Chapter-5

Study of cytokines in asthma
5.1 INTRODUCTION

Th-2 cells, mast cells and basophils play an important role in bronchial asthma (Marone, 1998). These cells upon interaction with allergen induced IgE synthesis can trigger the release of cytokines for the differentiation of Th cells to Th-2 cells to secrete a series of cytokines, like IL-4, IL-5 and IL-13. These 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 & Paul., 1994, Ennis et al., 2004). IL-5 has been suggested to be involved in the development of airway hyper-responsiveness (AHR). The present experiment was planned to evaluate the status of these cytokines in the mouse model of asthma. The study of these cytokines will throw light on the understanding which we will able to know better about their regulation and role in the disease onset and progression.

5.2 MATERIALS AND METHODS

5.2.1 Animals and treatments

Female BALB/c mice weighing approximately 25 g were divided into two groups of six each. The groups were as following,

I. Control mice (SAL/SAL mice)
II. Asthmatic mice (OVA/OVA mice)

5.2.2 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 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 microlitre of template RNA containing 50 ng of total RNA was used in a 50μL RT 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 Biotechnologies GmbH, Germany) used for RT-PCR were:

**IL-5**

Forward 5'-AAGGATGCTTCTGCACTTGA-3'  
Reverse 5'-TATCTCTCTGCAACTTG-3',

**IL-4**

Forward 5'-GACAAAAATCAGGTAAATCGAGAGAGA-3'  
Reverse 5'-ACGAGTAATCCATTGCATGAT-3';

**β-actin**

Forward 5'-GACATGGGAGATGTACTTCGAT-3'  
Reverse 5'-TCCAGACGCAGGATGGCGTGA-3'.

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IL-13
Forward 5'- TGGCTCTTGGCTGCTTTGG -3'
Reverse 5'- GTGATGGTTCAGCTCCTCAAT-3'

TGF-β
Forward 5' CCCCACTGATACCGCTGAGT 3'
Reverse 5' AGCAGTGAGCGCTGAATGG 3'

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

5.2.3 Collection of BALF and evaluation of cytokines

For collection of BALF, mice were anesthetized with ketamine hydrochloride (0.05 ml/mice), the trachea exposed by mid-line incision in the neck region and 1 ml of PBS was injected into lungs through trachea and withdrawn after 10 s. The fluid recovered from each mouse was centrifuged at 2,000 rpm, for 5 min at 25°C, and the supernatants used for Th2 cytokine analysis. IL-4, IL-5, IL-13, TGF-β and IFN-γ protein levels in BALF were quantified with commercial mouse ELISA kit (R&D system) according to manufacturer's instructions. Cell pellets were used for RNA isolation. The cell pellets were used for the isolation of RNA.

5.3 RESULTS

Except IFN-γ, IL-4, IL-5, IL-13 and TGF-β showed up-regulation in BALF and different tissues of asthmatic airways at mRNA as well as protein level (Fig.5.1, Table 5.1). IFN-γ levels in BALF, trachea and lung tissues of asthmatic mice were less than
the level seen in SAL/SAL mice. Thus the anti-inflammatory cytokine was found down regulated in asthmatic mice.
Fig. 5.1 Graphical representation of quantitative mRNA expression profile of various cytokines in OVA/OVA mice. Experiments were performed in Roche Light Cycler 480. C represent calibrator and T represent target gene (n=6).
### Table 5.1 Cytokine levels (pg/ml) in BALF of SAL/SAL and OVA/OVA mice

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>SAL/SAL</th>
<th>OVA/OVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>28.45 ±16.7</td>
<td>105.34 ± 30.8*</td>
</tr>
<tr>
<td>IL-5</td>
<td>23.86 ±13.9</td>
<td>70.63 ± 20.4*</td>
</tr>
<tr>
<td>IL-13</td>
<td>15.53 ±7.3</td>
<td>83.63 ± 10.21*</td>
</tr>
<tr>
<td>TGF-β</td>
<td>06.31 ±2.7</td>
<td>90.67 ± 8.34*</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>10.43 ± 4.1</td>
<td>8.23 ± 3.31</td>
</tr>
</tbody>
</table>

