INVESTIGATING TIGHT JUNCTION PROTEIN-RELATED REGULATION OF NASAL EPITHELIAL BARRIER INTEGRITY IN ALLERGIC RHINITIS PATIENTS AND NON-ALLERGIC INDIVIDUALS

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by

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AD	Atopic dermatitis
AEC	Absolute eosinophil count
AJs	Adherens junctions
ALI	Air liquid interface
APCs	Antigen presenting-cells
AR	Allergic rhinitis
ARIA	Allergic Rhinitis and its Impact on Asthma
B. tropicalis	*Blomia tropicalis*
BAT	Basophil activation test
BMI	Body mass index
CAR	Coxsackievirus and adenovirus receptor
cDNA	Complementary DNA
CO	Carbon oxide
CO_2	Carbon dioxide
CRS	Chronic rhinosinusitis
D. farinae	*Dermatophagoides farinae*
D. pteronyssinus	*Dermatophagoides pteronyssinus*
DCs	Dendritic cells
DEPs	Diesel exhaust particles
DM	Desmosomes
EMT	Epithelial-mesenchymal transition
ENT	Ear, nose, and throat
EoE	Eosinophilic esophagitis
FD4	Fluorescein isothiocyanate-dextran 4kDa
GUK	Guanylate kinase-like
HBECs	Human bronchial epithelial cells
HCC	Hepatocellular carcinoma
HDAC	Histone deacetylases
HDACi	Histone deacetylases inhibitor
HDM	House dust mite
HUSM	Hospital Universiti Sains Malaysia

IDT	Intradermal tests
IECs	Intestinal epithelial cells
IFN-γ	Interferon-gamma
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
JAMs	Junctional adhesion molecules
K2P	Two Pore Domain Potassium Channel
MAGUK	Membrane associated guanylate kinase
MHC	Major histocompatibility complex
NA	Not applicable
NHANES	National Health and Nutrition Examination Survey
NK	Natural killer
NO	Nitrogen oxide
NO_2	Nitrogen dioxide
ORL-HNS	Otorhinolaryngology-Head and Neck Surgery
PAR	Perennial AR
pHBECs	Primary human bronchial epithelial cells
PM	Particulate matter
PMNs	Polymorphonuclear leukocytes
PPR	Pattern recognition receptor
PS	Power and Sample Size
qPCR	Quantitative polymerase chain reaction
qRT-PCR	Quantitative real-time polymerase chain reaction
RT-qPCR	Reverse transcription-quantitative polymerase chain reaction
SAR	Seasonal AR
SCIT	Subcutaneous immunotherapy
SD	Standard deviation
SH3	Src-homology3
SIRT	Sirtuin
SLIT	Sublingual immunotherapy
SO_2	Sulphur dioxide
SPT	Skin prick test

ssIgE	Serum specific immunoglobulin E
TCR	T-cell receptors
TER	Transepithelial electric resistance
TGF-β	Transforming growth factor-beta
Th1	T helper 1
Th2	T helper 2
TJ	Tight junction
Trek-1	TWIK-related potassium channel-1
TRPV4	Receptor potential vanilloid 4
TSA	Trichostation-A
TSLP	Thymic stromal lymphopoietin
U	Unique
VAS	Visual analog scale
ZO	Zonula occludens

LIST OF APPENDICES

MENYIASAT PROTIN SIMPANGAN KETAT BERKAITAN PENGAWALAN INTEGRITI PENGHALANG EPITELIA HIDUNG DALAM PESAKIT RHINITIS ALERGI DAN INDIVIDU BUKAN ALERGI

ABSTRAK

Rhinitis alergi (AR) adalah penyakit alergi yang menjejaskan populasi yang besar di seluruh dunia. Walaupun tidak membawa maut, ia mempunyai kesan yang ketara terhadap kualiti hidup. Penghalang epitelia hidung dianggap penting sebagai barisan pertahanan pertama di saluran pernafasan atas kerana ia melindungi sistem imun perumah daripada terdedah kepada alergen. Kecacatan simpangan ketat (TJs) menyumbang kepada kegagalan berfungsi pengahalang epitelia hidung dalam pesakit AR sederhana-teruk. Walau bagaimanapun, perkaitan antara TJ epithelia hidung, protin zonula okluden (ZO) dan deacetylases histon (HDACs) dengan ciri demografi, klinikal dan persekitaran masih tidak jelas. Dalam kajian ini, kami bertujuan untuk menyiasat ekspresi mRNA *ZO-1*, *ZO-2*, *ZO-3*, *HDAC1*, dan *HDAC2* dalam sel epitelia hidung pesakit AR yang sensitif hama debu rumah (HDM) berbanding kawalan bukan alergi. Kami juga menkaji perkaitan antara tahap *ZO-1*, *ZO-2*, *ZO-3*, *HDAC1*, dan *HDAC2* dalam sel epitelia hidung dengan parameter demografi, klinikal dan persekitaran pesakit AR dan kawalan bukan alergi. Subjek yang direkrut terdiri daripada 28 orang pesakit AR dan 28 orang kawalan bukan alergi. Ujian tusukan kulit (SPT) telah dilakukan ke atas subjek untuk menentukan sama ada mereka sensitif secara alergi terhadap salah satu daripada alergen HDM. Kami memulakan kajian ini dengan mengumpul sampel sel epitelia hidung daripada semua subjek. Sampel RNA ditranskripsikan secara terbalik kepada cDNA untuk mengukur tahap ekspresi *ZO-1*, *ZO-2*, *ZO-3*, *HDAC1* dan *HDAC2* melalui tindak balas rantai polimerase masa nyata

kuantitatif (qRT-PCR). Ekspresi mRNA *ZO-1* menurun dengan ketara dalam pesakit AR berbanding kawalan bukan alergi (p=0.010). Tiada perbezaan yang ketara diperhatikan terhadap tahap ekspresi *ZO-2*, *ZO-3*, *HDAC1* dan *HDAC2* dalam pesakit AR berbanding kawalan bukan alergi. Walau bagaimanapun, kami mendapati perkaitan yang ketara antara tahap *HDAC2* yang tinggi dalam pesakit AR yang sensitif kepada *Dermatophagoides farinae* (*D. farinae*) (p=0.041). Kami juga mendapati perkaitan yang ketara antara tahap *HDAC2* yang tinggi dalam pesakit AR dengan kekerapan menukar cadar yang rendah (p=0.043). Ekspresi *ZO-2* yang tinggi diperhatikan dalam pesakit AR yang mempunyai haiwan peliharaan (p=0.007). Sebagai kesimpulan, data kami menunjukkan bahawa ekspresi *ZO-1* adalah rendah dalam pesakit AR yang menyumbang kepada penurunan integriti penghalang epitelia hidung. Penemuan ini menunjukkan hubungan atara ekspresi mRNA ZO-2 and HDAC2 dalam sel epitelia hidung dengan parameter persekitaran tertentu. Tambahan pula, kehadiran allergen dalam cadar juga membawa kepada peningkatan ekspresi HDAC2, yang mungkin menjejaskan ekspresi ZO dalam penghalang epithelia hidung. Menyasarkan penghalang epitelia hidung dengan memulihkan ekspresi *ZO-1* mungkin merupakan pendekatan terapeutik yang diharapkan untuk pesakit AR.

