC4	Leukotriene C4
MCs	Mast cells
MHCII	Major histocompatibility complex II
mLN	Mesenteric lymph nodes
moDC	Monocyte derived dendritic cells
MSC	Mesenchmymal stem cells
NFkB	Nuclear factor kappa B
NO	Nitric Oxide
NOS	Nitric oxide synthase

nTreg	Naturally regulatory T cells
PAMP	Pathogen associated molecular pattern
PAS	Periodic acid schiff
PBS	Phosphate buffer saline
pDC	Plasmacytoid dendritic cells
PGD2	Prostagladin D2
PGE2	Prostagladin E2
PRR	Pattern recognition receptors
RA	Retionoic acid
RBC	Red blood cell
ROS	Reactive oxygen species
RT	Room temperature
SC	Stem cell
SCF	Stem cell factor
SPP	Sodium pentaborate pentahydrate
SPT	Sodium perborate tetrahydrate
TCR	T cell receptor
TF	Tissue factor
Tfh	T follicular helper cells
TGF-β	Transforming growth factor- β
Th	T helper
Th-1	T-helper 1
Th-2	T-helper 2
Th-17	T-helper 17
TLR	Toll like receptor
TLR-4	Toll like receptor 4
TNF-α	Tumor necrosis factor α
Treg	Regulatory T cells
TSLP	Thymic stromal lymphopoietin

1. INTRODUCTION

1.1. ASTHMA

Asthma is a respiratory disease, which affects more than 350 million lives worldwide predominantly [1]. Asthma places a serious economic burden on countries with the high cost of treatment and the loss of labor. It involves thickening of airway walls and over production of mucus in airway passages in response to an allergen. As a result, a process known as atopy, which is the tendency of producing allergen specific immunoglobulin E (IgE) in response to an allergen exposure, initiates. Atopy is related to the advancement of allergic diseases, including food allergies, allergic rhinitis, atopic dermatitis, allergic asthma, and eczema. These atopy-related conditions are mostly encountered in genetically susceptible individuals [2]. There is no definitive treatment for asthma, only different treatment methods are used to reduce symptoms [3]. Approximately 50-70 percent of asthma patients have T helper 2 (Th2) immunity in asthma pathogenesis and dendritic cells (DCs) are in control of this immunity. The other type of immune cells, especially mast cells (MCs), eosinophils and T lymphocytes, play a role in this disease. The body exposed to the allergen produces allergen-specific IgE under the action of B lymphocytes, promoting elevated secretion of the interleukin-4 (IL-4), interleukin-5 (IL-5) and interleukin-13 (IL-13) cytokines by Th2. Activation of eosinophils and MCs increase airway hypersensitivity. Therefore, basal membrane thickness increases with increased airway inflammation, yielding tissue damage. In other words, a drastic airway remodeling takes places in the lung, resulting in coughing, shortness of breath, wheezing, and a feeling of tightness in the chest [4].

1.1.1. Asthma Epidemiology

Asthma is a common respiratory condition and affects millions of people around the world [5]. It causes 250,000-345,700 deaths each year in worldwide [6]. The prevalence of asthma varies significantly between the countries and their regions. Also, asthma symptoms and limitation of airflow seen are variable among the individual and patients.

Three different surveys are being used to identify childhood asthma epidemiology. These include International Study for Asthma and Allergies in Childhood (ISAAC) survey,

American Thoracic Society survey, and Aberg survey. In the United States, 20.1 million people suffer from asthma 6.3 millions being children. According to results of the survey, prevalence of asthma found ranges between percent of 2-15 ages in children. In the study of Huang et al., asthma prevalence is reported as 10.2 percent among preschool children in Shanghai, China [7]. According to another study, prevalence of life long cumulative asthma is reported as 13.7 percent in Istanbul, 8.1 percent in Ankara, Turkey, 9.4 percent in Antalya, Turkey and 2.1 percent in Gaziantep, Turkey amongst school children's parents [8–11].

1.1.2. Asthma Pathophysiology

Most asthmatic patients have persistent airway inflammation. Allergy is observed in 80 percent asthmatic children and most adults with asthma. It is characterized with type 2 inflammation [12–14]. Asthma pathogenesis can be consisted of four different fields that are airway inflammation of Th2 ^{high} and Th2 ^{low}, airway remodeling and airway hyperresponsiveness (AHR). The respiratory epithelium is known to play an important role both in initiating an adaptive immune response and in forming a first-line defence barrier that sensitizes the individual to the environmental stimuli such as infectious agents, pollutants, oxidants, and allergens, including pollen, fungi, particularly pets, and house dust mites (HDM) [12,14,15]. Allergen sensitivity depends on the processing of the allergen via antigen presenting cells (APC) such as DCs. Then, peptides can be presented by major histocompatibility complex II (MHC II) to the naïve T lymphocytes in the lymph nodes [16]. Interleukin-3 (IL3), IL4, IL5, and IL13 are cytokines that are secreted by the effector Th2 cells [14]. Also, naïve T cells can differentiate into other type of T lymphocytes which are also known as T follicular helper cells (Tfh) and these cells can migrate to the follicles of B lymphocytes in order to regulate immunoglobulin E (IgE) class switching from immunoglobulin M (IgM) and production of B lymphocytes [17]. Thanks to IgE antibodies, which are generated as a result of local MC activation via their binding to high affinity Fc receptor (FcεRI), allergens or stimuli can be recognized. Degranulation of MCs, which is IgE dependent, involves potent release of chemokines such as histamine, cysteinyl leukotrienes, and prostaglandins. Release of these chemokines promote bronchoconstriction and airway edema. Often times, these diseases lead to early asthmatic response (EAR) or early bronchoconstriction response in the later stages. Besides, some Th2 cells can migrate to the site of allergen exposure, thereby increase the existing inflamatory response to

generate a so called are type 2 inflammation, airway remodeling, and eosinophil recruitment under the action of secreted of cytokines. Migration of Th2 cells towards the allergen exposure sites in the airway mucosa depends on CCR4 ligands which are released by activated DCs [18].

1.1.3. Allergic Airway Remodeling

In asthma, the airway wall becomes thick depending on the severity and duration of the disease [19,20]. Because of the remodeling, the thickness of the airway smooth muscle (ASM) wall increases, sub epithelial reticular lamina becomes thick, matrix deposition concurrent with an increase in mucus production during the airway wall thickening. As seen in Figure 1.1., the data shows that histological lung tissue sections that are stained with hematoxylin eosin are taken from healthy patient as control and patient with asthma disease. Letters of a and c show non-asthmatic patient's lung sections that have epithelial integrity, normal basement membrane. Letters of b and d show asthmatic patients' lung sections that have loss of epithelial integrity, increasing of basement membrane density and amount of ASM contrast to healthy airways [23,24]. These changes lead to AHR, characteristic of which features asthma, involving increased sensitivity of the airways that become excessively narrow. Besides, mucus is created acting as a plug which promote air trapping and air hyperinflations in small airways [21,22].

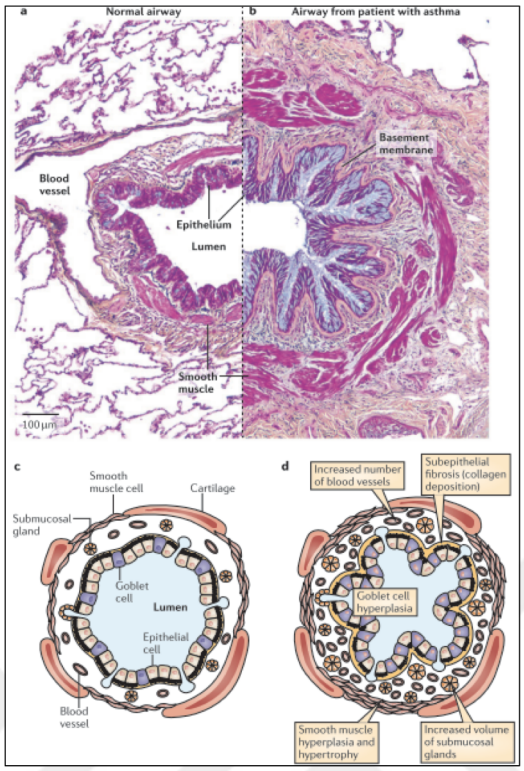

Figure 1.1. Histopathological feature of AAR

1.1.4. Asthma Phenotypes

There are five groups for classification of asthma phenotypes. These are early and late onset disease, obesity related, exercise-induced, and neutrophilic asthma. According to the National Institutes of Health-sponsored Severe Asthma Research Program (SARP) research, the first group of asthma phenotype is early onset asthma is related to atopy and allergic diseases [25,26]. Late onset disease that permanent eosinophilic asthma which are higher amount of eosinophils found into the sputum and peripheral blood [27]. It is less allergic than first group. Obesity, that is usually seen in adults, is also related to asthma phenotypes as controlling and severity for disease. Expression of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) increase in obesity [28]. Its non-atopic and eosinophilic inflammation cannot be observed in this phenotype. Neutrophilic asthma phenotype has increased air trapping, worsened lung functions and a thicker airway [29]. Exercise-induced asthma (EIA) has not been defined with genetic factors or some biomarkers. Secretion of histamine and prostaglandins are important for EIA [30].

1.1.5. Asthma Endotypes

Asthma can be divided into two different endotypes. One endotype involves type-2 inflammations which are related to allergy, conversely, the other which involves non type-2 inflammations that are not related to allergy. Allergic inflammation can be detected by the presence of high IgE, Th2 cytokines, low or high fractional excretion of nitric oxide (FeNO) (exhaled breath of nitroxide), and eosinophilic inflammations. Furthermore, an allergic inflammation can respond to glucocorticoids (GC). On the other hand, non-allergic asthma presents with neutrophilic inflammation and it can be detected with high levels of IL-6 and IL-17 and oxidative stress. Strikingly, the second endotype cannot respond to GC like as allergic phenotype [12,31,32]

1.1.6. Asthma Treatments

In general, asthma treatments are developed to minimize day and night symptoms and enable normal daily activities with the least side effects. There are two main types of pharmacological interventions. One of them is based on bronchodilators which aim at removal of the symptoms. For example, β2 agonists and anticholinergics are used as bronchodilators. β2 agonists are used in the management of crisis as a symptom reducing agent in all stages of asthma which can be administered intravenously, nebulously, orally, or using inhalers. They can activate adenyl cyclase, thereby cyclic adenosine monophosphate (c-AMP) is increased [33,34]. For example, ipratropium bromide, which is an anticholinergic drug, can mostly be used in an inhaler form. All anticholinergics can block muscarinic receptors of M2 and M3 that are present in the bronchial mucosa.

Second treatment type depends on anti-inflammatory approaches which use corticosteroids, leukotriene receptor antagonists, and long acting β2 agonists. These reagents can be used every day and for a long time periods to keep asthma symptoms under control. There are two types corticosteroids and the most effective type consist of inhaled corticosteroids (ICS) for relieving inflammation. They cross the cell membrane and interact with DNA by binding to receptor of cytoplasmic glucocorticoid. Thereby, they can reduce asthma symptoms, mucus secretion, and both frequency and severity of asthma attacks as well as regulation of nitric oxide (NO) release into epithelial cells. Also, ICS suppress the production of

chemotactic mediators and prevent the access of inflammatory cells into the airways [35–38]. Leukotrienes have a role in pathogenesis of asthma and promote an increase in edema, mucus secretion, and eosinophilic inflammation. Leukotriene antagonists show their effects by blocking cysteinyl leukotriene receptors [39,40]. Long acting β2 agonists do not affect airway inflammation and, therefore, they are used in combination with inhaled steroids in order to provide better control of asthma symptoms [41].

Also immunotherapies which are anti-IgE, anti-IL-5 and anti-IL-13, and/or anti-IL-14 therapy have been developed [42]. Omalizumab is used as an anti-IgE therapy modality. It can sequester free IgE antibodies and, thereby, blocks IgE binding to the FcεRI receptors that are present on the periphery of MCs and basophils. Hence, these cells become blunted to the stimulation by allergens [43,44]. Mepolizumab, Reslizumab, and Benralizumab are used in anti-IL-5 therapy-based approaches. These biological drugs target IL-5 signaling and, thereby, decrease number of eosinophils in blood and sputum [45,46]. In addition to these therapy modalities, immunomodulation capacity of MSCs has been tapped into as an innovative treatment approach for asthma. Due to their migratory properties, they can access to asthmatic niche and regulate the ratio of Th2 to Th1.

1.2. INFLAMMATORY CELLS IN ASTHMA

During the inflammatory phase, MCs, eosinophils, T lymphocytes, and neutrophils that are locally present in the structure of airway, as well as macrophages and DCs that extravasate from the circulation to the airways to the site of inflammation play crucial roles when asthmatic patients become symptomatic [12,14].

1.2.1. Mast Cells

Mast cells (MCs) are granular cells located in connective tissues. They differentiate from hematopoietic stem cells (HSC) in the bone marrow (BM) and circulate in the blood around the body as precursor cells. They migrate to some parts of the body such as lung and mucosa of the intestines to differentiate and mature. MCs are connected to extracellular matrix through matrix components such as fibronectin, vitronectin, and laminin that are ligands to different integrins [47]. Because of these interactions a signaling cascade involving post-

translation of modification such as protein phosphorylation trigger both release of histamine and cell motility.

Activation of MCs occurs in conjunction with the binding of an allergen to FcεRI receptors found in the MCs surface. This binding initiates the production and secretion of asthma related mediators and cytokines such as histamine, leukotriene C4 (LTC4), and prostaglandin D2 (PGD2) upon activation by osmotic stimuli and allergens [48,49]. These mediators play a role in the mucus secretion, regulation of bronchoconstriction, and vascular changes which lead to mucosal edema. In addition, MCs can increase IgE synthesis, promote ASM hyperresponsiveness to IL-4 and IL-13 cytokines and attract eosinophils in the airways with IL-5 cytokines [50]. MCs contribute to the remodeling of airways through secretion of proteases such as tryptase and chymase, transforming growth factor- β (TGF- β), and TNF-α which regulates AHR. According to the recent studies, there is a strong correlation between asthmatic airways and TNF-α. Thus, AHR is also associated with increasing MCs [50,51].