Values represent mean ± SD, n = 6 samples per group* Significant (P < 0.05) in comparison with SAL/SAL mice.
5.4 DISCUSSION

In our mouse model of asthma we observed heightened expression of IL-4, IL-5, IL-13 and TGF-β. IL-4, IL-5, and IL-13, are derived from T helper type 2 (Th2) cells, although they may also be derived from other cell types. Th2 cells are recognised by their secretion of IL-4, IL-5, and IL-13, as opposed to Th1 cells, which secrete IL-2 and IFN-γ, although a clear distinction between Th1 and Th2 cells is not as distinct in humans as in mice. Th2 cytokines may play an important role in the pathophysiology of allergic diseases, including asthma (Barnes., 2001). Interestingly IFN-γ expression in OVA/OVA mice was less than the basal level ie SAL/SAL Mice in BALF, trachea and lung. Thus Th1 cells may not be associated with asthma. Alternatively the balance between Th2 and Th1 cytokines decides the development of asthma. Active TGF-β has also been over expressed in the lungs of mice, with the development of severe interstitial and pleural fibrosis, consisting of excess collagen deposition, extracellular matrix proteins, fibronectin, elastin and the presence of myofibroblasts). TGF-β is an important fibrogenic and immunomodulatory factor that may also play a role in the structural changes observed in the asthmatic airways (Duvernelle et al., 2003) and we have observed heightened expression of this molecule in OVA/OVA mice. Thus in the mouse model of asthma TGF-β may be one of the cytokine which take part in airways remodeling in line with the observations made earlier by Makinde et al., 2007.
6.1 INTRODUCTION

Our results revealed low expression of Stat3 mRNA and protein in lungs of asthmatic mice. It appears that the low expression of Stat3 in asthmatic airways may be associated with inflammation. The functional role of Stat3 in asthma pathology is not clear as Stat3 regulates the negative feedback loop by inducing Socs3. Whether depleting Stat3 in vivo restores normal function in asthmatic mice or aggravate disease in normal mice is not known. To check this hypothesis we studied the in vivo effect of Stat3 siRNA on immunoglobulin, cytokines, eosinophils, AHR, and airways histopathology in asthmatic mice and normal mice.

6.2 MATERIALS & METHODS

Animals were grouped as follows. Each group contained three animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>OVA/OVA mice transfected with Scrambled sequence on day 26 (N=3)</td>
</tr>
<tr>
<td>Group II</td>
<td>OVA/OVA mice transfected with Stat3 siRNA on day 26 (N=3)</td>
</tr>
<tr>
<td>Group III</td>
<td>Normal mice transfected with Scrambled sequence on day (-) 2 and thereafter sensitized on day 0 and 14, and challenged on days 14, 21, 22, 23 and 26 (N=3)</td>
</tr>
<tr>
<td>Group IV</td>
<td>Normal mice transfected with Stat3 siRNA on day (-) 2 and thereafter sensitized on day 0 and 14, and challenged on days 14, 21, 22, 23 and 26 (N=3)</td>
</tr>
</tbody>
</table>

6.2.1 Determination of the minimum dose of Stat3 siRNA that inhibit > 80% of Stat3 mRNA

A pool of 3 strands of Stat3 siRNA duplexes (Santa Cruz Biotech, Calif., USA) complexed with 5% glucose solution was diluted in ExGen500 in vivo transfection reagent containing a cationic polymer, polyethyleneimine (Fermentas Life Sciences,
Harrington, Ont., Canada) and administered according to the manufacturer's instructions in varying concentrations on 26th day. Two days later, the mice were killed by cervical dislocation and the lungs were dissected out. 25–30 mg of each tissue was stored in RNA later (Sigma) for RNA extraction and one-step RT PCR analysis. The sense strand sequences of the siRNA (Santa Cruz Biotech) were as follows:

\[
\text{Stat3: } 5'\text{-GAGUGCAGGAUCUAGAACAtt-3';} \\
5'\text{-CAGGACGACUUUGAUUUCAAtt-3';} \\
5'\text{-GAAGACACUGACUGAUGAAAtt-3';} \\\n\]

scrambled siRNA 5'-UUCUCCGAACGUGUCACGUdttdt-3' (Qiagen).