INVESTIGATING TIGHT JUNCTION PROTEIN-RELATED REGULATION OF NASAL EPITHELIAL BARRIER INTEGRITY IN ALLERGIC RHINITIS PATIENTS AND NON-ALLERGIC INDIVIDUALS

ABSTRACT

Allergic rhinitis (AR) is an allergic disease affecting a huge population worldwide. Despite not being fatal, it has a significant impact on the quality of life. The nasal epithelial barrier is considered crucial for the first line of defence in the upper airway as it protects the host immune system from exposure to allergens. Defective tight junctions (TJs) contribute to nasal epithelial barrier dysfunction in moderate-severe AR patients. However, the association of nasal epithelial TJs, zonula occludens (ZO) proteins and histone deacetylases (HDACs) with the demographical, clinical and environmental characteristics of AR patients remains unclear. In this study, we aimed to investigate the mRNA expression of *ZO-1*, *ZO-2*, *ZO-3*, *HDAC1* and *HDAC2* in nasal epithelial cells of house dust mites (HDMs)-sensitised AR patients compared to non-allergic controls. We also examine the association between *ZO-1*, *ZO-2*, *ZO-3*, *HDAC1* and *HDAC2* levels in nasal epithelial cells with the demographical, clinical and environmental parameters of AR patients and non-allergic controls. The recruited subjects consisted of 28 AR patients and 28 non-allergic controls. A Skin prick test (SPT) was performed on the subjects to determine whether they were allergically sensitised to either one of the HDM allergens. We started this study by collecting nasal epithelial cell samples from all the subjects. The RNA samples were reverse transcribed into cDNAs for measurement of *ZO-1*, *ZO-2*, *ZO-3*, *HDAC1* and *HDAC2* expression levels by quantitative real-time polymerase chain reaction (qRT-PCR). The mRNA expression of *ZO-1* was significantly decreased in

AR patients compared to non-allergic controls (p=0.010). No significant difference was observed in the expression levels of *ZO-2*, *ZO-3*, *HDAC1* and *HDAC2* in AR patients compared to non-allergic controls. However, we found a significant association of higher *HDAC2* levels in AR patients sensitised to *Dermatophagoides farinae* (*D. farinae*) (p=0.041). We also found significant associations of higher *HDAC2* levels in AR patients with lower frequency of changing bedsheet (p=0.043). Higher expression of *ZO-2* was observed in AR patients who had pets (p=0.007). In conclusion, our data indicated that *ZO-1* expression was lower in AR patients, contributing to decreased nasal epithelial barrier integrity. In addition, we also demonstrated a correlation between the mRNA expression of *ZO-2* and *HDAC2* in nasal epithelial cells with specific environmental parameters. Furthermore, the presence of allergens in the bedsheet also leads to an increase in *HDAC2* expression, which may affect *ZOs* expression in nasal epithelial barrier. Targeting the nasal epithelial barrier by restoring *ZO-1* expression may be a promising therapeutic approach for AR patients.

CHAPTER 1

INTRODUCTION

1.1 Study Background

The immune system is essential to defend the body against microorganisms such as bacteria, viruses, and fungi for a human to stay healthy. The immune system is incredibly complex as it is made up of vital networks of cells and organs that protect the body from infections and other diseases (Descotes, 2014; Nicholson, 2016). Allergic disease is one of the diseases that results from the immune system's response to foreign substances. The immune system may overreact to foreign substances by developing antibodies, resulting in the clinical symptoms of allergic diseases.

The epithelial barrier is the first line of defence against the invasion of harmful pathogens. Tight junctions (TJs) are one mode of cell-cell adhesion that act as the first epithelial barrier to the diffusion of solutes through intracellular space and establish a boundary between the domains of the apical and basolateral plasma membranes (Tsukita, 2013).

Recently, it has been observed that reduced TJs expression is associated with the breakdown of the nasal epithelial barrier (Ayumi Fukuoka & Yoshimoto, 2018; Steelant et al., 2016). Zonula occludens (ZO) proteins form a complex structure with TJs between the epithelial cells and were found to be decreased in AR patients compared to healthy controls (Bauer et al., 2010; H.-J. Lee et al., 2016; Yılmaz et al., 2019). Moreover, overexpression of histone deacetylases (HDAC) in epithelial cells can repress the transcription of ZO genes (Jiang et al., 2015). These findings reflect an important understanding of the underlying mechanisms, demonstrating the role of ZO proteins and HDACs in AR pathogenesis.

This study focused on moderate-severe AR patients. They were sensitised to the most common types of house dust mite (HDM) in Malaysia, *i.e. Dermatophagoides farinae* (*D. farinae*), *Dermatophagoides pteronyssinus* (*D. pteronyssinus*), and *Blomia tropicalis* (*B. tropicalis*). Previously, a few studies have reported on *ZO-1* expression in AR patients, but there is a lack of literature on the expression of *ZO-2* and *ZO-3* in AR patients. Furthermore, research on *HDAC1* and *HDAC2* expression in AR patients is still limited.

Thus, this study was conducted to investigate the gene expression of *ZO-1*, *ZO-2*, *ZO-3*, *HDAC1* and *HDAC2* in the nasal epithelial cells of HDM-sensitised moderate-severe AR patients of a Malaysian population in comparison with non-allergic individuals. This study also aimed to provide data on the demographical, clinical and environmental characteristics of Malaysian AR patients.

1.2 Problem Statement

This study focused on the expression of the ZO and HDAC genes in HDM-sensitised moderate-severe AR patients attending the Otorhinolaryngology-Head and Neck Surgery (ORL-HNS) clinic of Hospital Universiti Sains Malaysia (HUSM) in Kelantan. HDMs have been identified as the major triggering allergens in Malaysia (Azid et al., 2019; Sani et al., 2019). Uncontrolled infiltrations of inhaled allergens into the nasal airways are thought to be caused by TJ dysfunction. ZO-1 was found to be defective in the nasal epithelium cells of AR patients, and ZOs are considered crucial molecules in the structure of TJ. Furthermore, HDAC1 was found to affect the regulation of ZO-1 proteins. Thus, both ZOs and HDACs might be important in TJ and AR. However, there is a lack of literature on TJ gene expression in AR patients.

