Histopathologic Changes of the Lung in Newborn Mice Born from Asthmatic Mothers

Astımlı Annelerden Doğan Yenidoğan Farelerde Akciğerdeki Histopatolojik Değişiklikler

Arzu BABAYİĞİT, MD,[a]
Duygu ÖLMEZ, MD,[a]
Güven ERBİL, MD,[b]
Özkan KARAMAN, MD,[a]
H. Alper BAĞRIYANIK, MD,[a]
Osman YILMAZ, MD,[c]
Nevin UZUNER, MD[a]

Departments of [a]Pediatrics, Division of Allergy, [b]Histology and Embriology, [c]Multidisciplinary Animal Laboratory, Dokuz Eylül University Hospital, İzmir

Geliş Tarihi/*Received:* 20.05.2008
Kabul Tarihi/*Accepted:* 29.06.2008

Yazışma Adresi/*Correspondence:*
Arzu BABAYİĞİT, MD
Dokuz Eylül University Hospital, Department of Pediatrics, Division of Allergy, İzmir
TÜRKİYE/TURKEY
arbabayigit@yahoo.com

ABSTRACT Objective: Asthma has its origins in early-life. Maternal asthma is an important risk factor, the mechanism of this effect is still unknown. The aim of this study was to evaluate the histopathologic changes of the lung in newborn mice born from asthmatic mother. **Material and Methods:** 28 BALB/c mice were divided into 4 groups; Group I (Astmatic mother mice), Group II (Control mother mice), Group III (Babies from asthmatic mother) and Group IV (Babies from control group). Mice in group I were sensitized with ovalbumin and mice in group II received saline. Mothers and 1-day-old baby mice were sacrified and airway histopathology was evaluated by using light and electron microscopy in all groups. **Results:** In asthmatic mother mice group (Group I), all histopathologic parameters including thickness of the epithelium, basement membrane and subepithelial smooth muscle were significantly higher when compared with the control group (Group II) (p= 0.000, p= 0.000, p= 0.000 respectively). When Group III and IV were compared with each other, the thickness of the epithelium, basement membrane and subepithelial smooth muscle were significantly higher in babies born from asthmatic mothers (p= 0.000, p= 0.000, p= 0.000 respectively). **Conclusion:** The results of this study suggest that structural changes of the lung may begin in the prenatal period in babies born from asthmatic mothers. Further studies are needed to clarify the histopathologic changes of the lung in childen of asthmatic mothers, which factors influence these changes and whether these changes are permanent or temporary.

Key Words: Asthma; animals; newborn; pathology; lung

ÖZET Amaç: Astım hayatın erken dönemlerinde başlar. Annede astım varlığı önemli bir risk faktörüdür ancak bu etkinin mekanizması halen bilinmemektedir. Bu çalışmanın amacı astımlı annelerden doğan yenidoğan farelerin akciğerlerindeki histopatolojik değişiklikleri değerlendirmektir. **Gereç ve Yöntemler:** 28 BALB/c fare 4 gruba ayrıldı; Grup I (Astımlı anne grubu), Grup II (Kontrol anne grubu), Grup III (Astımlı annelerden doğan yavru grubu) ve Grup IV (Kontrol grubundan doğan yavru grubu). Grup I'deki fareler ovalbumin ile duyarlılaştırıldı ve Grup II'deki farelere salin verildi. Anneler ve 1 günlük yavru fareler sakrifiye edildi ve tüm grupların havayolu histopatolojileri ışık ve elektron mikroskopi kullanılarak değerlendirildi. **Bulgular:** Astımlı anne fare grubu (Grup I), kontrol grubu (Grup II) ile karşılaştırıldığında epitel, bazal membran ve subepitelyal düz kas kalınlıkları dahil tüm histopatolojik parametreler önemli oranda yüksek bulundu (sırası ile p= 0.000, p= 0.000, p= 0.000). Grup III ve IV birbiri ile karşılaştırıldığında ise, astımlı anne yavrularında epitel, bazal membran ve subepitelyal düz kas kalınlıklarının önemli oranda yüksek olduğu görüldü (sırası ile p= 0.000, p= 0.000, p= 0.000). **Sonuç:** Bu çalışmanın sonuçları, akciğerdeki yapısal değişikliklerin astımlı anne bebeklerinde prenatal dönemde başlayabileceğini düşündürmektedir. Astımlı annelerin çocuklarında akciğerdeki histopatolojik değişiklikleri, bu değişiklikleri etkileyen faktörleri ve bu değişikliklerin kalıcı veya geçici olduğunu belirleyecek ileri araştırmalara ihtiyaç vardır.

