Mitochondrial Structural Changes and Dysfunction Are Associated with Experimental Allergic Asthma

Ulaganathan Mabalarajan, Amit Kumar Dinda, Sarvesh Kumar, Reema Roshan, Pooja Gupta, Surendra Kumar Sharma and Balaram Ghosh

*J Immunol* 2008; 181:3540-3548; doi: 10.4049/jimmunol.181.5.3540

http://www.jimmunol.org/content/181/5/3540

References

This article cites 41 articles, 17 of which you can access for free at:
http://www.jimmunol.org/content/181/5/3540.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Mitochondrial Structural Changes and Dysfunction Are Associated with Experimental Allergic Asthma

Ulaganathan Mabalirajan,*‡ Amit Kumar Dinda,† Sarvesh Kumar,* Reema Roshan,* Pooja Gupta,* Surendra Kumar Sharma,‡ and Balaram Ghosh2*

An imbalance between Th1 and Th2 immune response is crucial for the development of pathophysiological features of asthma. A Th2-dominant response produces oxidative stress in the airways, and it is thought to be one of the crucial components of asthma pathogenesis. Although mitochondrion is a crucial organelle to produce endogenous reactive oxygen species, its involvement in this process remains unexplored as yet. We demonstrate in this study that OVA-induced experimental allergic asthma in BALB/c mice is associated with mitochondrial dysfunction, such as reduction of cytochrome c oxidase activity in lung mitochondria, reduction in the expression of subunit III of cytochrome c oxidase in bronchial epithelium, appearance of cytochrome c in the lung cytosol, decreased lung ATP levels, reduction in the expression of 17 kDa of complex I in bronchial epithelium, and mitochondrial ultrastructural changes such as loss of cristae and swelling. However, there was no change in the expression of subunits II and III of cytochrome c oxidase. Interestingly, administration of IL-4 mAb reversed these mitochondrial dysfunction and structural changes. In contrast, IFN-γ mAb administration neither reversed nor further deteriorated the mitochondrial dysfunction and structural changes compared with control asthmatic mice administered with isotypic control Ab, although airway hyperresponsiveness deteriorated further. These results suggest that mitochondrial structural changes and dysfunction are associated with allergic asthma. These findings may help in the development of novel drug molecules targeting mitochondria for the treatment of asthma.

To understand this, we have checked ultrastructural changes of mitochondria of bronchial epithelium and several key functions of lung mitochondria related to COX$_{PTC}$ in asthmatic mice administered with either IL-4 mAb or IFN-γ mAb. In this study, we have shown that mitochondrial damage and alterations of its key functions are associated with experimental allergic asthma.

**Materials and Methods**

**Animals**

Male BALB/c mice (8–10 wk old) were obtained from National Institute of Nutrition (Hyderabad, India) and acclimatized for 1 wk prior to starting the experiments. All animals were maintained as per Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines, and protocols were approved by Institutional Animal Ethics Committee.

**Grouping of mice**

Mice were divided into five groups, and each group was named according to sensitization/challenge/treatment, as follows: SHAM/PBS/normal controls (N.CON), OVA/OVA/asthmatic controls (A.CON) (OVA, chicken egg OVA, grade V; Sigma-Aldrich), OVA/OVA/dexamethasone (DEX) (Sigma-Aldrich; n = 6 mice), OVA/OVA/IL-4 mAb (mAb against murine IL-4; R&D Systems; n = 6 mice), and OVA/OVA/IFN-γ mAb (mAb against murine IFN-γ; R&D Systems; n = 6 mice). SHAM/PBS/N.CON represented mice pooled from two subgroups (six mice in each subgroup) treated with either oral DMSO or i.p. injection of PBS. OVA/OVA/A.CON mice also were pool of two subgroups (six mice in each subgroup) treated with either DMSO or i.p. injection of rat IgG as isotypic (ISO) control Ab (OVA/OVA/ISO).