The scrambled siRNA duplex was used as the negative control. To determine the optimum siRNA concentration that inhibited >80% Stat3 mRNA expression, different concentration of Stat3 siRNA was transfected in SAL/SAL mice and Stat3-mRNA level measured by RT-PCR, 48h after transfection.

6.2.2 *In vivo* Inhibition of Stat3 gene expression by siRNA and its effect on immunoglobulin, cytokines, eosinophils, AHR, mRNA and airways histopathology in asthmatic and normal mice.

To assess the effect of Stat3 siRNA on total as well as OVA specific IgG and IgE levels in serum and BALF, airway hyperresponsiveness and lung histology, protocols described in earlier chapters were used. On 26th day Stat3 siRNA or scramble sequence (SS) was transfected in OVA/OVA mice. All the parameters were assessed on day 28. In another set of experiment, normal mice were transfected with Stat3 siRNA and thereafter asthma was induced in a similar way as described earlier. Animals in each group were subjected to first to plethysmography, thereafter
eosinophil counting, and IgG and, IgE detection. Airways tissues were collected after killing animals for histopathology

6.3 Results

6.3.1 Determination of the optimum dose of siRNA

It was revealed that 2 and 4 μg/mice of siRNA inhibited >80% expression of the Stat3 mRNA in lung of SAL/SAL mice. β-actin mRNA levels at all the doses were unaffected (Fig. 6.1). We adopted the minimum dose that inhibited > 80% Stat3 mRNA expression. The minimum optimal dose of Stat3 siRNA selected was 2 μg/mouse
Fig. 6.1 siRNA-mediated silencing of Stat3 genes. *In vivo* silencing of the Stat3 gene in SAL/SAL mice by siRNA. SAL/SAL mice were transfected with varying doses: 0 (transfection reagent control), 1, 2 and 4 μg of Stat3 siRNA/mouse.
6.3.2 Effect of Stat3 silencing on blood eosinophil

Silencing Stat3 on day 26 in OVA/OVA mice did not alter blood eosinophil counts in comparison to scramble sequence transfected OVA/OVA mice (Fig. 6.2). However, high eosinophil counts were observed in Stat3 silenced normal mice which are subsequently sensitized and challenged with OVA in comparison to scramble sequence control (Fig 6.3) indicating that knockdown of Stat3 gene in mice lead to severe eosinophilia in mice subjected to future allergen exposure.
Chapter-6

Stat3 Silencing in asthmatic mice: Effect on cytokines, immunoglobulins, eosinophils, AHR & airway structure
Fig: 6.2 Effect of Stat3 siRNA on eosinophil count in OVA/OVA mice. Each bar represents mean values from three (3).
Fig. 6.3 Effect of Stat3 siRNA on eosinophil count in SAL/SAL mice + Stat3 siRNA transfected + OVA/OVA as compared to SAL/SAL mice + scramble sequence transfected + OVA/OVA. Each bar represent mean of data from three (3) mice.
6.3.3 Effect of Stat3 silencing on sRaw

Silencing Stat3 on day 26 in OVA/OVA mice did not alter lung function in terms of sRaw) in comparison to scramble sequence transfected OVA/OVA mice (Fig.6.4). However, higher sRaw values were observed in Stat3 silenced normal mice which are subsequently sensitized and challenged with OVA in comparison to scramble sequence control (Fig 6.5) indicating that knockdown of Stat3 gene in mice lead to poor lung function in mice following allergen exposure.
Fig. 6.4. Effect of Stat3 siRNA on lung function in OVA/OVA + Stat3 siRNA transfected mice. Lung function was measured in terms of methacholine dependent sRaw. (N=3)
Fig. 6.5 Effect of Stat3 siRNA on lung function in SAL/SAL mice + Stat3 siRNA transfected + OVA/OVA as compared to SAL/SAL mice + scramble sequence transfected + OVA/OVA. Lung function was measured in term of Methacholine dependent sRaw. (N=3)
6.3.4 Effect on Stat3 silencing on total and OVA specific IgG, IgE in serum and BALF