1.2.2. Regulatory T Lymphocytes

Homeostasis in the lung is maintained by cells located in the lung that are balanced by inflammatory and modulatory cytokines and a subset of regulatory T lymphocytes (Tregs) [52,53]. These homeostatic mechanisms must distinguish harmless antigens from pathogens. Tregs have a crucial role in the maintenance of immunological tolerance and lung homeostasis. They also have an important role in controlling inflammatory responses through the regulation of Th1, Th2, and Th17 effector function [54]. There is a constitutive T lymphocyte population, so-called Th cells, both in mouse and humans, that constitute approximately 5-10 percent of peripheral T cells [55]. Tregs are generated via three different pathways, including their differentiation from naturally occurring Tregs (nTreg), derivation from the thymus, and induced Treg (iTreg).

1.2.3. Helper T Lymphocytes

Helper T cells which is also known as $CD4^+$ T lymphocytes play an important role in adaptive immune system cell regulation by responding to peptide antigens. Cell activation signals can be initiated as a result of the binding of the class MHC II molecule which is

presented by APC to the TCR of the Th lymphocyte. Upon, the activation of naïve T lymphocytes, transcription factors are activated in order to produce cytokines inside the cell. These produced cytokines can stimulate the proliferation and differentiation of other Th cells. Phenotype of Th lymphocytes can be determined by the identification of the secreted cytokines. CD 4^+ T lymphocytes, which have a role in initiation and maintenance of the allergic response, can be classified into three groups. The first group of cytokines, which include IFN-γ, IL-2, and IL-12, can be produced by the Th1 lymphocytes. Cytokines of IL-4, IL-5, and IL-13 can be secreted by Th2 lymphocytes, while IL-17A, IL-17F, and interleukin 22 (IL-22) are produced by Th17 lymphocytes.

Th1 cytokines are dominant in non-allergic individuals. After allergen immunotherapy is administered in allergic individuals, the amount of cytokines were observed to shift from Th2 to Th1 [56]. In the study of the biopsy and bronchoalveolar lavage (BAL) fluid of asthma patients, amount of IL-4 and IL-5 cytokines were increased, but no changes were observed in other T lymphocytes, eosinophils, and MCs. This shows that Th2 response plays a crucial role in the pathology of allergic asthma.

Th2 lymphocytes are responsible for humoral immune responses and allergic reaction [57]. Th2 can induce a class switching of B lymphocytes to produce IgE. As previously stated, these cytokines secrete IL-4, which regulate the production of IgE antibody, IL-5,which induce differentiation into eosinophils, and IL-13, which stimulate mucus secretion. The two main endotypes of asthma are distinguished based on the abundance of Th2 cells, as Th2 ^{high} and Th2 ^{low} asthma. In Th2 ^{high} asthma, amount of eosinophils released into the sputum increase. Whereas, Th2 ^{low} asthma is not related to eosinophil inflammation, it is related to neutrophil or paucigranulocyte [29].

Th17 lymphocytes were defined as a different subgroup of Th lymphocytes. These cells have an important role in mucosal defense and in the pathogenesis of autoimmune diseases. Th17 lymphocytes can secrete interleukin 17 (IL-17), especially IL-17A and IL-17F, and IL-22. According to the recent studies, Th17 lymphocytes also play a role in asthma [58–61]. IL-17A and IL-17F increase the production of NO and prostaglandin E2 (PGE2). They also regulate neutrophil homeostasis and induce their aggregation in tissue [61]. They can stimulate tissue cells and leukocytes to secrete cytokines which is important for the recruitment of neutrophils and monocytes to the site of infection [62]. Th17 lymphocytes promote neutrophilic inflammation as well as AHR with the help of Th2 lymphocytes.

Therefore, they are associated with neutrophilic asthma [63]. According to a recent study, Th17 lymphocytes increased in the peripheral blood of asthmatic patients and this increase was proportional to the severity of asthma [64].

1.2.3.1. *Th2 ^{low} Asthma*

Another form of asthma is also known as non-Th2 asthma. Two different subtypes of Th2 ^{low} asthma endotype are classified as neutrophilic and paucigranulocytic inflammation (Figure 1.2.). Instead of expressing type 2 cytokines, Th2 and Th17 cell dominance is observed in neutrophilic inflammation [14,65]. Thus, production of interleukin 8 (IL-8) increases. This inflammation is related with refractory asthma. Paucigranulocytic inflammation has eosinophils and neutrophils normally present in sputum without IL-8 or type 2 cytokines. To date, this type of inflammation have no reliable biomarkers [66].

1.2.3.2. *Th2 ^{high} Asthma*

Th2 ^{high} inflammation is crucial for allergic disease. It may develop into an atopic disease when the number of eosinophil cells in the sputum and airway increase. DCs constitute a bridge between the innate and adaptive immune responses. DCs, which have costimulatory molecules, promote stimulation of local cytokines which lead to differentiation of uncommitted naïve T cells to Th2 cells. Induced airway epithelial cells secrete to innate cytokines which interleukin 33 (IL-33), interleukin 25 (IL-25), and thymic stromal lymphopoietin (TLSP) to initial activation of Th2 cells and innate lymphoid cell types 2 (ILC2). Transcription factor of GATA-3, which is important for the development of Th2 and ILC2 cells, is known as a master regulator of this inflammation. Th2 cells can produce type 2 cytokines such as IL-4, IL-5, and IL-13. Furthermore, ILC2s can be an alternative to produce IL-5 and IL-13. When type 2 cytokines accumulate continuously, eosinophils, basophils, and MCs can be stimulated. All of these cytokines lead to mucous cell hyperplasia and fibrosis that aggravate the airway remodeling. Cytokines IL-4 and IL-13 can help to class switching of B cells for secreting IgE. Besides, cytokines of IL-5 support the survival of eosinophils and chemotaxis into blood vessels to the airways. That being said, type 2 inflammation inhibitors and glucocorticoids are effective in the control of Th2^{high} asthma [24,67]. Biological mechanisms and pathological features contrast between Th2 ^{high} asthma which is allergic and non-allergic eosinophilic inflammation, Th2 ^{low} asthma which is pauci-granulocytic and Th1/Th17 neutrophilic inflammation and healthy group. Five different

inflammations which are allergic eosinophilic inflammation, non-allergic eosinophilic inflammation, mixed granulocytic asthma, T1/T17 neutrophilic inflammation and pauci-granulocytic asthma shown in the Figure 1.2. depends on two main types of Th2 endotypes or health [68].

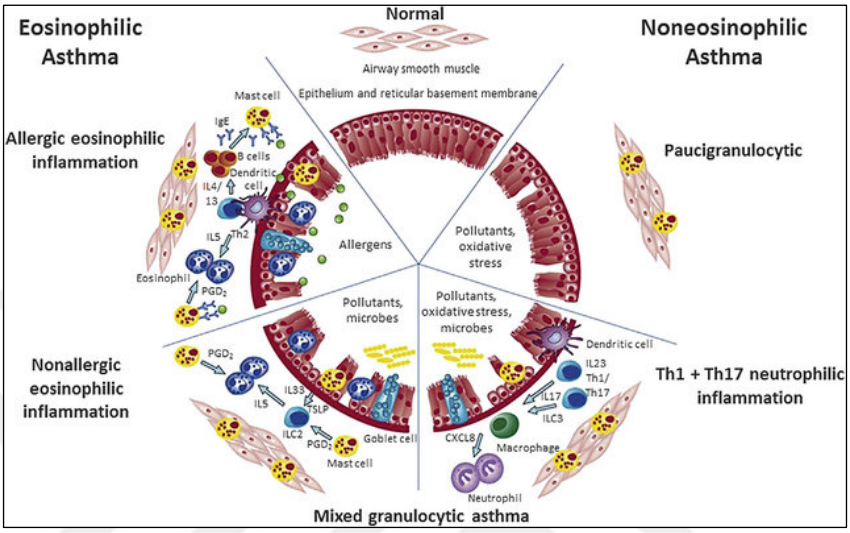

Figure 1.2. Asthma immunopathology [68]

1.2.4. Cytotoxic T Lymphocytes

Cytotoxic T lymphocytes (CTLs) are also known as $CD8^+$ T lymphocytes. CTLs induce apoptosis of infected cells and tumor cells. There is an immunological synapse structure between CTLs and the target cells. CTLs have specialized granules, which consist of effector proteins such as perforin and granzyme. They can release these granules into the environment when they come in contact with the impacted cell directly for destruction. Perforin makes pores on the target cell membrane and assists to passage of granzymes through the cell membrane. They also produce some cytokines such as IFN-γ, TNF-α, and IL-2. CTLs can recognize peptide antigens, which are presented by the class MHC I molecule [69,70].

1.2.5. Alveolar Macrophages

Alveolar macrophages (AMs), which are dominant immune effector cells that are located in the alveolar spaces and near to alveolar epithelial cells, are responsible for the activation of

inflammatory responses. AMs are characterized as CD11c^{high}, CD103^{low}, Ly6C ^{low}, MHCII^{low}, Siglec F^{high}, and CD11b^{low} [71–73]. AMs comprise of approximately 95 percent of the lung immune cells in homeostasis, therefore, they play a crucial role in maintaining homeostasis and host defense in the lung [74]. They are naturally suppressive and they eliminate pathogens while ignore harmless material. AMs express receptors for IL-10 and TGF- β that are secreted from alveolar epithelium for the maintenance lung homeostasis [75]. AMs also secrete proinflammatory cytokines such as macrophage inflammatory protein 1-α, TNF-α, granulocyte macrophage colony stimulating factor (GM-CSF), and CXCL-8, chemokines such as eotaxin and CCL5, prostaglandins, and LTB4. These secreted mediators play a role in the initiation and maintenance of asthma-related inflammation. AMs execute a function as an APC. In their manuscript Peter et al. propose AMs as forgotten cells of asthma. Although, DC and MCs have been studied heavily in asthma research, effect of AMs in this disease has been somewhat neglected despite their recently highlighted role in the pathology [76]. Lauzon-Joset et al. showed that a decrease in the number of AMs is associated with an increase in the activation of DCs in terms of their APC functions. That study claims that AMs may function in the regulation of DCs in triggering an allergic response. However, OVA model of AAD, which does not interact with inhaled allergens for humans, was used in this study and this model can be used in murine models [77].

1.2.6. Eosinophils

Eosinophils are the type of granulocytes that are formed in the BM. It is a hallmark of classical type 2 inflammation in this disease. Although, eosinophils have usually been proven to play an important role in maintaining immunity against helminthic parasites, recent studies, where phenotype of the eosinophil null mice suggested a more complex role for these cells than previously thought. Intriguingly, in another study, absence of eosinophil was associated with inhibition of parasitic growth [78]. In fact, more recent studies show that these cells have an important role in protection against viral infections, especially in the case of respiratory inflammations [79].

Lung and upper airways have a few eosinophils, however, significant increases in their numbers are observed during allergic inflammation. They migrate with other inflammatory cells and contribute to tissue injury, bronchoconstriction, and mucus secretion into the

airways. Inflammatory processes underlying allergic responses are managed differently in the sense that an elaborate network of cytokines and chemokines, which regulate IgE secretion and BM progenitor cell differentiation, controls the onset and progression of an asthmatic attack.

IL-5 and eotaxin proteins are the main regulators of eosinophil activation and recruitment [80]. These cells are a source of cationic granule proteins, which induce microvascular transmissivity and support inflammatory responses. Furthermore, due to being rich in cationic granule proteins and cysteinyl leukotrienes, they can induce bronchospasms. They can procure production of interleukin 18 (IL-18) and IL-25 which activate Th2 cells and due to this cells, releasing of thymic stromal lymphopoietin (TSLP) induce in allergen models. They have been acknowledged as origin of Th2 cytokines, which are IL-4, IL-5, and IL-13.

1.2.7. Neutrophils

Neutrophils have an important role in the innate immune system as pro-inflammatory cells for asthma. Neutrophilic inflammation is the another promoting factor of airway remodeling and tissue destruction [81]. They secrete antimicrobial and destructive enzyme and proteases. The phagocytotic capacity of neutrophils is limited, nevertheless they can phagocytose microorganisms in circulation and extravasate. They can survive in tissues for only a few hours. In other words, they can only respond to inflammation in the early phase, but cannot provide long term defense [62]. Neutrophils have two functions that are immunomodulatory and antimicrobial. During the early stages of asthma, they secrete several inflammatory mediators after airway infiltration [82]. Cells migrate from the circulatory system to the airways and their migration depends on chemoattractive signals. In the context of asthma, level of neutrophil chemotactic activity is increased compared to that seen in the cases of allergic rhinitis or healthy cells. This phenomenon is demonstrated with the increasing plasma levels of IL-8 in asthma patients [83].

Neutrophils can kill microbes by pumping up the levels of reactive oxygen species (ROS) as an oxidative mode of destruction or using of anti-microbial peptides and proteases as non-oxidative measure. They are a rich source of serine proteases and source of reactive nitrogen species, derived from NO, that is formed by nitric oxide synthase (NOS) on L-arginine [84,85]. Granulocytes may release ROS due to a stimulation by plant-derived stimulants or

immunoglobulins such as IgE by lipopolysaccharide (LPS) of bacterial origin. If their improvement and clearance encounter a problem, significant damage will occur at the site of inflammation. Also dysregulation in their recruitment is known to underlie the pathology of many diseases of the lung such as chronic obstructive pulmonary disease (COPD), bronchiectasis, and cystic fibrosis (CF). Patients who have neutrophilic asthma are less responsive to corticosteroids than eosinophilic asthma patients [86].

