CHAPTER – 1
INTRODUCTION, REVIEW OF LITERATURE & OBJECTIVES OF THE STUDY
1.1 INTRODUCTION

Asthma is a chronic disorder, characterized by recurrent symptoms of wheeze, breathlessness, chest tightness and cough, often associated with bronchial hyperresponsiveness (BHR), variable airflow limitation and chronic inflammation of the airways (Johansson et al., 2004). It usually begins in childhood, often in association with an inherited susceptibility to produce IgE to common environmental allergens, including house dustmite, animal protein, fungal spores, and pollens (Boushey, 1998). It is estimated that up to 80% of children with asthma may be atopic. Atopy is a personal or familial tendency to become sensitized and produce IgE antibodies in response to common environmental allergen exposures (Johansson et al., 2004). Approximately, 300 million people are affected worldwide causing 250,000 annual deaths by this airway disease (Bateman et al., 2008). Asthma reduces the quality of life for the affected individual, and places a burden on society as a whole due to elevated health care costs and decreased productivity of asthmatic individuals. The burden of asthma is heavy not only for the individual and the family but also for society (Cleemput and Kesteloot, 2002; von Mutius, 2000). Asthma can place considerable limitations on the physical, emotional, social, and professional lives of sufferers, and these may be greater when symptoms are not adequately controlled. Children can become very distressed by their disease, with considerable absences from school and reduced participation in family life.

The cardinal symptom of asthma is recurrent wheezing, but all that wheezes are not asthma and not all asthma wheezes. According to the National Heart, Lung, and Blood Institute (NHLBI) expert panel report, a history of recurrent wheezing, cough, breathlessness, or chest tightness suggests a possible diagnosis of asthma (NHLBI, 1997). Taking a good history of symptoms, precipitating factors, and development of the disease and its prior response to treatment are extremely important in understanding asthma. Several other demographic and environmental risk factors for asthma have been identified. Low socioeconomic status and non-white race have been linked to increased asthma prevalence. Male gender is a risk factor for asthma in early childhood. Viral infections of the lower respiratory tract resulting in wheezing during infancy, in particular respiratory syncytial virus, significantly increase the risk of developing asthma. Exposure to environmental
factors such as tobacco smoke, dust, or cockroaches plays a key role in the development of asthma. Prenatal exposure to environmental tobacco smoke has also been associated with recurrent wheezing and a physician diagnosis of asthma in young children, however, this may represent transient wheezing, as the association does not persist (Taussig et al., 2003; Burrows et al., 1989; Lannero et al., 2006). Weather change is also a commonly reported precipitating factor of the symptoms of asthma.

The diagnosis of asthma in childhood is primarily based on frequency, quality, and severity of symptoms in addition to family history and other allergic co-morbidities. Response to therapy can be especially helpful as a diagnostic tool in younger children where pulmonary function testing can be a challenge. In a school-aged child, the diagnosis of asthma is accomplished by obtaining pertinent information regarding type, frequency, and severity of symptoms in addition to determining the presence of risk factors, such as a parent with asthma or the coexistence of atopic dermatitis. Additionally, airflow limitation that improves following bronchodilator in a child with lower respiratory symptoms strongly supports the diagnosis of asthma. Optimal treatment of asthma requires an understanding of the central concept of asthma control and how this is used to modify treatment. Environmental control and intermittent reliever therapy is all that is necessary for intermittent asthma. Persistent asthma requires regular anti-inflammatory controller therapy. Inadequate adherence and poor technique are more usual causes for treatment failure than incorrect drug selection (Levin and Weinberg, 2011).

It is well known that the prevalence of asthma has been reported to increase in many places around the world during the last decades (Manning et al., 2007). The causes of asthma and why asthma seems to have increased is still not well understood. The increase in asthma prevalence has been suggested in some way to be related to western lifestyle factors, as most often increased prevalence rates are reported from westernized countries (Beasley et al., 2000; Britton, 2003). In the developing countries although the prevalence of childhood asthma is reported to be lower, there is growing evidence to suggest that the prevalence is increasing alarmingly as it did in the western countries over 2-3 decades ago. The International Study of Asthma and Allergy in Childhood (ISAAC) has shown that the prevalence of asthma and atopy in children from affluent countries is higher than in low-
income countries (Asher et al., 2006). The prevalence is variable in different regions and countries in the world. Aït-Khaled et al. (2007) described the prevalence of a wide range of atopic disorders throughout Africa. The highest prevalence of current asthma was observed in urban areas with a higher standard of living, but asthma also had a representative prevalence in endemic parasite and tuberculosis zones. In Latin America, the prevalence of asthma and allergic diseases in childhood is similar to that in industrialized countries, although great variability has been found. In a recent survey in Asia, a 16.1% prevalence of wheezing in the previous 12 months was found in rural children from Bangladesh; similar percentages were reported in other developing regions (www.isaac.auckland.ac.nz). In India, an investigation by Jain et al. (2010) in a cross sectional community based study on rural Indian children showed the prevalence of bronchial asthma to be 10.3%. Taken all together, the evidence shows the prevalence of asthma is high and is still increasing, mainly in developing countries, although a slightly upward trend has been also shown in high income countries (Pearce et al., 2007).

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role, in particular, mast cells, eosinophils, T lymphocytes, neutrophils, and epithelial cells. Jousilahti et al. (2002) showed the association of sensitive systemic inflammation markers (CRP, serum amyloid-A [SAA], and plasma fibrinogen) with asthma. That supports a hypothesis of persistent systemic inflammation in asthma in parallel with local inflammation. Several studies have shown the association of asthma with airway inflammation in which eosinophils and mast cells play an important role. The recent studies employing bronchoalveolar lavage in infants and young children have provided the supportive evidence of increased eosinophils in the lavage fluid of allergic asthmatics. Eosinophils are at relatively low levels in infants younger than 30 months. Furthermore, increased neutrophil numbers have been found in lavage fluid in children with asthma of greater persistence and longer duration. Inflammatory changes mainly affect the airway mucous membrane lining. In addition to the inflammatory changes, goblet cells in the mucous membrane produce increased amounts of thick sticky mucus. A third component influencing airway narrowing is smooth muscle contraction of the bronchial wall, leading to bronchospasm and bronchoconstriction. Airway inflammation is
responsible for the characteristic feature of bronchial hyperreactivity, making the child with asthma vulnerable to weather changes, humidity, cold air, mist, non-specific irritants, and exercise-induced bronchospasm (Levin and Weinberg, 2011).

C-reactive protein (CRP) is one of the most characteristic markers of the inflammatory process. The monitoring of CRP levels is a good diagnostic tool and is very useful for the assessment of early inflammation, treatment monitoring and acute-phase diseases (Tall, 2004). In recent years, there have been some reports concerning the measurement of serum levels of hs-CRP as a useful tool for detecting systemic inflammation in asthma (Takemura et al., 2006; Fujita et al., 2007). Several studies have indicated a positive correlation between asthma and increased CRP levels (Jousilahti et al., 2002; Ford, 2003; Olafsdottir et al., 2005).

Allergic diseases including asthma are characterized by an increase of serum IgE levels (Peng, 2009; Rage et al., 2009). IgE plays a central role in the initiation and the propagation of the inflammatory cascade and thus the allergic response (Buhl, 2005). Exposure to environmental factors, particularly inhalant allergens is commonly reported as a precipitant of acute exacerbations of asthma (Bacharier et al., 2003). IgE is implicated in airway inflammation and allergic reactions and may play a role in modulating the severity of asthma, because previous studies have found associations between high IgE levels and asthma severity, airway hyperresponsiveness, and lower baseline lung function (Naqvi et al., 2007).

Cytokines play a critical role in the orchestration of chronic inflammation in many diseases, including asthma. Multiple cytokines and chemokines have been implicated in the pathophysiology of asthma (Barnes et al., 1998; Chung and Barnes, 1999). With airway hyper-responsiveness being the physiological hallmark of asthma, it is also characterized by chronic inflammation of the respiratory tract, allergen-specific IgE production, infiltration of eosinophils, the recruitment of T cells into the airways, and alterations in the fine balance between type 1 helper T lymphocytes (Th1) and type 2 helper T lymphocytes (Th2) responses towards Th2 bias (Larche et al., 2003). Th2 cells secret a panel of cytokines with several overlapping functions including Interleukin (IL)-4,
IL-5, IL-13, and granulocyte-macrophage colony stimulating factor (GM-CSF). By mediating differentiation of the Th2 subpopulation and eosinophils, as well as modulating B-cell proliferation and IgE switching, the Th2 cytokines are thought to play a prominent role in asthma (Robinson et al., 1992; Wills-Karp and Finkelman, 2008). The sentinel Th1 cytokine, interferon gamma (IFN-γ) and IL-12 reciprocally stimulate their production and function during cell-mediated immunity and development of naive T lymphocytes into Th1 cells. Evidence suggests a contributory role of Th1 cells and their cytokines in asthmatic inflammation and airway hyperresponsiveness (Cooper and Khader, 2007; Kumar et al., 2006).

Asthma and allergy are complex conditions often present in the same family or closely related subjects. Genetic factors undoubtedly contribute to disease susceptibility but the expression of the disease can be modulated by environmental exposures and the interactions between the two. Candidate-gene and linkage studies followed by positional cloning have already provided a large number of susceptibility genes (Vercelli, 2008a). The last decade has been marked by the publication of more than 20 genome-wide association studies (GWASs) in asthma or allergy phenotypes. GWASs have reported novel and interesting genes but have also confirmed the role of some functionally relevant genes previously described. However, heritability of allergic diseases has not been elucidated completely so far (Kabesch, 2010).

The HLA genes map on chromosome 6p21 play an important role in the regulation of the immune system (Shiina et al., 2004). Many studies have documented that 6p21 region is strongly linked to atopic phenotype and asthma and it is considered a major locus influencing allergic diseases (Cookson, 2004; Moffatt et al., 2003; Hakonarson and Wjst, 2001). Numerous studies have investigated the association of HLA alleles and/or haplotypes with asthma. Some of the earlier studies have reported the association of various HLA class I alleles/haplotypes (Turton et al., 1979; Morris et al., 1980; Huang et al., 1981; Bondarenko et al., 1991; Blumenthal et al., 1992; Kim et al., 2006), while large number of studies have investigated the association of HLA class II alleles/haplotypes (Soriano et al., 1997; Lara-Marquez et al., 1999; Guo et al, 2001; Woszczek et al., 2002;
Torío et al., 2003; Movahedi et al., 2008; Hanchard et al., 2010) with childhood asthma in different populations.