Therefore, we set out to investigate the nasal epithelial barrier defect, specifically the ZO and HDAC genes expression in HDM-sensitised AR patients. First, we recruited adult AR patients who met the criteria for being classified as moderate-severe AR patients. Healthy adults who did not present with any allergic symptoms were recruited as controls. Secondly, the demographical, clinical and environmental characteristics of the recruited subjects were collected in this study. Finally, the expressions of ZOs and HDACs genes were measured and correlated with the demographical, clinical and environmental parameters of AR patients and non-allergic controls.

1.3 Research Questions

1. Is there a difference in *ZO-1*, *ZO-2* and *ZO-3* gene expression in HDM-induced AR patients compared to healthy non-allergic individuals?
2. Is there a difference in *HDAC1* and *HDAC2* gene expression in HDM-induced AR patients compared to healthy non-allergic individuals?
3. Does the expression of *ZO-1*, *ZO-2*, *ZO-3*, *HDAC1* or *HDAC2* associate with the demographical, clinical and environmental parameters of AR patients?

1.4 Objectives

1.4.1 General objective

To elucidate the expression levels of ZOs and HDACs genes in nasal epithelial cells of HDM-sensitised AR patients compared to non-allergic controls.

1.4.2 Specific objectives

1. To determine and compare the mRNA expression of TJ genes (*ZO-1*, *ZO-2* and *ZO-3*) in nasal epithelial cells of AR patients and non-allergic controls.
2. To determine and compare the mRNA expression of HDACs (*HDAC1* and *HDAC2*) in nasal epithelial cells of AR patients and non-allergic controls.
3. To examine the association of levels of TJ genes (*ZO-1*, *ZO-2* and *ZO-3*) and HDACs (*HDAC1* and *HDAC2*) in nasal epithelial cells with the demographical, clinical and environmental parameters of AR patients and non-allergic controls.

1.5 Hypothesis

1. The mRNA expression of TJ genes (*ZO-1*, *ZO-2* and *ZO-3*) in nasal epithelial cells are lower in AR patients compared to non-allergic controls.
2. The mRNA expression of HDACs (*HDAC1* and *HDAC2*) are higher in nasal epithelial cells of AR patients compared to controls.
3. There are associations between the levels of TJ genes (*ZO-1*, *ZO-2* and *ZO-3*) and HDACs (*HDAC1* and *HDAC2*) in nasal epithelial cells with certain demographical, clinical and environmental parameters of AR patients and non-allergic controls.

CHAPTER 2

LITERATURE REVIEW

2.1 Human Immune System and Allergy

2.1.1 The Innate and Adaptive Immune System

The immune system refers to a collection of cells, chemicals, and processes that protect the host from infection by foreign antigens, such as microbes, viruses, cancer cells, and toxins that are constantly evolving (Chaplin, 2010; Jean S et al., 2018). The immune system will respond to the invasion of microorganisms or their products into the epithelial cells of the mucosa and skin (Chase & Lunney, 2019). It plays a crucial role in maintaining human health and protecting the human body against the invasion of microorganisms (Abbas et al., 2021).

Innate immunity is the first line of defence against invading pathogens. It is a non-specific defence mechanism and does not have an immunologic memory of the reaction. Thus, it cannot recognise the same pathogen if the host is exposed to the same pathogen in the future. This protection is triggered as soon as an antigen is encountered (Jean S et al., 2018). While adaptive immunity is antigen-dependent, antigen-specific immunity develops through receptor recognition (Jean S et al., 2018; Smith, 2012). It stores memory of the infection which enables a more rapid response for subsequent exposure to the same antigen. However, the immune response will take time to develop upon the first exposure (Jean S et al., 2018). Both innate and adaptive immunity are complementary to each other, and any flaws in either of these defences will make the host vulnerable.

Innate immunity consists of four types of protective barriers: physical, physiologic, endocytic, phagocytic, and inflammatory (Chaplin, 2010; Jean S et al.,

2018). It relies on pattern recognition receptors (PPRs) to identify particular pathogens or pathogens with similar structures (Mogensen, 2009). Innate immunity is responsible for the recruitment of immune cells to infection and inflammation sites through the production of cytokines and chemokines. The release of cytokines will activate many cellular defence mechanisms all over the body (Sokol & Luster, 2015). However, dysregulation of cytokine activity may result in inflammatory or autoimmune disease.

Biochemical activity acts to complete the immune reaction by identifying and coating the pathogens to be ingested by the phagocytes, a process known as phagocytosis. The phagocytosis process will kill some pathogens and infected cells that are present in the tissues, organs, blood, and lymph (Jean S et al., 2018). Phagocytes, mast cells, dendritic cells (DCs), natural killer (NK) cells, basophils, eosinophils, macrophages, and innate lymphoid cells are immune cells participating in the first line of defence (Rivera et al., 2016). Macrophages are also involved in the presentation of antigens to T cells. However, innate immunity still has a limited range of pathogenic molecular patterns that can be recognised. This is due to the variability of pathogenic structures as well as the pathogens' ability to avoid host detection, which has driven the evolution of adaptive immunity (Jean S et al., 2018).

Adaptive immunity will be developed if innate immunity fails to defend against infectious agents or pathogens. The identification of "non-self" antigens, which are dependent on specific receptors, is the primary function of adaptive immunity. This will generate the pathogen-specific immunologic effector pathways to eliminate specific pathogens or pathogen-infected cells, and then the immunologic pathway will be further developed (Bonilla & Oettgen, 2010; Warrington et al., 2011). During the first infection, cells that express these immune receptors can remain in the host, providing immunologic memory to quickly eliminate a specific pathogen in subsequent infections

(Jean S et al., 2018). Adaptive immune responses are the basis for effective immunisation against infectious diseases. Antigen-specific receptors are expressed on the surfaces of T cells and B cells in the adaptive immune system (Chaplin, 2010).

Hematopoietic stem cells will produce T cells in the bone marrow, which then migrate and mature in the thymus. T cells aid in the recognition of infected hosts by expressing a series of specific antigen-binding receptors known as T-cell receptors (TCR) on their membranes (Chaplin, 2010; Jean S et al., 2018). Each T cell expresses a single form of TCR and can proliferate and differentiate rapidly when receiving the appropriate signal. Despite that, T cells require the action of antigen presenting-cells (APCs) such as DCs, macrophages, B cells, fibroblasts, and epithelial cells to help in recognising a specific antigen (Jean S et al., 2018). Major histocompatibility complex (MHC), a group of proteins that are expressed on the surface of the APCs will recognise and bind the antigen fragments to present them to the T cell (Bonilla & Oettgen, 2010; Wieczorek et al., 2017). TCR activation results in the production of cytokines such as interleukin-1 (IL-1, -2, -4, -5), transforming growth factor-beta (TGF-β), interferon-gamma (IFN-γ), and many more (Jean S et al., 2018). These cytokines will regulate the response of the cells involved in immunity and inflammation (Jean S et al., 2018; JL et al., 2000).