Silencing Stat3 on day 26 in OVA/OVA mice did not alter allergen specific IgG and IgE levels in comparison to scramble sequence transfected OVA/OVA mice (Table 6.1). However, elevated levels of OVA-specific IgE were observed in Stat3 silenced normal mice which are subsequently sensitized and challenged with OVA in comparison to scramble sequence control (Table 6.2) indicating that silencing of Stat3 gene in mice lead severe allergy following exposure to allergen.
Table 6.1

Immunoglobulins level in serum and BALF of OVA/OVA + scramble sequence transfected mice and OVA/OVA + Stat3 siRNA transfected mice

<table>
<thead>
<tr>
<th>Immunoglobulins</th>
<th>OVA/OVA +SS transfected</th>
<th>OVA/OVA+Stat3 siRNA transfected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total serum IgG, mg/ml</td>
<td>14.83</td>
<td>16.31</td>
</tr>
<tr>
<td>OVA-specific serum IgG, mg/ml</td>
<td>10.86</td>
<td>12.21</td>
</tr>
<tr>
<td>OVA-specific BALF IgG, µg/ml</td>
<td>175.56</td>
<td>187.32</td>
</tr>
<tr>
<td>Total serum IgE, µg/ml</td>
<td>40.58</td>
<td>48.28</td>
</tr>
<tr>
<td>OVA-specific serum IgE, µg/ml</td>
<td>52.35</td>
<td>57.12</td>
</tr>
<tr>
<td>Ova-specific BALF IgE, µg/ml</td>
<td>14.93</td>
<td>18.13</td>
</tr>
</tbody>
</table>

Data represent mean values from 3 mice in each group.
Table 6.2

Immunoglobulins level in serum and BALF of SAL/SAL mice + scramble sequence transfected mice + OVA/OVA and SAL/SAL mice +Stat3 siRNA transfected + OVA/OVA

<table>
<thead>
<tr>
<th>Immunoglobulins</th>
<th>SAL/SAL + SS transfected + OVA/OVA</th>
<th>SAL/SAL +Stat3 siRNA transfected + OVA/OVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total serum IgG, mg/ml</td>
<td>13.21</td>
<td>15.64</td>
</tr>
<tr>
<td>OVA-specific serum IgG, mg/ml</td>
<td>12.86</td>
<td>12.01</td>
</tr>
<tr>
<td>OVA-specific BALF IgG, μg/ml</td>
<td>165.56</td>
<td>167.32</td>
</tr>
<tr>
<td>Total serum IgE, μg/ml</td>
<td>40.58</td>
<td>108.28</td>
</tr>
<tr>
<td>OVA-specific serum IgE, μg/ml</td>
<td>32.45</td>
<td>87.16</td>
</tr>
<tr>
<td>Ova-specific BALF IgE, μg/ml</td>
<td>14.93</td>
<td>43.34</td>
</tr>
</tbody>
</table>

Data represent mean values from 3 mice in each group. Except total serum IgE and OVA-specific IgE (indication higher values) all other immunoglobulins showed mild elevation in immunoglobulins level in SAL/SAL mice + scramble sequence transfected + OVA/OVA mice as compared to SAL/SAL mice +Stat3 silenced + OVA/OVA
6.3.5 Effect of silencing Stat3 on cytokine profile