Anahtar Kelimeler: Astım; hayvanlar, yenidoğan; patoloji; akciğer

Turkiye Klinikleri J Med Sci 2008;28:606-611

Asthma is a respiratory disorder with origins in early life.[1-5] The origins of asthma in early life suggest that prenatal events may influence susceptibility to allergic airway disease.[1,4] Pathogenic mechanisms include roles for genetic susceptibility as well as exposure to environmental factors.[1,6-8] Epidemiologic studies have identified an increased risk for asthma in children of asthmatic mothers, but the mechanism for this effect has not been well characterized.[9-11]

Potential pathophysiological mechanisms include perinatal transfer of maternal mediators such as cytokines, across placenta or via breast milk.[12] Such mediators may polarize the newborn's immune system, increasing its susceptibility to develop asthma by skewing immune reactions towards a pro-allergic Th2-driven response, to an even greater extent than is normally found.[13,14]

In the current study, we investigated whether the offspring of asthmatic mother mice (sensitized and repeatedly exposed to ovalbumin (OVA) Ag) showed any histopathologic changes of the lung in the newborn period (First day of life).

MATERIAL AND METHODS

EXPERIMENTAL ANIMALS

Pathogen free, 10-12 week-old, female and male BALB/c mice, weighing 25 to 30 g, were purchased from Bornova Veterinary Control and Research Institute (İzmir, Turkey) and maintained in a pathogen-free laboratory of Dokuz Eylül University. They were kept in hygenic macrolene cages and in air-conditioned rooms at a 12 hour light/12 hour dark cycle. The offsprings of female mice which were 1-day-old were kept with their mothers. The Animal Ethics Committee of the Dokuz Eylül University approved the experimental procedures, and the maintenance of animals was in accordance with institutional guidelines.

STUDY GROUPS

28 mice were divided into 4 groups: Group I (Asthmatic mother mice), Group II (Control group, mother mice), Group III (Babies from asthmatic mice) and Group IV (Babies from control group), each group including 7 mice.

SENSITIZATION AND INHALATIONAL EXPOSURE

BALB/c mice are high responders to ovalbumin.[15] The female mice in Group I were sensitized via two intraperitoneal injections, on days 0 and 14 of the experiment, of 10 μg/0.1 mL chicken egg albumin (OVA, grade V, ≥98% pure; Sigma, St. Louis, MO, USA) with alum as an adjuvant. The mice in Group I were then exposed to aerosolized OVA for 30 minutes per day on 3 days of the week for 8 weeks beginning from the 21st day of the study. The mice in the control group (Group II) received normal saline with alum intraperitoneally on days 0 and 14 of the experiment and aerosolized saline without alum for 30 minutes per day on 3 days of the week for 8 weeks beginning from the 21st day of the study.[16,17] Exposures were carried out in a whole body inhalation exposure system. Temperature and relative humidity were maintained at 20-25°C and 40-60% respectively. A solution of 2.5% OVA in normal saline was aerosolised by delivery of compressed air to a sidestream jet nebuliser and injected into a chamber. The aerosol generated by this nebuliser comprised >80% particles with a diameter of <4 μm. Particle concentration was maintained in the range of 10-20 mg/mm^3.[16] Immediately after the last aerosol exposure, the female mice (Group I and II) were placed in cages with male mice to allow mating. At day 15 of pregnancy, mice in Group I were further exposed to an aerosol challenge of OVA for each of 3 consecutive days. The schematic summary of the experimental protocol were given in Figure 1.

