Introduction
Asthma is a chronic inflammatory disorder of the airways. In ancient Greek, the word asthma means "panting or short drawn breath". Patients suffering from this disease appear to be gasping for breath that is relieved either spontaneously or after appropriate treatment with bronchodilators or anti-inflammatory drugs. Asthma attacks may either be immediate, delayed or dual in onset. Asthma is characterized by three key phenotypes: intermittent airway obstruction, inflammatory cell infiltration, and airway hyper-responsiveness (AHR). Other symptoms of asthma include difficulty in breathing, wheezing, chest tightness and coughing. The worldwide incidence, morbidity and mortality of asthma are increasing dramatically. Asthma results from a complex interplay between genetic and environmental factors (Bousquet et al., 2000). It is one of the most common disorders encountered in clinical medicine in both children and adults. It affects nearly 155 million individuals the world-over (Hoffjan and Ober, 2002).

In an epidemiological study conducted in India, approximately 10-15% of the Indian population, particularly women and children (under 5 yr of age), were found to be affected by atopic asthma. The increase in asthma mortality is highest among children. In 1997, 7 million children had asthma and more than 2000 of them died of the disease. It has been estimated that around 34 percent of the total man-days are lost due to asthma and other airway disorders (Smith, 2000). These statistics are increasing every year. In addition to its human costs, asthma imposes high financial costs on society. The rising incidence of asthma over the past decades suggests that environmental and lifestyle factors are important (Hoffjan and Ober, 2002). Although insight into the pathophysiology of asthma has increased substantially over recent
years, a number of issues remain to be further clarified. The pathology of asthma is associated with reversible narrowing of airways, associated with prominent features that involve structural changes in the airway walls, extracellular matrix deposition, abnormalities of bronchial smooth muscle, and eosinophilic inflammation of the bronchial wall, hyperplasia and hypertrophy of mucous glands (Jurgen and Ulrich, 2005). This disease is clinically characterized by recruitment and activation of specific inflammatory cells, chemotaxis, bronchoconstriction, increased airway secretion (and mucus cell hyperplasia), plasma exudation, neural effects, hyperplasia and hypertrophy of airway smooth muscle cells and increased airway hyperresponsiveness which is defined as an exaggerated acute obstructive response of the airways to one or more non-specific stimuli, often associated with airway epithelial damage and disruption, a common feature of even mild asthma (Laitinen et al., 1985).

In addition, there are structural changes to the lung which are termed as airway remodeling. Studies of airways in chronic asthmatics by bronchoscopic methods and induced sputum have provided much helpful and insightful data. Experiments such as the bronchial challenge with allergen provide valuable insights into the allergic inflammatory response but no scientific reports shows the actual remodeling process. Airway structural changes are evident in human patients with chronic asthma (Jeffery et al., 1989; Roche et al., 1989). Remodeling responses in asthma are now being considered as potential targets for therapeutic intervention. The utility of this approach, however, will depend on a number of factors. Thus, the
process of airway wall remodelling is still not understood and requires investigation into its mechanisms and the role of drugs in its reversal and prevention.

Diagrammatic presentation of Airway remodeling

Development of airway remodeling

Chronic asthma is associated with structural changes to the airway wall which are termed as airway remodeling. These structural changes include hyperplasia of the bronchial epithelial cells, which leads to excess mucus secretion and might lead to occlusion of the airways. An imbalance in metabolism of extracellular matrix, either increased production or reduced breakdown of matrix proteins, leads to deposition of collagens and other matrix protein within the sub epithelial or sub mucosal areas. Myocyte muscle mass is increased by hyperplasia of airway smooth muscle and myofibroblast proliferation. The combination of these processes leads to airway narrowing and therefore, reduced lung function. Airway remodeling in asthma is recognized as irreversible structural changes. However, several recent reports have revealed that remodeling might be the process of repair from injury. Airway
remodeling is increasingly recognised to be a serious consequence of chronic asthma. Although the acute inflammation associated with asthma has been modelled extensively both in vitro and in vivo, the structural changes occurring in the lung have only been investigated in past few years. Airway remodeling is believed to be part of the pathophysiology of this disease. It has been suggested that remodelling leads to functional consequences (Roche et al., 1989; Chetta et al., 1997), such as irreversible airflow obstruction, but this is unproven. One hallmark of such airway structural change is airway thickening with deposition of sub epithelial matrix proteins, particularly collagen. Increased levels of collagen types I, III, and V have been reported in asthmatic subjects and in various animal models of asthma (Roche et al., 1989, Wilson and Li., 1997, Chu et al., 1998). To date, however, excess collagen production specifically associated with airway remodelling has not been rigorously quantified in an asthma model.

One of the striking advances in the last decade has been the recognition that cytokines play a critical role in orchestrating, perpetuating and amplifying the inflammatory response in asthma. Many cytokines and chemokines are involved in the pathophysiology of asthma (Barnes et al., 1998, Barnes et al., 1999). While some of these cytokines, such as interleukin (IL)-1, tumour necrosis factor-α and IL-6 are involved in many inflammatory diseases, including chronic obstructive pulmonary disease, rheumatoid arthritis and inflammatory bowel disease, others are more specific to allergic inflammation. Cytokines play a pivotal role in the development of allergic diseases by regulating the activation and proliferation of Th2 cells. Cytokine mediated signals on effector cells are primarily transduced by the Jak-Stat signalling
cascade (Darnell, 1997). The two major subsets of CD4+ T cells, Th1 and Th2 cells secrete distinct profiles of cytokines. Th1 cells secrete IL-2, interferon-γ and tumour necrosis factor-β whereas Th2 cells secrete IL-4, IL-5, IL-9, IL-10 and IL-13 (Sender and Paul., 1994). The Th2 cytokines (IL-4, IL-5 and IL-13) control the majority of the contributors to the airway inflammation, including IgE class switching, recruitment and activation of eosinophils and mucous hyperproduction (Sender and Paul, 1994, Ennis et al., 2004). IL-4 stimulates Jak1 and Jak3 to activate Stat6 (Pernis and Rothman, 2002), which plays an important role in regulating airway inflammation such as Th2 differentiation and IgE production (Kuperman et al., 1998, Akimoto et al., 1998, Miyata et al., 1999, Herrick et al., 2000, Tomkinson et al., 1999). Its role is confirmed in experiments with Stat6 deficient mice, which failed to develop allergen induced airway inflammation and airway hyperresponsiveness (AHR) (Kuperman et al., 1998). Sensitised and challenged Stat6 mice failed to develop a pulmonary Th2 response, AHR, goblet cell hyperplasia, eosinophilia, or IgE (Tomkinson et al., 1999). Presently several groups have investigated the expression and activation of Stat6 in asthmatics (Miller et al., 1998; Christodoulopoulos et al., 2001; Mullings et al., 2001) and have either found a high density of Stat6 expressing cells in the airways (Christodoulopoulos et al., 2001) or an elevated level of Stat6 secreted by bronchial epithelial cells (Mullings et al., 2001). Stat3, with diverse roles in biological processes including cell proliferation, survival apoptosis and inflammation is another cytoplasmic peptide belonging to the Stat family. It is activated by a large number of extracellular stimuli including IL-6, various other cytokines, granulocyte CSF, epidermal growth factor and IL-10 (Zhong et al., 1994; Darnell., 1997; Leonard and O’Shea., 1998,
Akira, 1997). However not much is known about its activation and function in the lung during airway inflammation. The functions of Stat3 are extensively studied in cell culture systems, immunological disorders and several inflammatory diseases. Its activation is implicated in the regulation of cell proliferation, differentiation, transformation and apoptosis (Bromberg and Darnell, 2000). In vivo aberrant expression of Stat3 is associated with immune tolerance (Cheng et al., 2003) acute phase response (Alonzi et al., 2001) septic shock (Andrejko et al., 1998) and constitutive activation in chronic inflammation. These observations suggest that Stat3 play important role during airway inflammation, especially asthma. Stat3 in macrophages play a negative role in inflammation (Takeda et al., 1999) and tissue specific disruption of Stat3 during haematopoiesis is associated with a lethal inflammatory bowel syndrome (Welte et al., 2003) suggesting that it regulates the induction of a distinct set of target genes in different cell types. Although these studies have indicated the importance of Stat3 in inducing inflammatory response, very little is known regarding its activation and function in the lung during acute inflammation. Gao et al., 2004 reported that the activation of Stat3 in the lungs is macrophage dependent and IL-6, IL-10 and C5a contribute to Stat3 activation in inflamed rat lung.