2.1.2 Allergy

Allergy can be defined as a hypersensitivity reaction and protective immunity initiated by allergen infiltration (Kay, 2009; Tanno et al., 2016). The immune system can cause excessive immunological and inflammatory responses, resulting in hypersensitivity reactions that are harmful to the human body (Abbas et al., 2021). Type I hypersensitivity is known as an immediate reaction and is characterised by the

overproduction of Immunoglobulin E (IgE) antibodies against the soluble allergen. These IgE will then bind to mast cells causing them to degranulate and release histamine and other inflammatory mediators, resulting in inflammation (Abbas et al., 2021; Vaillant et al., 2021).

There are four types of hypersensitivity reactions: Type I, II, III & IV hypersensitivity reactions. Type I hypersensitivity reaction is always mediated by IgE antibodies that are bound to the surface of mast cells against the soluble antigen. This interaction results in mast cell activation and further degranulate with the release of histamine and other inflammatory mediators (Abbas et al., 2023; T. C. King, 2007; Marwa & Kondamudi, 2023). Type II hypersensitivity is a cytotoxic reaction mediated by Immunoglobulin G (IgG) or Immunoglobulin M (IgM) antibodies. These specific antibodies will bind to antigens, leading to the complement system activation and cell damage or lysis (Abbas et al., 2023; T. C. King, 2007; Marwa & Kondamudi, 2023). Type III hypersensitivity is also known as immune complex injury and involves IgG and IgM antibodies (Mak & Saunders, 2006; Marwa & Kondamudi, 2023). The build-up of these immune complexes through the formation of antigen-antigen complexes that activate complement leads to polymorphonuclear leukocytes (PMNs) chemotaxis and eventually causing tissue damage (Abbas et al., 2023; Marwa & Kondamudi, 2023; Snyder, 2017). Lastly, type IV hypersensitivity typically a delayed reaction involved by cellular response (Marwa & Kondamudi, 2023). It involves an activated T cells that provoke an inflammatory reaction after being exposed to exogenous or endogenous antigens (Marwa & Kondamudi, 2023).

Allergens are foreign proteins that cause the IgE antibody response in humans (Woodfolk et al., 2015; Yoo & Perzanowski, 2014)). These allergens are derived from a variety of sources including hyphae, spores, pollens, molds and foods (Cooper et al.,

2021; Whitby et al., 2022). Atopy is the tendency to become sensitised and lead to IgE antibodies development in human as a response against the environmental allergen exposure (Cockcroft, 2009; Peebles et al., 2012; S. Dua et al., 2023). While, allergy refers to any immune response, hence all atopic reactions are considered as allergic but not all allergic reactions are atopy (S. Dua et al., 2023). Allergen sensitization is a host tendency for the immune system to react against allergens or antigens present in the environment that are ingested, inhaled or absorbed, which leads to the overproduction of IgE (Cooper et al., 2021; Justiz Vaillant et al., 2023; Yoo & Perzanowski, 2014). Allergen sensitization can be measured either by a skin prick test (SPT) or by the detection of specific IgE in blood samples (Cooper et al., 2021). Sensitization rates vary among populations, which it might be influenced by genetic or environmental factors that modify the expression of atopy (Cooper et al., 2021).

Allergies are classified into respiratory, skin and gastrointestinal allergies, depending on how symptoms manifest in allergic people. AR, chronic rhinosinusitis (CRS), and asthma are respiratory allergies manifested in the respiratory tract (Bradley F, 2010; Laulajainen-Hongisto et al., 2020; Rosati & Peters, 2016). Atopic dermatitis (AD) is the most common type of skin allergy (Kostner et al., 2017; Yu et al., 2016). Food allergy is classifies as a gastrointestinal allergy since the allergens enter the bloodstream through the gastrointestinal system (Hideaki et al., 2013; Valenta et al., 2015). These allergic disorders are known to commonly coexist (Bantz et al., 2014). AD is often the primary symptom of atopic disease in infancy or early childhood, followed by food allergy, allergic rhinitis (AR) and asthma (Andrew H et al., 2010; Yu et al., 2016). This demonstrates that allergic disorders are linked to one another.

2.2 Allergic Rhinitis (AR)

2.2.1 Definition and pathophysiology of Allergic Rhinitis (AR)

AR is an IgE-mediated inflammation disorder in the nasal mucosa induced by immune system dysregulation caused by infiltration of allergens and cytokine production imbalance (Laulajainen-Hongisto et al., 2020; Pawankar et al., 2011; Small et al., 2018; Varshney & Varshney, 2015; Wang MS et al., 2020). This is due to the host's exposure to allergens or any foreign organism (London Jr & Ramanathan, 2017; Steelant et al., 2016; Wang MS et al., 2020). Sneezing, rhinorrhoea, nasal blockage, nasal itching, and postnasal drip are all symptoms of AR (Laulajainen-Hongisto et al., 2020; Naclerio et al., 2010). AR may affect both men and women, and it affects people of all ages. AR may have a variety of negative consequences in one's life, including lowering quality of life, impacting mood and daily activities, reducing academic performance, restricting social interaction, and increasing financial costs (Elango, 2005; Léger et al., 2006; Roger et al., 2016).

AR was traditionally categorised as seasonal AR (SAR) or perennial AR (PAR) based on the allergen that causes the symptoms (G. Ciprandi et al., 2005; Dhong, 2013). Pollen allergens are the most common cause of SAR, which affects patients during certain seasons, while animal dander or HDM is the most common cause of PAR, which affects patients all year (Beard, 2014; Small et al., 2018). In Malaysia, the most common HDM allergens include *D. pteronyssinus*, *D. farinae* and *B. tropicalis* (Majdiah et al., 2011; Mariana et al., 2000; Sani et al., 2019). It had also been observed that cat dander is another major aeroallergen that causes AR among adults and children in Malaysia (Asha'ari et al., 2010; Gendeh et al., 2004). Furthermore, a research in Japan's elementary school found that 51% of children were sensitised to HDM and 39% were

sensitised to Japanese cedar pollen (Yamazaki et al., 2015). According to studies in Malaysia, food allergens especially shellfish such as shrimp and crab, also induce allergic sensitization in AR patients (Gendeh et al., 2000, 2004; Wan Majdiah et al., 2016).

According to the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines, AR is then divided into intermittent or persistent and mild or moderate-severe (Figure 2.1) (Dhong, 2013). Thus, AR is classified as intermittent mild, intermittent moderate-severe, persistent mild or persistent moderate-severe.