Childhood asthma was associated with the HLA-DP locus (HLA-DPA1 and HLA-DPB1) in Japanese and Korean populations (Noguchi et al., 2011). In that study, modest associations were shown for the 17q21 locus containing ORMDL3/GSDMB/GSDMA and 5q31 (IL5/RAD50/IL13), whereas there were no associations with PDE4D, DENND1B, IL18R1, and IL2RB (Binia and Kabesch, 2012). GWAS in Asian populations also confirmed genetic heterogeneity between children and adults. The largest GWAS so far published on an Asian population identified the most significant associations between adult asthma and the major histocompatibility complex region (Hirota et al., 2011).
1.2. REVIEW OF LITERATURE

1.2.1 Definition of asthma

Asthma is a common chronic disorder of the airways that is complex and characterized by variable and recurring symptoms, airflow obstruction, bronchial hyperresponsiveness, and an underlying inflammation. The interaction of these features determines the clinical manifestation and severity of asthma and the response to treatment (NHLBI, 2007).

1.2.2 Childhood Asthma

Asthma is the most common chronic disease of childhood and the leading cause of childhood morbidity from chronic disease as measured by school absences, emergency department visits, and hospitalizations (Masoli et al., 2004). Asthma typically begins in early childhood, with an earlier onset in males than females (Bisgaard and Szefler, 2007; Kuehni et al., 2007). Atopy is present in the majority of children with asthma over the age of 3, and allergen-specific sensitization is one of the most important risk factors for the development of asthma (Sly et al., 2008).

1.2.3 Symptoms of asthma

Wheeze, Cough, breathlessness (typically manifested by patterns of activity limitation) and nocturnal symptoms/awakenings are the common symptoms of asthma.

1.2.3.1 Wheeze

Wheeze is the most common symptom associated with asthma in children. It has been strictly defined as a continuous high-pitched sound, sometimes with musical quality, emitting from the chest during expiration (Elphick et al., 2001). Wheezing occurs in several different patterns but a wheeze that occurs recurrently, during sleep, or with triggers such as activity, laughing, or crying is consistent with a diagnosis of asthma.
1.2.3.2 Cough

Cough due to asthma is recurrent and/or persistent, and is usually accompanied by some wheezing episodes and breathing difficulties. Nocturnal cough (occurring when the child is asleep) or cough occurring with exercise, laughing, or crying in the absence of an apparent respiratory infection, strongly supports a diagnosis of asthma.

1.2.3.3 Breathlessness

Breathlessness that occurs during exercise and is recurrent increases the likelihood of the presentation being due to asthma. In infants and toddlers, crying and laughing are an exercise equivalent (GINA, 2009).

1.2.4 Risk Factors of Asthma in Children

The risk factors for the development of asthma in children can be divided as allergic and nonallergic environmental triggers.

1.2.4.1 Allergic Environmental Triggers

The allergic environmental triggers for the development of asthma in children include, house dust mites, Cockroaches, animal allergens and indoor fungi.

1.2.4.1.1 House Dust Mites

The house dust mites are the predominant indoor allergens. Their bodies and feces are the sources of indoor allergen. House dust mites infest fabrics, including mattresses, bedding, rugs, upholstered furniture, and carpets (Arlian and Platts-Mills, 2001). It is estimated that it takes 100 mites per gram of dust to produce sensitivity and 500 per gram of dust to cause wheezing. 50% of perennial asthma is due to dust mites (Paramesh, 2002).

1.2.4.1.2 Cockroaches

Exposure to cockroach allergen in the living quarters is associated with the development of sensitization, and sensitization to cockroach allergen is associated with an increased risk of developing asthma (Morgan et al., 2004).
1.2.4.1.3 Companion Animal Allergens

The relationship between exposure and sensitization to allergens from companion animals is not clear, and there are insufficient data to recommend for, or against, the presence of a pet in the home unless the child has become sensitized to the pet species (Bufford and Gern, 2007; Ownby et al., 2002; Platts-Mills et al., 2005; Platts-Mills et al., 2001).

1.2.4.1.4 Fungi

Sensitization to Alternaria is a major risk factor not only for the development of asthma in children, but also for its severity (O’Hollaren et al., 1991; Salo et al., 2006).

1.2.4.2 Nonallergic Environmental triggers

Nonallergic triggers of asthma exacerbations in children affect both atopic and non-atopic children. The triggers having the most concern for children: environmental tobacco smoke (ETS), viral infections, endotoxin, pollutants and microbes and their products are discussed here along with the other two risk factors viz. maternal diet during pregnancy and/or lactation and the psychosocial environment.

1.2.4.2.1 Environmental Tobacco Smoke (ETS)

ETS is a common indoor exposure, which can be assessed by measuring cotinine, a metabolite of nicotine, in urine or saliva. Thus, this exposure is unique in that there is a feasible, inexpensive means of measuring personal exposure over time. According to an Institute of Medicine report, smoking in the home is causally related to exacerbations of asthma in preschool aged children (Stark et al., 2003). ETS is associated with asthma in older children also (Johnston et al., 2000).

1.2.4.2.2 Viral Infections

Viruses are the most important cause of acute infection-induced wheezing in infants and children (Apter, 2003; Stein et al., 1999). Children with severe viral respiratory infections, particularly RSV infections, are at risk for the development of asthma (Apter, 2003; Castro-Rodriguez et al., 1999).
1.2.4.2.3 Endotoxin

Endotoxin, lipopolysaccharide (LPS), is a major component of the outer membrane of gram-negative bacteria. Exposure to endotoxin in infancy is theorized to be protective of the development of allergy and asthma (Gereda et al., 2001; Gehring et al., 2002). In light of endotoxin’s Th-1-inducing activity, this theory is consistent with the Hygiene Hypothesis. However, exposure later in life is proposed to increase acute and chronic inflammation (Braun-Fahrländer et al., 2002).

1.2.4.2.4 Pollutants

The pollutants cause the oxidative stress, airway inflammation and asthma in those who are genetically susceptible to oxidant stress exposures in addition to causing the direct toxicity on the lungs (Gauderman et al., 2005; Millstein et al., 2004). The effect of air pollution caused by traffic or industry on pediatric asthma has been extensively studied (Hirsch et al., 1999; D’Amato et al., 2005).

1.2.4.2.5 Microbes and their products

The impact of bacterial products and their relationship to the development of asthma is increasingly a focus of interest and forms part of the so called “hygiene hypothesis”. Exposure to farming environment in early life has been associated with a reduced risk of asthma and allergy in children compared to those who have not grown up on a farm (Braun-Fahrländer et al., 2002; von Mutius and Radon, 2008). Wheezing in early childhood is predominantly linked to viral infections, especially those due to rhinovirus, respiratory syncytial virus (RSV), Boca virus, and metapneumovirus (MPV) (Heymann et al., 2005; Jackson et al., 2008; Lee et al., 2007).

1.2.4.2.6 Maternal Diet during Pregnancy and/or Lactation

There are insufficient data to support a protective effect of any dietary intervention during pregnancy or lactation in preventing asthma atopic disease (Greer et al., 2008; Kramer and Kakuma, 2006). Although breastfeeding decreases early childhood wheezing associated with upper and lower respiratory infections, there is little evidence that breastfeeding
prevents development of persistent asthma (Gdalevich et al., 2001; Sears et al., 2002; Takemura et al. 2001; Wright et al., 2001).

1.2.4.2.7 Psychosocial Factors

A child’s social environment may play a role in the development and severity of asthma (Chen et al., 2004; Wright et al., 2002). Stress in family or other primary caregivers during the first year of life is associated with an atopic profile and wheeze in infants, and is also associated with asthma at age 6 to 8 years (Wright et al., 2005).

Besides, various other risk factors are responsible for the development of asthma. Children born by Cesarean section have a higher risk of asthma than those born by vaginal delivery (Tollanes et al., 2008), particularly children of allergic parents (Roduit et al., 2009). Paracetamol (acetaminophen) use during pregnancy (Rebordosa et al., 2008) and for fever in the child’s first year of life (Beasley et al., 2008) has been associated with increased prevalence of asthma in children.

1.2.5 Diagnosis of asthma in children

The diagnosis of asthma is challenging in preschool children for many reasons. There are no specific diagnostic tools or surrogate markers for detecting asthma in infancy. Many preschool children with wheezing will not persist to be diagnosed with asthma. Large birth cohorts have shown that approximately 50% of preschool children with recurrent wheezing episodes will have only transient wheezing of childhood (Martinez et al., 1995). The diagnostic evaluations using spirometry, exhaled nitric oxide, and sputum samples are not feasible in the preschool children.

Therefore, the diagnosis of asthma in young children can be done based on symptom patterns and on a careful clinical assessment of family history and physical findings. The presence of atopy or allergic sensitization provides additional predictive support, as early allergic sensitization increases the likelihood that a wheezing child will have asthma (Sly et al., 2008).
1.2.5.1 Clinical History

A clinical diagnosis of asthma is often prompted by symptoms such as episodic breathlessness, wheezing, cough, and chest tightness (Levy et al., 2006). For young children having a history of recurrent respiratory symptoms, a strong family history of asthma in first degree relatives (especially the mother), and/or atopy presenting as atopic dermatitis, food allergy, and/or allergic rhinitis also make a diagnosis of asthma more likely.

1.2.5.2 Therapeutic trial

A trial of treatment with short-acting bronchodilators and inhaled glucocorticosteroids for at least 8 to 12 weeks may provide some guidance as to the presence of asthma. Marked clinical improvement during the treatment and deterioration when it is stopped supports a diagnosis of asthma (GINA, 2009).

1.2.5.3 Test for IgE-mediated Allergy (Atopy)

It has been shown that allergic sensitization is the major risk factor for the development of asthma and for its persistence and severity (Illi et al., 2006; Sears et al., 2003). Sensitization to allergens can be assessed using either immediate hypersensitivity skin testing or an in vitro method that detects antigen-specific IgE antibody.

1.2.5.4 Chest Radiograph (X-ray)

A plain chest radiograph may help to exclude structural abnormalities of the airway (congenital malformations such as congenital lobar emphysema, vascular ring), chronic infection (e.g. tuberculosis), or other diagnoses. Radiographic studies such as chest X-rays are often performed in children with suspected asthma mainly to rule out other causes of cough or wheeze and have little diagnostic utility (Spahn et al., 2009).
1.2.5.5 Lung Function Testing

The diagnosis of asthma is usually based on the presence of characteristic symptoms. However, measurements of lung function, and particularly the demonstration of reversibility of lung function abnormalities, greatly enhance diagnostic confidence. This is because patients with asthma frequently have poor recognition of their symptoms and poor perception of symptom severity, especially if their asthma is long-standing (Killian et al., 2000).

1.2.5.5.1 Peak Expiratory Flow

The peak expiratory flow (PEF) is the maximum flow obtained within the first 200 milliseconds of a forced expiratory maneuver after inhalation to total lung capacity (TLC). Peak expiratory flow measurements are made using a peak flow meter and can be an important aid in both diagnosis and monitoring of asthma. Modern PEF meters are relatively inexpensive, portable, plastic, and ideal for patients to use in home settings for day-to-day objective measurement of airflow limitation. However, measurements of PEF are not interchangeable with other measurements of lung function such as FEV\(_1\) in either adults (Sawyer et al., 1998) or children (Eid et al., 2000).