Transfecting Stat3 siRNA on day 26 in OVA/OVA mice did not alter the cytokine profile in the airways of asthmatic mice. (Fig.6.6, Table 6.3) However, transfecting Stat3 siRNA in normal mice before sensitization and challenge with OVA leads to pronounced expression of IL-4, IL-5, IL-13 and TGF-β (Fig. 6.7, Table 6.4). Th1 cytokine IFN-γ was found down regulated in both Stat3 silenced asthmatic mice and normal mice. In Stat3 silenced asthmatic mice prominent over-expression of TGF-β was observed. In Stat3 silenced normal mice amplified over-expression of all the Th2 cytokines was seen.
Fig. 6.6 mRNA expression profile of various cytokines in OVA/OVA + Stat3 siRNA transfected mice as compared to OVA/OVA + scramble sequence transfected mice, graphical representation of Real Time Data (mean of 3 mice). C represents calibrator and T represents target gene.
Table 6.3
Cytokines level (pg/ml) in BALF of OVA/OVA + scramble Sequence and OVA/OVA + Stat3 siRNA transfected mice

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>OVA/OVA + SS transfected</th>
<th>OVA/OVA + Stat3 siRNA transfected</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>110.54</td>
<td>125.53</td>
</tr>
<tr>
<td>IL-5</td>
<td>68.62</td>
<td>74.62</td>
</tr>
<tr>
<td>IL-13</td>
<td>84.36</td>
<td>92.37</td>
</tr>
<tr>
<td>TGF-β</td>
<td>83.28</td>
<td>268.67</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>0.39</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Cytokines values represent mean of three mice per group. Mild increase in OVA/OVA + Stat3 siRNA transfected group in comparison with OVA/OVA + scramble sequence transfected mice except TGF-β (which showed exaggerated increase) (N=3).
Fig. 6.7 mRNA expression profile of various cytokines in SAL/SAL mice + scramble sequence transfected + OVA/OVA and SAL/SAL mice + Stat3 siRNA transfected + OVA/OVA. Graphical representation of Real Time Data (mean of 3 mice) C represents calibrator and T represents target gene.
Table 6.4
Cytokines level (pg/ml) in BALF of SAL/SAL mice + transfected with scramble sequence + OVA/OVA and SAL/SAL mice + Stat3 siRNA transfected + OVA/OVA

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Normal mice + SS transfected + OVA/OVA</th>
<th>Normal mice + Stat3 siRNA transfected + OVA/OVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>115.54</td>
<td>253.34</td>
</tr>
<tr>
<td>IL-5</td>
<td>57.52</td>
<td>175.22</td>
</tr>
<tr>
<td>IL-13</td>
<td>94.66</td>
<td>292.47</td>
</tr>
<tr>
<td>TGF-β</td>
<td>78.28</td>
<td>308.27</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>0.39</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Cytokines values represent mean of three mice per group. Higher values of all cytokine levels among SAL/SAL mice + Stat3 siRNA transfected + OVA/OVA in comparison with Sal/SAL + scramble sequence + OVA/OVA except IFN-γ. There is no change (N=3).
6.3.6 Effect on Stat3 silencing on airway structure

Necropsy examination of all the animals from OVA/OVA +SS and OVA/OVA + Stat3 silenced mice showed emphysematous lungs and patchy consolidation. Similar presentation of lungs was evident from Stat3 silenced normal mice which are subsequently exposed to OVA and Scramble sequence transfected normal mice which are subsequently exposed to OVA.

Histopathological examination of lungs from OVA/OVA +scramble sequence transfected mice depicted emphysematous alveoli, thickened alveoli and fibrous tissue proliferation (Fig.6.8 & 6.9). Interestingly similar histopathological observations were noticed in OVA/OVA + stat3 siRNA transfected mice (Fig.6.10 & 6.11). Trachea from OVA/OVA +scramble sequence transfected mice (Fig.6.12) and OVA/OVA + Stat3 siRNA transfected mice depicted submucosal infiltrations of inflammatory cells (6.13). It is also important to mention that transfecting Stat3 siRNA in normal mice subsequently sensitized and challenged with OVA showed thickened alveolar septa, infiltration of inflammatory cells and fibrous tissue (6.14 & 6.15), similar observations were observed in scramble sequence transfected OVA/OVA mice (Fig.6.16 & 6.17). As no change was not observed in lung gross pathology and histopathology tracheal histopathology was not conducted among Stat3 siRNA transfected normal mice sensitized and challenged with OVA and Scramble sequence transfected mice which subsequently sensitized and challenged OVA.
Fig. 6.8 Lung form OVA/OVA + scrambled sequence transfected mice depicting thickened alveolar septa, and infiltration of inflammatory cells and emphysema (arrow) similar to that of asthmatic mice, 125X, H&E