Stat3 has recently been implicated in the pathogenesis of asthma in a study showing that Stat3 dependent pathways induced by IL-13 in lung myofibroblast are inhibited by the administration of an inhaled corticosteroid, fluticasone (Cazes et al., 2001). The pharmacogenesis of asthma treatment reveals Stat3 as one of the candidate genes showing association in response to corticosteroid treatment (Tantisira et al., 2004). Stat3 polymorphism is significantly associated with baseline
FEV1 both in adults and children, suggesting that it may participate in inflammatory pathways and have an impact on the level of lung function (Litonjua et al., 2005). Thus there is growing evidence that Stat3 may also associated with airway inflammation and it would be interesting to know the status of this molecule in conjunction with Socs3 and IL-6 in airway inflammation as Socs3 is a negative regulator of Stat3 and gp130, the latter being the common signal transducing receptor chain for IL-6 type cytokines and ubiquitously expressed in most tissues. In the present study the role of Stat3 in airway remodelling has been studied in a mouse model of asthma. Alternatively, the fate of airways remodelling and the status of asthma associated cytokines by knockdown of Stat3 gene has been investigated.

Studies regarding the mechanisms underlying airway hyperresponsiveness are difficult to perform in human hence, animal models for allergic asthma are therefore required. The choice of the mouse for this type of studies has several advantages. First, the murine immune system is well characterized and many tools are available to manipulate immunological processes (Coffman et al., 1989; Scheynius et al., 1993; Van Oosterhout et al., 1995). Secondly, various mouse strains with mutations or genetic modifications are available (Galli and Kitamura., 1987, Tepper et al., 1990), which provide the opportunity to study certain aspects of immunological processes in more detail. Together, this makes the murine species very useful in investigating the role of immunological processes in the development of airway hyperresponsiveness. Studies in murine experimental models have contributed greatly to understanding the mechanisms of allergic inflammation underlying asthma. Modelling chronic asthma is the problematic because long term
antigen challenge triggers widespread pulmonary parenchyma inflammation or leads to eventual down regulation of inflammation and airway hyper-reactivity (Kumar and Paul., 2002).

Murine models have also facilitated the investigation of novel options for controlling allergic inflammation. These have included not only conventional pharmacologic approaches, using inhibitors of the synthesis of inflammatory mediators or functional antagonists (Fujitani et al., 1997, Kanehiro et al., 2001, Henderson et al., 2002, Oh et al., 2002), but also radical therapeutic options involving antisense oligonucleotides and DNA immunization (Finotto, 2001, Hertz, 2001). Numerous models of environmentally induced airway inflammation and AHR have been investigated in the inbred mouse. In comparison with outbred species, the inbred mouse represents a powerful tool for delineating the genetics of allergen-induced AHR. Because all animals in an inbred strain are genetically uniform, phenotypic variability is controlled solely by environmental influences. Furthermore, the short gestation period, large litter sizes, availability of numerous inbred mouse strains, well-defined genetic map, and ability to control the environment in which the animals are reared establish the inbred mouse as the ideal species for investigating both simple and complex genetic traits.

Although asthma is apparently restricted to the human species, animal models can be used to investigate particular aspects of this human disease. In order to investigate the mechanisms underlying airway hyperresponsiveness we developed murine model of asthma by sensitizing and challenging of BALB/c mice with Ovalbumin. The model exhibited airway-specific acute-on-chronic inflammation and
changes of airway wall remodelling as seen in human asthma, together with hyperreactivity. This is more realistic model of asthma offers a number of opportunities for investigation of pathogenic mechanisms and novel therapeutic agents. Chronic asthma with frequent symptoms need continuing treatment to control of symptoms and is resistant to steroids and is characterised by an incomplete reversibility of airflow obstruction despite treatment with bronchodilators and corticosteroids in high doses. The mainstay of treatment is inhaled corticosteroids. Although mild asthma can be safely treated with low doses of inhaled corticosteroids, patients with moderate and severe asthma are often receiving high doses of inhaled or systemic steroids, or both. The long term consequences of steroids given by mouth are well documented, with side effects such as osteoporosis, skin thinning, and hypertension.

It is evident from the current status of knowledge that Stat3 mRNA expression and protein activation are significantly low in inflammatory murine lung compared with non inflamed lung; blocking Socs3 gene expression resulted in heightened expression of IL-6 and Stat3 genes. Here the implications of these observations are discussed in the light of the pathogenesis of asthma. RNA interference (RNAi) can be exploited in medicine to find new therapies for diseases like asthma. RNAi can be thought of as an exciting discovery with many possible applications particularly in medicine at present and in the future. Lines of current research of this therapy include treatment of cancer, HIV, hepatitis B, influenza, Huntingdon’s disease, asthma and macular degeneration or blindness. The latter of which is currently undergoing clinical trials, demonstrating that RNAi therapy has genuine potential as a treatment in the
future. RNA interference is an innate mechanism for the silencing of genes, found to be active in many organisms including humans. This therapy involves injection of siRNAs (small interfering RNAs) for the genes of factor H and B as well as a serine protease (all proven to increase the risk of developing macular degeneration) so that they are recruited into the RISC complex and can degrade the target mRNA, greatly reducing chances of developing the disease or a deterioration. These principles relate in a similar way to other conditions, for example if siRNAs coding genes for proteins involved in tumour growth are injected into the cancerous site, silencing occurs, resulting in prohibited growth of tumour and obvious benefit to the patient. In the future, if genes could be silenced at an embryonic stage then hereditary diseases could be wiped out. There is always a risk that novel therapies could have unforeseen or long term side effects, although scrupulous experimentation and trials should reveal and prevail over any encountered. One study in mice suggested that RNAi affected some cell processes such as DNA repair, the cell cycle and even led to apoptosis (self destruction of cell). It may also prove a little unreliable to simply rely on animal experimentation because of genetic differences between animals and humans. A further setback of RNAi is its temporary nature, as mentioned previously. People will always prefer to have as long lasting medication as possible and avoid lifetime regular use although this is not a major inconvenience if the symptoms are cured. One possible line of investigation could be to insert plasmids that contain genes for the production of the desired siRNAs so that RNAi is continual or even lifetime. However insertion of plasmids is difficult even in the laboratory and can often lead to cell death, a risk that may be too great. Although, inevitably, there will be
drawbacks in RNAi therapies, future advancements and technology seems promising to conquer these and provide a new tool for the treatment and possible cure of many serious diseases including asthma.

Stat3 is the subject of intense scientific investigation, because it's known to be an important transcription factor that turns on genes required for the division, growth and death of cells. Small interfering RNA (siRNA) is a potent, selective, and easily-inducible method for specifically blocking expression of desired genes. siRNA is reported to be more efficient than traditional therapy such as antisense oligonucleotides. However, the therapeutic potential of siRNA for allergic diseases has not yet been investigated in detail. In the present study using murine model of asthma we have studied the role of Stat3 silencing in asthma. The study will also help in understanding the mechanism of disease onset and progression in the light of signaling molecules and pro-inflammatory cytokines. The study will also throw light on the therapeutic potential of siRNA and further to investigate the effects of it especially on airway inflammation, remodeling, hyperresponsiveness and infiltration and activation of eosinophils.
Review of Literature
Allergic asthma is a complex chronic inflammatory disease of the airways and its etiology is multifactorial. It involves the recruitment and activation of many inflammatory and structural cells, all of which release inflammatory mediators that result in typical pathological changes of asthma (Barnes et al., 1998, O'Byrne and Postma., 1999). Symptoms of asthma range from mild to life threatening. All these symptoms are caused because the airways become narrow and blocked during an asthma episode. In response to an allergic reaction the lining of the airways of asthmatic patient become very inflamed which means they swell with fluid and cells (lymphocytes, eosinophils and mast cells). The infiltration of leukocytes, particularly eosinophils, into the lungs and release of vasoactive mediators from mast cells set the stage for asthmatic inflammation. Many cells and cellular elements, including mast cells, eosinophils, T-lymphocytes, macrophages, neutrophils and epithelial cells are involved in the process. The cells that line the airway produce excess mucus, which builds up inside the airway passage. Increased mucus production in asthma is an important cause of airflow obstruction during severe exacerbations. Excessive mucus secretion from hyperplastic goblet cells is a feature of the asthmatic airway and might lead to the occlusion of the airways. Airway remodeling thus results in thickened airway walls in asthma, and is thought to be a determinant of airway hyper-responsiveness (AHR), as well as the accelerated loss of lung function over time described in asthma (Busse et al., 1999, Elias et al., 1999). The rings of muscle that wrap the airways constrict tighter and tighter, pinching the airways closed.
Normal and asthmatic airways. In the normal airway, there is a lot room for air to move back and forth during the breathing process. This is compared with an airway that is inflamed, constricted and filled with fluid where there is almost no room for air flow.