1.2.5.5.2 Spirometry

Spirometry is the recommended method of measuring airflow limitation and reversibility to establish a diagnosis of asthma. Measurements of FEV\(_1\) and FVC are undertaken during a forced expiratory maneuver using a spirometer. The degree of reversibility in FEV\(_1\) which indicates a diagnosis of asthma is generally accepted as 12% and 200 ml from the pre-bronchodilator value (Pellegrino et al., 2005). Spirometry is reproducible, but effort-dependent. Therefore, proper instructions on how to perform the forced expiratory maneuver must be given to patients, and the best of three recordings should be considered.

1.2.6 Treatment of Asthma

For all patients with a confirmed diagnosis of asthma, the goal of treatment is to achieve control of the clinical manifestations of the disease and maintain this control for prolonged
periods. Medications currently available for childhood asthma include: reliever medications (Short-acting inhaled $\beta_2$-agonists and other bronchodilators) and controller medications (ICS, LTRA, LABAs, Sustained-release theophylline, Cromolyn sodium, Oral steroids and Anti-IgE antibodies).

1.2.6.1 Reliever medications

1.2.6.1.1 Short-acting $\beta_2$ agonists

Rapid-acting inhaled $\beta_2$-agonists are the most effective bronchodilators available and therefore the preferred reliever treatment for asthma in children of 5 years and younger. An MDI with spacer is, in the most cases, an effective way for delivering reliever therapy (Castro-Rodriguez and Rodrigo, 2004; Cates et al., 2006). When delivery is not optimal because of lack of cooperation or distress, or when the child is hypoxic, nebulizer therapy is also an option. Oral therapy is not recommended due to its slower onset of action and its tendency to produce more side effects. The safety margin for dose range is wide and determination of the optimal dose can be difficult. The lowest effective dose that provides adequate clinical control and minimizes side-effects, such as tachycardia, dizziness and jitteriness, is recommended. Salbutamol, the most commonly used drug, has a favorable safety and efficacy profile in patients aged 2–5 years (Skoner et al., 2005). Terbutaline and formoterol also have safety and efficacy profiles comparable to that of salbutamol.

1.2.6.1.2 Ipratropium bromide

The only other reliever of any relevance is Ipratropium bromide. In acute asthma its combined use with $\beta_2$-agonists may result in favorable outcomes in children (Rodrigo and Castro-Rodriguez, 2005), although results were ambiguous in those less than 2 years of age (Everard et al., 2005).

1.2.6.2 Regular controller therapy

The main goal of regular controller therapy should be to reduce bronchial inflammation.
1.2.6.2.1 Inhaled corticosteroid (ICS)

Inhaled corticosteroid (ICS) is a first-line treatment for persistent asthma. It reduces the frequency and severity of exacerbations and should be introduced as initial maintenance treatment (200μg BDP equivalent) when the patient has inadequate asthma control. Atopy and poor lung function predict a favorable response to ICS (Szeffler et al., 2005). If control is inadequate on a low dose after 1–2 months, reasons for poor control should be identified. If indicated, an increased ICS dose or additional therapy with LTRAs or LABAs should be considered. A low-dose inhaled glucocorticosteroid is recommended as the preferred initial treatment to control asthma in children 5 years and younger (Guilbert et al., 2004; Szeffler et al., 2007).

Table 1. Low Daily Doses* of Inhaled Glucocorticosteroids for Children 5 Years & Younger

<table>
<thead>
<tr>
<th>Drug</th>
<th>Low Daily Dose (μg)</th>
</tr>
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<tbody>
<tr>
<td>Beclomethasone dipropionate</td>
<td>100</td>
</tr>
<tr>
<td>Budesonide MDI+Spacer</td>
<td>200</td>
</tr>
<tr>
<td>Budesonide nebulized</td>
<td>500</td>
</tr>
<tr>
<td>Ciclesonide †</td>
<td>NS</td>
</tr>
<tr>
<td>Fluticasone propionate</td>
<td>100</td>
</tr>
<tr>
<td>†Mometasone furoate</td>
<td>NS</td>
</tr>
<tr>
<td>†Triamcinolone acetonide</td>
<td>NS</td>
</tr>
</tbody>
</table>

* A low daily dose is defined as the dose which has not been associated with clinically adverse effects in trials including measures of safety.
†NS = Not studied in this age group. (Table adapted from GINA, 2009)

1.2.6.2.2 Leukotriene Receptor Agonist (LTRA)

It is an alternative first-line treatment for persistent asthma. Evidence supports use of oral montelukast as an initial controller therapy for mild asthma in children (Knorr et al., 2001), as it provides bronchoprotection, and reduces airway inflammation as measured by nitric oxide levels in some preschool children with allergic asthma (Straub et al., 2005). It is a therapy of choice for those who cannot or will not use ICS. LTRA is suggested as
treatment for viral-induced wheeze and to reduce the frequency of exacerbations in young children aged 2–5 years (Bisgaard et al., 2005).

### 1.2.6.2.3 Long-acting inhaled β2-agonists (LABAs)

Long-acting inhaled β2-agonists (LABAs) are bronchodilators, but as long-term therapy for asthma they are usually prescribed in combination with an inhaled glucocorticosteroid and are therefore considered controller medications. Efficacy is not well documented in children in contrast to adults, and use should be evaluated carefully (Verberne et al., 1998; Sorkness et al., 2007). Combination products of LABA and ICS may be licensed for use in children over 4–5 years, however, the effect of LABAs or combination products has not yet been adequately studied in children of 5 years and younger. Formoterol and salmeterol have shown long-lasting bronchodilatory and bronchoprotective in this age group (Nielsen and Bisgaard, 2001).

### 1.2.6.2.4 Oral theophylline

Theophylline is inexpensive, and in some countries, it is used for children whose families cannot afford ICS, LTRAs, or LABAs. There is anecdotal evidence that low-dose theophylline may be of benefit in select groups of children who remain uncontrolled on ICS, LTRAs or LABAs. Although a few studies in children 5 years and younger suggest clinical benefit from regular use of theophylline, the effects are small and mostly non-significant (Seddon et al., 2006).

### 1.2.6.2.5 Cromolyn sodium (nedocromil)

Cromolyn sodium can be prescribed for children as young as 2 years of age. It is less effective than ICS. It must be used frequently (four times per day), and may take up to 4 weeks to work (Guevara et al., 2006). It is free of side-effects. Cromolyn sodium is available as oral or nasal inhalers, nebulizer solution, and eye drops. In a Cochrane review, it has been shown that the cromolyn therapy has no beneficial effect on the preschool children (van der Wouden et al., 2003).
1.2.6.2.6 Anti-IgE antibodies

Patients aged ≤12 years may benefit if they have moderate-to-severe persistent atopic asthma that is inadequately controlled despite treatment with other therapies (Walker et al., 2006). Mode of application and cost will limit this intervention to patients who fail to respond to currently available therapies.

1.2.6.2.7 Oral glucocorticosteroids

Because of the side effects associated with prolonged use, oral glucocorticosteroids in young children with asthma should be restricted to the treatment of acute severe exacerbations. If used, oral glucocorticosteroids (syrup or tablets) are preferred to systemic (intramuscular or intravenous) administration, but are most effective when administered early in an exacerbation. A dose equivalent to prednisolone 1-2mg/kg/day, with a maximum of 20 mg in children under 2 years of age and 30 mg for children 2-5 years, is recommended. A 3-5 day course is sufficient in most children and can be stopped abruptly (GINA, 2009).

1.2.7 Pathophysiology of asthma

Asthma is an inflammatory disorder of the conducting airways which undergo distinct structural and functional changes, leading to non-specific BHR (bronchial hyperresponsiveness) and airflow obstruction that fluctuates over time (Holgate et al. 2010). The development of clinical asthma results from a complex biologic interaction between multiple gene products (one or more containing genetic variations that enhance susceptibility) and at least one environmental toxin (Los et al., 1999; Cookson, 1999).

1.2.7.1 Airway inflammation

Inflammation has a central role in the pathophysiology of asthma. As noted in the definition of asthma, airway inflammation involves an interaction of many cell types and multiple mediators with the airways that eventually results in the characteristic
pathophysiological features of the disease: bronchial inflammation and airflow limitation that result in recurrent episodes of cough, wheeze, and shortness of breath (Figure 1). This allergic inflammatory response is characterized by an infiltration with eosinophils and resembles the inflammatory process mounted in response to parasitic and worm infections. The inflammatory response not only provides an acute defense against injury, but is also involved in healing and restoration of normal function after tissue damage as a result of infection of toxins. In asthma, the inflammatory response is activated inappropriately and is harmful rather than beneficial (Barnes, 2003).

Figure 1. Inflammation in the airways of asthmatic patients leads to airway hyperresponsiveness and symptoms. (Figure taken from Barnes, 2003).

1.2.7.2 Bronchial hyperreactivity

Airway inflammation also leads to bronchial hyperresponsiveness, described as excess airway narrowing in response to stimuli. Depending on the degree of inflammation, the airways can close. The more severe the asthma, the more hyperreactive the airways. The ultimate result and significance is the degree of airflow obstruction resulting from trigger exposure (Conboy-Ellis, 2006). BHR is a fundamental abnormality in asthma which increases in proportion to disease severity and is functionally antagonized by $\beta_2$-
adrenoceptor agonists. The mechanisms underlying BHR are still not known for certain, but an increase in airway smooth muscle alterations to its physicochemical properties (An and Fredberg, 2007) and mast cell infiltration (Begueret et al., 2007) are considered important.

1.2.7.3 Airflow obstruction

Bronchospasm, edema, and mucus hypersecretion lead to airflow obstruction, but it is often reversible. Variable airflow obstruction is demonstrated by measuring forced expiratory volume (FEV1), peak expiratory flow (PEF), or hyperresponsiveness to methacholine challenge (Boulet et al., 1999). However, as the disease becomes more persistent and inflammation more progressive, other factors further limit airflow (Figure 2). These include edema, inflammation, mucus hypersecretion and the formation of inspissated mucus plugs, as well as structural changes including hypertrophy and hyperplasia of the airway smooth muscle. These latter changes may not respond to usual treatment.