**Sensitization, challenge, and treatment of mice**

As shown in Fig. 1, mice were sensitized by three i.p. injections of 50 μg of OVA in 4 mg of aluminum hydroxide (OVA groups such as OVA/OVA/CON, OVA/OVA/DEX, OVA/OVA/IL-4 mAb, and OVA/OVA/IFN-γ mAb) or 4 mg of aluminum hydroxide (SHAM/PBS/N.CON) on days 0, 7, and 14. Mice were challenged from days 21 to 32 (30 min per day) with 3% OVA in PBS (OVA groups) or PBS (SHAM/PBS/N.CON) using a nebulizer with a flow rate of 9 L/min (OMRON CX3 model). DMSO or DEX (0.75 mg/kg) had been given orally from days 19 to 32. Normal rat IgG, IL-4 mAb, or IFN-γ mAb was administered to respective groups i.p. on days 13, 20, and 30. AHR measurement and sacrifice were performed on day 33.

**AHR measurement**

AHR to methacholine (MCh; Sigma-Aldrich) was determined in unrestrained and restrained conscious mice by single-chamber plethysmography (SCP) and double-chamber plethysmography (DCP), respectively (model PLY 3211 and PLY 4451; Bu xo Electronics), as described previously (14–16). SCP was performed for the estimation of enhanced pause (Penh), and DCP was for the estimation of both specific airway conductance (sGaw) and specific airway resistance (sRaw). We have taken two mice at a time for the measurement either in SCP or in DCP. Totals of 50 μl of PBS/each MCh dose with 15% duty cycle for SCP and 100 μl of PBS/each MCh dose with 30% duty cycle for the DCP have been applied. These variations in dose-volume and instrument setup for SCP and DCP methods might produce variation in dose-response curve. It has been reported that lung resistance strongly correlates with Penh in BALB/c mice (17). Penh has been shown to be useful and reliable in BALB/c mouse model of asthma in which predominant AHR is due to peribronchial inflammation rather than parenchymal inflammation compared with other strains (18). Also, restrained DCP technique has been performed, because this technique is soundly based on the first principles of physics of the lung similar to invasive techniques and allows nonterminal measurements. It has recently been shown that there was an excellent correlation between sRaw-DCP method and airway resistance by the forced oscillation technique (19). These measurements were estimated on day 33 for all mice. Final results

**FIGURE 1.** Experimental protocol for the induction of allergic asthma. Eight- to 10-wk-old male BALB/c were grouped, sensitized, and challenged, as described in Materials and Methods. Vehicle (DMSO) or DEX has been given orally from days 19 to 32. Normal rat IgG, IL-4 mAb, or IFN-γ mAb was administered to respective groups i.p. on days 13, 20, and 30. AHR measurement and sacrifice were performed on day 33.

**FIGURE 2.** Effect of IL-4 mAb or IFN-γ mAb on AHR to MCh. Mice were randomly divided and named as per status of sensitization/challenge/treatment. AHRs were determined on day 33. A, Absolute values of Penh (unitless parameter) with increasing concentrations of MCh were estimated. *, p < 0.05 vs SHAM/PBS/N.CON; †, ‡, and ¶, p < 0.05 vs OVA/OVA/A.CON. B and C, Absolute values of sGaw and sRaw with increasing concentrations of MCh were estimated. *, p < 0.05 vs SHAM/PBS/N.CON; †, ‡, and ¶, p < 0.05; and **, NS vs OVA/OVA/A.CON.
for SCP were expressed in absolute Penh (unitless) values with each concentration of MCh. For sGaw and sRaw, measurements were performed with increasing concentrations of MCh, and results were expressed in absolute values (cm H$_2$O s$^{-1}$/cm H$_2$O H$_{100}$s$^{-1}$, respectively).