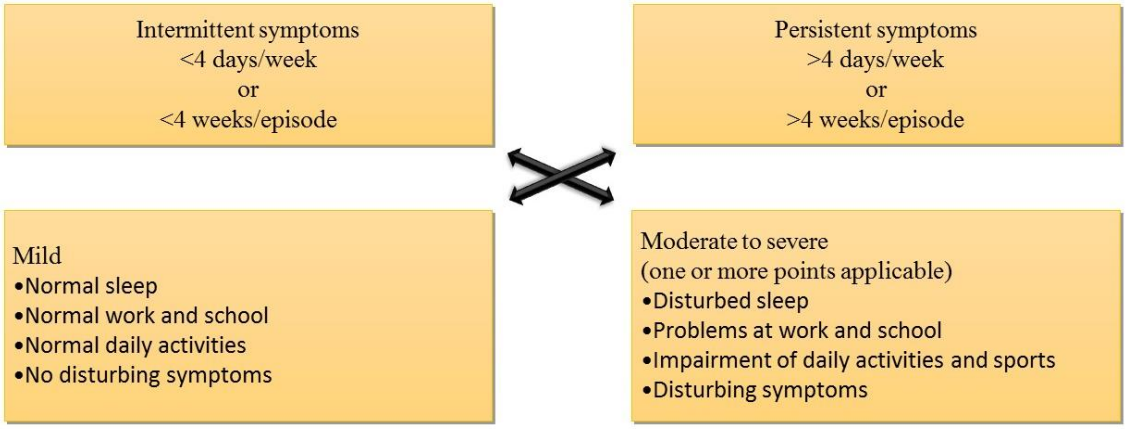

Figure 2.1 Summary of AR severity and classification symptoms based on ARIA guidelines (Dhong, 2013).

The pathogenesis of AR begins with allergen sensitization. Allergen infiltration will induce the production of IgE antibodies and trigger the humoral response (Akuthota & Weller, 2012; M. Han et al., 2021). The invaded allergens are processed by APCs such as DCs at a mucosal site, leading to the activation of naive $CD4^+$ T cells to become T helper 2 (Th2) cells (M. Han et al., 2021; Y.-Y. Han et al., 2016; Watts et al., 2019; Wheatley & Togias, 2015). Simultaneous activation of epithelial cells by non-antigenic pathways (*i.e.* proteases) can lead to the release of epithelial cytokines such as thymic stromal lymphopoietin (TSLP), IL-25, and IL-33, which can polarise the sensitization

process into a Th2 cell response. This induces Th2 cells to secrete cytokines such as IL-4 and IL-13 (M. Han et al., 2021; Wheatley & Togias, 2015). This process drives the B cells into allergen-specific IgE-producing plasma cells, which then produce IgE antibodies (Wheatley & Togias, 2015). These IgE antibodies will bind to mast cells and circulating basophils (M. Han et al., 2021).

Upon re-exposure, the allergen binds to these IgE antibodies on mast cells and basophils in the nasal mucosa (M. Han et al., 2021; Watts et al., 2019; Wheatley & Togias, 2015). In the early phase, the interaction will trigger a cascade of reactions in which mast cells and basophils release mediators such as histamine, chemokines, cytokines, and adhesion molecules that encourage increased leukocyte production in the bone marrow (Watts et al., 2019; Wheatley & Togias, 2015). Typical AR symptoms like nasal congestion, rhinorrhoea or itching are produced by the substances that act on the vessels and glands of the nose (M. Han et al., 2021).

Late phase happened after four to six hours of allergen exposure, local activation of Th2 lymphocytes by DCs results in the release of chemokines and cytokines that orchestrate the influx of inflammatory cells such as eosinophils, basophils, neutrophils, T cells, and B cells to the mucosa, providing more allergen targets (Wheatley & Togias, 2015). Th2 inflammation renders the mucosa more sensitive to allergens as well as environmental irritants. Furthermore, allergen exposure further stimulates IgE production (Wheatley & Togias, 2015). In a series of time-dependent phases, these effector cell types, mediators, and cell signalling molecules work in a complex network of interactions that results in specific symptoms and the inflammatory morphology of AR (Watts et al., 2019). The pathophysiology of AR is presented in Figure 2.2.