Figure 2. Factors limiting airflow in acute and persistent asthma. Key: GM-CSF, granulocyte-macrophage colony-stimulating factor; IgE, immunoglobulin E; IL-3, interleukin 3 and TNF-α, tumor necrosis factor-alpha. (Taken from: Holgate & Polosa, 2006).
1.2.7.4 Airway Remodeling

Airway remodeling involves an activation of many of the structural cells, with consequent permanent changes in the airway that increase airflow obstruction and airway responsiveness and render the patient less responsive to therapy (Holgate and Polosa 2006). These structural changes can include thickening of the sub-basement membrane, subepithelial fibrosis, airway smooth muscle hypertrophy and hyperplasia, blood vessel proliferation and dilation, and mucous gland hyperplasia and hypersecretion. On the basis of a large number of converging observations, it is suggested that in asthma a structurally and functionally defective lower airways epithelium underlies abnormal responses to the inhaled environment leading to enhanced signalling between the airway epithelium and underlying structural (the epithelial–mesenchymal trophic unit, EMTU) and immune cells. This would promote a microenvironment that facilitates allergic sensitization, supports different types of inflammation and predisposes the airways to exacerbations leading to persistence of asthma during childhood (Holgate et al., 2010). Activation of the EMTU might also be responsible for driving tissue remodelling that progressively leads to a loss of reversibility, reduced lung function and refractoriness to treatment in adults (Figure 3).

Figure 3. Chronic asthma is characterized by enhanced epithelial–mesenchymal communication with the release of a range of different growth factors linked to remodeling. (Taken from: Holgate et al., 2010). Key: Ar, amphiregulin; EGF, epidermal growth factor; ET-1, endothelin-1; FGF, fibroblast growth factor; IGF, insulin-like growth factor; KGF, keratinocyte growth factor; PDGF, platelet-derived growth factor; NGF, nerve growth factor; VEGF, vascular endothelial growth factor.
1.2.7.5 Effector Cells of Inflammation and Remodeling in Asthma

Normally there is a fine balance between immune cells, the epithelium, and the host immune response in the healthy human airway. Airway inflammation in asthma reflects a distortion of this balance and is orchestrated through complex interplay between multiple effector and target components (Murphy and O’Byrne, 2010).

1.2.7.5.1 Mast Cells

Mast cells are critical in mediating the acute response in asthma. While classically, mast cell activation occurs following the binding of antigens to FceRI-bound, antigen-specific IgE, they may also be activated through other mechanisms, including stimulation of complement receptors, FcγRI, and via TLRs (Nigo et al., 2006). Activated mucosal mast cells release bronchoconstrictor mediators (histamine, cysteinyl-leukotrienes, prostaglandin D2) (Robinson, 2004). Mast cells also can release a large number of cytokines to change the airway environment and promote inflammation even though exposure to allergens is limited. Mast cells are generally considered proinflammatory and mediators of tissue destruction, they may conversely help limit airway damage (Kalesnikoff and Galli, 2008).

1.2.7.5.2 Basophils

Basophils have a crucial role in initiating allergic inflammation through the binding of antigen-specific IgE antibodies at the FceRI (Obata et al., 2007). Using a basophil-specific marker a small increase in basophils has been documented in the airways of asthmatic patients, with an increased number after allergen challenge. However, these cells are far outnumbered by eosinophils (approximately 10:1 ratio) and their functional role is unknown (Macfarlane et al., 2000).

1.2.7.5.3 Eosinophils

Increased numbers of eosinophils exist in the airways of most, but not all, persons who have asthma (Chu and Martin, 2001; Williams, 2004). These cells contain inflammatory
enzymes, generate leukotrienes, and express a wide variety of pro-inflammatory cytokines. Increases in eosinophils often correlate with greater asthma severity. Increased numbers of eosinophils present in the airways release basic proteins that may damage airway epithelial cells. Eosinophils may also have a role in the release of growth factors and airway remodeling (Larche et al., 2003).

1.2.7.5.4 Neutrophils

Neutrophils are increased in the airways and sputum of persons who have severe asthma, during acute exacerbations, and in the presence of smoking. Inhaled corticosteroids reduce airway eosinophils, but increase airway neutrophils and increase the expression of the neutrophil chemoattractant IL-8, which is associated with loss of asthma control (Maneechotesuwan et al., 2007).

1.2.7.5.5 T lymphocytes

Increased numbers of T lymphocytes in the airways release specific cytokines, including IL-4, IL-5, IL-9, and IL-13, that orchestrate eosinophilic inflammation and IgE production by B lymphocytes (Akbari et al., 2006). An increase in Th2 cell activity may be due in part to a reduction in regulatory T cells that normally inhibit Th2 cells.

1.2.7.5.6 Dendritic Cells

These cells function as key antigen-presenting cells that interact with allergens from the airway surface and then migrate to regional lymph nodes to interact with regulatory cells and ultimately to stimulate Th2 cell production from naïve T cells (Kuipers and Lambrecht, 2004).

1.2.7.5.7 Platelets

There is some evidence for the involvement of platelets in the pathophysiology of allergic diseases. After allergen challenge there is a significant fall in circulating platelets (Sullivan et al., 2000) and circulating platelets from patients with asthma show evidence of increased activation and release the chemokine RANTES (Moritani et al., 1998).
1.2.7.5.8 Macrophages

Macrophages are the most numerous cells in the airways and they can be activated by allergens through low-affinity IgE receptors to release inflammatory mediators and cytokines that amplify the inflammatory response (Peters-Golden, 2004). Macrophages may both increase and decrease inflammation, depending on the stimulus. Alveolar macrophages normally have a suppressive effect on lymphocyte function, but this may be impaired in asthma after allergen exposure (Spiteri et al., 1994). One anti-inflammatory protein secreted by macrophages is IL-10 and its secretion is reduced in alveolar macrophages from patients with asthma (John et al., 1998). Macrophages from normal subjects also inhibit the secretion of IL-5 from T-lymphocytes, probably via the release of IL-12, but this is defective in patients with allergic asthma (Tang et al., 2001). Macrophages may therefore play an important anti-inflammatory role, by preventing the development of allergic inflammation.

1.2.7.5.9 Epithelial cells

Airway epithelium is another airway lining cell critically involved in asthma (Polito and Proud, 1998). The generation of inflammatory mediators, recruitment and activation of inflammatory cells, and infection by respiratory viruses can cause epithelial cells to produce more inflammatory mediators or to injure the epithelium itself. The repair process, following injury to the epithelium, may be abnormal in asthma, thus furthering the obstructive lesions that occur in asthma.

1.2.8 Prevalence of Childhood Asthma

International Study of Asthma and Allergies in Childhood (ISSAC) is a systematic approach to recording the worldwide prevalence of pediatric asthma. Key findings from ISAAC Phase One (1994–1996) included large variations in the worldwide prevalence of symptoms of asthma which were found even among genetically similar populations (ISAAC Steering Committee, 1998a; ISAAC Steering Committee, 1998b) suggesting that environmental factors play an important role. Further study of the global prevalence and
severity of asthma symptoms was undertaken in ISAAC Phase Three, conducted between 2000 and 2003, involving 798,685 adolescents from 233 centres in 97 countries, and 388,811 children from 144 centres in 61 countries (Lai et al., 2009). As in ISAAC Phase One, wide variations in prevalence were found around the world. The prevalence of wheeze in the past 12 months in adolescents varied from 32.6% in Wellington (New Zealand) to 0.8% in Tibet (China), and in children from 37.6% in Costa Rica to 2.4% in Jodhpur (India). The prevalence of symptoms of severe asthma (defined as ≥4 attacks of wheeze, or ≥1 night per week sleep disturbance from wheeze, or wheeze affecting speech in the past 12 months) varied from 16% in Costa Rica to 0.1% in Pune (India) in adolescents, and from 20.3% to 0% in the same two centres in children (Asher, 2010).

The prevalence of asthma is increasing in both the developed and developing countries of the world. Prevalences are high (>10%) in developed countries and also increasing in developing regions as they become more westernized. The highest asthma prevalences are found in the United Kingdom (>15%), New Zealand (15.1%), Australia (14.7%), the Republic of Ireland (14.6%), Canada (14.1%), and the United States (10.9%) (Masoli et al., 2004).

In developing regions (Africa, Central and South America, Asia, and the Pacific), asthma prevalence continues to rise sharply with increasing urbanization and westernization. High prevalences have been reported in Peru (13.0%), Costa Rica (11.9%), and Brazil (11.4%). In Africa, asthma prevalence is highest in South Africa (8.1%), perhaps the most westernized of the African countries (Masoli et al., 2004).

In India, large numbers of studies have reported the varying rates of asthma prevalence in pediatric population. A study has reported a wide variation (4-19%) in the prevalence of asthma in school-going children from different geographic regions of India (ISAAC Steering Committee, 1998a). A recent study reported the prevalence of bronchial asthma to be 10.3% in rural Indian children (Jain et al., 2010). Another study on school-going children in Lucknow showed the prevalence of asthma to be 2.3% in age the group of 6-7 years and 3.3% in age group of 13-14 years (Awasthi et al., 2004).
1.2.9 Economic Burden of Asthma

The economic burden of pediatric asthma may be divided into the direct costs and indirect costs of care. Data from the National Heart, Lung and Blood Institute (NHLBI) and the American Lung Association (ALA) estimated the total direct cost of asthma in 2010 at more than $15 billion, with prescriptions accounting for more than one-third of expenditures. Indirect costs from lives lost and lost productivity exceed $5 billion, with total costs related to asthma recently reaching $20.7 billion (American Lung Association, 2010). In addition, Kamble & Bharmal calculated total expenditures per person with asthma, estimating an annual cost to treat the disease of $1,004.60 per child (Kamble and Bharmal, 2009).

1.2.10 Social Impact of Asthma

Asthma attacks and exacerbations pose strain on the health care system and affect school and job performance. Because of the chronic nature of asthma the lives of sufferers are affected in a multitude of ways including sleeplessness, daytime fatigue, reduced levels of activity and work and school absenteeism. This can result in life-long detrimental effects including adverse outcomes on early education in children, reduced fitness, weight gain and the inability to concentrate while at work. A study of caregivers of children with asthma in France indicated that during a 12-month period 30% of caregivers missed work overall and 13% missed more than 5 days of work because of their child’s asthma (Laforest et al., 2004).

1.2.11 C-Reactive Protein (CRP)

C-reactive protein is considered as the prototypic marker of inflammation in humans and a member of a highly conserved family of proteins called the pentraxins (Figure 4). The human crp gene is located on chromosome 1q23 (Walsh et al., 1996) and consists of two exons and one intron (Lei et al., 1985; Woo et al., 1985). CRP is synthesized as a 206 amino acid polypeptide and secreted by hepatocytes as an approximately 23 kDa, non-
glycosylated monomer, which non-covalently associates to form the homopentameric ring structure characteristic of pentraxin family members (Thompson et al., 1999).

Figure 4. Three-dimensional structure of human C-Reactive Protein (CRP). CRP is synthesized as a 206 amino acid polypeptide that folds to form a flattened jellyroll structure, which then assembles into a radially symmetrical pentamer. (Taken from: Thompson et al., 1999).