**Bronchoalveolar lavage (BAL) and sera separation**

On day 33, each mouse was sacrificed, BAL was performed, and BAL fluids were processed to separate cell pellets and supernatants, as described earlier (20). Briefly, collected BAL fluid was centrifuged at 1000 rpm, for 10 min at 4°C. The cell pellets were washed three times with PBS and resuspended in PBS; a small portion was taken to evaluate total cell number; and differential counts were estimated after staining with Leishman’s stain (20). The absolute number of cells was calculated by multiplying the percentage of each subset in an individual sample by the total number of cells in that sample. Blood was withdrawn by cardiac puncture, and serum was separated, as described previously (20).

**Combined in situ perfusion and immersion fixation for transmission electron microscopy**

Whole body fixation was performed at room temperature, as described earlier (21). Lungs were removed and immersed again in the fixative. The fixed lungs were dissected under dissection microscope (SZX-12; Olympus) to locate the first generation bronchi, and those were cut into many slices of same thickness. Each slice was made into three blocks, and they were further processed; the stained sections were viewed under transmission electron microscope, as described previously (22).
Table I. Effects of IL-4 mAb, IHN-γ mAb, and DEX on perivascular (PV), peribronchial (PB), total lung inflammation scores, airway mucin content, bronchial epithelial thickness (BET), L-OOH, and 13-S-HODE levels

<table>
<thead>
<tr>
<th></th>
<th>Inflammation Score</th>
<th>Airway Mucin Content (nM/mm²)</th>
<th>BET (μm)</th>
<th>Lipid H₂O₂ (nm/25 mg lung)</th>
<th>13-S-HODE (ng/50 μg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PV</td>
<td>PB</td>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHAM/PBS/N.CON</td>
<td>2.9 ± 0.2</td>
<td>3.5 ± 0.2</td>
<td>6.4 ± 0.4</td>
<td>1.0 ± 0.2</td>
<td>7.3 ± 1.0</td>
</tr>
<tr>
<td>OVA/OVA/A. mAb</td>
<td>13.7 ± 0.7a</td>
<td>17.5 ± 1.0</td>
<td>1.0 ± 0.4</td>
<td>0.84 ± 0.04</td>
<td>119.8 ± 10.5a</td>
</tr>
<tr>
<td>OVA/OVA/IL-4 mAb</td>
<td>2.9 ± 0.3b</td>
<td>7.5 ± 1.0</td>
<td>0.65 ± 0.0</td>
<td>0.55 ± 0.10</td>
<td>99.9 ± 11.3b</td>
</tr>
<tr>
<td>OVA/OVA/IHN-γ mAb</td>
<td>6.8 ± 1.6</td>
<td>17.1 ± 0.6</td>
<td>0.79 ± 0.06</td>
<td>139.2 ± 10.9</td>
<td></td>
</tr>
<tr>
<td>OVA/OVA/DEX</td>
<td>1.5 ± 0.1a</td>
<td>9.8 ± 0.7b</td>
<td>0.59 ± 0.07</td>
<td>102.8 ± 8.2</td>
<td></td>
</tr>
</tbody>
</table>

* Value of p < 0.05 vs SHAM/PBS/N.CON.

** Value of p < 0.05 vs OVA/OVA/A.mAb.

** Lipid hydroperoxide (L-OOH) and hydroxyoctadecenoic acid (HODE) assay

Lipid peroxidation was determined in lung tissues by measuring L-OOH with L-OOH assay kit (Cayman Chemical), as per instructions. Briefly, 50 mg of lung tissue was homogenized in 250 μL of PBS and centrifuged at 5000 rpm for 10 min. Resultant supernatant was further used for the extraction of L-OOH with deproteination procedure (Cayman Chemical). Resultant chloroform fractions were used for measurement of L-OOH using Microplate Reader (Bio-Rad), as per instructions. The 13-hydroxyoctadecenoic acid was used as the standard. The level of HODE (13-S type) was measured in CYTO by ELISA without extraction (Assay Designs). Briefly, 100 μL of CYTO in duplicate was taken for the measurement by competitive ELISA (13-S-HODE enzyme immunoassay kit from Assay Designs). Results were expressed in ng/100 μg CYTO.