1.2.8. Dendritic Cells

DCs are formed from HSC in the BM and they are found in all tissues except for the eyes, brain, and testis. DCs are one of the important professional APCs and play a crucial role in the regulation of immune response and activating an adaptive immune response. DCs are the main APCs to stimulate naïve T lymphocytes for primer immune response. There are three DC signal for T lymphocytes stimulation (Figure 1.3.). Signal 1 which is antigen specific signal formed between TCR and MHC class II peptides which present pathogens after internalization into pattern recognition receptors (PPRs). Signal 2 which is co-stimulate signal arise between cytokines of CD28 and CD86 that is secreted by DCs after ligation of PRRs as TLRs which are detect to infection with pathogen associated molecular pattern (PAMPs) and tissue factors (TFs). Signal 3 which is polarizing signal depends on activation of PRR release of cytokines such as interleukin 12 (IL-12) promote to development from naïve Th cell to Th1 or Th2 cells [87]. So far, no unique cell surface marker, that can identify a DC specifically, could not be detected. Therefore, various surface markers based either on their presence or absence must be determined to confidently identify pure DC populations. DCs require several stimulating factors such as TNF-α, stem cell factor (SCF), and GM-CSF for maturation.

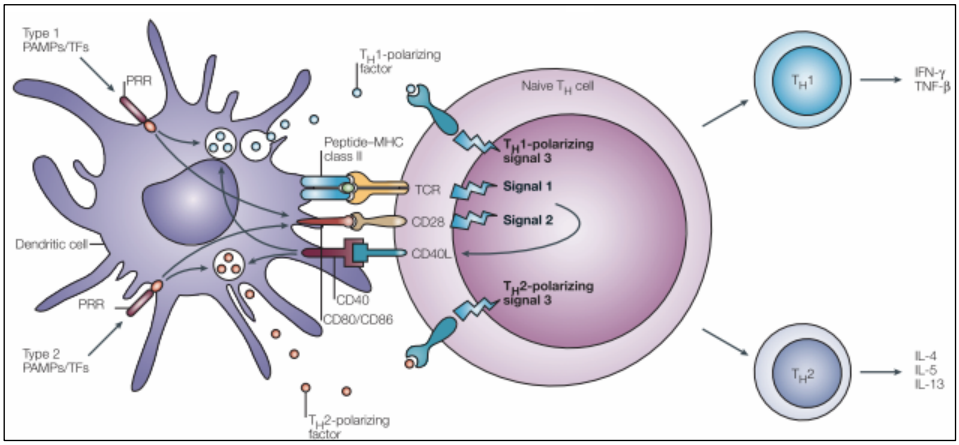

Figure 1.3. T lymphocytes activation and Th1-Th2 cell polarization with DC [87]

Typically, patients who have slight to moderate asthma experience Th2-mediated eosinophilic inflammation. On the other hand, patients, who have acute asthma, experience Th17-related neutrophilic inflammation sometimes in combination with Th2-related eosinophilic inflammation. Differentiation of these mediator cells are stimulated by the allergen-exposed DCs, which can migrate toward the lung or to the lymph nodes where the differentiation of Th cells from naïve T lymphocytes takes place in mediastinal lymph node (mLN). DC lineages are heterogeneous cell populations that include conventional DCs (cDCs), which are also known as myeloid DCs. These lineages of DCs consist of $CD11b^+$DCs (cDC2) as well as $CD103^+$DCs (cDC1), plasmacytoid DCs (pDCs), and monocyte derived DCs (moDCs).

1.2.8.1. *Conventional Dendritic Cells*

DC1s are located between the airway epithelium and vascular endothelial cells. DC1s express CD103, which is an alpha integrin and beta-7 integrin that binds E-cadherin expressed by the epithelial cells. This interaction is imported function and localization of DC1s. This DC subset have highest express tight junction than other DC subsets [71]. However, these surface markers are not specific enough for precise localization, because they are also found higly expressed in moDCs [71,88,89].

DC1 has the ability of cross presentation of antigen for CTL, but they have lower capacity for taking up allergens compared to that of other DC subsets [72,90]. Beside promoting CTL response, DC1 are related to tolerogenic function because of their capacity of induction.

They also can stimulate Tregs differentiation after the HDM exposure when stimulated by agents such as retionoic acid (RA) and peroxisome [91,92]. They can restrict Th2 response during airway inflammation induced by allergens such as OVA or HDM and helminth infections [93,94]. They are also responsible for the maintenance of homeostasis by controlling T helper 17 (Th17)- mediated immune response in *Aspergillus* infection [95]. Besides, cDC1 play a role in removal of apoptotic cells. cDC1s are characterized by $CD11b^{low}$, $CD11c^{high}$, $MHCII^{high}$, $CD103^{high}$, and absence of CD64, LY6C and SiglecF [71–73].

Another subset of DCs comprise of DC2s, which is different abilities such as taking up allergen efficiently, inducing T lymphocyte proliferation, and migrating to mLN [72,96]. DC2s are important for inducing Th2 lymphocytes differentiation during lung inflammation. They can also induce Th17 cell differentiation in the gut [96,97]. The main marker of DC2 is CD11b, which is used for the localization of cDC2s. cDC2s are characterized by the presence of $CD11b^{high}$, $CD11c^{high}$, $Ly6C^{low}$, $MHCII^{high}$, and absence of CD64, CD103, and Siglec F [71–73].

1.2.8.2. *Monocyte Derived Dendritic Cells*

After an infection or allergic inflammation, monocytes can be differentiated into moDCs at the site of the infection or inflammation. moDCs are divided into two different populations based on the status of their Ly6C expression. $Ly6C^{high}$ moDCs are known as inflammatory moDCs. During an inflammation some $Ly6C^{high}$ moDCs decrease their $Ly6C^{high}$ expression and differentiate into $Ly6C^{low}$ moDCs.

The phagocytotic capacity of moDCs is better than that for other DCs subsets. However, their capacity to stimulate Th cell proliferation is more limited compared to that of cDC2s. They can produce cytokines and chemokines for recruitment and activation of Th2 cells upon exposure to allergens such as HDMs. Following the entry of these allergen into the airway, they accumulate within 48 hours in the lungs and their maximal abundance, especially in the mLN, is observed at 72 hours [72]. moDCs are typically characterized as $CD11b^{high}$, $CD11c^{high}$, MHC II^{high}, $LY6C^{low}$, FcεRI ^{high}, $CD64^{high}$, and absent of CD103 and Siglec F [71–73].

1.2.8.3. *Plasmacytoid Dendritic Cells*

pDC differentiate directly from CDPs in the BM. Their differentiation depends on several factors such as FIt3L and signal transducers. They can detect viral infections through the activation of TLR7 and TLR9 and product of type I interferon (IFN-α). Thus, pDCs play a role in respiratory viral infections, especially allergic asthma [98,99]. They are found in a less numbers in the lung. However, the number pDCs steeply increases upon an exposure to an allergen and airway ECs secrete of IL-15 [100,101]. pDCs are characterized by the presence of $CD11b^{high}$, $CD11c^{low}$, MHC II^{low}, $LY6C^{low}$, and absence of CD64, CD103, and Siglec F [71–73].

1.3. HDM-INDUCED ALLERGIC ASTHMA MODEL

Respiratory epithelial cells (RECs) are the first barrier to protect our body against airborne pathogens, allergens, and dust such as HDM. RECs express PPR that recognize molecular patterns of foreign antigens such as PAMPs. PPR also recognize damage-associated molecular patterns (DAMP), which are produced by the damaged tissues. When allergens that cause allergic asthma come in contact with the RECs in the respiratory tract, they are detected by the toll like receptor 4 (TLR4) present in the periphery of the RECs and this detection triggers the initiation of the NFkB-dependent signaling pathways. As result of this stimulation, RECs activate both the innate and adaptive immune systems by releasing cytokines, including IL-33, IL-1α, IL-1β, GM-CSF, and TSLP [102–104].

In addition to the REC-triggered stimulation of an immune response, DCs, that localize just under the epithelial layer, can detect foreign antigenic structures in the airway due to their cytoplasmic extensions. Following the uptake of the HDM particles by the DC's, they migrate to the major lymph node, which is the mLN, to stimulate the naïve $CD4^+$ T lymphocytes, which -in turn- becomes primed to differentiate into Th2 cells. Th2 cells, then secrete IL-4, IL-5, IL-13 or Tfh that play a role in the B lymphocyte-driven generation of IgE. Next, allergen specific IgE binds $FcεRI^{high}$ on MCs and eosinophils, which marks the onset of the so-called *Sensitization Phase*.

If the allergen is encountered again, *Challenge Phase* reaction takes place, in which case, Th2 cells can migrate to the lung EM with help of CCR4 ligands, which are secreted by the

DCs. In this phase, Th2 cells secrete IL-4, IL-5, and IL-13 cytokines in the lymph node and lung, thereby, induce B cell-driven synthesis of IgE. The consequential burst in the IgE levels end up activating eosinophils and trigger their differentiation. Meanwhile, MCs, which are coated with allergen-specific IgEs, activate and release the granules formed inside the cell, when allergen binds IgE on the MCs and eosinophils. The latter two, key events, that occur in eosinophils and MCs, underly the clinical presentation of a *bona fide* asthma attack involving symptoms such as excessive amount of mucus production and thickening of airway smooth muscle at varying severity [105–109].

In experimental animal models of DC's, which have an important role in the development of Th2-type asthma, it has been observed that asthma symptoms decrease with the depletion of DCs in these mice [110,111]. As a result of these recent findings, DC's are revealed as a promising therapeutic target among the immunotherapeutic approaches for the treatment of asthma (Figure 1.4.).

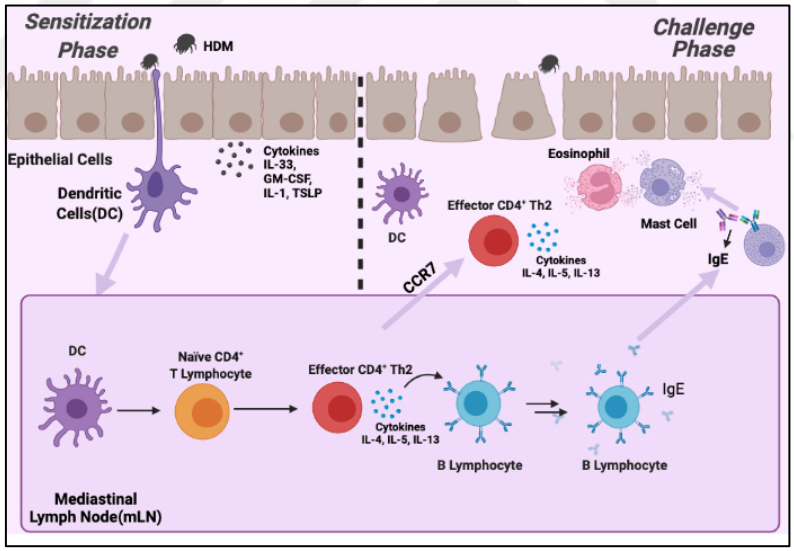

Figure 1.4. Immunological mechanism of asthma symptoms in the experimental HDM model of asthma. Figure generated from Biorender

1.4. STEM CELLS

Stem cells (SCs) are the main source of all cells that are found within the body. These cells are the first form of all cells in any tissue and organ before specialization. It means that SCs can become cells of the blood tissue when needed, and can be transformed into muscle tissue

cell as needed. In any tissue damage, these unspecified cells allow the tissue to regain its old functionality by specializing to attain the necessary properties of the damaged tissue. The ability to differentiate with the ability to divide without limitation paved the way for the use of stem cells for therapeutic purposes [112]. This grade of stem cells appropriate for treatment stage is only possible, should the isolated pool of healthy stem cells retain the desired properties in adequate number. The categorization of a cell as a SC depends on the three required stages. These are the ability to be transformed into different cell types, capable of performing the necessary functionalities of the tissue to be applied *in vivo* and self-renewal. SCs can be classified as totipotent, pluripotent, and multipotent according to their intrinsic differentiation capacity. Totipotent stem cells, which are the most capable of differentiation, are capable of forming a whole new organism with all of its tissues and organs. Although, the pluripotent stem cells cannot form the whole organism like totipotent cells, they can form many tissue types and organ systems such as the bone and cartilage tissue, adipose tissue, connective tissue, and nerve tissues. Moreover, pluripotent SCs have the characteristic of self-renewal, like in the case of embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) [113]. Multipotent stem cells have partially lost their ability to differentiate, nevertheless, they can still provide the formation of specialized groups of cells. Self-renewal capacity of multipotent stem cells are lower compared to that of the ESCs.

1.4.1. Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are multipotent stem cells that have the potential to self-renew and differentiate into a variety of specialized cell types such as osteoblasts, chondrocytes, adipocytes, and neurons. MSCs are easily accessible, expandable, immunosuppressive, they offer the advantage of not eliciting an immediate immune response. Therefore, MSCs have become an attractive tool of cell therapy and source for tissue engineering. MSCs can be isolated from various sources such as adipose tissue, BM, tendon, peripheral blood, and cord blood. BM is the most common source of MSCs [114]. They have three main functions, which include matrix construction (they have the ability to synthesize extracellular matrix proteins), cytokine support (they support hematopoietic cells with cytokines), and immunosuppressive characters (they have immunomodulatory effects on dendritic cells and other immune system cells, especially on T lymphocytes).

MSCs have several advantages, including their ability to differentiate into the cells of other tissue types such as muscle, bone and tendon, neuron and pancreatic cells, successful homing to site of damage, and are usually immunosuppressive or non-immunogenic making them amenable for clinical use [115]. MSCs migrate to the site of inflammation or injury of tissue by increasing expression of CxCR4 receptor and they only target to damaged tissue, do not migrate to healthy tissue [116]. MSCs are defined as hypoimmunogenic, because they express the MHC class I molecule on their surface instead of MHC class II. Therefore, they can be used both as autologous and allogeneic tissue source [117]. BM-MSCs are usually isolated and purified through their physical adherence to the plastic cell culture plate and can be identified with CD marker expression such as $CD44^{+}$, $CD90^{+}$, and $CD45^{-}$ [118].