C-reactive protein (CRP) is an acute-phase reactant secreted in response to circulating inflammatory cytokines. Interleukin-6 plays a critical role in CRP induction (Castell et al., 1990). CRP was initially described in 1930 by Tillet and Francis as the serum factor responsible for the precipitation of acute phase sera with the C-substance (C-polysaccharide, CPS) of pneumococcal cell walls. Although CRP is structurally distinct from the immunoglobulins, it shares with them the ability to initiate several biological functions including precipitation (Tillet & Francis, 1930), opsonization (Ganrot & Kindmark, 1969), capsular swelling (Hedlund, 1961) and agglutination (Patterson & Higginbotham, 1965). Two major biological activities of CRP have been well defined: first, it is able to bind several biological substrates that are distributed widely in nature (Gotschlich et al., 1982). Second, it has significant activation capabilities, in particular to activate the complement system (Kaplan & Volanakis, 1974) and to bind to and modulate the function of phagocytic leukocytes (Kindmark, 1971). These effects support the concept that this serum protein may have a potentially central role in host defense mechanisms.
CRP has now emerged as a circulating marker for cardiovascular diseases and asymptomatic atherosclerosis (Ridker et al., 2000; Wang et al., 2002). It has long been used clinically to evaluate the presence and degree of inflammation because CRP blood levels increase as much as 1,000-fold within 24 hours after the onset of inflammation (Kushner et al., 1981). It has been demonstrated that CRP levels are associated with age, sex, race (African-American), body mass index (BMI), smoking, serum lipids, blood pressure, presence of diabetes mellitus, 2-h post-challenge glucose, frequency of exercise, and cardio-respiratory fitness (Folsom et al., 2002; Hashimoto et al., 2004).

1.2.11.1 C-reactive protein in asthma

An epidemiological study showed that elevated levels of hs-CRP correlate significantly with respiratory symptoms and with prevalence of non-allergic asthma (Olafsdottir et al., 2005). Various publications suggest that CRP could be taken into consideration as a simple, cheap and reliable marker for monitoring asthmatic inflammation (Kony et al., 2004; Olafsdottir et al., 2005). In a study, the higher plasma CRP level was reported in asthma independent of various other factors (Kasayama et al., 2009). Fujita et al. (2007) reported that increased hs-CRP levels may be associated with allergic inflammation, particularly eosinophilic inflammation, and the degree of airway obstruction in asthmatic patients. In another study, Szalai et al. (2002) suggested that an increase in CRP concentration may accompany the acute phase of allergic inflammation.

Elbehidy et al. (2010) showed significantly higher concentration of hs-CRP in three different groups of asthmatics than in controls. Further, serum hs-CRP levels were significantly higher in patients with uncontrolled asthma than in the two groups with controlled disease and hs-CRP correlated negatively with FEV1% and positively with sputum eosinophil%. A recent study has shown significantly higher level of serum hs-CRP in patients with acute asthma compared to controls (Razi et al., 2012). Kilic et al. (2012) have also shown significantly higher levels of serum hs-CRP in asthmatic than in controls. They have also shown significantly higher level of hs-CRP in moderate asthmatics compared to controls.
1.2.12 Immunoglobulin E (IgE)

Immunoglobulin E (IgE) is set apart from other immunoglobulin isotypes because of its very short half-life (<1 day) and very low concentrations in the circulation (Oettgen and Geha, 1999). In part, this is because some proportion of the circulating amount is continually removed and destroyed in endosomes (in the endosomes IgG is protected by FcγRn). IgE is extremely biologically active despite the low concentrations in the circulation. This is because IgE antibodies bind to high-affinity receptors on the surface of mast cells and basophils, so that these cells may be highly sensitive to allergens even when the concentration of IgE in the circulation is very low. In addition, the expression of the high-affinity receptors is upregulated during allergen-induced rhinitis (Rajakulasingam et al., 1997), probably by IgE itself (Yamaguchi et al., 1997; Williams and Galli, 2000). In addition to triggering immediate-hypersensitivity reactions and late-phase responses, there is accumulating evidence that preformed IgE can augment humoral and cellular immune responses to allergens (Oettgen and Geha, 1999).

1.2.12.1 Pathophysiologic Role of IgE in Asthma

IgE plays a key role in the pathogenesis of allergy. The genetic predisposition to mount a local mucosal IgE response, known as atopy, is one of the strongest risk factors for developing asthma (Nelson, 2001; Karjalainen et al., 2003). The majority of asthma is associated with atopy (Pearce et al., 1999), however there are also clinically defined variant forms of the disorder which are independent of atopy, i.e. do not mount an IgE response to environmental allergens (Macfarlane et al., 2000). Th2 associated inflammation and IgE production are also the features of non-atopic or intrinsic asthma, although what drives this process remains unknown (Humbert et al., 1999). Once synthesized, IgE antibodies circulate in the blood before binding to the high-affinity IgE receptor FcεRI that is present on mast cells in tissue or on peripheral blood basophils. Subsequent allergen exposures cause inflammatory-cell recruitment, activation and mediator release. IgE-sensitized mast cells expressing the high affinity IgE receptor (FcεRI) degranulate, releasing both pre-formed and newly synthesized mediators including histamine, leukotrienes, prostaglandins (PGs) and cytokines (Figure 5). Chemokines
released by inflammatory and resident cells direct recruitment of inflammatory cells, eosinophils and Th2 cells. Eosinophils release an array of pro-inflammatory mediators, including leukotrienes and basic proteins and mediators such as, IL-5 (Murdoch and Lloyd, 2010). These mediators cause the so-called early-phase asthmatic reaction (EAR), which is characterized by constriction of airway smooth muscle (ASM) cells, vascular leakage, mucus production, enhanced airway hyperresponsiveness (AHR) and recruitment of inflammatory cells (Bradding and Holgate, 1999). This EAR is immediate, lasting 30–60 min and 4–6 h later followed by the late-phase asthmatic reaction (LAR) (Baraniuk, 1997). The late-phase is characterized by excessive inflammation of the airways, resulting in structural changes, including airway wall thickening, subepithelial fibrosis, goblet cell hyperplasia, myofibroblast hyperplasia, ASM cell hyperplasia and hypertrophy, and epithelial hypertrophy. This is collectively known as airway remodeling (Bloemen et al., 2007).

Figure 5. Immune cells and the inflammatory cascade in asthma. Initial exposure to allergen leads to the activation of allergen-specific Th2 cells and IgE synthesis (sensitization). Subsequent allergen exposures cause inflammatory-cell recruitment, activation and mediator release. IgE-sensitized mast cells expressing the high affinity IgE receptor (FceRI) degranulate, releasing both pre-formed and newly synthesized mediators including histamine, leukotrienes and cytokines, which promote vascular permeability, smooth muscle contraction and mucus production. Key: APC, antigen-presenting cell; ASM, airway smooth muscle; EpC, epithelial cell; GM-CSF, granulocyte monocyte colony stimulating factor; MHC, major histocompatibility; TCR, T cell receptor; TSLP, thymic stromal lymphopoietin. (Taken from: Murdoch & Llyod, 2010)
1.2.12.2 IgE in asthma

Atopy is a nearly universal finding in children with asthma which is described as a tendency to produce excessive amounts of IgE antibodies when exposed to allergens (Burrows et al., 1989). Estimation of total IgE level provides evidence in support of atopy. IgE concentration at birth is about 0.22 IU/ml. It reaches the adult value at 14 years of age. Markedly raised IgE levels have been reported in cases of parasitic infestations, wiskott–Aldrich syndrome, alcoholism, HIV and severe burns cases etc. Healthy, non allergic adults have an expected IgE concentration of up to 120 IU/ml (Witting et al., 1980). IgE concentrations may vary as a result of diet, genetic background, geographical location and other influences (Chowdary, 2003). The concentration of IgE in serum is age dependent and normally remains at levels less than 10 IU/ml in most infants during the first year of life. There is a wide distribution of expected serum IgE values in healthy individuals of same age group (Kjellman et al., 1976).

Allergic diseases including asthma are characterized by an increase in serum IgE levels (Peng, 2009; Rage et al., 2009). IgE plays a central role in the initiation and the propagation of the inflammatory cascade and thus the allergic response (Buhl, 2005). Exposure to environmental factors, particularly inhalant allergens is commonly reported as a precipitant of acute exacerbations of asthma (Bacharier et al., 2003). IgE is implicated in airway inflammation and allergic reactions and may play a role in modulating the severity of asthma, because previous studies have found associations between high IgE levels and asthma severity, airway hyperresponsiveness, and lower baseline lung function (Naqvi et al., 2007).

Various population studies have shown an association between the prevalence of asthma/bronchial hyperresponsiveness and the total serum IgE levels (Freidhoff and Marsh, 1993; Grainger et al., 1990; Sears et al., 1991), independent of specific reactivity to common allergens or symptoms of allergy. Burrows et al. (1989) found a close correlation between serum IgE levels and the self-reported asthma. Borish et al. (2005) have reported higher IgE levels in severe asthmatics compared to moderate and mild asthmatics. Afshari et al. (2007) have reported considerably higher levels of serum IgE and IL-4 in asthmatics.
than in non-asthmatic controls. In addition, several investigators have reported the elevated levels of total serum IgE in asthmatics (Anupama et al., 2005; Sharma et al., 2006; Sandeep et al., 2010).

1.2.13 Cytokines

Cytokines are low-molecular weight regulatory proteins or glycoproteins secreted by white blood cells and various other cells in the body in response to a number of stimuli. Their secretion is typically transient. The cytokine network is a complex and dynamic system, involved in numerous biological responses in the human body (Joseph et al., 2002). Although an understanding of cytokine biology may appear daunting, it has many worthwhile utilities. Role of cytokines in disease pathogenesis unfolds the disease course in an explicit way. It also highlights the impact of molecular pathology on the practice of medicine and it has many practical therapeutic applications. Understanding cytokines physiology is an important step in optimizing their therapeutic use and furthering our knowledge of the biogenesis of different disorders (Ikram et al., 2004).

1.2.13.1 CD4+ T cell subsets and Cytokine profile

In 1986, Mosmann et al. described two types of T helper (Th) clones in mice, Th1 and Th2 cells, which were distinguishable by the profile of cytokine production. Effector Th1 cells are involved in delayed-type hypersensitivity through their production of interferon (IFN)-γ and interleukin (IL)-2, whereas Th2 cells secrete IL-4, IL-9, IL-10 and IL-13, and promote antibody-mediated humoral immune responses (Brown and Ennis, 2005). Several studies suggest that polarized human Th1 and Th2 cells produce a relatively similar pattern of cytokines, compared to their mouse analogs (Romagnani, 1991), and numerous immunological diseases in humans have been associated with a Th1/Th2 cytokine imbalance (Romagnani, 1994). The cytokine profile of human CD4+ Th cells is elaborated in Table 2. Upon activation, naïve T helper cells become an uncommitted T cell termed Th0 cell. These Th0 cells secrete multiple varieties of cytokines, and in response to stimulation, differentiate into either Th1 or Th2 cells, distinguishable by their cytokine repertoire.
Table 2. Cytokine profiles of human CD4+ T cell subsets

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*Cytokines highlighted in italics represent very low levels (if present at all). (Taken from: Brown and Ennis, 2005).