Total cytochrome c oxidase activity measurement

COX₁ETC activity and total citrate synthase activity were measured and calculated, as per instructions (Sigma-Aldrich). Briefly, COX₁ETC activity was measured based on the oxidation of ferrocyanochrome c to ferricytochrome c by COX₁ETC present in the mitochondria treated with n-dodecyl-β-D-maltoside. This was quantified by the decrease in absorbance at 550 nm. Total citrate synthase activity was measured by the hydrolysis of acetyl CoA to thios, which reacts with 5,5′-dithio-bis(2-nitrobenzoic acid) to form 5-thio-2-nitrobenzoic acid. This was detected colorimetrically by following the absorption at 405 nm. Positive control experiments were performed with pure cytochrome c oxidase or citrate synthase (Sigma-Aldrich). The ratio between total COX₁ETC and net citrate synthase activities was calculated.

Immunohistochemistry (IHC)

Commercial goat polyclonal Abs for COX₁ETC subunits II and III (Santa Cruz Biotechnology), mouse mAbs for COX₁ETC subunit I, and 17-kDa subunit of complex I (Mitosciences and Molecular Probes, respectively) were used as primary Abs, and respective HRP-conjugated secondary Abs (Sigma-Aldrich) were used for IHC (26). Negative control experiments were performed by using either γ-globulin as isotype controls (Jackson ImmunoResearch Laboratories) or omission of primary Abs.

Cytochrome c estimation

The levels of cytochrome c were measured in CYTO and BAL fluid by ELISA, as per instructions (R&D Systems). Briefly, 5 μg of CYTO protein or 100 μL of BAL fluid supernatants per well in duplicate was used to measure cytochrome c with rat/mouse cytochrome c enzyme immunoassay kit. For Western blot, cytochrome c was separated by 12% SDS-PAGE and transferred onto polyvinylidene difluoride membranes (Millipore). Transferred membrane was blocked with blocking buffer (5% skim milk), incubated with cytochrome c Ab (1:2500; Invitrogen), followed by HRP-conjugated anti-mouse secondary Ab, and developed with diaminobenzidine-H₂O₂, α-Tubulin was used as a loading control. Signals were detected by spot densitometry (Alpha EaseFC software from Alph Innotech).
cance was set at decrease in sGaw was found with IL-4 mAb administration or mAb, or isotypic Ab, and AHRs were determined, as described in Materials and Methods. IL-4 mAb administration or DEX treatment attenuated the increase in Penh and sRaw compared with control asthmatic mice (Fig. 2, A and C). In contrast, the attenuation of the decrease in sGaw was found with IL-4 mAb administration or DEX treatment. In contrast, IFN-γ mAb administration worsened the condition by further increasing the Penh and decreasing sGaw compared with control asthmatic mice. Almost similar results were also obtained in increasing sRaw values (Fig. 2, A and C).

**Table II. Effects of IL-4 mAb, IFN-γ mAb, and DEX on total, differential, and absolute cells in BAL fluid**

<table>
<thead>
<tr>
<th></th>
<th>Differential count in percentage (×10⁴/ml)</th>
<th>Absolute cells (×10⁴/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TCC Macro Mono Neutro Eosino</td>
<td>Macro Mono Neutro Eosino</td>
</tr>
<tr>
<td>SHAM/PBS/N.CON</td>
<td>7.5 ± 1.1</td>
<td>6.3 ± 0.9</td>
</tr>
<tr>
<td>OVA/OVA/A.CON</td>
<td>26.6 ± 2.9b</td>
<td>11.0 ± 1.4</td>
</tr>
<tr>
<td>OVA/OVA/IL-4 mAb</td>
<td>13.9 ± 2.1b</td>
<td>7.0 ± 1.3</td>
</tr>
<tr>
<td>OVA/OVA/IFN-γ mAb</td>
<td>31.0 ± 5.8</td>
<td>13.0 ± 2.0</td>
</tr>
<tr>
<td>OVA/OVA/DEX</td>
<td>9.0 ± 2.7c</td>
<td>4.5 ± 1.5</td>
</tr>
</tbody>
</table>