1.4.2. Mesenchymal Stem Cells in Asthma

MSCs have been used in the treatment studies of many diseases, including asthma due to their therapeutic potential. They migrate to asthmatic niche and can regulate Th2 to Th1 ratio, IgE secretion, mucus and IL-4, IL-5, and Il-13 synthesis. They also can inhibit allergic response through increasing levels of both TGF-β and IFN-γ. Cytokines of IL-1, Il-L-6, TNF-α and IFN-γ are secreted as inflammatory response, while TGF-β and IL-10 are secreted as anti-inflammatory response. As seen in Figure 1.5., MSCs can secrete TGF-β, indoleamine 2,3-dioxygenase (IDO), prostaglandin E2 (PGE2) and nitric oxide (NO) for regulating immune system. They can inhibit eosinophil, Th2 cells which are secretion of IL-4, IL-5 and IL-13 for mucus production, induction of eosinophil and IgE production by B lymphocytes. Also, MSCs can promote to Treg cells for inhibition of airway remodeling [119–121]. Baraza et al. performed experiments to demonstrate the therapeutic potential of BM-MSC on the HDM model of allergic asthma in mice. They observed a significant decrease in the airway responsiveness and bronchial contraction following BM-MSC treatment [122]. In another study, Habibian et al. used BM-MSCs for the treatment of OVA model of allergic asthma model in mice and they observed a decrease in the IgE secretion, eosinophilia, and Th2 and Th17 cytokines upon the BM-MSC treatment [123].

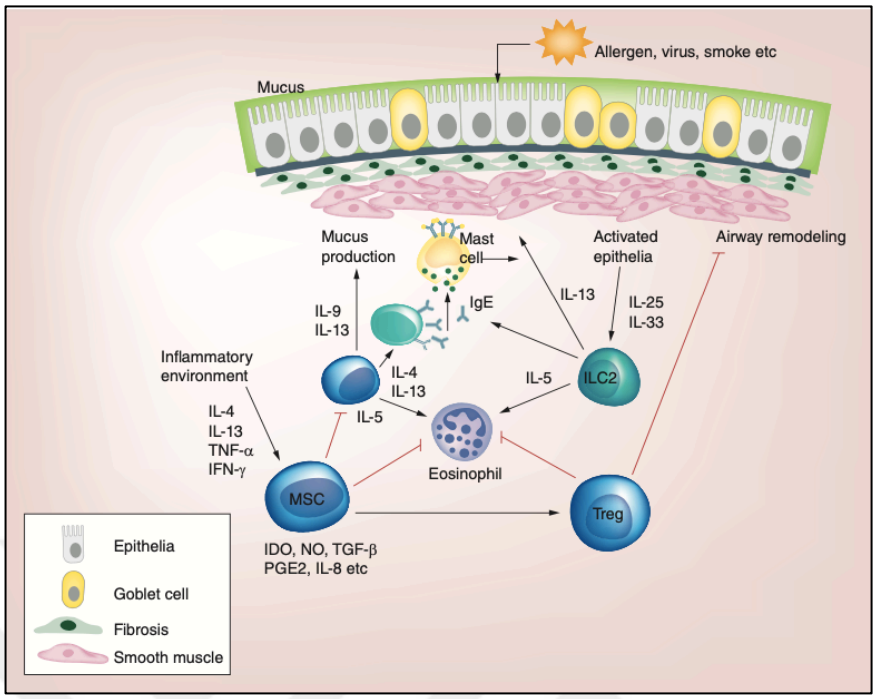

Figure 1.5. Immunomodulation of MSCs on immune response in asthma [119]

1.5. BORON DERIVATIVES

Micronutrients are a nutrient type that are required in small amounts throughout life for organisms to maintain healthy physiological functions [124]. There are several type of micronutrient such as copper, selenium, zinc and boron. As far as their impact on asthma is concerned, several studies point to either adverse or favorable effects of certain micronutrients on asthma. For example, both cooper and zinc are known to aggravate asthma symptoms through pumping inflammatory responses. While micronutrients such as selenium are shown to dampen asthma symptoms through down-modulation of immunological responses [125–127]. However, any information have been found about boron on asthma.

Boron which is atomic number of 5 is a bioactive food component. It is the essential molecule used in many organisms in the development such as fungi, green plants, and animals. Boron is found in several human tissues and can involve in embryogenesis in zebra fish. Some studies show that boron is beneficial for bone growth and maintenance, perhaps reducing of cancer risk [128]. Boron compounds have a potential for being used in the treatment of several types of cancers such as breast, lung, colon, and prostate [129–132]. It can regulate bone tissue metabolism as well as others such as on steroid hormone metabolism

together with calcium, vitamin D, and magnesium [133]. Also, boron supplement improve deficiencies of vitamin D, magnesium, and calcium [134]. Several boron derivatives are shown to have anti-inflammatory effects. Boron can stabilize release of insulin and fix damaged lipid metabolism.

There are different forms of boron-based compounds such as SPT, and SPP. Abdik et al. studied obesity in mice and they observed that SPP can reduce weight gain if the animals are administered the compound for 90 days [135]. In addition, boron can help reducing the number of circulating NK cells. Low dose of boron treatment can change antioxidant enzyme activity. It is also crucial for releasing of insulin and enzyme adjustment in the immune system [136]. On the other hand, some boron-containing compounds have been used in antiseptic solutions. Boron containing molecules in bacteria can enhance quorum sensing signal ability. They can also enhance the Fc receptor-mediated signaling in macrophages and increase secretion of IL-6 and IL-1β. Boron derivatives have an effect in decreasing TNF-α [137,138].

In this thesis, the therapeutic potential of SPT, SPP, and BM-MSC lysate on HDM-induced allergic asthma in mice was investigated. To determine their therapeutic potential, SPT, SPP and BM-MSC lysate were delivered intranasally on HDM induced allergic asthmatic mice. Firstly, histopathological therapeutic effect was determined with H&E staining in the lung. Followed by determination of immune cells such as dendritic cell, T lymphocytes, alveolar macrophage, and neutrophils in terms of number and/or percentage in the lung and/or mLN via flow cytometry, changes in the IgE levels of serum were analyzed with ELISA test.

2. METHODS

2.1. PREPARATION OF HDM EXTRACT

House dust mite (HDM, *Dermatophagoides Pteronyssinus)* (#361862, GREER) which is 155.80 mg dry wt/vial and 45.80 mg protein/vial was dissolved with phosphate buffered saline (PBS) (REF #14190-094, Gibco) as 1 mg/ml stock solution. The prepared solution was aliquoted into 1 ml Eppendorf tubes and stored in -20^{o}C in the freezer. During the HDM challenge time course, it can be stored at 4^{o}C for 4 days.

2.2. PREPARATION OF BORON DERIVATIVES

IC_{50} value (1.25 mg/ml) of SPT (#71840, Sigma-Aldrich) and IC_{50} value (5.5 mg/ml) of SPP (NaB, National Boron Research Institute-BOREN) were found in previous studies of genetic bioengineering department of Yeditepe University. In this thesis, 1:10 diluted IC_{50} of SPT and 1:10 diluted IC_{50} value of SPP were prepared with PBS and filtered through a 0.20 μm filter mesh. It must be prepared freshly for using in the treatment of HDM asthma model.

2.3. PREPARATION OF MESENCHYMAL STEM CELL LYSATE

2.3.1. Bone Marrow Isolation from Mouse

The experimental animal which is BALB/c mouse was sacrificed by CO_2 inhalation and/or cervical dislocation. Then, mouse was sterilized with 70 percent ethanol in order to prevent contamination and transferred to the cabinet which is laminar flow hood cabinet (Class II, Biobase). Bone of femur, tibia and humeri were taken by sterile surgical tools. Cleaned from muscle and fat bones were transferred to a petri dish which consists of 10 ml complete minimum essential medium (MEM) (REF #M7278, Sigma-Aldrich) that contain 15 percent fetal bovine serum (FBS) (REF #10270-106, Gibco) and 1 percent PSA. The bones were washed 2 times with PBS solution which is containing 1 percent PSA. The bone ends were cut and 5 ml of complete MEM was passed through the bone 2 times by using insulin syringe.

Cell was counted by hemocytometer (Bright-Line, Sigma) with trypan blue stain (REF #15250-061,Gibco) under inverted microscope (Cat #4155101901000, Carl Zeiss).

2.3.2. Culture of BM and Obtaining BM-MSC

The obtained BM cells were cultured in the tissue culture dish 100 (Cat #93100, TPP®) and/or T25 tissue culture flasks (Cat #90025-90075, TPP®) and they were incubated in the cell culture CO_2 incubator (at 37°C with 5 percent CO2) (Heal force) for 5 days. During these days, method of Huant et al. was used and no treatments such as cytokines were not added [118]. Every 4-6 days, cells will be passaged at ratio of 1:3. When BM-MSCs reached in the passage number 3, they were used as lysate in HDM asthma model.

2.3.3. Mesenchymal Stem Cell Lysate Production

MSCs which are obtained from BM were dissolved with PBS as 10^4cells/ml. Lysate was obtained by freezing and thawing five times at -80°C refrigerator (New Brunswick Scientific) and 57°C heat block. The concentration of BM-MSC lysate was determined with Bradford assay (Cat #R1271, Fermentas) and NanoDrop (#ND-2000, Thermo Scientific) as 3 mg/ml. The lysate which is diluted as 1 mg/ml was stored at -80°C for being used in the treatment of HDM asthma model.

2.4. HDM CHALLENGE

HDM challenge has been done in two different ways which are the minimum and most effective dose of HDM and effectiveness of therapeutic agents that are SPP, SPT, and BM-MSC lysate determination. Firstly, three groups of BALB/c female mice were used for first step of HDM challenge. One of them is control group which was only given 30 µl PBS. Other two groups are experimental groups which are given 50 µg HDM extract, and 100 µl HDM extract. During first 4 days, 50 µg and 100 µg HDM extract that were dissolved in 30 µl were given intranasally (i.n.) to mice under inhalation of isoflurane anesthesia. Same process repeated between 15-18th days. Blood and lungs of mice were collected. Any therapeutic agents were used in dose trials.

Secondly, BALB/c female mice were divided into 5 different groups. These are control mice which were only given PBS, HDM mice which were asthmatic, HDM+SPT mice which were asthmatic and treated with SPT, HDM+SPP which were asthmatic and treated with SPP, and lastly HDM+BM-MSC lysate mice which were asthmatic and treated with BM-MSC lysate. During in the first 4 days, 50 μg HDM extract was given i.n. to asthmatic mice in 30 μl and 30 μl PBS solution was given i.n. to control mice. On the 10^{th} days, blood was collected from the ear vein of the mice. Therapeutic agents which are SPT, SPP, and BM-MSC lysate were given i.n. asthmatic group, except HDM group, on 12^{th} and 14^{th} days as 0.55 mg SPP, 0.125 mg SPT and 1 mg BM MSC lysate. In between 15-18^{th} days, 50 μg HDM extract was given i.n. to all mice, except control, in 30 μl. During these days, 30 μl PBS solution was given to control mice. On the 19^{th} day, the experiment of HDM challenge was ended and samples of mLN and lungs were harvested and blood were collected.

2.5. COLLECTION SAMPLES OF MLN, LUNG AND BLOOD FROM HDM CHALLENGE MICE

2.5.1. Preparation of Lung Single Cell Suspension

All group of mice were sacrificed with CO_2 inhalation and/or cervical dislocation. 10 ml 1mM PBS-EDTA solution was inserted into the right ventricle of hearth by 25 G needle syringe for lung perfusion. Lung tissue with heart and lymph node were collected into the falcon tube which has mixture that consists of 10 mL wash buffer that is Dulbecco's Modified Eagle's Medium (DMEM) (REF #41966-029, Invitrogen, Gibco) with 5 percent of FBS on the ice. Each lung lobes were transferred to new tissue culture dish 60 mm (Cat #93060, TPP®) which contains 3 ml 0.5 percent of BSA+PBS solution and 500 mg DNase I (REF #10104159001, Roche). The lung lobes were cut into small pieces using scissors and forceps. 1 mg/ml Liberase™ Research Grade (REF #05401127001) solution was added and they were incubated for 60 minutes at 37°C. After incubation, lung pieces were transferred onto 70 μM filter (REF #542040, Greiner) into 100mm Tissue culture dish (Cat #93100, TPP®). The remaining tissues in the filter were smashed with cap of syringe on the filter. Cells were transferred into falcon tube and were centrifuged (Cat #1580R, Gyrozen) for 5 minutes at 1500 x rpm at 4°C. Cells were washed with cold wash buffer and were centrifuged

again. Supernatant was discarded and pellet was dissolved with 2 ml 1x red blood cell lysing buffer was added to cells for 2.5 minutes at room temperature (RT). 12.5 ml cold wash buffer was added into falcon tube and centrifuged. This step was repeated twice. Supernatant was discarded and pellet was dissolved with 10 ml cold wash buffer. Single cell suspension of lung was counted by hemocytometer with trypan blue stain under inverted microscope. This process was applied simultaneously for each mouse.

2.5.2. Preparation of Lymph Node Single Cell Suspension

mLN was separated from thymus, heart under the microscopy in petri dish. mLN was transferred onto 40 μM filter (Cat #PESS330455) in 60mm petri dish which contains 1 ml wash buffer. mLN in the filter was smashed with cap of syringe on the filter. Filter was washed with 7 ml wash buffer. Pipet up and down was done for creating single cell suspension. Cells were transferred to 15 ml falcon tube. They were centrifuged for 5 minutes at 1500 x rpm at 4°C. Cells were resuspended with 7 ml wash buffer. Single cell suspension of mLN was counted by hemocytometer with trypan blue stain under inverted microscope.

2.5.3. Blood and Getting Plasma

Blood was collected from each mouse at 10^{th} and 19^{th} days. 50 μl blood was added into tubes which contain 50 μl sodium citrate. Samples were centrifuged for 5 minutes at 3000 x g. Supernatant was taken and transferred to new 0.2 ml Eppendorf tube. These tubes were kept in refrigerator at -20°C.