**Prevalence**

Asthma affects about 10% of children and 5% of adults. More than 300 million people suffer from asthma worldwide. This disease causes considerable mortality and morbidity, with 2000 potentially avoidable deaths each year. The prevalence of asthma in a population is approximately 4–8% (using symptom based questionnaires); this increases to 20–25% in those affected with first degree relatives. This measure can be expressed as the risk ratio ($\lambda_s$), which is defined as the prevalence of a disease in first degree relatives of an affected subject divided by the prevalence in the general population. In asthma $\lambda_s$ is approximately 5–6, compared with 15 in type-I diabetes mellitus, 8 in schizophrenia, and 3.5 in type-II diabetes mellitus (Anderson and Morrison., 1998).
Etiology of asthma

The central tenet of research in asthma genetics is that clinical disease occurs in a subject with a genetic susceptibility, which becomes expressed after exposure to an environmental trigger. Several environmental factors have been proposed, including house dust mites, cigarette smoke, viral respiratory tract infections, and atmospheric pollution. Unfortunately, the elements of the genetic susceptibility have proved to be more elusive (Anderson and Morrison, 1998). Agents that cause respiratory allergy have broadly been categorized into low-molecular-weight chemical haptens (molecular weight of 1000 Da) and high-molecular weight agents that include proteins from diverse sources, i.e., plants, bacteria, arthropods or animals. The low molecular-weight agents are reactive chemicals and act as allergen when bound to appropriate carrier molecules, such as autologous proteins. The incriminated etiologic low-molecular-weight agents share a common toxicological characteristic of being irritant in nature. In some cases, these agents are present as a gas, in others the inciting agent is an aerosol or represent mixtures of a volatile hapten partitioned with the aerosol phase. High-level exposure to irritants may cause airway hyperreactivity and considered to be different from typical occupational asthma because of its rapid onset, specific relationship to a single environmental exposure, and no apparent pre-existing period of sensitization to occur with the apparent lack of an allergic or immunologic etiology. Hence, this illness is termed reactive airways dysfunction syndrome (RADS). Mechanisms to explain the development of RADS focus on the toxic effects of the irritant exposure on the airways and may be attributed to neurogenic effect (Brooks et al., 1985). This implies some uncertainty as to whether
exogenously administered agents exert similar effects when the exposure is direct (by inhalation) or via systemic routes. To date more than 50 different mediators have been identified in asthma, although, at the same time, current gene technology identifies an ever increasing range of molecules that could be involved in the sensitization process to an allergen and/or the development of a chronic inflammatory process in the mucosa of the lower airways. This evolution tends to confer an image of overwhelming complexity. The reason(s) for the induction of immediate hypersensitivity to the antigen mediated by a predominant IgE and IgG1 response may reside in properties of the antigen or allergen itself, the time frame investigated, or the dose and route of sensitization.

Pathogenesis

Cytokines play a critical role in the normal development and function of the immune system. On the other hand, many rheumatologic diseases are characterized by poorly controlled responses to or dysregulated production of these mediators. Over the past decade tremendous strides have been made in clarifying how cytokines transmit signals via pathways using the Janus kinase (Jak) protein tyrosine kinases and the Signal transducer and activator of transcription (Stat) proteins. Antigen-induced airway hyperresponsiveness and airway inflammation are features of both human asthma and animal models of this disease. The genesis of these key asthma phenotypes represents the summation of a complex cascade of immune responses. It is hypothesized that multiple cell types are involved in the induction, propagation, and maintenance of these immune processes. Several molecules have been reported to
be essential for cell-cell interactions, inflammatory cell recruitment, and effector functions leading to the overall expression of the asthmatic phenotype.

An important goal of asthma research is to understand the genetic and environmental triggers for asthma and the factors that lead to variations in its natural history. It is known that inflammation is a key element in the diathesis (Wills-Karp and Ewart, 2004). Past decade studies have demonstrated that airway inflammation is a principal feature in the pathophysiology of asthma (Gern et al., 1999). The disorder is multifactorial. It is (in both initiation and progression) because of the involvement of numerous resident and recruited inflammatory cells. T cells and IgE-mediated responses are known to be a key factor in the allergic response (Elias et al., 2003). The dendritic cells are the bridge between allergens and T cells through antigen-processing events. In response to allergens the T lymphocytes produce a restricted array of cytokines. In particular, the pro-inflammatory cytokines are synthesized by the Th2 subtype of T helper cells. The other subtype, the Th1 cells are involved in virus defence and antagonize the allergic response. An imbalance in the expression of T cell phenotype is thought to play an important role in the pathophysiology of asthma (Yazdanbakhsh et al., 2002). T helper cells are not the only source of proallergic cytokines; mast cells, basophils, eosinophils, CD8+ T cells and bronchial, fibroblast and smooth muscle cells can also produce inflammatory molecules as those encoded by the 5q cytokine cluster, including IL-4, IL-5, IL-9 and IL-13 (Renauld, 2001). Cross-linking to the IgE on mast cells ε subunit of the high affinity IgE receptor (FceRI) triggers the release of preformed vasoactive mediators, synthesis of prostaglandins and leukotrienes, and transcription of cytokines (Wenzel
et al., 2003). The allergen-specific IgE are also induced by the interaction of the IgE with the FcεRII receptor (CD23) on B cells. IgE production is associated with allergic hypersensitivity responses to inhaled allergens. The release of neutral protease from mast cells, through the IgE receptor, can activate the receptors of endothelial and epithelial cells that in turn lead to the production of cytokines and adhesion molecules that selectively recruit eosinophils and neutrophils, cells relevant to the inflammatory manifestations (Cohn et al., 2004). Eosinophils play a key role in the pathogenesis of allergic asthma (Bandeira-Melo and Weller, 2003). They secrete a number of inflammatory mediators including cytokines, tumor necrosis factor-alpha (TNF-α), transforming growth factor-β (TGF-β), prostaglandin E2 (PGE2), cysteinyI leukotrienes and platelet activating factor (PAF), as well a number of reactive oxygen intermediates, cytotoxic peptides and degradation enzymes such as elastase and collagenase (Chanez et al., 1990; Behm and Ovington, 2000; Carey et al., 2003).

Thus, the inflammatory response is also involved in the damage and repair of the host tissue. Along with cytokines, chemokines play a major role in asthma pathogenesis as they are potent leukocyte chemoattractants, cell activating factors, and histamine-releasing factors. In particular, the eotaxin subfamily of chemokines and their receptor CC chemokine receptor 3 (CCR3) have emerged as central regulators of the asthmatic response (Saito et al., 2002, Scheerens et al., 2002). It can be anticipated that further insight into the functional role of cytokines will result in novel therapeutic perspectives. For some cytokines, specific inhibitors are currently being developed for human use. It will be interesting to see how they lead to better
understanding of the disease process in the individual patient and allow for fine-tuning of treatment regimens.

Airway inflammation and remodeling in asthma

Schematic section through airway wall. EGF: Epidermal growth factor, GM-CSF: Granulocyte macrophage colony stimulating factor, TNF-α: Tumor necrosis α factor; VCAM-1: Vascular cell adhesion molecule-1; ICAM-1: Intercellular adhesion molecule-1; MCP-1: Monocyte chemotactic protein-1; Histamine; LT: Leukotriene; TFG-β: transforming growth factor β; PDGF: Platelet derived growth factor.

Biochemical pathways involved in the pathogenesis of asthma

The biochemical pathways involving atopic asthma have been studied in great detail. Basically, two types of airway responses are initiated on allergen challenge of an appropriately sensitized asthmatic individual (Busse and Rosenwasser, 2003). The early phase is characterized with an acute bronchospasmodic event that begins 15-30
min after exposure and resolves over time. The process initiates with the recruitment of a subtype of CD4+ T cells, Th2, which produce predominantly interleukin-4 (IL-4), interleukin-5 (IL-5) and interleukin-13 (IL-13), at the site of immune activation (Martinez and Holt, 1999, Busse and Rosenwasser, 2003). IL-4 along with IL-13 induces B cells to produce IgE (Holberg et al., 1999). IL-13 also induces mucus secretion from the goblet cells (Kibe et al., 2003, Kondo et al., 2002). IL-5 in association with interleukin-13 (IL-13) and granulocyte macrophage colony stimulating factor (GMCSF) helps eosinophils to grow, mature and infiltrate into the lungs (Yamashita et al., 2002, Matsumoto et al., 2003, Nag et al., 2003). Thus, asthma is mainly associated with an increase in Th2 cytokines both in the bronchoalveolar lavage (BAL) and serum and with increased IgE levels in the sera (Brown et al., 2003, Larche et al., 2003). Cross linking of IgE receptor present on mast cells by fresh exposure of allergens initiates this acute phase. The late phase response begins 4-6 h after the initial insult and causes prolonged symptom. The infiltration of leukocytes, particularly eosinophils, into the lungs and release of vasoactive mediators from mast cells set the stage for asthmatic inflammation. Along with cytokines, chemokines play a major role in asthma pathogenesis as they are potent leukocyte chemoattractants, cell activating factors, and histamine-releasing factors. In particular, the eotaxin subfamily of chemokines and their receptor CC chemokine receptor 3 (CCR3) have emerged as central regulators of the asthmatic response (Saito et al., 2002; Scheerens et al., 2002). Past few year studies have provided an integrated mechanism for understanding the coordinate interaction between IL-13 and chemokines in the pathogenesis of asthma (Zimmermann et al., 2003). Finally,
structural alterations, including airway wall thickening, lung fibrosis, mucus metaplasia, hyperplasia and hypertrophy of the myocyte are certain features which are generally observed in the airway of asthmatics (Elias et al., 1999; Holgate et al., 1999).