FIGURE 1: Schematic summary of the experimental protocol. BALB/c female mice received intraperitoneal (ip) injections of OVA (10 μg) and alum (1 mg) at 0 and 14 days and were exposed to aerosols of OVA (2.5%) for 30 minutes on 3 consecutive days at 3-10 weeks, followed by mating. At day 15 of pregnancy, mice in Group I were further exposed to an aerosol challenge of OVA for each of 3 consecutive days. Babies from asthmatic mother mice were sacrified one day after birth and histopathological analysis were done.

The mothers and baby mice were sacrificed by an overdose of ketamin 24 hours after birth and histopathological specimens were collected. Two investigators who were interpreting the histopathology were blinded to the treatment groups. Samples were fixed in 10% formaline for light microscopic evaluation. After overnight fixation, the longitudinally oriented a horizontal slice (bu ifadede bir yanlışlık var) from the mid zone of the left lung was embedded in paraffin. Serial sections of 5 μm were stained with Toluidine Blue (for routine histopathological examination). Photomicrographs were taken by JVC TK-890-E camera (Japan) which was adapted on Olympus BH-2 RFCA model microscope (Olympus Optical Co. Ltd, Tokyo, Japan). Blind histological analysis was carried out with UTHSCSA Image Tool for Windows Version 3.00 software (http://ddsdx.uthscsa.edu/dig/itdesc.html/; provided in the public domain by the University of Texas Health Sciences Center San Antonio, TX) after the images were transferred from a light microscope onto a computer.

Samples were fixed in 2.5 % glutaraldehyde for electron microscopic evaluation. Tissues were embedded in EPON after follow-up process of electron microscopic evaluation. Respiratory tracts were marked from the semithin sections. Ultrathin sections were stained with uraniyl acetate and lead citrate. Libra 120 Carl Zeiss electron microscope (Oberkochen, Germany) was used for this evaluation. Photomicrographs were taken by JVC TK-890-E camera. Basal membrane thicknesses of samples of the respiratory epitelium were examined with electron microscopy by using ITEM version 5.0 (Olympus Soft Imaging Solutions Gmbh Copyright © 1986-2007) program.

Statistical analysis

SPSS 11 package program was used in the statistical analysis. All results were presented as mean±SD from the number of experiments indicated. For all histopathologic parameters differences between the four groups were determined by the Kruskal-Wallis. Differences between 2 groups were analyzed by the Mann-Whitney U test. A $p< 0.05$ was considered statistically significant.

RESULTS

When compared with the control mother group (Group II), the asthmatic mother mice group (Group I) had significantly increased thicknesses of epithelium, basement membrane and subepithelial smooth muscle layers ($p= 0.000$, $p= 0.000$, $p= 0.000$ respectively). These results revealed that the asthma model was successfully established. Table 1 presents the mean±SD, range and p values of histopathologic parameters evaluated.

When histopathologic parameters of babies from asthmatic mother mice (Group III) were compared with the babies from control mother mice

TABLE 1: Comparison between histopathologic parameters of asthmatic mother mice and control mother mice.

	Asthmatic mother group (Group I) Mean ± SD (Range) (n: 7)	Control mother group (Group II) Mean ± SD (Range) (n: 7)	p value
Basement membrane thickness (nm)	1244.95 ± 400.69 (734.45-2172.7)	777.60 ± 330.25 (353.61-1668.29)	0.000
Epithelium thickness (μm)	24.37 ± 3.48 (19.1-29.29)	18.59 ± 1.8 (14-21.5)	0.000
Subepithelial smooth muscle layer thickness (μm)	8.89 ± 1.78 (5.3-12.23)	5.95 ± 1.75 (2.65-8.95)	0.000

TABLE 2: Comparison between histopathologic parameters of babies from asthmatic mother mice and control mother mice.