2.6. FLOW CYTOMETRY ANALYSIS

Lung and single cell suspension were counted and divided into three groups for lung, one group for mLN, and a few cells from them were separated for compensation analysis in flow cytometry. Divided cells were resuspended with 200 μl FACS buffer which is consist of PBS, two percent of FBS and 0.05 percent of sodium azide and were replaced into 96 cell culture plates (REF #3799, Costar). Plate was centrifuged for 5 minutes at 1500 x rpm at 4°C. supernatant was discarded and Fc block (CD16/32, Cat #101320, Biolegend) was

added. Cells were incubated for 5 minutes at 4ºC. Antibodies which are used for determination population of DCs, T lymphocytes and neutrophils were added in 100 µl FACS buffer into plates and were incubated for 15 minutes at 4ºC. Centrifuge and washing with FACS were performed twice. Cells were fixed with fixation buffer (Cat #420801, Biolegend). After centrifugation, pellets were resuspended with 200 µl FACS buffer. Cells were analyzed by flow cytometry (CytoFLEX S). The obtained data from flow cytometry were analyzed by using FlowJo™10 (BD).

Table 2.1. Antibodies used in flow cytometry analysis

Antibody	Fluorochrome	Clone	Cat No	Company
Anti-Mouse I-A/I-E	APC	M5/114.15.2	107614	Biolegend
Anti-Mouse Ly-6G	PE-Cy™7	1A8	560601	Biolegend
Anti-Mouse Siglec F	APC-Cy™7	E50-2440	565527	BD Biosciences
Anti-Mouse CD11C	PE	N418	117307	Biolegend
Anti-Mouse CD103	PE/Dazzle™594	2E7	121430	Biolegend
Anti-Mouse CD172a (Sirpα)	PE/Cy7	P84	144007	Biolegend
Anti-Mouse CD3	APC	17A2	100236	Biolegend
Anti-Mouse CD4	Alex Fluor 700	GK1.5	100429	Biolegend
Anti-Mouse CD8a	PE	53-6.7	100707	Biolegend
Anti-Mouse CD45	redFluor™710	30-F11	80-0451-U100	TONBO biosciences
Anti-Mouse CD16/CD32(Fc Block)	Unlabeled		101320	Biolegend

2.7. HISTOPATHOLOGICAL EVALUATION

After the two methods of HDM challenge applied, mice were sacrificed and their every lobe of lung were dissected and fixed in percent of 10 neutral buffered formalin solution (#HT501128, Sigma Aldrich) in shaker for 48 hours. Tissue processing was done by tissue processor (REF #TP1020, Leica). Lung tissues were embedded in paraffin wax by paraffin embedding station (REF #EG1160, Leica). 5 μm thick sections were taken from embedded lung tissues by microtom (#RM2245, Leica) and put on the microscope slides which are positive charged (REF #EG-SP4572, Epiglass)

2.7.1. Hematoxylin and Eosin Staining

Sections of lung were incubated in 60^{o}C incubator for 1 hour for removing paraffin. Sections were kept in xylene for 10 minutes for removing the remaining paraffin. They were passed through increasing alcohol series for 5 minutes each. Then, they were washed by distilled water. Sections were stained by hematoxylin (REF #06014/L, Bio-optica) for 8 minutes. They were washed with tap water and were dipped in alcohol series. Slides were stained by eosin solution (REF #10003/L, Bio-optica) for 1 minute. Then, they were passed though decreasing alcohol series for 1 minute each. Sections were kept in the xylene for 30 minutes and were covered with mounting medium (REF #H5000, VectaMount). Image was taken by the light microscope (REF #DM6000, Leica)

2.8. IGE ENZYME LINKED IMMUNOSORBENT ASSAY

Mouse IgE ELISA kit (REF #RAB0799-1KT, Sigma Aldrich) were used as sandwich assay procedure for IgE ELISA assay. Briefly, 100 μl of each samples and standards were covered in 96 well plate and incubated at +4^{o}C with gentle shaking during overnight. Solutions were discarded and well plate washed with wash buffer four times. Detection antibody was added to each well and incubated for 1 hour at RT. Washing step was repeated. Streptavidin solution was added and incubated for 45 minutes at RT. Washing step was repeated. TMB substrate reagent was added and well plate incubated for 30 minutes at RT. Stop solution

was added. Absorbance of each well was measured by microplate reader (Varioscan^{TM} Lux Multimode reader, Thermo Fisher) at 450 nm

2.9. STATISTICAL ANALYSIS

The statistical analyses were analyzed with GraphPad Prisim 8 (GraphPad Software). Most of experiment were analyzed with at least three replicates. To compare healthy, asthmatic and treated groups were compared by one way ANOVA with a Tukey' multiple comparison test. Error bars were represented the SEM. The values were accepted significant as $p<0.05$ and more significant as $p<0.01$.

3. RESULTS

3.1. EFFECTIVE DOSE OF HOUSE DUST MITE

To determination of the most effective dose of HDM for asthma model in mice two different amount (50 μg and 100 μg) of HDM were used for intranasal delivery. IgE level in plasma and histopathological differences were determined by ELISA and hematoxylin eosin staining, respectively. It was found that IgE level increased in 50 μg and 100 μg HDM delivered mice (Figure 3.1.). According to evaluation of lung histopathological, lymphocyte infiltration and the lumen of bronchiole with mucus were observed in mice which was given 50 μg HDM (Figure 3.2.). Therefore, effective dose was selected as 50 μg and it was used for experimental setup.

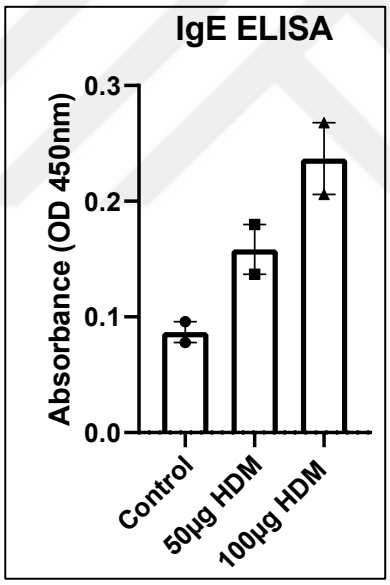

Figure 3.1. Immunoglobulin E secretion compared with control and 50 μg -100 μg HDM mice $p<0.05$ n=2

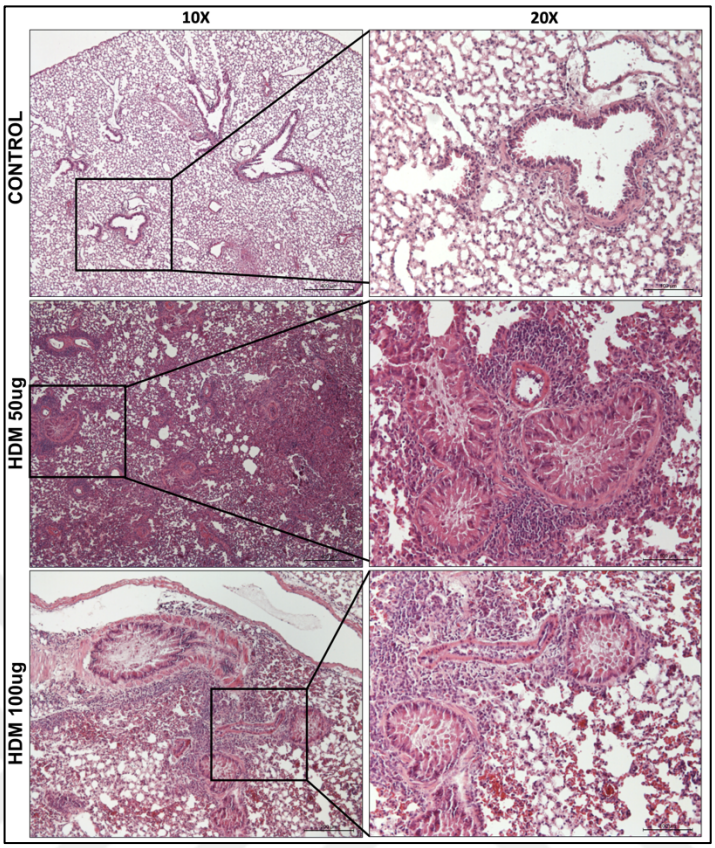

Figure 3.2. Lung histopathological evaluation of control and asthmatic mice groups with H&E by light microscope under 10x and 20x

3.2. BM-MSC GENERATION FROM BONE MARROW

BM was isolated from the mouse as mentioned in the method section. Then, obtained BM cells were cultured till passage 3. At third passage, MSC formation was controlled and morphology of cells were photographed (Figure 3.3.). BM-MSC lysate was obtained from BM-MSC at passage number of 3 and protein concentration was measured with NanoDrop and Bradford assay as 3 mg/ml.

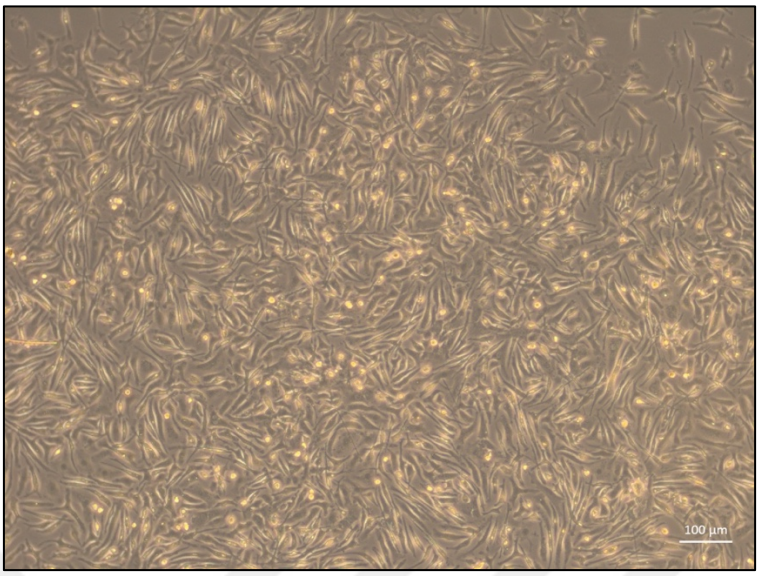

Figure 3.3. BM-MSCs obtained from BM in at passage number 2 were photographed by using light microscope under 10x

3.3. THERAPEUTIC EFFECT OF BORON DERIVATIVES AND BM-MSC LYSATE ON ASTHMA HDM MODEL IN MICE

After determination 50 µg/mouse was effective dose for HDM asthma model in mice. Therapeutic effect of SPP, SPT and BM-MSC lysate were determined. SPP and SPT dose were used according their 1/10 diluted IC_{50} value. 30 ul of 1 ug/ml BM-MSC was used in the experiment. Mice were given HDM extract in the first 4 days for the sensitization phase. On the 10^{th} days, blood was collection from the ear vein of the mice. Therapeutic agents which were SPT, SPP and BM-MSC lysate were delivered by i.n. on day 12^{th} and 14^{th} days. HDM extracts were given in next 4 days for challenge phase. On the 19^{th} day, the experiment was terminated and samples which are lung, mLN, and blood were collected. Immunological and pathological parameters were evaluated to find therapeutic effect of these three agents (Figure 3.4.).

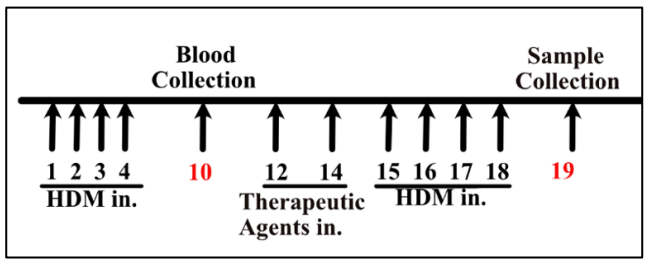

Figure 3.4. HDM mediated asthma model

3.4. THERAPEUTIC EFFECT OF BORON DERIVATIVES AND BM-MSC LYSATE ON LUNG

3.4.1. Lung Histopathological Evaluation of Boron Derivatives and BM-MSC Lysate Therapeutic Effects

Lung sections were stained with hematoxylin and eosin. According to histopathological of lung sections, cell infiltration in peribronchial area and increasing mucus secretion were observed in HDM (Figure 3.5.), BM-MSC lysate and SPT treated mice (Figure 3.6). However, very similar results that are decreasing cell filtration in this area and decreasing mucus secretion were observed in SPP treated mice on Figure 3.6.