**Role of genes in the pathogenesis of asthma**

The past few years have witnessed remarkable progress in unravelling the genetics of asthma using both genome wide linkage and candidate-gene-association approaches. Genetic association studies in asthma, using candidate gene approaches, have also flourished in recent years, as both genomic tools for comprehensively surveying variation across genes and high throughput genotyping platforms have become available. Some studies have further demonstrated that genetic susceptibility is both contexts dependent and developmentally regulated. Asthma is a common, chronic disease with a complex etiology. A review of the literature revealed >120 genes correlating with asthma in humans in association studies and >150 genes in animal model (Szalai et al., 2008). These results indicate that asthma is a polygenic disease and its complexity originates from interaction of an unknown number of genes and environmental factors.

Atopic asthma is found to be strongly familial, however the mode of inheritance is controversial. A large number of studies have been carried out and a number of candidate genes have been identified. In addition, a number of chromosomal regions have been identified using genome-wide scans, which might contain important unknown genes. Atopic asthma in children is found to be strongly familial and a genetic basis is indicated by familial aggregation and the identification
of candidate genes and chromosomal regions linked to asthma risk (Burke et al., 2003). The risk of a first-degree relative of an asthmatic individual being asthmatic is two to almost six times higher than the risk for an individual from the general population to develop the disease (Lander and Schork, 1994, Koppelman et al., 1999). Both shared genes and shared environment account for such a huge risk.

Studies with twins have shown that the incidence of asthma is significantly higher in monozygotic twins than dizygotic twins (Edfors-Lubs, 1971, Skadhauge et al., 1999). It has earlier been shown that atopic asthma was influenced by a few genes with moderate effects (Jenkins et al., 1993). Similarly few other studies have implicated the maternal inheritance of atopy (Moffatt and Cookson, 1998). A previous study has suggested that early breastfeeding may increase the risk of allergic disease in genetically susceptible children (Duffy, 2001). Although asthma has a significant heritable component, the mode of inheritance is controversial due to the complex nature of the disorder. In a study conducted in Taiwan, it was concluded that a history of asthma in parents is a strong risk factor for asthma in the offspring (Wang et al., 2000). Under the assumption of applied segregation, it was reported that at least one major gene exists that could be involved in the development of allergy. In addition, a polygenic/multifactorial (genetic and environmental factors) influence with a recessive component inheritance may be involved in the pathogenesis of asthma (Wang et al., 2000). Further, there are gene-gene interactions that may lead to increased risk of developing asthma (Barnes, 1999, 2000, Hoffjan and Ober, 2002). Polymorphisms in several candidate genes have been found to be associated with asthma and allergic disorders.
In different independent studies, polymorphism in the \( \beta \)-chain of high-affinity receptor for IgE (FceRI\( \beta \)) in the same chromosomal location was found to be associated with asthma, atopy, bronchial hyperresponsiveness and severe atopic dermatitis (Cookson et al., 1989, Kinet, 1999). A significant association of total serum IgE concentrations and asthma with genetic markers within the IL-4 gene cluster (5q31.1) has been established (Marsh et al., 1994, Meyers et al., 1994). Interestingly, this region contains a large number of important candidate genes that encode IL-4, IL-13, IRF1, IL-9, CD14, IL-12\( \beta \) and \( \beta_2 \)-adrenergic receptor. In past decade, polymorphisms have been recognised in several of these genes which may contribute to the pathophysiology of allergic diseases (Baldini et al., 1999; Gao et al., 1999). It has been proposed that these genes are co-ordinately expressed due to the presence of some common regulatory motifs; therefore, polymorphisms within this cluster could be due to linkage disequilibrium with other known or unknown genes (Marsh et al., 1994).

Chromosome 12q is another interesting region for both asthma and atopy because of the presence of several candidate genes encoding IFN-\( \gamma \) (Nakao et al., 2001, Nagarkatti et al., 2002) signal transducer and activator of transcription 6 (STAT 6) (Djukanovic., 2001, Suminami et al., 2002, Herbon, 2002) a mast cell growth factor and a \( \beta \)-subunit of nuclear factor-\( \gamma \). Studies with Afro-Caribbean and Caucasian populations found an association of serum IgE and asthma to markers on chromosome 12q (Hizawa., 1999). Earlier studies in several populations have observed that IFN-\( \gamma \) gene was linked to atopy and asthma (Nakao et al., 2001, Nagarkatti et al., 2002). Past few year studies carried out in the Indian population
have shown a significant positive association of (CA)n repeat in IFN-γ with asthma phenotype and serum IgE levels (Nagarkatti et al., 2002). Stat6 plays a major role in the initiation of signals from activated Th2 cells, specifically through IL-4 and IL-13 receptors (Mullings et al., 2001). In a study conducted in the Indian population, novel polymorphisms in the Stat6 gene had been identified (Nagarkatti and Ghosh, 2002). A polymorphism in the IL4Ra coding region has been associated with asthma (Mitsuyasu et al., 1999). Also, polymorphism in TNF-α has been found to be associated with asthma (Moffatt and Cookson., 1998). There is a significant difference in the linkage in candidate genes among various ethnic populations. Studies of asthma conducted in Japan, UK, and USA have implicated chromosome 5q as the region containing one or more susceptibility genes for asthma (Doull et al., 1996, Noguchi et al., 1997, Hizawa et al., 1998, Ober et al., 1998).

However, in studies conducted in Australian, Finnish, British, Scottish and German populations, chromosome 5q did not be appeared to be linked with asthma or atopy (Kamitani et al., 1997, Laitinen et al., 1997, Ulbrecht et al., 1997, Mansur et al., 2000, Van Eerdewegh et al., 2002). These studies on candidate genes have been mostly done on limited sample sizes. For the utility of these studies a large scale epidemiological study is required to classify various classes of allergies and asthma. In addition to studies on candidate genes, several genome-wide searches have been carried out. In this approach, genetic markers throughout the genome are mapped in family members and are used to identify chromosomal regions that are co-inherited with a particular phenotype such as asthma, bronchial hyperresponsiveness (BHR), or a positive SPT.
Gene expression studies

DNA microarray techniques can be used to study gene expression of a large number of loci. The expression can be monitored simultaneously and the expression profiles may be compared in different samples. The difference in the sample may be due to the time interval at which the gene expression is observed, exposure to a treatment, cell types studied and other causes. The heterogeneity of disease phenotype may be studied with many different methods in order to collect information that may contribute to the overall understanding of the disease. This technology has been used in asthma with different intents and reveals to be a reliable tool to confirm previous observations or to provide new clues for metabolic pathways involved in the pathophysiology of the disease.

Role of environment in the pathogenesis of asthma

In addition to genes, environmental factors, such as allergens, food, childhood viral infection etc., also play significant roles in causing asthma. The incidence of asthma is rising with an alarming rate in developed as well as in the developing countries. It has been postulated that the immune deviation resulting in asthma takes place much earlier in utero (Prescott et al., 1998). Depending on the genetic status of the mother during pregnancy and exposure to various allergens, it is possible that the child may be born with an intrinsic propensity to be atopic. Genetically predisposed children when exposed to environmental allergens develop asthma even in very early phase of life (Liu and Szefler, 2003). Evidence of polymorphism in the CD14 (LPS receptor) gene supports this hypothesis (Baldini et al., 1999). In a recent study conducted in Canada, it has been shown that daily visits to a local hospital due to
asthma increased significantly with increases in level of pollens and pollution in the air (Dales et al., 2003). Similarly, in a study carried out in US, it has been shown that with increase in air pollution levels in Cincinatti, Cleveland and Columbus, the visits to the asthma clinic increased significantly (Jaffe et al., 2003). In a study carried out among Palestinian children it has been shown that familial atopic diseases are predictors of asthma in children, however the indoor environment, such as the presence of cats, dogs, etc., also play a major role (El-Sharif et al., 2003). In contrast, it has also been shown that the prevalence of asthma in the western countries is increasing even though the environment is cleaner than earlier (Richter et al., 2003). For example, the incidence of atopic disorders including asthma in East Berlin increased after the unification of Germany (Nicolai and Mutius, 1997, Heinrich et al., 1998, Von et al., 1998, Weiland et al., 1999).