	Babies of asthmatic mother group (Group III) Mean ± SD (Range) (n: 7)	Babies from Control mother group (Group IV) Mean ± SD (Range) (n: 7)	p value
Basement membrane thickness (nm)	1384.5 ± 360.5 (820.75-2030.6)	509.14 ± 116.67 (335.2-845.4)	0.000
Epithelium thickness (μm)	14.68 ± 2.53 (9.46-20.54)	10.52 ± 2.05 (7.85-16.55)	0.000
Subepithelial smooth muscle layer thickness (μm)	6.72 ± 1.16 (4.48-9.15)	4.66 ± 0.77 (3.55- 6.56)	0.000

(group IV), Group IV had statistically significant increase in the thicknesses of epithelium, basement membrane and subepithelial smooth muscle layers (p= 0.000, p= 0.000, p= 0.000 respectively). The mean±SD, range and p values of histopathologic parameters evaluated were given in Table 2.

Light and electron microscopic findings of the lung (group III and IV) were shown in Figure 2.

DISCUSSION

Epidemiologic studies have identified an increased risk for asthma in children of asthmatic mothers.[9-11] This study sought to test the hypothesis that a child from an asthmatic mother can have increased susceptibility to develop allergic asthma and the remodeling process in children from asthmatic mothers may begin in the prenatal period. We used a mouse model of asthma, in which airway hyperresponsiveness, allergic pulmonary inflammation and remodeling were seen after sensitization and aerosol challenge with the allergen OVA. The results of this study did indeed show structural changes of the lung in the offspring of asthmatic (but not normal) mother mice. The data provide the direct demonstration that transfer of asthma susceptibility can occur in this model.

There is evidence that immune system changes in utero or in early postnatal life can cause persistent or significant alterations in allergic susceptibility. These studies include ideas of systemic maternal-mediated skewing of fetal and early neonatal immunity toward Th 2 responses such as the requirement for a successful pregnancy of increased Th2/Th1 cytokines ratio, the demonstrated synthesis of Th2 cytokines at the placental interface, and the observation of increased production of Th2 cytokines by fetal or neonatal T cells.[13,17-20] The current study suggested that histopathologic changes of the lung in the babies of asthmatic mothers had begun in the prenatal period and that they had significantly increased thickness of epithelium, basement membrane and subepithelial smooth muscle in their first day of life when compared with the control group.

There were some limitations of the study such as the lack of evaluation of cytokine levels which have an important role in asthma pathogenesis, small number of animals used (possibility that type 1 and type 2 errors may exist) and the results found in our study may not translate to positive findings in human clinical trials. Also, there is no follow-up period to see if histopathologic changes of babies from asthmatic mothers are permanent.

In conclusion, the results of the current study suggested that histopathologic changes of baby mice which were born from asthmatic mothers might begin in the prenatal period and that they had increased thickness of epithelium, basement membrane and subepithelial smooth muscle in thier first day of life. Further studies are needed to evaluate the histopathologic changes of the lung in babies of asthmatic mothers.