1.2.13.2 Role of cytokines in the pathogenesis of asthma

Asthma is a common respiratory disorder characterized by recurrent episodes of coughing, breathlessness and wheezing. Cytokines play a key role in orchestrating the chronic inflammation of asthma and chronic obstructive pulmonary disease (COPD) by recruiting, activating, and promoting the survival of multiple inflammatory cells in the respiratory tract (Figure 5). Epithelial cells play an important role in orchestrating the inflammation of asthma through the release of multiple cytokines, including SCF (which maintains mast cells in the airways), TSLP (which acts on DCs to release the Th2 chemoattractants CCL17 and CCL22, which act via CCR4), and several chemokines that attract eosinophils by activating CCR3. Th2 cells orchestrate the inflammatory response in asthma through the release of IL-4 and IL-13 (which stimulate B cells to synthesize IgE), IL-5 (which is necessary for eosinophilic inflammation), and IL-9 (which stimulates mast cell proliferation). Mast cells are thus orchestrated by several interacting cytokines and play an important role in asthma through the release of the bronchoconstrictor mediators.
histamine, cysteinyl-leukotrienes (Cys-LTs), and PGD2 (Barnes, 2008). Type 1 cytokines (IFN-γ, IL-12 and TNF-β) promote pro-inflammatory immune responses, whereas type 2 cytokines (IL-4, IL-5, IL-10, and IL-13) promote anti-inflammatory, antibody-dependent immune responses (Mosmann and Coffman, 1989). Dysregulated type 1/type 2 cytokine production and skewed development of memory Th1 or Th2 subsets, which secrete type 1/type 2 cytokines, respectively, have been implicated in the progression of multiple immune disorders including asthma (Steinke and Borish, 2001; Mazzarella et al., 2000), leukemia (Zhang et al., 2000), and other cancers (Skinnider and Mak, 2002). As a result, there is a great interest in using type 1 and type 2 cytokines as markers of human immune function. It is well established that a strong correlation exists between the presence of eosinophils and the presence of Th2 cells in the asthmatic airways and that classical Th2 cell-derived cytokines, namely IL-4, IL-5, IL-9 and IL-13, play critical roles in orchestrating and amplifying allergic inflammation in asthma (Nakajima and Takatsu, 2007). However, accumulating evidence suggests that the regulation of allergic inflammation is more complex. Although insight into the pathophysiology of asthma has increased substantially over recent years, a number of issues remain to be further clarified. These include a better understanding of the exact functional role of each cytokine in the sensitization process and in the complex relationship between inflammation, remodeling and altered airway behavior (Kips, 2001).

1.2.13.3 Role of Th2 Cytokines in Allergic Inflammation

1.2.13.3.1 IL-4

Eosinophilic inflammation is the characteristic of allergic disorders including asthma. Allergic diseases including asthma are characterized by inflammation with pronounced infiltration of eosinophils (Kay, 2001; Cohn et al., 2004). An essential biological activity of IL-4 in the development of allergic inflammation is to drive the differentiation of naïve Th0 cells into Th2 cells, which secrete IL-4, IL-5, IL-9 and IL-13 but not IFN-γ (O’Garra and Arai, 2000; Murphy and Reiner, 2002). Various studies have suggested that IL-4 is essential for the initial differentiation and/or expansion of antigen-specific Th2 cells but
may not be essential for the induction of allergic airway inflammation at an effector phase (Coyle et al., 1995; Brusselle et al., 1994). On the other hand, some studies have indicated the importance of IL-4 in promoting allergic inflammation at an effector phase by inducing the recruitment of Th2 cells in part via vascular cell adhesion molecule-1/very late antigen-4-dependent mechanisms (Cohn et al., 1997). Therefore, IL-4 could play a role in the induction of allergic inflammation in a sensitized individual, but the relative importance of IL-4 depends on the state of sensitization and/or genetic background.

1.2.13.3.2 IL-5

IL-5 is a key Th2-type cytokine that plays an important role in the differentiation, maturation, and survival of eosinophils (O’Byrne, 2007). It has been demonstrated that the expression of IL-5 mRNA in bronchial biopsies of asthmatic patients is increased as compared with healthy volunteers and that the predominant source of IL-5 mRNA is CD4+ T cells (Robinson et al., 1992). Indeed, CD4+ T cell activation in asthma is accompanied by increased serum concentrations of IL-5 (Corrigan et al., 1993). IL-5 mRNA and protein are also found in mast cells located within allergen-challenged tissues.

1.2.13.3.3 IL-9

Over-expression of IL-9 in mice induces inflammation mediated by eosinophils, mucus hyperplasia, mastocytosis, AHR, and increased expression of other Th2 cytokines and IgE. Asthmatic patients show increased expression of IL-9 and its receptor in the airways (Zhou et al., 2001). IL-9 plays an important role in differentiation and proliferation of mast cells and interacts synergistically with SCF (Barnes, 2008).

1.2.13.3.4 IL-13

Accumulating evidence suggests that IL-13 plays a key role in the allergic response via its actions on epithelial and smooth muscle cells and not through traditional effector pathways involving eosinophils and IgE-mediated events (Wills-Karp, 2004; Wynn, 2003). The importance of IL-13 was evidenced by the finding that neutralization of endogenously released IL-13 with a soluble form of IL-13Rα2, which binds IL-13 but not IL-4, during
antigen exposure largely inhibited the characteristics of asthma in murine asthma models (Wills-Karp et al., 1998). The cytokines involved in asthma are shown in Figure 6.

![Cytokines involved in asthma](image)

**Figure 6. Cytokines involved in asthma. (Taken from: Barnes, 2008).**

### 1.2.13.4 Cytokines in asthma

With airway hyper-responsiveness being the physiological hallmark of asthma, it is also characterized by chronic inflammation of the respiratory tract, allergen-specific IgE production, infiltration of eosinophils, the recruitment of T cells into the airways, and alterations in the fine balance between type 1 helper T lymphocytes (Th1) and type 2 helper T lymphocytes (Th2) responses towards Th2 bias (Larche et al., 2003). Many investigators have studied the serum cytokines in asthmatic subjects (Daher et al., 1995; Litonjua et al., 2003; Silvestri et al., 2006). In another study, the production of IL-4 and IFN-γ in phytohaemagglutinin (PHA)-stimulated peripheral blood mononuclear cell cultures from atopic children was examined. The result of the study showed that highly atopic children with IgE $> 600$U/ml produced significantly more IL-4 and less IFN-γ in
vitro than age-matched non-atopic controls (Tang et al., 1993). Lee et al. (2001) investigated the serum levels of IL-4, IL-5, IL-13 and IFN-\(\gamma\) in asthmatics and showed that acute asthmatics had significantly increased levels of circulating IL-4, IL-5, and IL-13, although the differences were of borderline significance in serum IFN-\(\gamma\) when compared with control group. Smart and Kemp (2002) showed that atopic children had significantly reduced IFN-\(\gamma\) and increased IL-4 and IL-5 but not IL13 production to staphylococcal superantigen (SEB) stimulation when compared with non-atopic children. Bogic´ et al. (2004) have reported significantly higher IL-4 and IL-5 serum concentrations in asthmatic group compared to control and these were significantly higher in patients with moderate and severe asthma compared to mild asthmatics. Joseph et al. (2004) studied the serum level of IL-5 in asthmatics and the result of their study showed the elevated levels of IL-5 in mild and moderate persistent asthmatics compared to controls. Various studies have reported the elevated levels of Th-2 cytokines and reduced levels of Th-1 cytokines in allergic and asthmatic subjects (Cohn et al., 2004; Akpinarli et al., 2002; Robroeks et al., 2007; Shahid et al., 2002; Pukelsheim et al., 2010).

1.2.14 Human Leukocyte Antigen (HLA)

The genetic loci involved in the rejection of foreign organs are known as the major histocompatibility complex (MHC), which encodes the highly polymorphic surface molecules. The human MHC is called the HLA (Human Leukocyte Antigen) system because these antigens were first identified and characterized using alloantibodies against leukocytes (Terasaki, 1990). The HLA, located on the short arm of Chromosome 6, is one of the most extensively studied regions in the human genome because of the contribution of multiple variants at this locus in autoimmune, infectious, and inflammatory diseases and in transplantation (Fernando et al., 2008). The HLA system has been well known as transplantation antigens, but the primary biological role of HLA molecules is in the regulation of immune response (Bjorkman et al., 1987). The importance of these MHC antigens in the immune response was first described by Baruj Benacerraf (Benacerraf, 1981). MHC molecules act as antigen-presenting structures, the particular set of MHC
molecules expressed by an individual influences the repertoire of antigens to which that individual’s TH and TC cells can respond.

1.2.14.1 General Organization of the HLA system

The classical MHC encompasses approximately 3.6 megabasepairs (Mb) on 6p21.3 and is divided into three subregions: the telomeric class I, class III, and the centromeric class II regions (Figure 7). The HLA Class I genes and Class II genes each spread over approximately one third of this length. The class III region does not encode HLA molecules but contains genes for complement components (C2, C4, and factor B), 21-hydroxylase, tumor necrosis factors (TNFs), and some others (Beck and Trowsdale, 2000). Thus, the Class III region is not actually a part of the HLA complex, but is located within the HLA region, because its components are either related to the functions of HLA antigens or are under similar control mechanisms to the HLA genes (De Jong et al., 2003). Based on the similarity of structure and their function, HLA-I and HLA-II molecules are described below.

![Chromosome 6](image)

Figure 7. Genetic map of human leukocyte antigen (HLA) region. (Adapted from: Westover et al., 2011).
1.2.14.2 HLA Class I molecules

Class I MHC genes encode glycoproteins expressed on the surface of nearly all nucleated cells, the major function of the class I gene products is the presentation of peptide antigens to TC cells. The class I genes code for a polypeptide chain of the class I molecule, the β chain of the class I molecule is encoded by a gene on chromosome 15, the beta 2-microglobulin gene. The α chain has five domains: two peptide-binding domains (α1 and α2), one immunoglobulin-like domain (α3), the transmembrane region, and the cytoplasmic tail (Figure 8). There are some 20 class I genes in the HLA region; three of these, HLA-A, B, and C, the so-called classic, or class Ia genes, are the main actors in the immunologic theater (Klein and Sato, 2000). Immunological studies indicate that HLA-B (which is also the most polymorphic) is the most significant HLA Class I locus, followed by HLA-A and then HLA-C. There are other HLA Class I loci (e.g. HLA-E, F, G, H, J, K and L), but most of these may not be important as loci for “peptide presenters” (Lotteau, 1992).