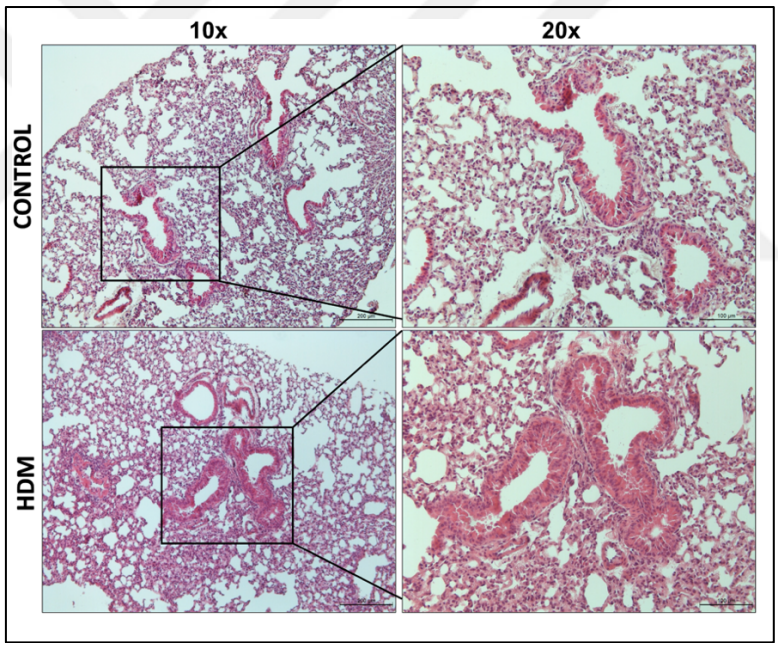

Figure 3.5. Stained lung section of control and asthmatic mice groups with H&E by light microscope under 10x and 20x

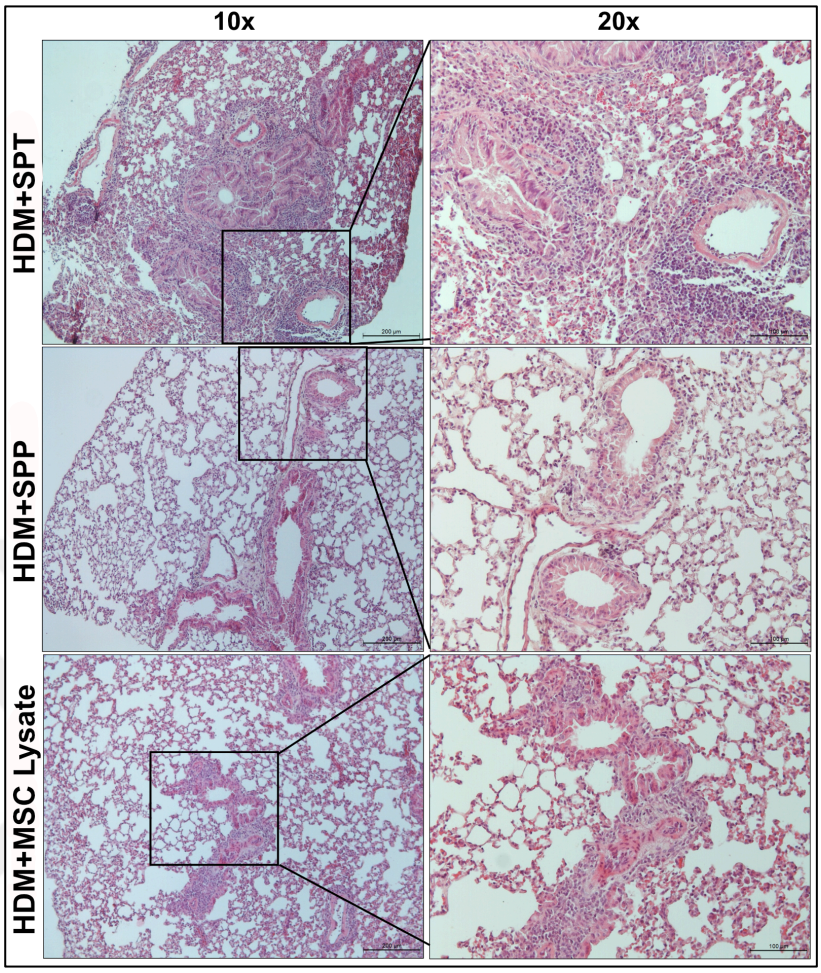

Figure 3.6. Stained lung section of SPT, SPP and BM MSC Lysate treated asthmatic mice groups with H&E by light microscope under 10x and 20x

3.4.2. Dendritic Cell Population

To show therapeutic agents effect of SPP, SPT and BM-MSC lysate in HDM mediated asthmatic mice with another parameter. Lung single cell suspension was prepared after treatment completed. Firstly, total cell number was counted with trypan bleu staining. It was found that HDM asthmatic mice and SPT treated mice had increased total lung cell number but SPP and BM-MSC lysate treated mice had decreased cell number when compared HDM asthmatic mice (Figure 3.7.). This result correlated with H&E staining. Following that single cells were stained with I-A/I-E (MHC II ,APC), CD11c (PE), CD103 (PE/Dazzle^{TM}594), Siglec F (APC-Cy^{TM}7) and CD172a (Sirpα,PE/Cy7). After the discarding cell debris, percentage of 87.4 alive cells were found. Siglec F^{high} and CD11c^{high} cells were accepted as AM and their percentage were 6.63. Siglec F^{-} cells were selected. CD11c^{high} and MHC II^{high}

cell population were accepted as total DC from Siglec F^{-} cells and their percentage were 1.87. CD11b^{high} and CD103^{low} cells were accepted as CD11b^{+} DC populations and percentage of their were 84.7 on Figure 3.8. CD11b^{low} and CD103^{high} cells were accepted as CD103^{+} DC populations and percentage of their were 15.45 in HDM asthmatic mice

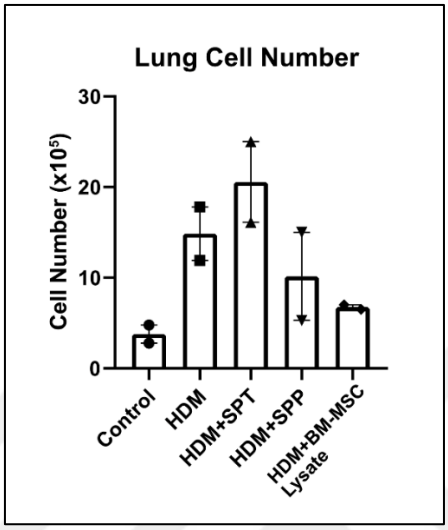

Figure 3.7. Single cell suspension number of lung

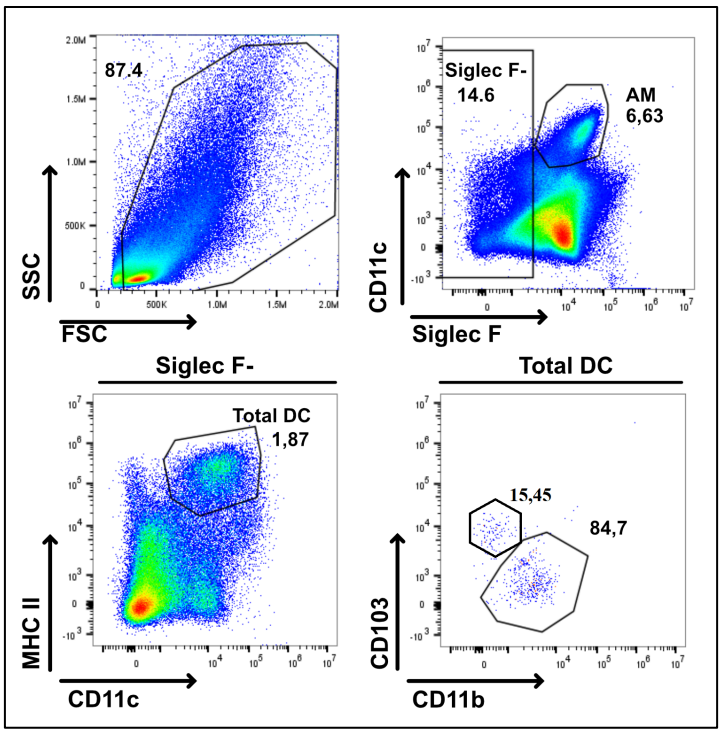

Figure 3.8. The gating strategy of DC populations as pseudocolor plots

Pseudocolor plots were used to analyze the total number of DC. It was found that HDM groups had induced total DC infiltration in lung and only SPP treated groups had decreased total DC percentage whereas SPT or BM-MSC lysate treated group did not have any as shown in Figure 3.9

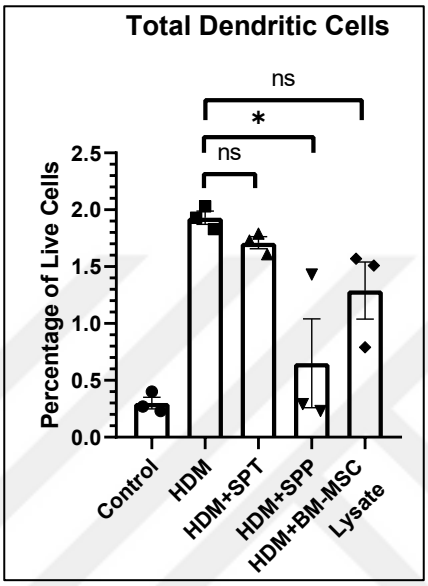

Figure 3.9. Percentage of total DC in live cell ($p<0.01$, $n=3$)

After the DCs subset percentage were determined among the live cells. DC subset percentages were determined. Firstly, $CD11b^+$ DCs which are DC2 subset percentages were evaluated. It was found that HDM induced infiltration $CD11b^+$ DC in HDM asthmatic mice and only SPP treatment decreased $CD11b^+$ DC percentage in the lung. But the other groups did not induce to decrease of $CD11b^+$ DC population (Figure 3.10.) Any differences were found in $CD103^+$DC population which is the another DC subset shown in Figure 3.11.

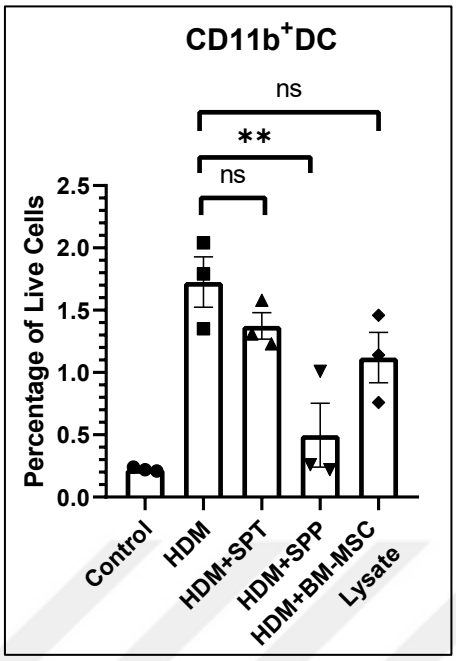

Figure 3.10. Percentage of $CD11b^+$ DC in live cell with control mice, asthmatic mice and treated mice ($p<0.001$, $n=3$)

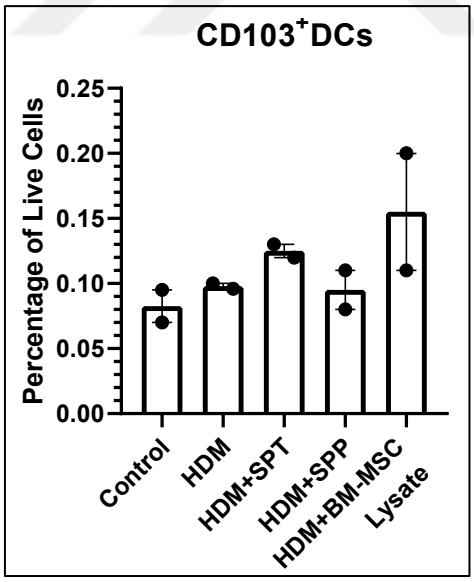

Figure 3.11. Percentage of $CD103^+$ DC in live cell

3.4.3. Alveolar Macrophage Population

The data obtained from flow cytometric analyses was interpreted as bar graphs to analyze AMs was shown in Figure 3.12. It was found that HDM asthmatic mice had increased percentage of AM. SPP, SPT, and BM-MSC treatment decreased AM percentage in lung.

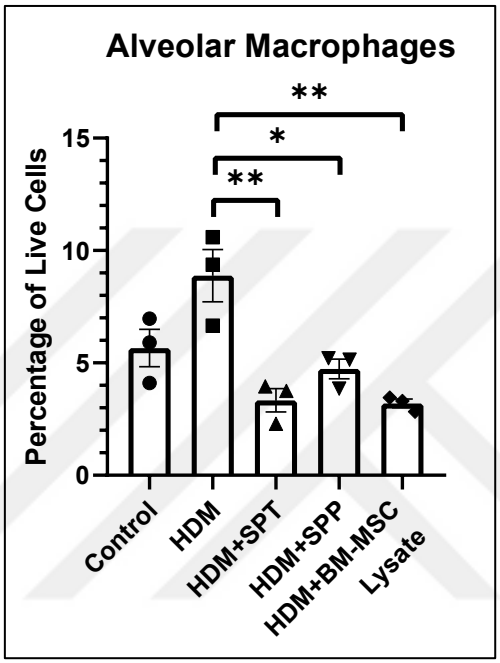

Figure 3.12. Percentage of AMs in live cell comparison with control mice, asthmatic mice and treated mice. *p<0.05,**p<0.01

3.4.4. T Lymphocyte Population

To determine how T lymphocyte percentage was affected after SPP, SPT and BM-MSC lysate treatment, single cell suspension of lung and mLN were obtained from mice and cells were stained with CD3 (APC), CD4 (Alex Fluor 700) and CD8 (PE). Cell debris was discarded and live cells were found as percentage of 61.9. $CD3^{high}$ population was accepted as $CD3^{+}$ T lymphocytes. $CD8^{high}$ and $CD4^{low}$ lymphocytes were accept $CD8^{+}$ T lymphocytes and $CD8^{low}$ and $CD4^{high}$ lymphocytes were accepted $CD4^{+}$ T lymphocytes in mice lung (Figure 3.13.)

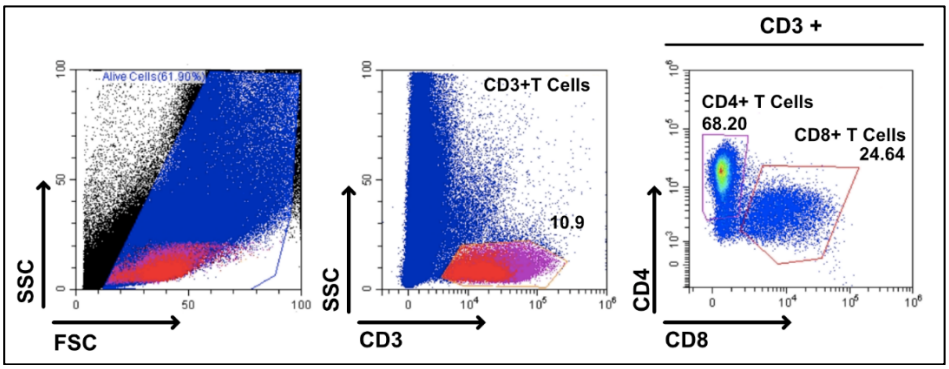

Figure 3.13. The gating strategy of T lymphocyte populations

According to percentage of live cell numbers on T lymphocytes population in lung, HDM treated group induce $CD4^+$ T lymphocyte percentage but after SPP, SPT, and BM-MSC lysate treated groups slightly decreased its percentage in the lung. Although HDM asthmatic mice had similar level of total $CD3^+$ T lymphocytes and $CD8^+$ T lymphocytes decreased after SPP, SPT, and BM-MSC lysate treatment showed in Figure 3.14.