Similarly, many surveys have identified an inverse relationship between prior microbial exposure and the development of atopy. Further, it has been seen that respiratory allergy appears less frequently in people exposed to orofaecal and food-borne microbes. Thus, improved hygiene, early infection and antibiotic use, and semi-sterilized diet may facilitate atopy by influencing exposure to commensals and pathogens that stimulate cell populations such as gut associated lymphoid tissue (Heaton et al., 2003; Chu et al., 2003). It is, therefore, proposed (hygiene hypothesis) that the cleaner environment in the western countries is not favourable for providing signals for Th1 development, especially in children born of atopic parents. The underlying reason of these apparently contradictory observations is not understood as yet. Nevertheless, it seems very likely that environment is only a triggering factor. A
genetically predisposed individual will develop the disorder anyway once the ‘proper’
environmental exposure is provided irrespective of the specific nature of the trigger.
Therefore, the identification of the environmental factors that trigger asthma offers the
possibility of prevention of disease.

Endpoints of respiratory allergy

As detailed above, a common pathologic accompaniment or cause of
increased airway hyperresponsiveness is a prolonged airway inflammation and
remodelling after inhalation of specific haptens or antigens. It is suggested that the
inflammation and/or remodelling are responsible for the change in increased agonist
responsiveness. In contrast with the assays relying upon an induction of a specific set
of characteristic immunologic biomarkers, some models do not depend upon a
preconceived mechanism of sensitization and response. Rather, they function by
reproducing the characteristics, which typify the hypersensitivity reactions, i.e., the
immediate-onset physiologic response of the airways which is bronchoconstriction.
The clinical manifestation of respiratory allergy is not characterized by one single,
unique endpoint, except anaphylaxis that occurs in guinea pigs. Depending on the
type of plethysmography applied, an analysis of additional characteristics typifying a
change in breathing patterns is useful. In spontaneously breathing animals, in
addition to measurement of respiratory rate, flow-derived endpoints, such as peak
inspiratory and expiratory flows and respiratory-pattern related endpoints, e.g.
inspiratory, expiratory, and relaxation times, enhanced pause (Penh) have been
shown to improve the sensitivity of analysis.

Structural changes of the lung: airway remodeling and pathobiology
of chronic asthma
As already alluded to above, one of the hallmarks of asthma is chronic inflammation and airway remodelling. In humans, pulmonary function measurements and sometimes also histopathology (lung biopsies) conveniently addresses these changes. Although changes in pulmonary function are not pathognomonic for specific lesions, much about lung structure can be implied from functional changes. In such cases, a battery of tests of different facets of lung function are usually applied, and results are expressed as classes of function disorders (e.g. obstructive or restrictive) that are consistent with classes of morphological changes (e.g. emphysema, bronchitis, fibrosis) (Pauluhn, 2000., Pauluhn et al., 2001).

Asthma in BALB/c mice is marked eosinophilic infiltration in lung parenchyma and in large and distal airways. They also found subepithelial fibrosis, myocyte hypertrophy and hyperplasia, elastic fibre fragmentation and increased numbers of myofibroblasts in airways and lung parenchyma. They reported infiltration of neutrophils, lymphocytes, and dendritic cells in the lungs and increased collagen fibre content in the alveolar walls. Histopathological studies of the airway inflammation in asthma have found increase in eosinophils and lymphocytes. The resultant phenomena of mucosal oedema, vascular congestion, bronchoconstriction, increased mucus production, and impaired ciliary function are thought to be a consequence of the interaction and activation of these inflammatory cells. Bronchial biopsy samples and broncho-alveolar lavage cell studies have shown an increased number of eosinophils, lymphocytes, and mast cells. In addition, the eosinophils showed evidence of activation with increased concentrations of major basic protein, eosinophilic cationic protein, and other granular proteins (Anderson and Morrison.,
Yamauchi et al., 2008 evaluated the structural changes in lung tissue in OVA-inhaled mice at day 50 (4-week exposure). They showed a markedly increased deposition of collagen around the airway wall compared with that of saline-inhaled mice. Masson-trichrome staining of lung tissue at day 29 demonstrated highly increased collagen in OVA-inhaled mice which was not observed in the airway wall of saline-inhaled mice. Furthermore, in OVA-inhaled mice, abundant inflammatory cell infiltration and goblet cell hyperplasia were detected. However, at present the clinical importance and mechanism of airway remodeling in asthma remains controversial.

Janus Kinases

There are only four known mammalian Jaks: – Jak1, Jak2, Jak3, and Tyk2 – which were identified in the early 1990s by techniques that capitalized on homology of their kinase domains to other tyrosine kinases (Krolewski et al., 1990, Wilks et al., 1991; Harpur et al., 1992 & 1994). Since the discovery of these family members, no new mammalian members have been identified, suggesting that they may comprise the entire family. Teleost and avian Jaks have been identified, as has a single Drosophila Jak; thus, these critical signaling molecules are highly conserved throughout evolution (Binari and Perrimon, 1994; Harrison et al., 1995; Sofer et al., 1998). Shortly after their discovery, their functional importance of IFN and cytokine signaling was established (Darnell et al., 1994, Ihle, 1995). It was first shown that Jaks are essential for IFN signalling using a panel of cell lines that were resistant to IFNs (Velazquez et al., 1992; Watling et al., 1993) and subsequently, type I cytokines were also found to activate Jaks; in fact, all type I and II cytokines activate Jaks in some combination (Witthuhn et al., 1993, Argetsinger et al., 1993, Witthuhn et
It was also shown that Jaks physically associate with cytokine receptors.

**Diagrammatic Structure of Janus kinases (Jaks)**

![Diagram of Jaks Kinases](image)

**Signal transducers and activators of transcription (Stats), and suppressors of cytokine signaling (SOCs).** Regions of homology shared by Jaks have been termed Jak homology (JH) domains. JH1 is a kinase domain and JH2 is a pseudo-kinase domain. The amino-terminus of the Jaks appears to be important for association with cytokine receptors subunits.

**Stat structure**

In contrast to the Jaks, the structure of the Stat molecules has been reasonably well characterized (Becker *et al.*, 1998; Chen *et al.*, 1998). Overall, the structure of the Stats is similar to that of other transcription factors such as nuclear factor-kB and p53. The dimeric molecule forms a C-clamp structure around the DNA, but, unlike nuclear factor-kB and p53, there are fewer direct contact sites with the DNA backbone. Rather, the nutcracker-like structure of the Stats is largely dependent upon SH2–phosphotyrosine interactions.

Stats have a conserved amino-terminal protein–protein interaction domain, followed by a segment (the coiled-coil domain) with multiple protruding a-helices. This is followed by the actual DNA binding domain, a linker domain, the SH2 domain, a conserved site of tyrosine phosphorylation, and a variable carboxy-termini transcriptional activation domain. Amino-terminal dimer–dimer interaction domain
with the exception of Stat6, Stats bind somewhat indiscriminately to the same consensus sequences; it is notable that clustered imperfect Stat binding sites are found in a number of relevant cytokine inducible promoters. Even though Stats bind poorly to these sites, cooperative dimer–dimer interactions can occur (Xu et al., 1996). This is mediated by the conserved amino-termini of Stats, which consists of eight helices that form hook-like structures, facilitating these interactions. Perhaps unimportant for Stat binding to a single consensus binding site (Mikita et al., 1996), these domains appear critical for binding to imperfect sites (John et al., 1999).

Diagrammatic Structure of Stat

Stats have a conserved tyrosine residue, phosphorylation of which allows Stat dimerization; a src homology (SH2) domain that mediates the dimerization; and an amino-terminal region that is known to play a role in the dimerization of Stats dimer. The amino-terminal, carboxy-terminal and coiled-coil regions of Stats can interact with other transcription factors.

Stat3

Stat3 was first identified as a factor activated by cytokines signaling through gp130 (IL-6, leukaemia inhibitory factor, and ciliary neurotropic factor). Stat3 deficiency is embryonically lethal, perhaps due to the absence of leukocyte inhibiting factor function, as well as its role in maintaining stem cell pluripotency (Takeda et al., 1997; Escary et al., 1993). In contrast, gene targeting of Stat3 only in myeloid cells
produced an exaggerated inflammatory response, resulting in premature death largely due to impaired IL-10 function (Takeda et al., 1999). These animals became highly susceptible to endotoxic shock with increased production of inflammatory cytokines such as tumor necrosis factor-α, IL-1, IFN-γ, and IL-6. The suppressive effects of IL-10 on the production of inflammatory cytokines by macrophages and neutrophils were completely abolished, and these mice developed chronic enterocolitis with age. Additionally, these mice manifested an exaggerated Th1 response, which may also help to explain the inflammatory bowel disease seen. These results might suggest a role for abnormal Stat3 signaling in other autoimmune processes. Although it is clear that Stat3 is essential for appropriate IL-10 signaling, its function for other cytokines remains unclear because of the embryonic lethality seen in Stat3 knockout embryos.