The HLA Class I antigens comprise a 45-kilodalton (kDa) α chain associated noncovalently with a 12-kDa β2-microglobulin molecule, which plays an important role in the structural support of the heavy chain. The HLA Class I molecule is assembled inside the cell and ultimately sits on the cell surface with a section inserted into the lipid bilayer of the cell membrane and has a short cytoplasmic tail (Figure 8). The full 3-dimensional structure of HLA Class I molecules has been determined from X-ray crystallography (Browning and McMichael, 1996). This has demonstrated that the molecule has a cleft on its outermost surface, which holds a peptide. In fact, if a cell becomes infected with a virus, the virally induced proteins within the cell are broken down into small peptides and these are then inserted into this cleft during the synthesis of HLA Class I molecules. The role of HLA Class I molecules is to take these virally enhanced peptides to the surface of the cell and by linking them to the T-Cell receptor of a cytotoxic (CD8) T cell, demonstrate the presence of the virus. The CD8+ T cell will now be “educated” and it will be able to initiate the process of killing cells which subsequently have that same viral protein/HLA Class I molecule on their surface. This role of HLA Class I molecules in identifying changed cells (e.g. virally infected) is the reason why they must be present on all cells (Roitt et al., 1998).
1.2.14.3 HLA Class II Molecules

The class II genes code for the two different polypeptide chains, a 33-kDa α chain and a 28-kDa β chain, which associate by non-covalent interactions (Figure 9). The DR gene family consists of a single DRA and up to nine DRB genes (DRB1 to DRB9). The DRA gene encodes an invariable α chain and it binds various β chains encoded by the DRB genes. The DQA1 and DQB1 gene products associate to form DQ molecules, and the DPA1 and DPB1 products form DP molecules (Choo, 2007). Like class Iα chains, class II MHC molecules are membrane-bound glycoproteins that contain external domains, a transmembrane segment, and a cytoplasmic anchor segment. Each chain in a class II molecule contains two external domains: α1 and α2 domains in one chain and β1 and β2 domains in the other. The membrane-distal portion of a class II molecule is composed of the α1 and β1 domains and forms the antigen-binding cleft for processed antigen (Kindt et al., 2007).

Class II MHC genes encode glycoproteins expressed primarily on antigen-presenting cells (macrophages, dendritic cells, and B cells), where they present processed antigenic peptides to TH cells. Thus the “education” process, which occurs from HLA Class II presentation, involves the helper-function of setting up a general immune reaction that will involve cytokines, cellular and humoral defense against the bacterial (or other) invasion.
This role of HLA Class II molecules in initiating a general immune response is the reason why they need only to be present on “immunologically active” cells (B lymphocytes, macrophages, etc.) and not on all tissues (Roitt et al., 1998).

Figure 9. Structure of a class II MHC molecule showing the extracellular, transmembrane and cytosolic domains. (Taken from: http://pathmicro.med.sc.edu/bowers/mhc.htm).

1.2.14.4 Antigen Processing and Presentation

MHC-I and MHC-II molecules play different roles in T-cell-mediated adaptive immunity (Janeway et al., 2005; Lund et al., 2005). The MHC class I molecules present the peptide antigen derived from the endocytic pathway of antigen processing to cytotoxic T cells. In the endocytic pathway of antigen processing, endogenous antigens are first cleaved into peptide fragments by the proteasome, which are then generally translocated by the transporter associated with antigen processing (TAP) into the endoplasmic reticulum (ER). In the endoplasmic reticulum, various molecular chaperones, namely calnexin, calreticulin and tapasin, play their significant roles in the assembly of MHC-I molecule and the peptide binding to it. Finally, MHC-I molecules bind certain peptides and present them to cytotoxic T lymphocytes (CTL) stimulating cellular immunity. On the other hand, in the MHC-II pathway, exogenous antigens are first taken into the cell through endocytosis, and then degraded to peptides within endosomes and lysosomes mainly by aspartic and cysteine proteases (e.g. cathepsin). MHC-II molecules are synthesized in the ER, form complexes with invariant chain (Ii), which blocks the peptide binding cleft of MHC-II, and facilitates MHC-II entering into golgi from ER. Later MHC-II complex fuses with
endosome containing exogenous peptides. Finally, with the help of another MHC-like molecule, such as HLA-DM in humans, MHC-II can bind exogenous peptides and present them to T helper cells. Both antigen processing and presentation are important in the process of T-cell-mediated adaptive immunity, but peptide binding to MHC molecules is the most selective step (Zhang et al., 2012). The antigen processing and presentation by MHC-I and MHC-II molecules are depicted in Figure 10.

![Figure 10](image)

Figure 10. Antigen processing and presentation by: MHC-I molecules (left) and MHC-II molecules (right). *(Taken from: Neerinck et al., 2013).*

**1.2.14.5 Genetics of HLA**

Routine tissue typing identifies the alleles at the three HLA Class I loci (HLA-A, -B, and -C) and the three Class II loci (HLA-DR, -DP and -DQ). Thus, as each chromosome is found twice (diploid) in each individual, a normal tissue type of an individual will involve 12 HLA antigens (Sullivan and Amos, 1986). These 12 antigens are inherited codominantly that is to say, all 12 antigens are recognized by current typing methods and the presence of one does not affect our ability to type for the others. There are a number of
genetic characteristics of HLA antigens, they are: Polymorphism, Inheritance, Linkage disequilibrium and cross-reactivity (Browning and McMichael, 1996).

**1.2.14.5.1 Polymorphism**

MHC molecules are highly diverse because of an extensive range of MHC polymorphism. In fact, the IMGT/HLA database of June 2011 (Robinson et al., 2011) contains over 6000 HLA alleles, which include 4946 HLA-I and 1457 HLA-II alleles. Each encoded MHC molecule binds to a distinct set of peptides, but binding preferences of most alleles have not yet been experimentally characterized, mainly because of two reasons. First, biological experiments require immense amount of time and financial cost. Second, the number of possible peptides derived from pathogens is huge, while binding peptides (binders) will be merely a tiny fraction of all those possible peptides (Assarsson et al., 2007; Yewdell and Bennink, 1999).

The HLA polymorphism is not evenly spread throughout the molecule, but is clustered in the antigen–binding groove (Bjorkman and Parham, 1990; Klein and Sato, 2000). Amino acid variations in several regions change the fine shape of the groove and thus alter the peptide-binding specificity of HLA molecules (Falk et al., 1991). The distribution and frequency of HLA antigens vary greatly among different ethnic groups. It has been postulated that this diversity of HLA polymorphism has evolved under unique selective pressure in different geographic regions. This could be related to the role of the HLA molecules in the presentation of prevalent infectious agents in the different areas of the world.

**1.2.14.5.2 Inheritance of HLA**

HLA genes are closely linked and the entire MHC is inherited as an HLA haplotype in a Mendelian fashion from each parent. The segregation of HLA haplotypes within a family can be assigned by family HLA studies. This way of presenting the HLA type is referred to as a phenotype (Thomas et al., 1998). This is illustrated in the figure below. Two siblings have a 25% chance of being genotypically HLA identical, a 50% chance of being HLA
haploidentical (sharing one haplotype), and a 25% chance that they share no HLA haplotypes.

![Figure 11. Mendelian inheritance of HLA haplotypes demonstrated in a family study. (Taken from: Choo, 2007).](image)

**1.2.14.5.3 Linkage disequilibrium**

Basic Mendelian genetics states that the frequency of alleles at one locus does not influence the frequency of alleles at another locus (Law of independent segregation). However in HLA genetics this is not true. Certain HLA haplotypes are found more frequently in some populations than expected by chance. This phenomenon is called the linkage disequilibrium. The most extreme example is in Caucasians where the *HLA-A1, B8, DR3 (DRB1*O301), DQ2 (DRB1*0201)* haplotype is so conserved that even the alleles at the complement genes (Class III) can be predicted with great accuracy. Similar haplotypes are observed in selected caste groups and tribal groups of India (Tiercy et al., 2002). Because of linkage disequilibrium, a certain combination of HLA Class I antigen, HLA Class II antigen and Class III products will be inherited together more frequently than would normally be expected. It is possible that these “sets” of alleles may be advantageous in an immunological sense, having a positive selective advantage.
1.2.14.5.4 Cross-reactivity

Cross-reactivity is the phenomenon whereby one antibody reacts with several different antigens, usually at one locus. The term CREG is often used to describe “Cross reacting groups” of antigens. It is useful to think in terms of CREG’s when screening sera for antibodies, as most sera found are “multi-specific” and it is rare to find operationally monospecific sera. The rarity of monospecific sera means that most serological tissue typing uses sera that detects more than one specificity, and a typing is deduced by subtraction. For example, a cell may react with a serum containing antibodies to HLA-A25, A26, and A34 and be negative for pure A26 and pure A25 antisera. In this case, HLA-A34 can be assigned, even in the absence of pure HLAA34 antisera (Ferrer et al., 2005).

1.2.14.6 HLA and Disease Susceptibility

Some HLA alleles occur at a much higher frequency in those suffering from certain diseases than in the general population. The diseases associated with particular MHC alleles include autoimmune disorders, certain viral diseases, disorders of the complement system, some neurologic disorders, and several different allergies. The MHC was first associated with disease in 1967 when HLA B antigens were found at increased frequency in patients with Hodgkin’s lymphoma (Amiel, 1967). Since then, variation within the MHC has been found to be associated with almost every autoimmune disease, as well as several infectious and inflammatory diseases. However, because of the extensive LD that exists among alleles throughout this locus, the causal MHC variants have remained elusive for the great majority of diseases (Fernando et al., 2008).

There are two general explanations for HLA and disease associations (McDevitt, 1985). Firstly, there may be a linkage disequilibrium between alleles at a particular disease associated locus and the HLA antigen associated with that disease - this is so for HLA-A3 and Idiopathic Haemochromatosis. Another possible explanation for these associations is that the HLA antigen itself plays a role in disease, by a method similar to one of the following models: a) by being a poor presenter of a certain viral or bacterial antigen, b) by providing a binding site on the surface of the cell for a disease provoking virus or
bacterium, c) by providing a transport piece for the virus to allow it to enter the cell, d) by having such a close molecular similarity to the pathogen, that the immune system fails to recognize the pathogen as foreign and so fails to mount an immune response against it. It is most likely that all these mechanisms are involved, but to a varying extent in different diseases (Thorsby, 1997).

1.2.14.7 Association of HLA with Asthma

Asthma is a heterogeneous disease for which a strong genetic basis is firmly established. Asthma and its associated trait ‘‘atopy’’ were some of the first complex diseases for which a strong genetic basis was established (Barnes, 2001). In the early 1990s, the genome-wide linkage approach, whereby the inheritance patterns of chromosomal regions using highly polymorphic, genetic (‘‘microsatellite’’) markers evenly spaced across all chromosomes were genotyped in large samples of families, identified 10 chromosomal regions for which novel genes were subsequently identified by positional cloning i.e., DPP10 (Allen et al., 2003).