Fig. 6.9 Lung form OVA/OVA + scrambled sequence transfected mice depicting thickened alveolar septa, and infiltration of inflammatory cells and fibrous tissue (arrow) similar to that of asthmatic mice, 125X, H&E
Fig. 6.10 Lung form OVA/OVA + Stat3 siRNA transfected mice depicting thickened alveolar septa (arrow) and infiltration of inflammatory cells similar to that of asthmatic mice, 125X, H&E.

Fig. 6.11 Lung form OVA/OVA + Stat3 siRNA transfected mice depicting presence of fibrous tissue (arrow) similar to that of asthmatic mice, 125X, H&E.
Fig. 6.12 Trachea from OVA/OVA +scrambled sequence transfected mice showing submucosal infiltrations of inflammatory cells (arrow). 125X, H&E.

Fig. 6.13 Trachea from OVA/OVA +Stat3 siRNA transfected mice showing submucosal infiltrations of inflammatory cells (arrow). 125X, H&E.
Fig. 6.14 Lung from SAL/SAL mice + scramble sequence transfected + OVA/OVA mice showing submucosal infiltrations of inflammatory cells (arrow). 125X, H&E.

Fig. 6.15 Lung from SAL/SAL mice + scramble sequence transfected + OVA/OVA mice showing fibrous tissue (arrow). 125X, H&E.
Fig. 6.16 Lung from SAL/SAL mice + Stat3 siRNA transfected + OVA/OVA mice showing submucosal infiltrations of inflammatory cells (arrow) 125X, H&E.

Fig. 6.17 Lung from SAL/SAL mice + Stat3 siRNA transfected + OVA/OVA mice showing fibrous tissue (arrow) and fibrous tissue(arrow) 125X, H&E.
6.4 DISCUSSION