Cytokine signaling though Jaks and Stats

Immune and inflammatory systems are controlled by multiple cytokines, including interleukins and interferons. Many of these cytokines exert their biological functions through JAKs and Stats (Yoshimura et al., 2005).
Cytokines associate with cytokine receptor subunits and activate Janus kinases (Jaks). The Jaks in turn phosphorylate tyrosine-based docking sites on the receptor. Signal transducers and activators of transcription (Stats) then bind via their src homology (SH)2 domains. The Stats are then phosphorylated by the JAKs, form homo-hetero-dimers and then translocate into the nucleus, where they bind target sequences like gamma-activated sequence (GAS) motif. Transcriptional activation of genes typically requires the coordinated function of multiple accessory transcription factors. Additionally, serine phosphorylation of some Stats may be important for maximal transcription of target genes.
Activation of JAKs and Stats by cytokines: A schematic diagram showing the steps involved in regulation of gene expression by cytokines through the JAK/STAT pathway

Cytokine signaling through the JAK/STAT pathway is initiated upon cytokine binding to its receptor causing receptor oligomerization. This is followed by a coordinated series of tyrosine phosphorylation events that lead to activation of a variety of signaling molecules, including the Signal Transducers and Activators of Transcription (Stats).

**Socs3**

The suppressor of cytokine signaling (Socs) protein family represents a novel group of cytoplasmic negative feedback regulators of type I and II cytokines. Several of the Signaling pathways regulated by Socs proteins are important in allergic immune responses. Thus, Socs proteins may be important regulators of atopy (Rothman et al., 2007). The study of mice lacking genes encoding Socs proteins has
demonstrated the important role that these proteins perform in regulating the activity of an array of cytokines. Many of the pathways regulated by Socs proteins are important for the initiation of propagation of allergic immune responses. New data suggest that variants of Socs genes are present in human beings and that subtle alteration in the levels or structure of Socs proteins may affect their function. One of the essential properties of cytokines is their limited duration of action. This property leads to the effective curtailment of immune responses once the allergen is removed from the responding organ. Some studies have demonstrated that cytokine signaling is limited by several mechanisms. The SH2 domains of Socs3 and Socs1 share 40% homology. Multiple studies have demonstrated the capacity of both of these proteins to inhibit JAK/STAT signaling downstream of multiple cytokines. However, the mechanisms by which they alter signaling appear to differ. Whereas Socs1 directly binds to and inhibits activated JAK kinases, Socs3 inhibits JAK kinases when Socs3 is bound to phosphorylated cytokine receptors (Zhang et al., 2008). The role of Socs3 in the regulation of cytokines in allergic responses is complex. Initially, Socs3 was shown to inhibit IL-4-dependent Stat6 activation in 293T cells. However, B-cell lines that stably express Socs3 do not display any alteration in IL-4 signaling (Rothman et al., 1999). In mice, increased Socs3 expression correlates with the pathology of allergic immune diseases.
Socs proteins share a similar structure with a central SH2 domain, a region at the amino-terminus that is variable in both length and in amino acid sequence, and a region of homology at the carboxy-terminus termed the ‘Socs box’.

The molecular mechanism by which Socs proteins negatively regulate cytokine signaling

Cytokine stimulation activates the JAK-STAT pathway, leading to the induction of CIS, Socs1, Socs2, and/or Socs3. These Socs proteins then inhibit the signaling pathways that initially led to their production. Socs proteins therefore act in part of a negative feedback loop. CIS, Socs1, Socs2, and Socs3 appear to inhibit signaling by different mechanisms: Socs1 binds to the JAKs and inhibits catalytic activity, Socs3 binds to JAK-proximal sites on cytokine receptors and inhibits JAK activity, and CIS blocks the binding of Stats to cytokine receptors. The mechanism of Socs2 action remains to be determined.
ANIMAL MODELS OF ASTHMA

Numerous models of acute or chronic asthma have been developed in several animal species. Protocols leading to manifestations mimicking human asthma have been reported in rodents (guinea pig, rat and mouse), rabbit, cat, dog, sheep, pig, horse and non-human primates (Szelenyi, 2000). However, mouse models have proven to be very efficient for the study of detailed mechanisms because of the abundant knowledge accumulated on mouse immunology and the large size of the 'toolbox' available. Techniques in genetic engineering have considerably improved and allow the generation of gene-targeted mice at low cost, most valuable for studying detailed mechanisms.

Induction of asthma in the mouse is a sequential protocol including the systemic priming of the immune system against an antigen (sensitization), followed by the triggering of an eosinophilic allergic reaction by a second application of the antigen in the airways (challenge). The route of administration varies between models (intraperitoneal, subcutaneous, intranasal, intratracheal) and can lead to different manifestations (Repa et al., 2004) as well as the dose of antigen. The most widely used antigen is the chicken Ovalbumin (OVA), in combination with an adjuvant. The most commonly used adjuvant is aluminium hydroxide, alum.

Availability of transgenic and gene-targeted animals, and in part because of the variety of specific reagents available for phenotypic and functional analysis of the cellular and mediator response (Pauwels et al., 1997; Wills-Karp., 2000), are the reasons for extensive use mice model. Murine models of allergic bronchopulmonary inflammation have proved to be extremely useful for examination of the basic
mechanisms of allergic inflammation and the underlying immunologic response. The key contribution of CD4 T-lymphocytes to the pathogenesis of asthmatic inflammation, as well as the potentially crucial roles of so-called Th2 cytokines, has been highlighted by several studies in mice (Gavett et al., 1994; Garlisi et al., 1995; Kaminuma et al., 1997; Kaminuma et al., 1998). In particular, studies on interleukin (IL)-5 (Nakajima et al., 1992; Nagai et al., 1993) and IL-13 (Grunig et al., 1998) in murine models have led to an understanding of the cellular sources of these mediators and the signalling pathways involved. Other noteworthy mechanistic studies have focused on mediators and adhesion molecules involved in leukocyte recruitment (Nakajima et al., 1994; MacLean et al., 1996; Henderson et al., 1996; Mould et al., 1997; Gonzalo et al., 1998; Campbel et al., 1998; Wolyniec et al., 1998; Hisada et al., 1999 [a]; Hisada et al., 1999 [b]), IgE-independent mechanisms of allergic inflammation (Mehlhop et al., 1997; Hogan et al., 1997; Korsgren et al., 1997; Korsgren et al., 1997), the role of eosinophils in the afferent limb of the allergic response (Shi et al., 2000, MacKenzie et al., 2001, Mattes et al., 2002).

Murine models have also facilitated the investigation of novel options for controlling allergic inflammation. These have included not only the conventional pharmacologic approaches, using inhibitors of the synthesis of inflammatory mediators or functional antagonists (Cohn et al., 1997, Kuperman et al., 1998), but also the radical therapeutic options involving antisense oligonucleotides and DNA immunization (Finotto et al., 2001).
**In silico models**

It is currently not possible to achieve mathematical modeling of the immune system in its whole complexity. However, some groups have designed computer models reflecting the behaviour of individual parts of the immune response, for example the dynamic of proliferation and differentiation of cytotoxic T lymphocytes during the course of a viral infection. More related to the field of allergy, Kumar and coworkers 2004, described a fairly simple model of acute inflammation in a system composed of one pathogen and two inflammatory mediators. As far as asthma is concerned, the available in silico models only consist of mechanistic approaches, where the morphology of the airways is reproduced. These models can take into account bronchoconstriction and airway remodelling and associated to simulations of drug particles deposition in the airways, are mainly used in pharmacological studies on delivery of antiasthma inhaled drugs (Martonen et al., 2003).

**In vitro models**

Although in vitro technology allows studying separately the cellular actors of the asthmatic response like mast cells, bronchial epithelial and smooth muscles cells or T cells, no integrated in vitro model exists that can reproduce such a complex disease as asthma. However, mechanistic studies of bronchoconstriction, one of the hallmarks of asthma, can be done Ex vivo using isolated human or animal airways segments or bronchial rings (Frossard et al., 2005; Liu et al., 2006) or even whole mouse lungs (Witzenrath et al., 2006).