A variety of review papers describe genes associated with allergy/asthma (Peden, 2002; Ober and Hoffjan, 2006; Holloway et al., 2010; Vercelli, 2008a; Vercelli, 2008b). Ober and Hoffjan (2006) listed genes associated with asthma or atopy in more than 10 studies. This study evaluated 8 of these genes (IL4, IL13, TNF-a, HLA-DRB1, HLA-DQB1, FCER1B/MS4A2, CD14, ADAM33) as well as 3 glutathione-s-transferase genes (GSTM1, GSTP1, and GSTT1) for association with asthma/allergy among urban-residing African Americans. HLA class II genes relate to non-specific modulation of inflammation. HLA-DRB1 and HLA-DQB1 SNPs and haplotypes have been associated with a higher risk of toluene diisocyanate-induced occupational asthma (Choi et al., 2009), total serum IgE in Iranian subjects (Movahedi et al., 2008), atopy in Northern Chinese (Gao et al., 2003), Dermatophagoides species-sensitive asthma in Venuezuelan individuals (Lara-Marquez et al., 1999), and asthma severity in Whites in the United States (Juhn et al., 2007), suggesting a broad role for these genes in asthma pathogenesis across different ethnic groups. A study on Greek children with allergic asthma revealed that DRB1*04 and
DQA1*0301 might be important factors in susceptibility to asthma with sensitivity to mites (Parapanissiou et al., 2005).

Numerous earlier studies have investigated the association of HLA with asthma. There are large numbers of studies which have shown the association of many different HLA class II alleles with asthma among diverse ethnic groups. Many earlier studies have also investigated the association of HLA class I alleles and/or antigens with asthma. Table 3 & 4 show the HLA class I and HLA class II alleles/haplotypes reported to be associated with asthma in different populations by various investigators, respectively.
Table 3. Association of HLA class I alleles/haplotypes with asthma as reported by various investigators

<table>
<thead>
<tr>
<th>Study population</th>
<th>No. of subjects</th>
<th>HLA allele/haplotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extrinsic &amp; intrinsic adult asthmatic patients</td>
<td>100 (61 atopic &amp; 39 non-atopic)</td>
<td>HLA-B8</td>
<td>Morris et al., 1977</td>
</tr>
<tr>
<td>Population &amp; family study</td>
<td>122 (41 intrinsic, 40 extrinsic &amp; 41 ABPA)</td>
<td>No association</td>
<td>Turton et al., 1979</td>
</tr>
<tr>
<td>Chinese asthmatic children</td>
<td>99</td>
<td>HLA-B<em>5 HLA-B</em>17</td>
<td>Huang et al., 1981</td>
</tr>
<tr>
<td>Korean population</td>
<td>-</td>
<td>HLA-B<em>08 HLA-A</em>03</td>
<td>Bondarenko et al., 1991</td>
</tr>
<tr>
<td>Asthmatics with ragweed Pollen allergy (Case-Control)</td>
<td>52 patients &amp; 27 Controls</td>
<td>HLA-B*7, SC31, DR2</td>
<td>Blumenthal et al., 1992</td>
</tr>
<tr>
<td>Greek asthmatics patients</td>
<td>76 (35 children &amp; 41 adults) &amp; 400 controls</td>
<td>HLA-B5-B35 HLA-B8 HLA-A10</td>
<td>Apostolakis et al., 1996</td>
</tr>
<tr>
<td>Asian population</td>
<td>55 TDI exposed asthmatics, 47 asymptomatic &amp; 95 controls</td>
<td>A<em>02-DRB1</em>15, A<em>02-DQB1</em>06, B<em>62-C</em>09 &amp; A<em>02-DRB1</em>15-DQB1*06</td>
<td>Kim et al., 2006</td>
</tr>
<tr>
<td>Study population</td>
<td>No. of subjects</td>
<td>HLA allele/haplotype</td>
<td>Reference</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>-------------------------------------</td>
<td>----------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>Intrinsic &amp; allergic Asthmatics (Case-Control)</td>
<td>103 patients and 100 controls</td>
<td>HLA-B*12 ↑ A3/B7/DRw2 ↓</td>
<td>Morris et al., 1980</td>
</tr>
<tr>
<td>Croatian children with Allergic asthma</td>
<td>143 allergic asthmatic children &amp; 163 controls</td>
<td>HLA-B*08 ↑</td>
<td>Ivković-Jureković et al., 2011</td>
</tr>
</tbody>
</table>

► Increased  
↓ Decreased
Table 4. Association of HLA-class II alleles/haplotypes with asthma in various populations as reported by various investigators

<table>
<thead>
<tr>
<th>Study population</th>
<th>No. of subjects</th>
<th>HLA allele/haplotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spanish Soyabean-</td>
<td>78 soybean</td>
<td>DRB1<em>13, DRB1</em>05-05,</td>
<td>Soriano et al., 1997</td>
</tr>
<tr>
<td>epidemic asthmatics</td>
<td>67 nonepidermic</td>
<td>DRB1<em>05-06, DRB1</em>06-06, &amp; DRB1*06-06↑</td>
<td></td>
</tr>
<tr>
<td>epidemic asthmatics, &amp; 168</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venezuelan</td>
<td>20 atopic</td>
<td>DRB1*11↑</td>
<td>Lara-Marquez et al.,</td>
</tr>
<tr>
<td>Population</td>
<td>64 controls (41</td>
<td>HLA-DRB1*1101</td>
<td>1999</td>
</tr>
<tr>
<td>Non-atopic + 23 healthy)</td>
<td></td>
<td>-DQA1<em>0501-DQB1</em>0303↑</td>
<td></td>
</tr>
<tr>
<td>Chinese asthmatic population</td>
<td>98 asthmatics</td>
<td>DQA1*0101↑</td>
<td>Guo et al, 2001</td>
</tr>
<tr>
<td></td>
<td>and 67 controls</td>
<td>DQA1<em>0601, DQB1</em>0303 &amp; DQB1*0601↑</td>
<td></td>
</tr>
<tr>
<td>Caucasians with red</td>
<td>56 asthmatics</td>
<td>DQB1<em>0603 &amp; DQB1</em>0302↑ &amp; DQB1*0501↓</td>
<td>Horne et al., 2000</td>
</tr>
<tr>
<td>Cedar asthma</td>
<td>and 63 controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case-Control</td>
<td>102 asthmatics(57</td>
<td>HLA-DR7- DQA1*0201-</td>
<td>Bede et al., 2002</td>
</tr>
<tr>
<td>Hungarian mite-</td>
<td>mite-sensitive &amp;</td>
<td>DQB1<em>0202↑ &amp; HLA-DR4 - DQA1</em>0301-</td>
<td></td>
</tr>
<tr>
<td>Sensitive asthmatics</td>
<td>45 non-mite</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sensitive) &amp; 57 controls</td>
<td>DQB1*0302↓</td>
<td></td>
</tr>
<tr>
<td>Grass allergy patients of Poland</td>
<td>82 atopic (40</td>
<td>DRB1<em>02-B5</em>↑</td>
<td>Woszczek et al., 2002</td>
</tr>
<tr>
<td></td>
<td>with Asthma/rhinitis, 42</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>with rhinitis only &amp; 52 nonatopic controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study population</td>
<td>No. of subjects</td>
<td>HLA allele/haplotype</td>
<td>Reference</td>
</tr>
<tr>
<td>------------------------------------------------------</td>
<td>----------------------------------------</td>
<td>----------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>Taiwanese asthmatic Children</td>
<td>80 allergic asthmatics 69 non-asthmatics</td>
<td>HLA-DR13</td>
<td>Lin et al., 2002</td>
</tr>
<tr>
<td>Spanish <em>Artemisia vulgaris</em>-allergic asthmatics</td>
<td>213 asthmatics and 150 controls</td>
<td>DRB1<em>01 &amp; DQB1</em>0501</td>
<td>Torío et al., 2003</td>
</tr>
<tr>
<td>Korean Aspirin-intolerant Asthmatics</td>
<td>76 Aspirin-intolerant, 73 Aspirin-tolerant &amp; 91 controls</td>
<td>DPB1<em>0301 &amp; DRB1</em>0901- DQB1<em>0303- DPB1</em>0501</td>
<td>Choi et al., 2004</td>
</tr>
<tr>
<td>Asian TDI-induced Asthmatics</td>
<td>55 TDI-induced asthmatics, 47 asymptomatic and 95 healthy controls</td>
<td>DRB1<em>15-DPB1</em>05 &amp; HLA-A<em>02-DRB1</em>15, A<em>02-DQB1</em>06, B<em>62- C</em>09 &amp; A<em>02-DRB1</em>15-DQB1*06</td>
<td>Kim et al., 2006</td>
</tr>
<tr>
<td>Retrospective cohort study</td>
<td>340 children</td>
<td>HLA-DRB1*03</td>
<td>Juhn et al., 2006</td>
</tr>
<tr>
<td>Iranian children with allergic asthma</td>
<td>112 (75 males and 37 females) and 80 controls</td>
<td>DQB1<em>0603 &amp; DQB1</em>0602</td>
<td>Movahedi et al., 2008</td>
</tr>
<tr>
<td>Caucasian, African-American, Hispanic children with <em>Alternaria</em>-sensitive asthma</td>
<td>60 moderate-severe asthmatics &amp; 49 mild asthmatics</td>
<td>DQB1*03</td>
<td>Knutsen et al., 2010</td>
</tr>
<tr>
<td>Study population</td>
<td>No. of subjects</td>
<td>HLA allele/haplotype</td>
<td>Reference</td>
</tr>
<tr>
<td>------------------</td>
<td>----------------</td>
<td>----------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Retrospective cohort Study</td>
<td>383 children</td>
<td>Role of HLA DRB1*03 in asthma susceptibility independent of ancestral-haplotype-mediated linkage disequilibrium</td>
<td>Hanchard et al., 2010</td>
</tr>
<tr>
<td>Croatian children with allergic asthma</td>
<td>143 asthmatics and 163 healthy controls</td>
<td>HLA-DRB1*03 ( \uparrow ) ( \downarrow )</td>
<td>Ivković-Jureković et al., 2011</td>
</tr>
</tbody>
</table>

\( \uparrow \) Increased \( \downarrow \) Decreased
1.3 OBJECTIVES OF THE STUDY

1. To estimate the prevalence of asthma in children aged between 3-12 years and to investigate the associated risk factors.

2. To determine the serum C-reactive protein (CRP) concentration in asthmatic children to understand the inflammatory process in asthma.

3. To estimate the levels of total serum IgE in asthmatic and control subjects and to investigate the relationship of various demographic and clinical characteristics with the level of total serum IgE in asthmatics.

4. To determine the serum levels of Th1 (IFN-γ) and Th2 (IL-4) cytokines in order to investigate any alteration in Th1/Th2 balance in asthma.

5. To determine the frequency of some of the selected HLA class I and class II allelic groups in asthmatic and control groups to correlate the association, if any.