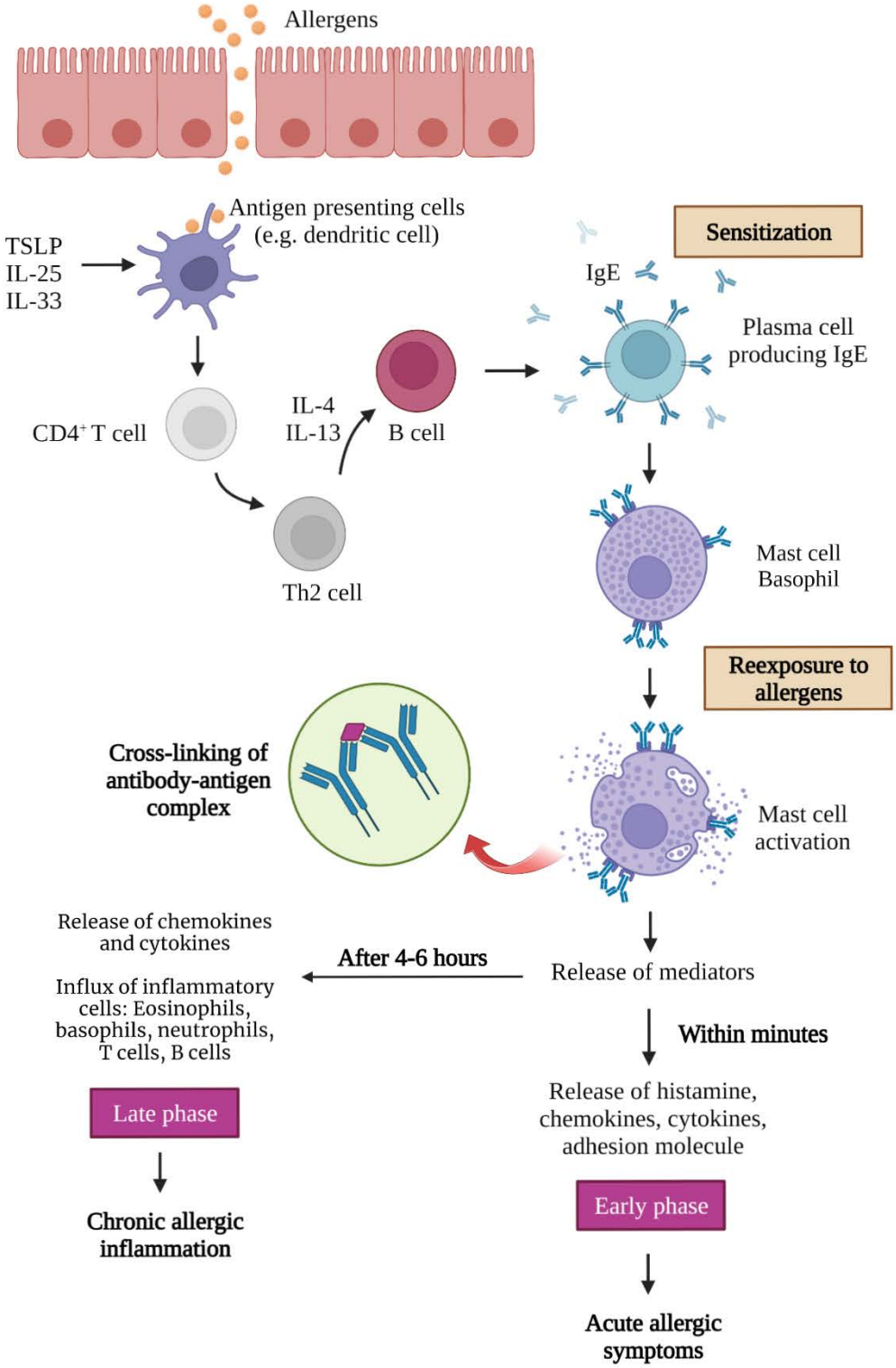

Figure 2.2 Pathophysiology of AR. Created with BioRender.com

2.2.2 Epidemiology of Allergic Rhinitis (AR)

AR is the most common of all allergic diseases, affecting around 10% to 40% of the population worldwide (Brożek et al., 2017). AR prevalence in the Western population is believed to be 30% (Wang MS et al., 2020). It has been estimated that 25% of children and 40% of adults worldwide suffer from AR (Brożek et al., 2017). The prevalence of AR in Malaysia has been reported to be 7.1% (Katelaris et al., 2011). A study conducted in Kota Kinabalu, Malaysia, found that 11.2% of Chinese between the ages of 12 and 20 were diagnosed with AR (Katelaris et al., 2012). According to a survey conducted across the Asia-Pacific regions, 2.5% in Philippines and 13.2% in Australia had a physician diagnosis of AR. The average age of a doctor's diagnosis of AR in adults was 26 years and 9 years for the children included in the survey (Katelaris et al., 2011).

In the United States, allergic illness is the third most prevalent chronic disease among children and adolescents (Galli et al., 2008). The prevalence of AR in the United States ranges from 9% to 42%, which is approximately 58 million of people (Settipane & Charnock, 2007). In the United Kingdom, the prevalence reaches 26% in adults, with an observed peak in the third and fourth decades of age (Bauchau & Durham, 2004; Eriksson et al., 2012). AR affects a huge population in Asia, ranging from 27% in South Korea (Chong & Chew, 2018) to 32% in the United Arab Emirates (Alsowaidi et al., 2010). There are differences between countries, possibly due to differences in allergens and immune reactions.

2.2.3 Predisposing factors of Allergic Rhinitis (AR)

AR is a disease that is commonly found in all age groups from childhood to adolescence to late adulthood (Blomme et al., 2013; Brożek et al., 2017; Eriksson et al., 2011). The prevalence of AR has been found to peak between the ages of 16 and 24 years old and it significantly decreased in the 65-84 age group in both men and women (Cazzoletti et al., 2015; Droste et al., 1996; Olivieri et al., 2002; Pakkasela et al., 2020). A systematic review and meta-analysis on AR found that the prevalence of AR in adults was not sex-specific (Pinart et al., 2017). However, there is a male predominance of AR in childhood, but females are more affected after the age of fifteen (Ford et al., 2003; Jensen-Jarolim & Untersmayr, 2008; Keil et al., 2010). Findings from a survey among university students in Turkey with a mean age of 20.71 ± 3.12 years showed that female students had a 42.8% higher risk of having AR than male students (Kef & Güven, 2020). These findings indicate that gender may influence the occurrence of AR in different age groups.

It is known that the increasing prevalence of asthma and allergies in recent years has been associated with the increasing prevalence of obesity (Noal et al., 2011; Peters et al., 2018). In the United States, a cross-sectional study of 5218 adults showed that being overweight or obese was associated with an increased risk of having AR (Gogna et al., 2015). Studies demonstrated that leptin, which is an adipokine and a fat-related hormone, was associated with allergen exposure and the severity of AR in AR patients (Giorgio Ciprandi et al., 2009; Hsueh et al., 2009).

Cigarette smoke exposure reportedly worsens allergic airway inflammation (H. Mehta et al., 2008). Previous studies revealed that cigarette smoke can exacerbate nasal allergic responses while increasing the level of serum IgE and the production of IL-5 (Y. S. Kim et al., 2017; Oryszczyn et al., 2000; Saulyte et al., 2014). In addition,

cigarette smoke directly affects the epithelial cells and results in increased permeability, the release of cytokines and chemokines and disturbed lymphocyte balance towards Th2 cells (Strzelak et al., 2018)

2.2.4 Clinical symptoms of Allergic Rhinitis (AR)

When the nasal mucosa is exposed to allergens, this will result in the production of IgE, activation of eosinophils and degranulation of mast cells and basophils, which will induce clinical symptoms (Meng et al., 2019). This is caused by the secretion of histamine, leukotrienes and prostaglandins by mast cells (Min, 2010). These nasal symptoms include sneezing, watery anterior rhinorrhoea, nasal itching, nasal obstruction and postnasal drip (London Jr & Ramanathan, 2017; Min, 2010; Naclerio et al., 2010). Additionally, AR patients often have non-nasal symptoms, including eye symptoms, ear symptoms, throat symptoms, chronic cough, headache and mental function (cognitive) impairment (Brożek et al., 2017; Spector et al., 2003). The assessment of these symptoms is used to diagnose the severity of AR.

2.2.5 Laboratory characteristics

Typically, the presence of total IgE in the serum supports the diagnosis of AR. It is supported by a previous study that proved AR patients had higher total IgE levels compared to non-allergic individuals (H.-J. Lee et al., 2016; Shirasaki et al., 2011). Multiple tests, including *in vivo* skin tests such as SPT and intradermal tests (IDT) and *in vitro* serum-specific immunoglobulin E (ssIgE) assays, can be used to determine the specific allergen that causes the development of IgE antibodies in AR (Ansotegui et al., 2020; Wongpiyabovorn et al., 2018). SPT and ssIgE assays are the most common

diagnostic approaches and have a strong correlation with specificity and sensitivity in the diagnosis of sensitization to common allergens (Nam & Lee, 2017; Wongpiyabovorn et al., 2018).

SPT remains the first-line approach for diagnosing allergic sensitivities (Ansotegui et al., 2020). This method is the most frequently used and effective diagnostic test for IgE antibody detection due to its rapidity, simplicity, and low cost (Ansotegui et al., 2020; Bousquet et al., 2012). This test is performed by introducing a specific allergen into the skin of allergic individuals using a lancet. Cross-linking of allergen-specific IgE bound to their membrane receptors causes dermal mast cells to begin to degranulate (Ansotegui et al., 2020). Degranulation causes the immediate release of histamines and other mediators, inducing a response that is clinically characterised by a wheal and flare that can be measured to determine the degree of cutaneous sensitivity (Ansotegui et al., 2020).