As we have seen it, mouse models are the main source of knowledge concerning the role of T cells in asthma. However, these models are not perfect. In
general, numerous differences exist between the immune systems of mice and humans (Mestas and Hughes, 2004), and regarding asthma in particular, some manifestations of human asthma cannot be reproduced in mice and some are triggered by different mechanisms. Specifically, the main difference lies in the fact that mice only develop transient and not chronic AHR (Epstein., 2004)

**Current mode of asthma therapy**

A large number of drugs are available for asthma therapy. Epinephrine, Isoproterenol, Theophylline/Aminophylline, Beclomethasone, Dexamethasone and Nedocromil sodium can be given through various routes like oral, subcutaneous, intravenous and inhalation route for therapy. These drugs help to control the signs and symptoms of asthma. The anti-leukotrienes are the newest class of anti-asthmatic drugs available. Although, they do not provide any quick relief, they help to control the symptoms of asthma in the longterm. Despite the introduction of such new agents, corticosteroids are the anti-inflammatory drugs of choice for the majority in the treatment of asthma (O'Byrne., 1989). Both intravenous and oral forms are available and are equally effective in the treatment of mild to severe asthma (O'Byrne., 1989). However, when inhaled, the dose is not sufficient to cause complete relief. Moreover, the therapy is associated with side effects like kidney, liver failure, increased hunger, compromised immune system, high blood pressure, etc. Additionally, in 25 per cent of the cases there may be resistance to treatment with the intensity of side effects increasing. A recent novel approach to treatment of asthma through the destruction of airway smooth muscle (ASM) has been reported. The technique called bronchial thermoplasty is similar to current ablation procedures popular in cardiology for the
destruction of aberrantly conducting myocardial tissues. Bronchial thermoplasty employs radiofrequency waves to heat the airway tissues via a catheter placed within the airway during bronchoscopy. Experiments on dogs have demonstrated an apparently selective destruction of ASM (Danek et al., 2004). To date, results in humans have been promising (Cox et al., 2006) and support the notion of ASM as an important cause of airway hyperresponsiveness in asthma.

Some unanswered questions

Asthma is described as an inflammatory disease that predominantly involves the large airways mainly because of scanty investigation of the small airways due to the difficulty of in vivo sampling. However, pathologic and physiologic evidence have emerged in the last few years suggesting that the inflammatory process extends beyond the central airways to the distal airways and the lung parenchyma (Tulic and Hamid, 2003; Tulic et al., 2001). Several studies have shown that the distal airways are responsible for airflow limitation and airway hyperresponsiveness (Sekizawa et al., 1986; Yanai et al., 1992; Kuwano et al., 1993; Carroll et al., 1993; Wagner et al., 1998). The availability of a realistic model of chronic human asthma offers additional opportunities to address a number of key questions relating both to pathogenetic mechanisms of human asthma and to potential novel approaches to therapy. Issues that might be of interest include the following:

1. Role of signalling molecules in asthma pathogenesis

2. Whether Stat3 siRNA is associated with airway remodeling.
Mice model also provides a very useful system for assessment of novel therapeutic agents, both conventional- and immunomodulatory-drugs. We believe that a valid model is most likely to realize its potential. We recognize the limitations of murine models for the study of chronic asthma. For example, there is little, if any, evidence of mast cell recruitment into the airway wall or epithelium (Kumar et al., 2000), which may reflect the paucity of mast cells in the airways of mice; the phenotype of the epithelium of the proximal intrapulmonary airways in mice is markedly different from humans, necessitating assessment of most epithelial responses in the trachea; and there is no increase in airway smooth muscle mass in this species (Kumar and Foster., 2001). Nevertheless, the advantages of the improved model considerably outweigh its disadvantages and facilitate the translation of basic research on the immunobiology of allergic asthma into clinical practice.

Future perspective in the treatment of asthma

The goal of current therapy for asthma is to render the patient as symptom-free as possible and to reduce or eliminate the need for rescue therapy and hospitalization. Even with the availability of a large range of drugs, most patients show considerable heterogeneity in terms of the type and extent of inflammatory response, response to environmental triggers and degree of atopy. Efforts in molecular and cellular biology over the past two decades have resulted in the discovery and development of breakthrough drugs that have started to fulfil the promise of the biotechnology industry. In addition to products already on the market, large numbers of additional agents are in clinical trials, or awaiting approval. Moreover, with the revolution in genomic capabilities, the pace of target discovery has
increased further which should lead to the discovery of many more therapeutically useful medicines with novel mechanisms of action. Various clinical trials in asthma treatment have shown that there is considerable variation in the treatment response from individual to individual. These differences may be due to genetic variations between individuals along with variable expression of metabolic enzymes and receptors for drugs. Several interesting trials of SOCS, *in vitro* and *in vivo* are under way, it is too early to discuss its application in humans. Another breakthrough feature in asthma treatment would be RNAi. The term RNAi was coined by Fire and Mello and their work gained them the Nobel Prize in 2006. RNAi is initiated by exogenous (foreign, eg. viral) double stranded RNA. On entering the cell cytoplasm the dsRNA activates dicer, a ribonuclease protein, which slices the dsRNA first into fragments of length ~25 base pairs, known as short interfering RNA or siRNA, and then into two separate single strands of RNA. It is to be noted that in humans, siRNA must be used for the process to occur since longer dsRNA causes an immune response in the cell involving the production of interferons, and thus preventing translation and other cell activities including RNAi in an attempt to stop viral replication. After the dicer enzyme cleaves the dsRNA, one of these single strands is degraded whilst the other is incorporated into a RISC (RNA-induced silencing complex) which will pair up to a molecule of mRNA with the complementary base pair sequence. Components of RISC called argonaute proteins act catalytically to destroy this mRNA. Effectively, translation of the protein encoded by the mRNA cannot take place once it has been destroyed and so the specific target gene is silenced. It is to be noted that RNAi does not always cause complete silencing of the target gene a few mRNA may escape the
RISC, still reaching the ribosome to be translated. For this reason RNAi is said to have ‘knockdown’ effects rather than ‘knockout’ of the target mRNA. However the ‘knockdown’ of the gene is sufficient to have a significant effect on protein production/gene translation. RNAi also takes place with endogenous dsRNA, known as micro RNAs, which are formed in the cell and are thought to help regulate gene expression and the differentiation of cells such as stem cells. In addition to RNAi applications in medicine, research around this topic is looking at producing lower levels of plant toxins or allergens in plants like tomatoes and decreasing levels of known carcinogens in the tobacco plant. Research has also been centred on the creation of libraries detailing precise gene functions and pathways.
Materials and Methods
Animals

Female BALB/c mice weighing 25-30g at the age of 8-10 weeks were obtained from NABL compliant animal facility of Indian Institute of Toxicology Research, Lucknow. Animals were housed under standard environmental conditions of temperature (22 ± 2°C), relative humidity (50-55%) and illumination (12 h light/12 h dark cycles). All animals were fed with standard pellet diet and had access to drinking water ad libitum. Experiments were carried out with the consent of the Institutional Animal Ethics Committee.

Development of mouse model of asthma

The mice were sensitized to OVA by intraperitoneal (i.p.) injection of 100μg Ovalbumin (OVA; grade V; Aldrich-Sigma Chemical) emulsified in 2% alum solution on days 0 and 14 or sham-sensitized with saline (SAL). On day 14, 30 min after OVA sensitization, the animals were challenged with OVA through the nasal route. A total of 50 μg OVA in sterile SAL was applied on the snout or the nostril in 5 cycles, 2 μl containing 10 μg OVA in SAL at each cycle at an interval of 2 min. On days 21, 22, 23 and 26, the mice were similarly challenged with 50μg OVA through the nasal route.

Measurement of airway hyper-responsiveness

AHR induced by different concentrations of Mch (0–40 mg/kg) was measured by using a double-chambered whole-body plethysmograph (Buxco; model No. PLY 3351). The raw flow curves were acquired by sampling the signals at 2 kHz. The regularity of the breathing patterns was first assessed by qualitatively monitoring the constancy of peak flows. Irregularities in the breathing pattern were often encountered due to movement of the neck in most of the cases and to a lesser
extent, sigh-breath and cough. Data arising from these irregularities in breathing pattern were not considered for quantitative analyses. Before the start of the experiments, the animals were conditioned to stay in the plethysmograph chamber for at least 3 or 4 sessions of 10 min duration each. It was necessary to precondition the animals to minimize irregularities in the breathing pattern during data acquisition and achieve constancy in peak thoracic and nasal flows in the shortest possible time. Both OVA sensitized and challenged mice (OVA/OVA) and saline sensitized and challenged mice (SAL/SAL) were conditioned in a similar way to nullify the effect of preconditioning of animals on the measurement of sRaw (specific resistance of airways). For each mouse, the protocol consisted of breathing in the plethysmograph for 10 min and the mean value was considered for analysis. sRaw is monitored with the Acknowledge software program version 3.1 (Biopac, USA).

**Eosinophil counting in blood**

The important clinical haematological variable in asthma episode i.e. eosinophils were measured using Abacus Haematology Analyser (model: Abacus junior 5). Blood was collected in anticoagulant coated vials and subjected to eosinophil analysis.