A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z, a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r, s, t, u, v, w, x, y, z

**Results**

**OVA-sensitized and challenged mice developed AHR, airway inflammation, mucus hypersecretion, BAL fluid eosinophilia, and increased OVA-specific IgE**

As shown in Fig. 2, A and C, OVA/OVA/A.CON mice have shown MCh dose-dependent increase in Penh and sRaw compared with SHAM/PBS/N.CON mice. In contrast, OVA/OVA/A.CON mice have shown dose-dependent decrease in sGaw (Fig. 2B), indicating that OVA-sensitized and challenged mice developed significant AHR. Furthermore, histopathological analysis of lung sections of OVA/OVA/A.CON mice showed dense infiltration of inflammatory cells, including eosinophils in perivascular and peribronchial regions as well as increased mucus secretion and bronchial epithelial hypertrophy (Fig. 3, A and B; Table I). BAL fluid analysis showed that there was a significant increase in the number of infiltrated cells, including eosinophils, in OVA/OVA/A.CON (Table II). Sera analysis showed a significant increase in the levels of OVA-specific IgE and IgG1 in OVA/OVA/A.CON compared with SHAM/PBS/N.CON mice (Fig. 3C). These results indicated that OVA/OVA/A.CON mice developed characteristic features of asthma, such as AHR, airway inflammation, bronchial epithelial hypertrophy, mucus hypersecretion, and increased allergen-specific IgE synthesis.

**IL-4 mAb and DEX reduced AHR**

To determine the effect of IL-4 on AHR, OVA-sensitized and challenged mice were administered with IL-4 mAb, IFN-γ mAb, or isotypic Ab, and AHRs were determined, as described in Materials and Methods. IL-4 mAb administration or DEX treatment attenuated the increase in Penh and sRaw compared with control asthmatic mice (Fig. 2, A and C). In contrast, the attenuation of the decrease in sGaw was found with IL-4 mAb administration or DEX treatment. In contrast, IFN-γ mAb administration worsened the condition by further increasing the Penh and decreasing sGaw compared with control asthmatic mice. Almost similar results were also obtained in increasing sRaw values (Fig. 2, B and C).

**ATP assay**

This was done with ATP Bioluminescence Assay kit, as described (Sigma-Aldrich), after TCA extraction. Briefly, 50 mg of weighed tissue was homogenized with 500 μl of 5% TCA and centrifuged at 10,000 × g for 20 min at 4°C. The resultant supernatant was optimally diluted with Milli-Q water to estimate the ATP using Bioluminometer (Berthold Technologies), and the amount of bioluminescent signal was recorded and expressed as relative light units.

**Statistical analysis**

Data are expressed as mean ± SEM. Significant differences between two groups were estimated using unpaired Student’s t test. Statistical significance was set at p ≤ 0.05.