In vitro laboratory tests for ssIgE antibodies are performed using commercially available test panels, which are more costly than SPT (Bousquet et al., 2012; Bulat Lokas et al., 2017). The presence of IgE antibodies capable of binding an extract allergen or molecular component is detected using the ssIgE test (Ansotegui et al., 2020). Furthermore, ssIgE assay can be useful for patients when skin testing is not available or cannot be performed due to extensive skin disease or other issues that complicate skin testing (Cox et al., 2011; Mansfield et al., 2012). Also, ssIgE assays are not affected by antihistamine drugs, and this can be useful for patients who are unable to stop medication (Ansotegui et al., 2020).

Other tests to consider include IDT, which can detect IgE-mediated allergies (Ansotegui et al., 2020). In IDT, a small amount of allergen is injected intradermally with a needle to form a small bleb, and the outcome measure is an increase in the size

of the wheal with a flare reaction after 20 minutes (Ansotegui et al., 2020; Tanno et al., 2016). In addition, the basophil activation test (BAT) can be used in cases where *in vitro* and *in vivo* tests yield negative results, as basophils are recognised as important effector cells in the immediate hypersensitivity response (Ansotegui et al., 2020). The degranulation of basophils can be detected and quantified by flow cytometry (Ansotegui et al., 2020; Bridts et al., 2014).

2.2.6 Diagnostic criteria of Allergic Rhinitis (AR)

Diagnostic criteria are a set of signs, symptoms, and tests for use in routine clinical care of patients. They are generally broad and must reflect the different characteristics of a disease (heterogeneity) in order to accurately identify as many people as possible who have the condition (Aggarwal et al., 2015). Diagnostic criteria are difficult to establish because there are no gold standards for diagnosing AR (Aggarwal et al., 2015).

However, AR patients usually have clinical symptoms and possess laboratory characteristics as described in the previous section. AR patients must encounter two or more of the clinical symptoms for more than one hour per day, which are watery rhinorrhoea, sneezing, nasal obstruction, or nasal itching (Bousquet et al., 2020; Okubo et al., 2020). Pollen-sensitised AR individuals may present with oral symptoms of pollen food syndrome, such as itchy mouth and throat after ingestion of food (Bousquet et al., 2020). Following the presentation of clinical symptoms of AR, allergy laboratory tests can be conducted for confirmation.

2.2.7 Treatment of Allergic Rhinitis (AR)

Current therapeutic strategies include exposure controls, pharmacotherapy (intranasal corticosteroids and antihistamines), and immunotherapy (subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT)). Exposure controls by using mite-proof bedding covers have been shown to reduce HDM exposure, but no significant improvement in the clinical symptoms of AR patients was observed (Terreehorst et al., 2003). Several studies showed that the major cat allergen, FeI d 1, was found in the floor dust at homes (Custis et al., 2003; Fahlbusch et al., 2002) and hospitals (Custovic et al., 1998) even in the absence of cats in both places. These observations suggest that not having a pet was not effective in managing AR. Therefore, standard exposure controls alone were no longer considered the cornerstone in the management of most cases of AR (Solelhac & Charpin, 2014).

The common pharmacotherapy for AR patients is intranasal corticosteroids and antihistamines. Intranasal corticosteroids commercially available to treat AR in adults and children are beclomethasone dipropionate, fluticasone propionate, flunisolide, budesonide, triamcinolone acetonide, and mometasone furoate (Braido et al., 2008). Intranasal corticosteroids have been proven to improve the symptoms of AR by reducing nasal mucosa hyperactivity through the anti-inflammatory action exerted by these medications (Bousquet, Khaltaev, Cruz, Denburg, Fokkens, Togias, Zuberbier, Baena-Cagnani, Walter, et al., 2008).

Next, antihistamines targeting the histamine H_1 receptor play an important role in improving and maintaining the quality of life of AR patients. Antihistamines such as loratadine, fexofenadine, rupatadine, levocetirizine, desloratadine, and bilastine are widely used in treating AR (Kawauchi et al., 2019). These drugs have been shown to

rapidly reduce the nasal and ocular symptoms of AR with an improvement in the quality of life in most AR patients (Demoly et al., 2014).

Those patients who do not have an adequate response may also consider immunotherapy as an effective treatment option (Lin et al., 2013; London Jr & Ramanathan, 2017). SCIT is a process of repeated doses of a specific, relevant allergen. The conventional SCIT protocol, which uses unmodified allergen extracts, involves a weekly dose build-up via subcutaneous injections, followed by maintenance doses every 4 or 8 weeks (Tsabouri et al., 2017). Fewer build-up doses are possible with the use of modified allergenic extracts or adjuvants (Tsabouri et al., 2017), which are substances or compounds that are co-administered with allergen extracts and have the ability to modulate the immune response (Zubeldia et al., 2018). SCIT has been shown to be highly effective for treating SAR, as significant reductions in symptom scores have been observed in SAR patients (Hagen et al., 2010). It has also been observed to be effective and safe to use in PAR adults and children with HDM sensitization (Y. Huang et al., 2019).

SLIT is one of the immunotherapy treatments that has gained interest in the US. This treatment involves placement of the allergen under the tongue for local absorption to desensitise the allergic individual over an extended treatment period to diminish allergic symptoms (Lin et al., 2013). SLIT is easy to administer, does not involve the administration of injections, and may be administered at home (Lin et al., 2013). SLIT is also safe and effective to treat SAR (Canonica et al., 2014) but its efficacy in treating HDM allergy in PAR patients, especially in children, is less convincing (Calderon et al., 2013).

2.3 Nasal Epithelial Barrier in Allergic Rhinitis (AR)

The nasal epithelial barrier plays an important role in both innate and adaptive mucosal immunity through the activation of functional molecules (e.g. pro-inflammatory cytokines, chemokines and growth factors) (Toppila-Salmi et al., 2015). This epithelial barrier functions by sealing the nasal passage and underlying tissue from any invading foreign pathogens as well as restricting the intercellular passage of fluid by connecting the epithelial cells to each other (London Jr & Ramanathan, 2017; Steelant et al., 2016). Airway epithelial cells also respond to inhaled pathogens by the producing nasal mucus, in which these pathogens are trapped in the mucus and transported through mucociliary clearance conducted by beating ciliated epithelium (Bergougnan et al., 2020; Tomazic et al., 2020).