Th2 cytokines may play an important role in the pathophysiology of allergic diseases, including asthma (Barnes, 2001). Transforming growth factor-β (TGF-β) is an important fibrogenic and immunomodulatory factor that may also play a role in the structural changes observed in the asthmatic airways (Duverrielle et al., 2003) and we have observed heightened expression of this molecule in OVA/OVA mice. It means TGF-β may be one of the cytokine which take part in airways remodeling as reported by Makinde et al., 2007. Silencing of Stat3 gene in asthmatic mice did not affect the Th2 cytokines along with TGF-β. Silencing of Stat3 gene in normal mice followed by allergen exposure increases TGF-β expression by several folds in comparison to SS transfected control. IL-4 and IL-5 secreted by Th-2 cells are also involved in inflammation and activation of eosinophils in the airways (Kaminuma et al., 1999; Yessel & Groux, 2000) while IL-13 induces airway hyperreactivity and allergic inflammation (KleinJanuary et al., 1999). Several groups have independently stressed the specific role of IL-13 in the effector phase of asthma in mice. In immunised mice, the neutralisation of IL-13 leads to reduction of airway hyper-responsiveness and pulmonary mucus formation. In addition administration of IL-13 to non-immunised mice, or selective expression of IL-13 in the lungs of transgenic mice leads to the entire asthmatic phenotype. It is revealed as a potent mediator of tissue fibrosis in asthma and a key regulator of the extra cellular matrix (Wynn, 2003). Interestingly, unlike IL-13 overexpression, TGF-β does not recruit inflammatory cells or enhance mucus secretion in the lung, suggesting that TGF-β can directly induce fibrosis in the absence of significant inflammation. TGF-β is secreted by most cell
lineages derived from the bone marrow including T cells, macrophages, eosinophils, and neutrophils and is one of the most widely studied pro-fibrotic cytokines. IL-5 and IL-13 are cytokines that are secreted by Th2 cells and regulates the allergic phenotype (Maeda & Yanagihara, 2001). Shaoping et al., 2009 has reported TGF-β in the airways and may be important in the airway remodeling changes observed in chronic idiopathic cough patients, which could in turn lead to activation of the cough reflex. IFN-γ is a signature cytokine of Th1 cells and is not affected in asthmatic mice. It appears that TH2 cytokines are crucial in the onset and progression of asthma. Our mouse model was successful in mimicking the clinically important human disease. Sensitization and challenge protocol adopted in this experiments revealed elevation of Th2 cytokines, eosinophil counts, the presence of OVA-specific IgE and IgG, alteration in the airway architecture and AHR in OVA/OVA mice and suggests the pathological and physiological states of allergic asthma.
In this study we investigated the role of Stat3 in pathogenesis of asthma and demonstrate low Stat3 mRNA and protein in the lungs of mice with inflamed and allergic airways and symptoms of bronchoconstriction. This is in contrast to the data obtained in the lungs without inflammation and allergy, indicating that repeated sensitization and challenge with an allergen reduces the elicitation of Stat3 molecules in the host and reflects the augmentation of a desensitizing mechanism in airways during the development of asthma. In our experiments IL-4, IL-5 and IL-13 show heightened expression, and basal expression in case of IFN-γ. IL-4, IL-5, and IL-13, are derived from T helper type 2 (Th2) cells, although they may also be derived from other cell types. Th2 cells are recognised by their secretion of IL-4, IL-5, and IL-13, as opposed to Th1 cells, which secrete IL-2 and interferon-γ, although a clear distinction between Th1 and Th2 cells is not as distinct in humans as in mice. A number of novel therapeutic targets have been identified and drugs are being developed for better efficacy with less side-effect for treatment of asthma. A major challenge in asthma therapy has therefore been the identification of novel therapeutic targets, which are safer and more specific in their action. With the rapid progress in the identification of genes involved in various ethnic populations combined with the availability in future of well-targeted drugs, it will be possible to have appropriate medicine as per the genetic make-up of an individual. Onset of inflammation by Ovalbumin challenge and subsequent series of changes at lung and other airways sets initiation of remodelling. We suppose that probable tissue damage in the background of asthma is important factor in the entire remodelling process. Our study pertaining to IgG and IgE levels in OVA/OVA mice hints about the correlation of these markers in the asthma. Gene
identification analyses have indicated several chromosomal regions and genes, involved in the causation and progression of asthma symptoms. We have observed significantly low expression of Stat3 gene in asthmatic mice and knockdown of Stat3 by antisense technology did not yield any change in the key molecules associated with asthma. However, the negative association of Stat3 in asthma pathophysiology may further add to the complex interactions of genetic and environmental determinants of the disease. On the other hand, induction of asthma in Stat3 knockdown mice showed increase secretion of TH2 cytokines in comparison to scramble sequence-transfected asthmatic mice indicating the anti-inflammatory property of Stat3. Tyrosine phosphorylated Stat3 is known to dimerize and subsequently translocate to the nucleus where it activates various transcription factors. In mice with inflammatory airways and symptoms of asthma, the latter mechanism is possibly operative. As a result of negative feedback loop, the Stat3 expression is low in OVA/OVA mice and knockdown of Stat3 in normal mice followed by induction of asthma results in vigorous onset of asthma in terms of AHR, immunoglobulin and cytokine profiles. Thus functionally Stat3 is an anti-inflammatory molecule and its depletion did not restore normal lung physiology and structure; it aggravated pulmonary damage.

It appears from our investigation that more studies need to be directed at the cellular, molecular, and genetic levels to know and understand the negative association of Stat3 in asthma pathogenesis. Understanding the negative association may help to devise strategies for intervention and therapy.