**IL-4 mAb reduced airway inflammation and BAL fluid eosinophilia**

To determine the effect of IL-4 on airway inflammation, OVA-sensitized and challenged mice were administered with IL-4 mAb,
IFN-γ mAb, or isotypic Ab, and the features of airway inflammation were determined, as described in Materials and Methods. Although OVA/OVA/A.CON mice showed dense infiltration of inflammatory cells around the bronchovascular regions along with significant epithelial cell damage, including denudation and/or ulceration of bronchial mucosa, OVA/OVA/IL-4 mAb mice showed focal inflammation around bronchi and vessel, and OVA/OVA/DEX mice showed the features almost similar to N.CON mice. It was also noted that IL-4 mAb or DEX treatment caused least ulceration of epithelium (data not shown) and significant reduction of goblet cell metaplasia (Fig. 3B). Also, inflammation score and morphometric analysis showed that OVA/OVA/IL-4 mAb and OVA/OVA/DEX had significant reduction in airway inflammation, airway mucin content, and thickness of bronchial epithelium (Table I). It was also noted that IL-4 mAb or DEX treatment caused least ulceration of epithelium (data not shown) and significant reduction of goblet cell metaplasia (Fig. 3B). Also, inflammation score and morphometric analysis showed that OVA/OVA/IL-4 mAb and OVA/OVA/DEX had significant reduction in airway inflammation, airway mucin content, and thickness of bronchial epithelium (Table I). However, OVA/OVA/IFN-γ mAb mice showed features similar to OVA/OVA/A.CON mice (Fig. 3B). As shown in Table II, IL-4 mAb or DEX treatment showed the reduction in total cell counts in BAL fluid, as well as absolute numbers of macrophage, neutrophil, and eosinophil.

As IgE class switching is controlled by IL-4 and IFN-γ, OVA-specific IgE levels were measured in sera. As shown in Fig. 3C, OVA-specific IgE levels were found to be elevated both in OVA/OVA/A.CON and OVA/OVA/IFN-γ. In contrast, it was significantly reduced in OVA/OVA/IL-4 mAb. However, IL-4 mAb did not affect OVA-specific IgG1 levels much (Fig. 3Cii). This finding is similar to earlier studies (27), and it could be due to IL-4-independent regulation of IgG1. Interestingly, OVA-specific IgG2a levels were significantly elevated in OVA/OVA/IL-4 mAb compared with OVA/OVA/A.CON (Fig. 3Ciii).

**IL-4 mAb efficiently reduced the L-OOH and HODE**

Because airway inflammation produces oxidative microenvironment in the local milieu, we measured the L-OOH levels in the lung tissue and HODE, one of the markers of lipid peroxidation, in the lung cytosol. IL-4 mAb administration significantly reduced the elevated L-OOH and HODE levels (Table I). In contrast, IFN-γ mAb administration did not further increase L-OOH, although HODE levels increased compared with OVA controls.

**IL-4 mAb and DEX increased the activity of COX<sub>ETC</sub>**

Because peroxynitrite and NO specifically inhibit COX<sub>ETC</sub> (8), we measured its activity, and it was normalized by respective citrate synthase activity. The results indicated that the COX<sub>ETC</sub>/citrate synthase was decreased in OVA/OVA/A.CON mice compared with SHAM/PBS/N.CON (Fig. 4A). Interestingly, IL-4 mAb or DEX treatment significantly restored the activity of COX<sub>ETC</sub>. However, IFN-γ mAb administration decreased the activity further, but it was not statistically significant compared with OVA controls.

**IL-4 mAb and DEX restored the reduction in expression of COX<sub>ETC</sub> subunit III**

Because subunits I, II, and III among 13 total subunits of COX<sub>ETC</sub> are critically important for its activity, IHC was performed for...
fluid supernatants were significantly increased in OVA/OVA/A.CON mice and SHAM/PBS/N.CON mice (Fig. 4B). In contrast, subunit II was found to be predominantly expressed in bronchi and alveolar epithelial cells compared with other structural cells of the lungs. It was not found to be expressed in infiltrated inflammatory cells. However, no significant difference was observed in its expression in OVA/OVA/A.CON mice compared with SHAM/PBS/N.CON mice. Interestingly, subunit III expression was also found to be predominant in bronchial epithelium and scarcely in alveolar epithelial cells compared with other structural cells of the lungs. It was not found to be expressed in infiltrated inflammatory cells.

Because the ATP levels have been found to be decreased in OVA/OVA/A.CON and OVA/OVA/IFN-γ mAb mice, whereas OVA/OVA/A.ON mice showed a significant reduction of “I” (Fig. 5, C and D). Western blot analysis also showed multiple bands other than the 13-kDa band. We have labeled these different forms as I, II, and IV (26, 30, and 50 kDa, respectively). Interestingly, IL-4 mAb and DEX treatment reduced all multimeric forms (Fig. 5, C and D).