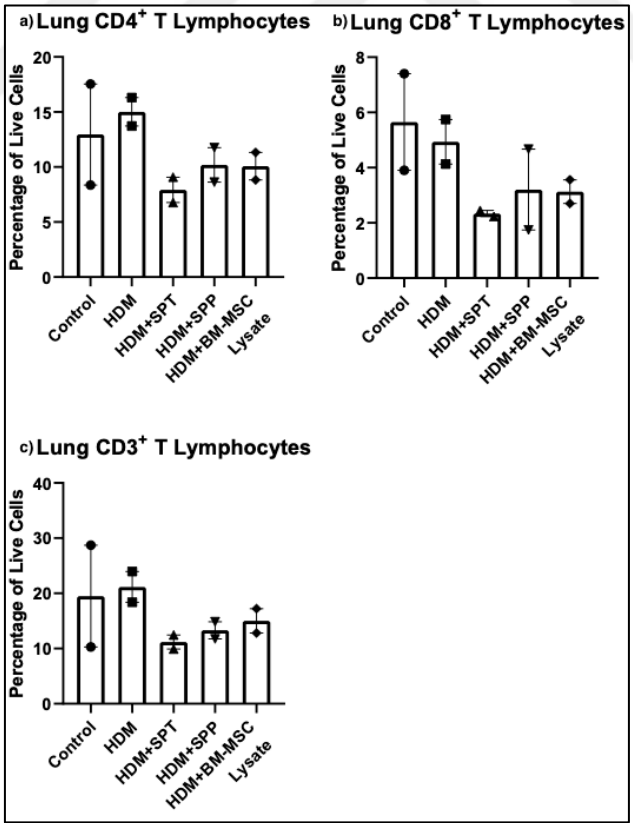

Figure 3.14. Percentage of T lymphocytes populations in live cell with control mice, asthmatic mice and treated mice. a) $CD4^+$ T lymphocytes, b) $CD8^+$ T lymphocytes and c) $CD3^+$ T lymphocytes

3.4.5. Neutrophil Population

Neutrophils are another immune cells which increased in some asthma patients. To show therapeutic effect of agents on asthmatic mice, neutrophil percentage were determined after treatment. Single cell populations of lung were obtained and these cells stained with Ly6G (PE-Cy^{TM}7), CD45 (redFluor^{TM}710), CD11c (PE), MHC II (APC) and Siglec F (APC-Cy^{TM}7). Firstly, cell debris was discarded and alive cells were observed as percentage of 81.9. Respectively, CD45^{high} population which is 17.4 percentage, CD11c^{low} population which is percentage of 25.8, Siglec F^{low} population which is percentage of 56.4 and MHCII^{low} population which is percent of 62 were selected. CD45 ^{high} , Siglec F^{high}, MHCII^{high}, CD11c ^{high} and Ly6G ^{high} population were accepted as neutrophil population on Figure 3.15.

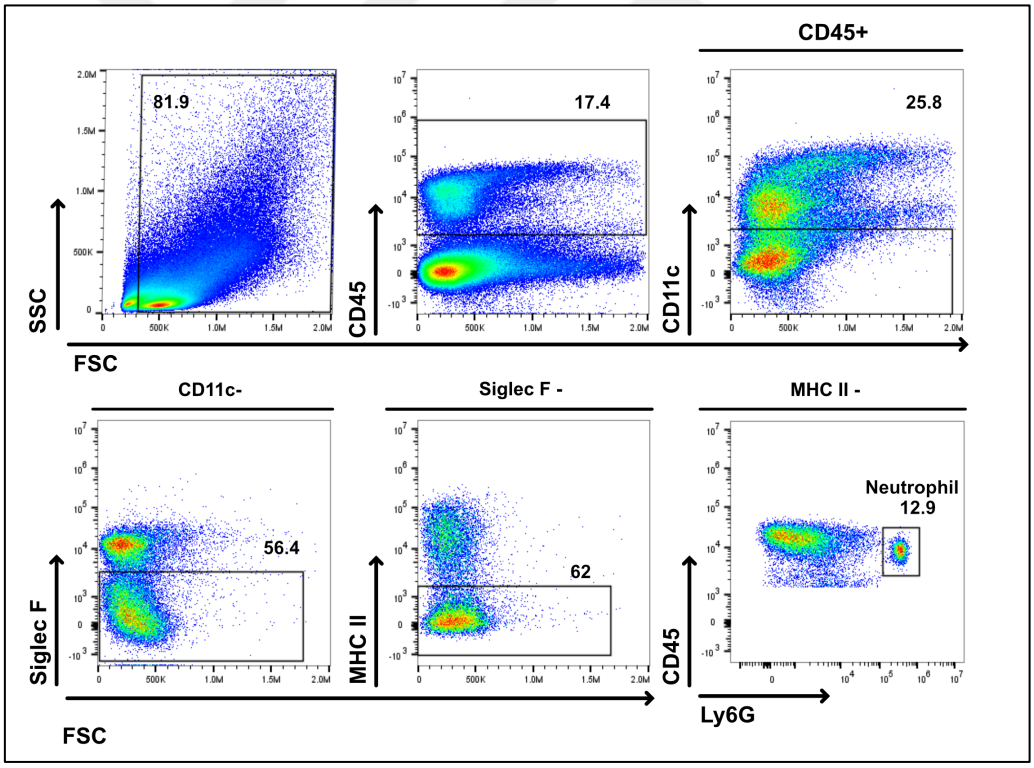

Figure 3.15. The gating strategy of neutrophil populations

It was found that after HDM asthmatic mice had increased neutrophil percentage. Although SPP treatment slightly decreased neutrophil percentage in the lung, SPT treatment increased and BM-MSC lysate did not change neutrophil percentage in lung (Figure 3.16.)

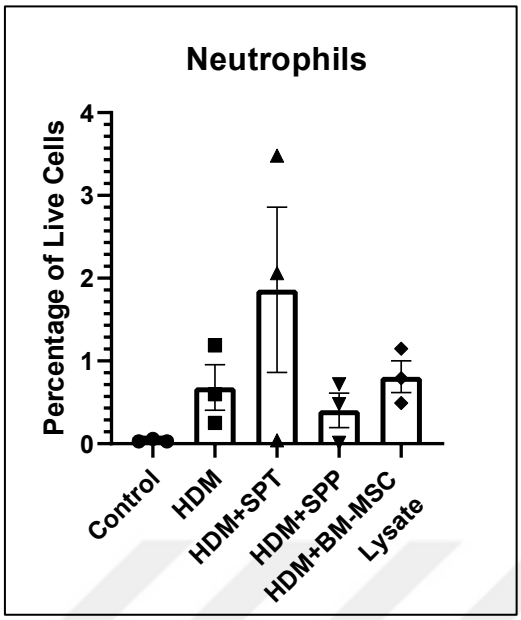

Figure 3.16. Percentage of neutrophil populations in live cell

3.5. THERAPEUTIC EFFECT OF BORON DERIVATIVES AND BM-MSC LYSATE ON MLN

As a result of antigen presenting cells migration to mLN after the allergic inflammation and/or infection in order to induce naïve T lymphocytes activation, the number of T lymphocytes in lymph nodes increases. Thus, the total mLN numbers were counted with trypan blue staining and total $CD3^+$ T, $CD4^+$ T and $CD8^+$ T lymphocyte percentages were determined via flow cytometric analyses to evaluate the therapeutic effects of these three agents. It was found that the total mLN cell number was increased in HDM asthmatic mice but on the other hand SPP, SPT, and BM-MSC treatments decreased the mLN cell numbers as shown in Figure 3.17.

During the flow cytometric analyses, at first debris were discarded and live cell percentage was found 93.8 percent. $CD3^{high}$ population was accepted as CD3 T lymphocytes and its percentage was 40.17. $CD8^{high}$ and $CD4^{low}$ cell were accept $CD8^+$ T lymphocytes and its percentage was 40.13. $CD8^{low}$ and $CD4^{high}$ cell were accepted $CD4^+$ T lymphocytes and its percentage was 58.61 in HDM mLN Figure 3.18.

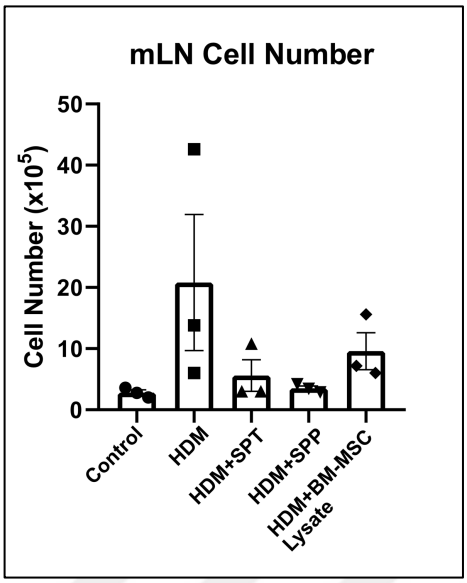

Figure 3.17. Single cell suspension of mLN cell number

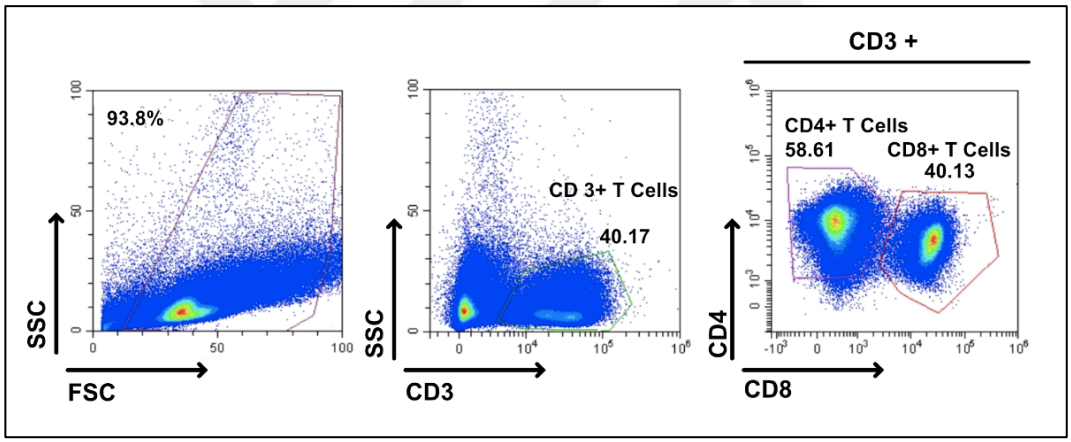

Figure 3.18. The gating strategy of T lymphocytes populations on mLN

According flow cytometry analysis, total percentages of $CD3^+$, $CD4^+$, and $CD8^+$ T lymphocyte increased in HDM asthmatic mice mLN. The highest reduction was observed on the percentage of $CD4^+$ T lymphocytes in SPP treated group although other groups also resulted in a reduction for total percentages of $CD3^+$, $CD4^+$, and $CD8^+$ T lymphocytes (Figure 3.19.).

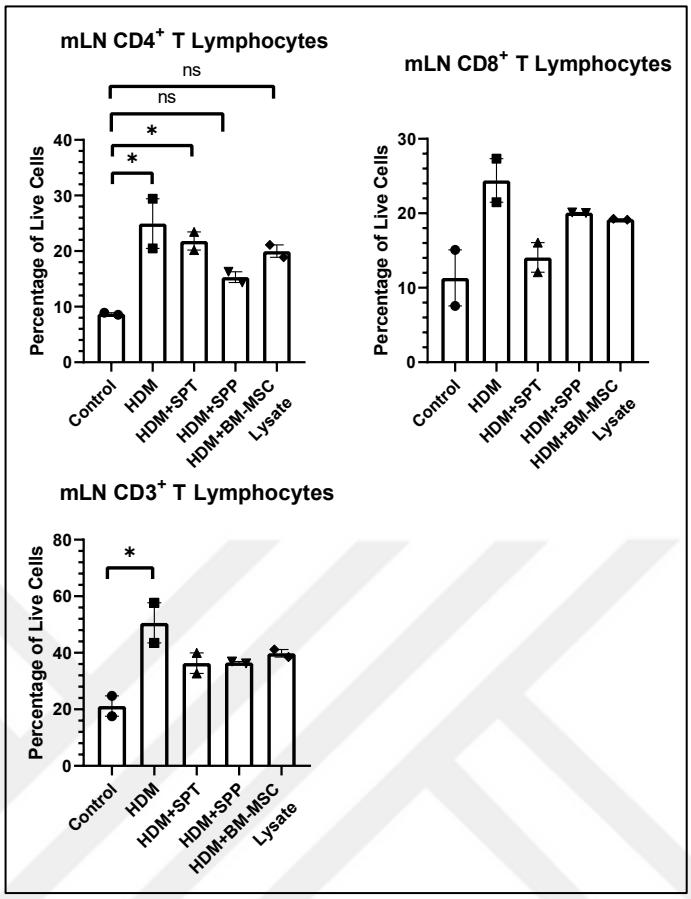

Figure 3.19. Percentage of T lymphocytes populations which are $CD3^+$,$CD4^+$ and $CD8^+$ T lymphocytes in live cell comparison with control mice, asthmatic mice and treated mice. *$p<0.01$

3.6. ENZYME LINKED IMMUNOSORBENT ASSAY

IgE level increases in asthmatic patients and it was shown in the literature as decreasing its level may be used to determine the therapeutic effects. Thus IgE levels in the serum was determined with ELISA after completion of treatments with SPP, SPT, and BM-MSC lysate. It was found that HDM asthmatic and SPT treated mice had high level IgE in their serum. SPP and BM-MSC lysate treatments decreased IgE level compared to HDM asthmatic mice as seen in the Figure 3.20. However, IgE level could not be determined in the serums collected on the 10^{th} day.