**Collection of serum and broncho-alveolar lavage fluid (BALF)**

Blood was collected from the retro-orbital sinus of anaesthetized mice with ketamin hydrochloride (0.05ml/mice) post last OVA challenge (on 28th day) and kept at room temperature for 3h. Sera obtained after centrifugation at 3000 rpm for 5 min were stored at -20°C for ELISA. After collection of blood, the mice were sacrificed by an overdose of anaesthesia, and dissected to expose the trachea and bronchi. The
left bronchi and the anterior end of the trachea were tied with surgical threads. Trachea was cannulated with 20 gauge catheter and 1 ml of PBS was injected into the left lung through trachea. The injected PBS was withdrawn after 10 sec. The collected BALF was centrifuged at 2000 rpm for 5 min at 4°C to separate the leucocytes. The supernatant was subjected to antibody and cytokine analysis. (Sureshkumar et al., 2005). IL-4, IL-5, IL-13, TGF-β and IFN-γ proteins in BALF were quantified with commercial mouse ELISA kits (R&D system, Minneapolis, USA) according to manufacturer’s instructions. Cell pellets were used RNA isolation.

**Evaluation of total and antigen specific IgG and IgE**

Total and antigen specific IgG and IgE antibodies in the BALF and sera were evaluated by quantitative ELISA following the manufacturer’s protocol (Mouse IgG ELISA Quantitation kit; Bethyl Laboratories, Inc; Montegomery, Tex, USA). The plates were coated with goat antimouse IgG antibody at a concentration of 10 μg/ml in a coating buffer (0.05 M carbonate bicarbonate, pH 9.6) and incubated for 60 min. Free sites were blocked with 50mM Tris, 0.14 M NaCl, 1% BSA, pH 8 for 30 min. Goat antimouse IgG conjugated with HRP was used for the detection of mouse IgG at a dilution of 1:10,000. Colour was developed by adding tetramethyl benzidine substrate for 10 min. The reaction was stopped by adding 100μl of 2 M H₂SO₄. The intensity of the colour developed was measured by an ELISA plate reader at 450 nm. Antigen specific IgE was determined by coating the plate with OVA (10 μg/ml ) in 0.05 M carbonate bicarbonate buffer, pH 9.5, and incubated at 4°C overnight. After incubation, the wells were washed with PBST and blocked with 200μl blocking reagent (50 mM Tris, 0.14 M NaCl, 1% BSA, pH 8) for 30 min. After three washes
with wash buffer, 100 μl analyte was added and incubated for 2 hrs at room temperature. IgE antibody in the analyte was detected with the help of goat antimouse IgE antibody conjugated with HRP. Development of colour and stopping of enzyme substrate reaction were performed in the same way as total IgG estimation.

IL-4, IL-5, IL-13, TGF-β, and IFN-γ proteins in BALF were evaluated by solid phase sandwich ELISA using ELISA kits according to the manufacturer's instructions (R&D System, Minneapolis, USA).

RNA isolation and quantification

Total RNA was isolated from liver, lung, tracheal tissues and BALF cells using RNeasy mini kit as per the manufacturer's instructions (RNeasy mini kit; Qiagen, Hilden, Germany).

The isolated RNA was quantified with the help of a UV spectrophotometer at 260 nm (A_{260}). Deionized water served as a blank. The difference of the absorbance of the sample and the blank was calculated and the concentration of total RNA was determined using the relationship between absorbance and concentration (1OD at A_{260} = 40 μg/ml RNA).

One step RT-PCR

The RT-PCR was carried out using one step RT-PCR kit (Qiagen, Germany). The RT-PCR conditions were 50°C for 30 min for reverse transcription; initial PCR activation at 95°C for 15 min followed by 30 cycles of denaturation at 94°C for 45s, annealing at a 5°C below the 7m of primers for 45s and extension of 1 min at 72°C. The reaction was stopped after a final 10 min extension at 72°C. 1μl of template RNA containing 50 ng of total RNA was used. RT-PCR products were run on 1.2%
agarose gel containing ethidium bromide, viewed and analysed on Gel documentation system using Gene snap and gene tool software for image acquisition and gel analysis, respectively (SynGene, Cambridge, UK). Molecular weight markers (0.5 μg) containing a defined quantity of each band was loaded and electrophoresed simultaneously. The SynGene analysis tool was used to extrapolate the cDNA quantity from the input quantity of standard molecular weight markers.

Primers (Operon Biotechnologies GmbH, Germany) used for RT-PCR were,

Stat3:  
5'GAAGACCAAGTTCATCTGTGTG-3' (forward)  
5'GTAGCACACTCCGAGGTCAGAT-3' (reverse)

β- actin:  
5'GACATGGAGAAGATCTGGCAC3' (forward)  
5'TCCAGACGCAGTAGGTGCTGA3' (reverse)

IL-5
Forward  
5'-AAGGATGCTTCTGCACCTTA-3'

Reverse  
5'-TATC TCTCTGAAAGACTTCTGG-3'

IL-4
Forward  
5'-GACAAAAATCACCTTGAGAGAGA-3'

Reverse  
5'-ACGAGTGATTTCCATATGAT-3'

β-actin
Forward  
5'-ACATGGAGAAGATCTGGGAG-3'

Reverse  
5'-GACAGTAATCCATTTGCATGAT-3'

IL-13
Forward  
5'-TGGCTCTTGCTGCTGTGCT-3'

Reverse  
5'-GTGATGTTGCTCAGCTCCTCAAT-3'
TGF-β
Forward  5' CCCCACTGATACGCCTGAGT 3'
Reverse  5' AGCAGTGAGCGCTGAATCG 3'

IFN-γ
Forward  5' TCTGGATCCATGAACGCTACACAGTG 3'
Reverse  5' CACCTCGAGTCAGCAGCGAC 3'

Cytokines expression in asthmatic mice

Total RNA was isolated from lung, trachea and BALF cells using RNeasy mini kit as per the manufacturer’s instruction (Qiagen, Hilden, Germany). RT PCR was carried out using a QuantiTect SYBR green RT PCR kit (Qiagen, Germany). The RT-PCR conditions were 50°C for 30 min for reverse transcription; initial PCR activation at 95°C for 15 min followed by 40 cycles of denaturation at 94°C for 15 s, annealing at a 5°C below the Tm of primers for 30 s and extension of 30 s at 72°C. Melting curve analysis (not shown) was performed to verify the specificity and identity of the RT-PCR products. One micro litre of template RNA containing 50 ng of total RNA was used in a 50μL Real Time PCR cocktail. All reactions were performed in triplicates. Relative quantifications were performed in Light Cycler 480 calibrator normalised relative quantification assay in which the target concentration is calculated relative to a non-regulated reference. The results are expressed as the target/reference ratio of each sample normalised by the target/reference ratio of the calibrator using Light Cycler 480 relative quantification software release 1.2.0625. Primers (Operon Biotechnologis Gmbh, Germany) used for RT-PCR amplification.
Histopathology

The tissues from mice after sacrifice were fixed immediately in 10% neutral buffered formal saline for 72 h. Fixation was followed by washing in running water for 6-8 hrs. Fixed tissues were dehydrated by graded series of alcohol, cleared in xylene and embedded in paraffin wax. Paraffin blocks were sectioned in microtome to generate 4-5μm thick sections and collected on egg albumin coated slides. After deparaffinization sections were stained by haematoxyline and eosin (Qualigens). Histopathological studies of the sections were carried out under light microscope (Nikon, Japan).

Silencing of Stat3 in mice

The mice were in vivo transfected with a set of three different Stat3 duplexes (Santa Cruz Biotech, California). These Stat3 siRNA duplexes were given to mice by complexing them with 5% glucose solution in ExGen 500 (in vivo transfection reagent Fermentas Life Sciences, Harrington, Ont., Canada) as per the methodology of the manufacturer. ExGen 500, polyethlenimine belongs to a new category of non viral non liposomal gene delivery reagents. It has better in vivo transfection efficiency as compared to other cationic lipids and polymers. Stat 3 siRNA of mouse is a pool of three different strands of siRNA. The sense strand sequences of the Stat3 siRNA (Santa Cruz Biotech, USA) were:

5’GAGUGCAGGACUCAGAACAtt3’,
5’CAGGACGACUUUGAUUUCAAtt3’,
5’GAAGACACUGACUGAUGAAAtt3’
and the scrambled siRNA sequence was 5' UUCUCCGAACGUGUCACGUdtdt 3' (Qiagen, Germany). The scrambled siRNA duplex was used as the negative control.

In vivo transfection was achieved by diluting 10 µg of siRNA in 50 µl of sterile 5% glucose solution and then mixed thoroughly by vortexing. About 1.8 µl of ExGen solution was diluted in 50 µl of sterile 5% glucose solution and vortexed. After that, 50 µl of diluted ExGen solution was added to 50 µl of diluted siRNA solution. After appropriate mixing the mixture was incubated at room temperature for 10 min. This siRNA mixture was administered in mice through intranasal route. Different concentrations of siRNA was administered to each mouse on day 26. Measurement of AHR, histopathology studies, RNA quantification and assessment of IgG & IgE levels were carried out on 28th day in each study.

**Statistical analysis**

Data shown represents mean values (± SD). Student’s t-test with p- value <0.05 taken as indicator of statistical significance was used for comparing activities of different treatments.