IL-4 mAb and DEX increased ATP levels in the lung

Furthermore, to evaluate one of the vital functions of mitochondria, we measured ATP levels in lung tissue. Interestingly, OVA/OVA/A.CON and OVA/OVA/IFN-γ mAb mice showed significant reduction in ATP levels compared with SHAM/PBS/N.CON mice, and significant reversal of ATP levels was found with IL-4 mAb or DEX treatment (Fig. 6A).

IL-4 mAb and DEX restored the reduction in expression of 17-kDa subunit of complex I

Because the ATP levels have been found to be decreased in OVA/OVA/A.CON and the expression of 17-kDa subunit of complex I is related with energy demand (28), IHC was performed for this subunit. We found that this subunit expressed exclusively in the bronchial epithelium of N.CON mice, whereas it was significantly decreased in OVA/OVA/A.CON mice. In contrast, IL-4 mAb treatment significantly restored the expression (Fig. 6B).
were seen in mitochondria of SHAM/PBS/N.CON. In contrast, the matrix was less dense with loss of cristae in OVA/OVA/A.CON mice due to the ballooning of mitochondria. Interestingly, when mitochondrial morphology was evaluated after IL-4 mAb or DEX treatment, it significantly restored the mitochondrial structural changes. However, IFN-γ mAb-treated mice showed the features similar to OVA controls.

**Discussion**

Earlier epidemiologic and genetic evidence suggested that maternal inheritance is one of the substantial risk factors for the development of asthma (29). Mitochondrial haplogroup has been shown to be associated with total serum IgE levels in asthmatics (29). In addition, endogenous reactive oxygen species and/or mitochondrial metabolism has been found to be involved in many phases of immune responses, which are critical in asthma pathogenesis, such as dendritic cell differentiation (30); proliferation of T and B cells; T cell activation; B cell differentiation, which is prerequisite for IgE synthesis; and release of mast cell mediators, including IL-4, which is essential for naïve T cell polarization toward Th2 phenotype (29). Thus, mitochondrial oxidative events are thought to be crucial in Ag presentation and in the generation of immune response that may lead to asthma pathogenesis. However, direct demonstration of the involvement of mitochondria in this process is lacking.

In this study, we have shown that mitochondrial dysfunction in experimental allergic asthma is IL-4 dependent. IL-4 is known to cause apoptosis in lung epithelial cells through STAT-6 phosphorylation by up-regulating 15-lipoxygenase (12), which has been shown to cause mitochondrial degradation by oxidizing mitochondrial membranes (31). Reduction in the levels of 13-S-HODE and L-OOH by IL-4 mAb (Table I) supports earlier observation that lipid peroxidation is a definite consequence of airway inflammation (2) and is one of the critical prior events for peroxidation of mitochondrial membrane to potentiate its dysfunction (32). HODE is a known lipid oxidative marker in the cytosol, and is one of the peroxide products of linoleic acid, a polyunsaturated fatty acid. Linoleic acid-rich phospholipids such as cardiolipin have a tight physical association with COXETC, and this association is needed for its maximal activity. Our observations that COXETC activity was reduced significantly by OVA sensitization and challenge and this decrease was sufficient to cause mitochondrial collapse (11).

The authors have no financial conflict of interest.

**Acknowledgments**

The scientific help provided by Dr. Arjun Ram and technical help provided by Tanvesh Kumar, Kameshwar Singh, Shashi Kant Singh, Jyotsna Batra, Jyotirmoi Aich, and Neha Gupta are greatly acknowledged. We thank Dr. Anurag Agrawal for his critical comments and suggestions during the preparation and revision of the manuscript, and also for helping us in quantitative morphometry. We also acknowledge the help provided by Sophisticated Analytical Instrument Facility, All India Institute of Medical Sciences, New Delhi.

**Disclosures**

The authors have no financial conflict of interest.
References


