Chapter-2

Development of mouse model of asthma: Eosinophils and airway remodeling in asthma
Chapter 2

2.1 INTRODUCTION

It is well known that eosinophils play an important role in the pathogenesis of allergic airway diseases because they contribute to the initiation and maintenance of the allergic response (Lee et al., 2001). There has been a lot of attention on the role of eosinophils in the pathophysiology of airway remodeling (Kay et al., 2004). Mathematical modeling studies have provided evidence that these alterations contribute to the symptoms and physiologic dysregulation seen in asthma.

The present study was performed to investigate the variations in blood eosinophils, as it is the most important clinical marker in inflammation also to study the changes in airway at tissue level to understand the airway remodeling in asthma.

2.2 Experimental plan

Mice were divided into two groups of 6 animals each as given below:

Group 1: Control mice treated with normal saline (SAL/SAL mice)

Group 2: Mice sensitized and challenged with OVA (OVA/OVA mice)

The eosinophils were measured using Abacus Haematology Analyser (model: Abacus junior 5) Variation in eosinophils counts on day 0 and 28 were monitored and graph plotted. Animals were sacrificed on 28th day after collecting blood and lungs and trachea were collected. Gross pathological investigation was done by naked eye while for histopathological investigations were done after fixing airway tissue in 10% formal saline tissues. Fixed tissues were processed to stain them by Hamatoxylene & Eosin method as described earlier.
2.3. RESULTS

2.3.1 Blood eosinophil

OVA/OVA mice showed significant (P<0.05) increase in eosinophil counts in comparison to SAL/SAL group on 28th day. While on day 0 there was no change in OVA/OVA mice when compared to SAL/SAL (Fig. 2.1).
Fig. 2.1 OVA/OVA mice showed significant (*P<0.05, N=6) increase in eosinophil counts in comparison to SAL/SAL group.
2.3.2 Gross pathology

On examination of the respiratory system by naked eyes (gross pathology), lungs from SAL/SAL group animals showed normal pale appearance and absence of froth in the tracheal lumen. In contrast, OVA/OVA lung showed mild to mild patchy congestion.

2.3.3 Histopathological studies of lungs from SAL/SAL and OVA/OVA mice

Histological examination of the lungs from control mice revealed structure comprised of bronchioles and alveoli. Alveoli formed gas exchange unit of the lung, consisting of alveolar duct, alveolar sacs and alveoli. Inter-alveolar septa were extremely thin. (Fig.2.2 & 2.3). Heavy infiltration of inflammatory cells into the alveoli was prominent in OVA/OVA mice. The blood capillaries presented congestion. Alveolar septa were thickened. Peribronchiolar lymphoid proliferation was present. Few alveoli depicted emphysema and majority of the alveoli were collapsed. (Fig.2.4 & 2.5).

2.3.4 Histology of trachea from SAL/SAL and OVA/OVA mice

Trachea is longest extrapulmonary conducting portion of the respiratory tract. While entering into the lungs it bifurcates distally into the left and right primary bronchi of the lungs. It is mucosal lined hollow structure consisting of C-shaped rings of fibroelastic tissue. The epithelium of trachea is typically pseudostratified ciliated columnar cells. The greater portion of the cells lining this epithelium is of non ciliated Clara cells. Trachea from SAL/SAL mice did not show submucosal infiltrations of inflammatory cells (Fig.2.6). However, trachea from OVA/OVA mice revealed submucosal infiltration.
of inflammatory cells. However, mucosal epithelium had apparently normal histological orientation. Cartilaginous part too, had normal morphology. (Fig. 2.7).
Fig: 2.2 Normal lung parenchyma from SAL/SAL mice showing normal histology of alveoli (arrow). 125X, H &E.

Fig: 2.3 Lung from SAL/SAL mice depicting normal bronchiole (arrow). 125X, H &E.
Fig: 2.4 Lung from OVA/OVA mice showing thickened alveolar septa (thin arrow), heavy infiltrations of inflammatory cells, emphysema (thick arrow). 125X, H &E.

Fig: 2.5 Lung parenchyma from OVA/OVA mice showing fibrous tissue proliferation around bronchiole (arrow). 125X, H &E.
Fig: 2.6 Trachea from SAL/SAL mice showing normal histology (arrow). 125X, H&E.

Fig: 2.7 Trachea from OVA/OVA mice showing moderate submucosal infiltrations of inflammatory cells (arrow). 125X, H&E.
2.4 Discussion

Defining the mechanisms of asthmatic lung remodeling can have critical clinical consequences as tissue remodeling and fibrosis are important pathological features of lung disease. Airway remodeling is a complex process that involves all of the component tissues of the airway from the epithelium to the adventitia. Each of the changes has the potential to alter airway physiology so as to promote airway narrowing, hyperresponsiveness and inflammation. In this study we studied the extent of gross and histological damages in the respiratory system. Additionally, blood eosinophils were monitored. We found typical inflammatory lesions in the lungs of OVA/OVA group mice while SAL/SAL group showed normal presentation. Histopathological studies of trachea and lungs from all the control mice showed normal histology while in OVA/OVA mice trachea revealed inflammatory cells influx at the submucosa which is indicative of inflammation. Lungs from OVA/OVA mice showed typical lesions of asthma, airway fibrosis, emphysema and thickened alveolar septa are indicative of airway remodeling. Reduction in the active respiratory alveoli might have forced other alveoli to respire more in order to compensate and this could have resulted in the emphysema. From the normal gross pathology of trachea in OVA/OVA mice and absence of any inflammatory lesions it appears that to develop lesions in the major conducting tubes of airway, a long time challenge may be required. Busse et al., 1999 and Bousquet et al., 2000 opined that airway remodeling is the important contributor to reduced lung function in asthmatics, and develop as a result from repeated cycles of airway injury induced by inflammatory responses followed by processes inherent in the lung to repair the damaged airway. Holgate et
al., 1999 reported that the narrowing of the airways is associated with smooth muscle contraction, airway wall thickening, edema and increased mucus secretion. Our findings in the present study showed typical inflammatory lesions in the trachea and lungs in asthmatic mouse. These findings are in accordance to many researchers (Hogg, 1984, Broide et al., 1991, Arm & Lee, 1992, Frigas et al., 1992, Persson & Erjefal, 1997, Bousquet et al., 1998). Elias et al., 1999 and Gibson., 2000 reported that, the infiltration of leukocytes, particularly eosinophils, into the lungs and release of vasoactive mediators from mast cells set the stage for asthmatic inflammation. It was concluded that remodeling in the lung parenchyma may have been induced by factors secreted both by inflammatory cells and by structural cells, the latter frequently under the influence of the former. The structural alterations caused by this response play an important role in generating the manifestations of the disorder. Among the inflammatory cells eosinophils might be more being the key player in allergenicity. Bates et al., 2004, examined the time course of changes in respiratory input impedance during induced bronchoconstriction in BALB/c mice sensitized and challenged with Ovalbumin and found that bronchoconstriction in mice is accompanied by complete closure of substantial regions of the lung and that closure increases markedly when the lungs are allergically inflamed. On day 28 post OVA exposure, significant increase (P<0.05) in the eosinophil count was observed in mice blood as compared to SAL/SAL group mice. Clinical and experimental investigations have shown strong correlation between the presence of eosinophils and AHR. It can be concluded from the present study that in asthmatic mouse model there is a typical remodeling of the airways.