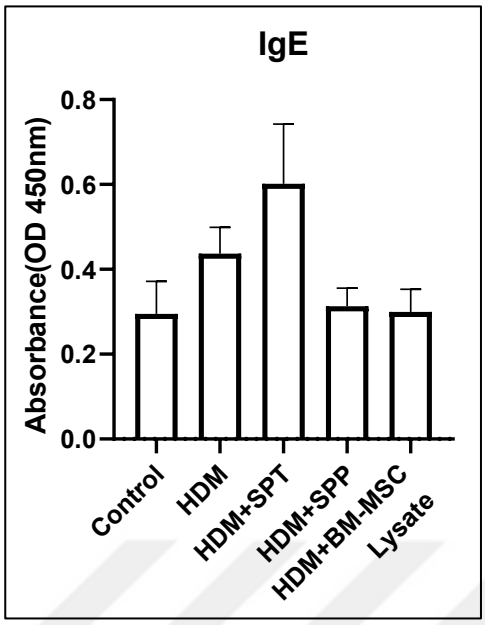

Figure 3.20. Immunoglobulin E secretion from control, asthmatic and treated mice plasma $p<0.001$, $n=3$

4. DISCUSSION

Asthma is a well-known respiratory disease that is prevalent worldwide, and related to AHR, allergic inflammation, airway remodeling, and mucus secretion. Clinical and preclinical studies showed that allergen-specific Th2 immune response is related asthma pathogenesis. 80 percent of children and 60 percent of adults with asthma involves Th2 immune response [139–141]. There are two types of pharmacological treatments, which include bronchodilators (that are used for elevation of symptoms) and anti-inflammatory drugs (that are used for controlling asthma symptoms). Beside these treatments, antibody treatment such as anti-IgE and anti-IL-5 and cell-based therapy approaches have also been studied [42]. MSC-based therapy is one of the important novel cellular therapeutic approaches. MSCs have immunomodulatory effects on both innate and adaptive immune cells, including DC, T and B lymphocytes. Bonfield et al. demonstrated that chronic airway inflammation can be reduced via human BM-MSCs on OVA mediated asthma model [142]. Sun et al. demonstrated that human iPS-MSC and BM-MSC-dependent treatments on OVA asthma model reduced the allergen specific pathological changes in mice [143]. Although current treatments can reduce asthma inflammation or asthmatic symptoms, still there is no treatment that completely cures this disease or prevents airway remodeling. Therefore, new treatments are needed [144,145].

In our study, therapeutic potential of three different boron-based agents which are SPP, and SPT, and BM-MSC lysate were investigated for their therapeutic potential on the HDM allergic asthma model in mice. Firstly, the effective dose of HDM to induce asthma model in mice was determined. After that mice were treated with SPP, SPT, and BM-MSC lysate. To evaluate the therapeutic effects such as histopathological effect, number and/or percentage of immune cells, and serum IgE level were determined.

Five lung sections from each mouse were examined by H&E. According to the histopathology, SPT treated mice showed increased cell infiltration, mucus secretion and erythrocyte in interalveolar area. Especially, alveolar congestion was increased specifically. Observation of SPP treated mice showed decreasing cell infiltration and mucus secretion which was very similar results with control groups.

Furthermore, lung immune cell percentage were determined with flow cytometric analyses following the observation that decreased immune cell infiltration was detected in the lungs of SPP-treated group. It is known that lung contains several immune cell populations such as AMs, interstitial macrophages, and DCs in mouse. DCs have a crucial role in several functions especially in initiation and maintenance of allergic asthma inflammation by controlling Th2 cells [146,147] . Given the data, the percentage of total DC in SPP-treated mice was decreased compared untreated HDM induced asthmatic mice. Tendency of decreasing was noticed in BM-MSC lysate, but no significant differences were observed in it and SPT treatment. This result was in correlation with the literature. Moller et al. showed that the number of DCs increase during the asthmatic inflammation and decrease after the treatment with corticosteroids [148].

DC subsets, which are $CD103^+$DCs and $CD11b^+$, were investigated in mice regarding the decrease in their percentage in total DC pool in SPP-treated mice. $CD103^+$ DCs, which are also as known as DC1s, are important for anti-tumor immunity and Th1 response [149]. Furuhashi et al. showed that $CD103^+$ DCs in lung reveal Th1 and Th17 responses predominately, but $CD11b^+$ DCs trigger to Th2 response [96]. As asthma is Th2 related disease, it was found that percentage of $CD103^+$ DCs were not significantly change within the groups.

Second DC subset is $CD11b^+$ DCs, which are also known as DC2. They play a role in the differentiation of effector Th2 cells from naïve T lymphocytes and affect the antibody response by stimulating Tfhs. It was found that percentage of $CD11b^+$ DCs were increased in asthmatic, SPT and BM-MSC treated mice, but these subset of DCs were decreased in SPP treated mice as control group. Therefore, it is thought that asthmatic inflammation was continuing in HDM, SPT and BM-MSC lysate treatment mice.

It can be said that the decreasing percentage of $CD11b^+$ DC2 in SPP-treatment mice, may help suppression of HDM-dependent asthma via Th2 immune response. Rijt et al. showed that $CD11b^+$ DCs in the lung are necessary for stimulation of Th2 cells during the airway inflammation [110]. It is also known that the classical $CD11b^+$ cDCs secrete CCL17 and CCL22 chemokines, which are important for Th2 lymphocytes to migrate into the lung [150,151]. Depending on the decreasing percentage of $CD11b^+$ DCs in SPP treated group, lung had less concentration of CCL17 and CCL22, which may have resulted in a reduced $CD4^+$ T lymphocytes percentage in the lungs of SPP-treated mice. Based on this knowledge,

the total T lymphocytes, $CD4^+$ T lymphocytes, and $CD8^+$ T lymphocytes were determined in the lung and mLN. SPP-treated mice had less $CD4^+$ T lymphocytes compared to other groups in mLN, although all groups had decreased total T lymphocytes and $CD4^+$ T lymphocytes percentage after the treatment.

More than 60 percent of adult asthma patients' sputum contain neutrophils [152]. In addition, oral corticosteroids can induce airway neutrophilia [153]. Thus, this situation explains why new treatments are necessary. According to our results, SPT induces the number of neutrophilia in the lung, whereas SPP showed a slight decrease on the neutrophil number compared to control group.

AMs can remove pathogens for maintaining lung homeostasis without inducing inflammation. But in the asthma, AMs act as pro-inflammatory cells and induce allergic reactions [154]. Decreasing AM percentage is important for treatment of asthma as shown in Hothersall et al. study for chronic obstructive pulmonary disease (COPD) on asthmatic patients [155]. In this thesis, it was found that the percentage of AMs was decreased in all SPP, SPT, and BM-MSC lysate-treated groups compared to untreated HDM induced asthmatic mice.

The decrease in IgE levels in serum may be another marker of asthma treatment. Th2 cells secrets IL-4 and IL-13 cytokines and these cytokines induce B lymphocytes to produce allergen specific IgE. The study of Buhl et al. on omalizumab which is used for anti-IgE immunotherapy and showed that it enhances the control of asthma disease by getting lower doses of corticosteroid [156]. Also, Abramson et al. showed that HDM-mediated immunotherapy can improve lung function in allergic asthmatics with HDM [157]. Blumberga et al. demonstrated that this immunotherapy can decrease HDM specific IgE levels [158]. In this thesis, total IgE secretion was examined and any significant differences were observed. However, tendency of IgE secretion decreasing in SPP and BM MSC lysate-treated mice were observed with contrast to asthmatic mice. It was also found that IgE levels increased in serum of SPT-treated mice which indicates that SPT has the potential of triggering asthma in mice.

It is a well-known fact that boron and boron derivatives have positive effects on wound healing. They can also act as anticancer agents in lung, prostate, and breast cancers [132,159]. There are myriad of new studies based on boron and diseases caused by their

deficiency. In addition to their therapeutic effects, boron derivatives can be used anti-oxidant and anti-inflammatory treatments because of their influence on increasing estrogen expression in the lung cancer. Therefore, it was expected that SPP and SPT have therapeutic potential. On the contrary, two boron derivatives displayed two different results. The collected data indicate that the new studies are needed to explain exact effect and mechanism of action for SPP and SPT. Dogan et al. showed that SPP can induce TGF-β expression which is the immune suppressive cytokine and cannot change TNF-α expression [160,161]. Based on the data in the literature, it can be hypothesized that demonstrated therapeutic effect of SPP may be the result of suppressed inflammation as a result of induced TGF-β expression.

In another study, Royce et al. showed that asthma symptoms regress with adoptive transfer of MSCs [162]. MSCs can reduce asthma symptoms by suppressing the function of effector T lymphocytes, increasing number of Treg, decreasing capacity of DC and secreting IL-10, TGF-β [163,164]. For this reason, MSC therapy has been used in preclinical and clinical trial for the treatment of asthma. Therapeutic potential of MSC lysates have been investigated on HDM asthma model. It was expected that BM-MSC lysate has therapeutic potential. However, the difference between the percentages of total DCs, $CD11b^+$ DCs and $CD103^+$ DCs were found non-significant in BM-MSC lysate and asthmatic mice, respectively. BM-MSC lysate cannot lower the number of DC, but it can decrease the percentage of AM more significantly. The data indicate that it can only be effective on AMs. According to histopathology assessments, an increase on cell infiltration in peribronchial area, mucus secretion, and alveolar congestion were observed. These results are similar with the results obtained in HDM group. The reason for inefficient treatment or not having the expected therapeutic effect for the BM-MSC lysate may be caused by preparation method where a freeze-thaw cycle used. Some immune suppressive molecules may be degraded and some DMAP molecules may be released due to the nature of the process. The destruction of the immune suppressive molecules may explain the results of BM MSC treatment for HDM induced asthma.

5. CONCLUSIONS

In conclusion, boron derivative of SPP (NaB) has a therapeutic potential for the treatment of asthma by decreasing mucus secretion, immune cell infiltration, and inflammation in the lung and $CD4^+$ T lymphocytes in mLN, and IgE levels in serum. However, another boron derivative of SPT and BM-MSC lysate did not change pathological and immunological parameters of asthma therapeutically. Even though the boron is widely investigated compound, there is still a huge gap for detailed studies of its derivatives such as SPT. This thesis is the first in literature for investigation of the therapeutic effects of boron derivatives and BM-MSC lysate on the allergic asthma.

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APPENDIX A : ETHIC STATEMENTS

T.C. YEDİTEPE ÜNİVERSİTESİ
Hayvan Deneyleri Yerel Etik Kurulu (HADYEK)

ETİK KURUL KARARI

Protokol No	Toplantı Tarihi	Toplantı Sayısı	Karar No	Proje Yürütücüsü
2020-880	30.11.2020	2020/11	2020/11-4	Dr. Öğretim Üyesi İbrahim Hatipoğlu
'Farelerde House Dust Mite (HDM) Astım Modeli Oluşturmak ve Semptomların Azaltılması Üzerine Yapılan Çalışmalar' isimli proje oy birliğiyle etik açıdan uygun görülmüştür.				

Hayvan Türü / Irkı	Toplam Hayvan Sayısı	Hayvanın Cinsiyeti
Fare / BALB/C	40	Dişi

Görevi	Adı Soyadı	Katılım Durumu
Başkan	Prof. Dr. Bayram YILMAZ	
Başkan Vekili	Prof. Dr. Erdem YEŞİLADA	
Üye	Dr. Veteriner Hekim Engin SÜMER	
Üye	Prof. Dr. M. Ece GENÇ	
Üye	Prof. Dr. Rukset ATTAR	
Üye	Prof. Dr. Gamze TORUN KÖSE	
Üye	Doç. Dr. Ediz DENİZ	
Üye	Doç. Dr. Aylin YABA UÇAR	
Üye	Doç. Dr. Burcu GEMİCİ BAŞOL	**Katılmadı**
Üye	Hakan GÖKSEL	
Üye	Ahmet ŞENKARDEŞLER	

T.C. YEDİTEPE ÜNİVERSİTESİ
Hayvan Deneyleri Yerel Etik Kurulu (HADYEK)

ETİK KURUL KARARI

Protokol No	Toplantı Tarihi	Toplantı Sayısı	Karar No	Proje Yürütücüsü
2021-053	14.10.2021	2021/10	2021/10-4	Prof. Dr. Fikrettin Şahin
'House Dust Mite (HDM) Astım Modeli Oluşturulmuş Farelerde Bor Türevlerinin ve Mezankimal Kök Hücre Lizatının Terapötik Potansiyelinin Araştırılması' isimli proje oy birliğiyle etik açıdan uygun görülmüştür.				
Hayvan Türü / Irkı		**Toplam Hayvan Sayısı**		**Hayvanın Cinsiyeti**
Fare / BALB/c		27		Dişi

Görevi	Adı Soyadı	Katılım Durumu
Başkan	Prof. Dr. Bayram YILMAZ	
Başkan Vekili	Prof. Dr. Erdem YEŞİLADA	
Üye	Dr. Veteriner Hekim Engin SÜMER	
Üye	Prof. Dr. M. Ece GENÇ	
Üye	Prof. Dr. Rukset ATTAR	
Üye	Prof. Dr. Gamze TORUN KÖSE	
Üye	Prof. Dr. Özlem MALKONDU	
Üye	Doç. Dr. Aylin YABA UÇAR	
Üye	Doç. Dr. Burcu GEMİCİ BAŞOL	
Üye	Atakan Mücahit YAVUZ	
Üye	Ahmet ŞENKARDEŞLER	