The physical barrier consists of different junctional complexes that connect the epithelial cells to each other (Steelant et al., 2017). TJs are the most apically located epithelial junctions, which are composed of transmembrane proteins such as claudins, occludin, junctional adhesion molecules (JAMs), and intracellular proteins, such as ZO-1, ZO-2, ZO-3, and tricellulin as well as C-interacting proteins to seal off the paracellular space between the epithelial cells (London Jr & Ramanathan, 2017; Steelant et al., 2017). Adherens junctions (AJs) are composed of E-cadherin and the cytoplasmic p120-, β- and α-catenin (Hartsock & Nelson, 2008; Steelant et al., 2017). They form an apical junctional complex together with TJs, which controls epithelial cell-to-cell contact, actin cytoskeleton regulation, intracellular signalling pathways and transcriptional regulation (Steelant et al., 2017). Desmosomes (DM) are found beneath the apical junctional complex, which is specialised for strong adhesion (Garrod & Chidgey, 2008; Steelant et al., 2017). These cell junctions work together to form the intracellular connection between the cells to limit the passage of foreign molecules and

protect the underlying tissue from exposure to harmful and allergenic stimuli (London Jr & Ramanathan, 2017; Steelant et al., 2016). The structure of the epithelial barrier is illustrated in Figure 2.3.

Dysfunction of these epithelial barriers may contribute to allergic disease through the increasing exposure of the underlying tissue to the environmental allergens (London Jr & Ramanathan, 2017). This has been linked to multiple chronic inflammatory diseases such as AD, asthma, inflammatory bowel disease, CRS and as well as AR (De Boer et al., 2008; London Jr & Ramanathan, 2017; Soyka et al., 2012). For example, single nucleotide polymorphisms in claudin-1, E-cadherin, and protocadherin-1 have been linked to asthma, AD, and Crohn disease (De Benedetto et al., 2011; Mortensen et al., 2014; Muise et al., 2009). Decreased barrier function and decreased expression of ZO-1, claudin-1, and occludin have been reported in biopsy specimens from patients with CRS with nasal polyps (Rogers et al., 2011; Soyka et al., 2012).

Screening studies using microarray gene expression, RNA-seq, and nasal mucus proteomics have suggested a role for barrier dysfunction in AR (Steelant et al., 2016; Tatsuta et al., 2019; Wang MS et al., 2020; Zhao et al., 2018). Multiple studies have found decreased epithelial cell junction protein expression in AR (Steelant et al., 2016; Wang MS et al., 2020). Immunohistochemical and immunoblotting results reported that there was a decrease in E-cadherin expression in the nasal epithelium of patients with AR (H.-J. Lee et al., 2016). Steelant and colleagues reported decreased ZO-1 and occludin expression as measured by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and weak immunofluorescence staining in AR biopsy specimens (Steelant et al., 2016). As a result of the breakdown of these TJs, inflammatory cells may influx into the lumen, causing tissue injury or inflammation (Soyka et al., 2012).

There are two hypotheses regarding the destabilisation of these TJs: 1) Direct proteolysis or 2) Disruption by inflammatory cytokines-mediated (Figure 2.2) (London Jr & Ramanathan, 2017). Invading allergens, such as HDM allergens, contain protease, which is capable of disrupting the TJ molecules (Okano, 2009; Roche et al., 2000; Hong Wan et al., 1999). Proteolytic activity can be explained by the action of the HDM cysteine proteinase antigen in *D. pteronyssinus* (Hong Wan et al., 1999). *D. pteronyssinus* has been reported to cleave extracellular domain sites in occludin and in claudin-1, resulting in increased epithelial permeability and *D. pteronyssinus* transit through the epithelial barrier (Hong Wan et al., 1999). Furthermore, *D. pteronyssinus* has been shown to cause a time-dependent breakdown of TJ as well as ZO-1 mislocalization from TJ (H Wan et al., 2000).

The high allergenicity of HDM proteases is also associated with their ability to mediate Th2-biassed immune responses by reducing T helper 1 (Th1) polarisation (Chapman et al., 2007; Schulz et al., 1998). For example, *D. pteronyssinus* promotes Th2-biassed proliferation by deconstructing the IL-2 receptor, which is involved in mediating Th1 proliferation (Schulz et al., 1998). In addition, the proteolytic activity of *D. pteronyssinus* promoted DC activation and induced Th2 polarisation via reduced release of IL-12, a pivotal cytokine in Th1 polarisation and differentiation (Ghaemmaghami et al., 2002). DCs that mature in the presence of proteolytically active *D. pteronyssinus* reduce the release of INF-γ, but increase the production of IL-4 by $CD4^+$ T cells, thus promoting Th2 polarisation (Ghaemmaghami et al., 2002). Inhibition of the protease activity of *D. pteronyssinus* as a therapeutic strategy for reducing HDM-induced barrier dysfunction has been reported (John et al., 2000).

IL-33 is one of the inflammatory cytokines that have been implicated in AR pathogenesis and is constitutively expressed and localised in the nucleus of nasal

epithelial cells. IL-33 is known to induce Th2 cytokine production in Th2 cells, eosinophils, and mast cells (Haenuki et al., 2012). Increased IL-33 expression has been found in the serum of SAR patients in Japan and revealed the association between IL-33 and AR (Sakashita et al., 2008). Using the ragweed pollen-induced murine model, IL-33 was constitutively expressed in nasal epithelial cells and showed an accumulation of eosinophils and basophils (Haenuki et al., 2012). In addition, IL-33 levels were increased in the sinus mucosa and significantly correlated with the total nasal symptom score in HDM-sensitised AR patients (Asaka et al., 2012). Intranasal administration of IL-33 showed decreased expression of occludin and ZO-1 in control mice (Sugita et al., 2018).

In addition, IL-13 and IL-4 stimulation increased the expression of transient receptor potential vanilloid 4 (TRPV4) channels in cultured normal epithelial cells (K. Lee et al., 2020). TRVP4 is a calcium-permeable channel found in respiratory epithelial cells (Cao et al., 2018; K. Lee et al., 2020). TRVP4 expression was found to be higher in AR patients compared to the healthy control group (K. Lee et al., 2020). Der p 1 reduced the expression of ZO-1 and E-cadherin after 24 h of stimulation in the presence of the TRVP4 agonist GSK1016790A. This indicated that the destruction of the epithelial barrier because of HDMs mediated by TRPV4 activation may be aggravated by increased TRVP4 in AR patients (K. Lee et al., 2020).

Particulate matter (PM) exposure may exacerbate the breakdown of the epithelial barrier in AR (Zhao et al., 2018). PM contains redox-active chemicals and transition metals that may exert their disruptive effects through the generation of reactive oxygen species (London et al., 2016). Human nasal epithelial cells grown at an air-liquid interface were exposed to $PM_{2.5}$ to observe if it could cause epithelial barrier dysfunction *in vitro*. There was a significant decrease in barrier function, as reflected