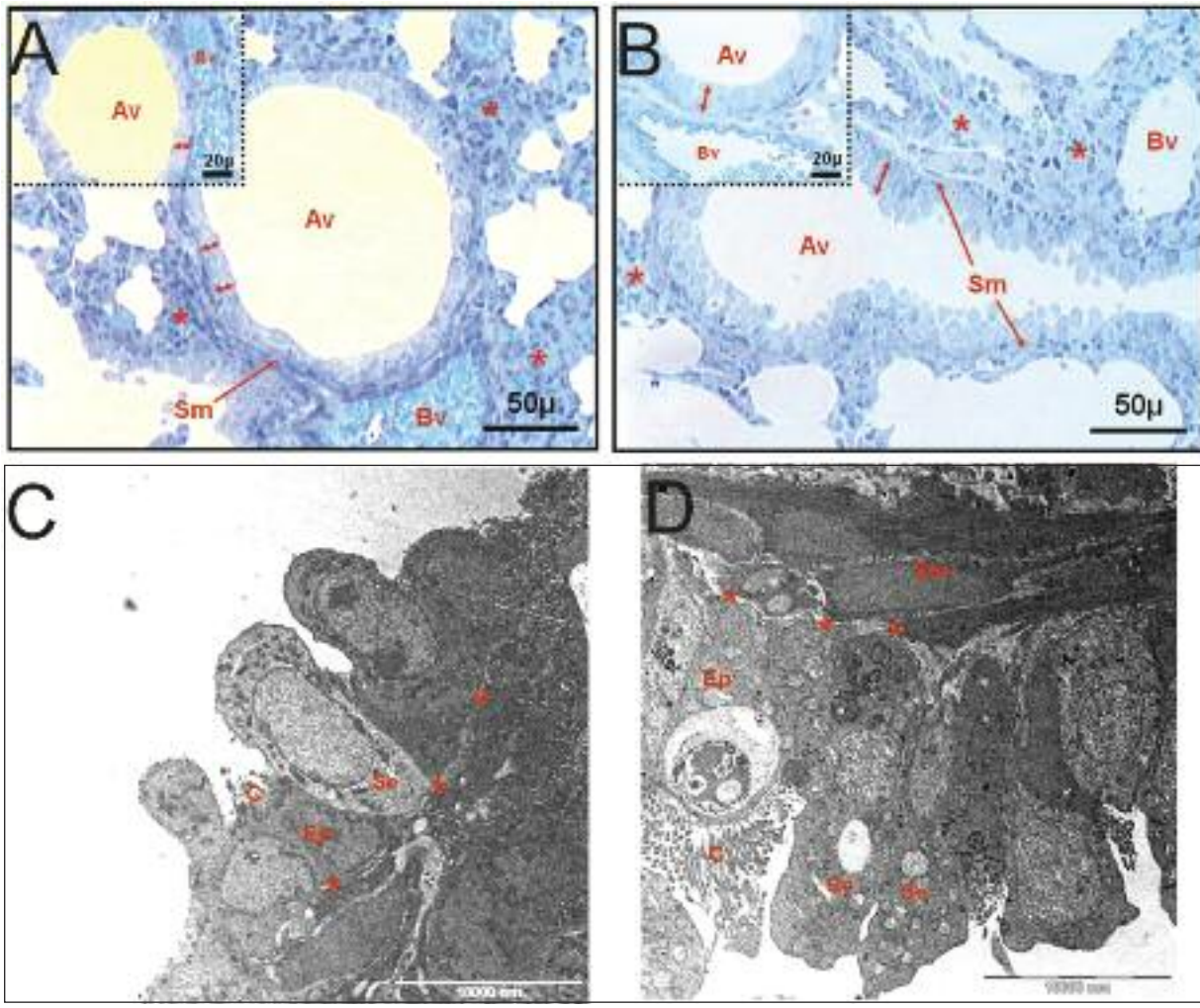

FIGURE 2: Light and electron microscopic views of the airways (Group III and IV).
A (Group IV): In babies from control mother group, light microscopic findings showed that respiratory epithelium (arrows) was regular. Blood vessels (Bv) and peribronchial lung parencyma (*) was seen as normal. Smooth muscle layer (Sm), airways (Av). [stained with Toluidine Blue, maginification: X66 (132 for small figures)].
B (Group III): In babies from asthmatic mother group, light microscopic findings revealed irregular respiratory epithelium. Thicknesses of epithelium, smooth muscle (Sm) (which were point out with arrows) and basement membrane (*) were increased. Basement membrane under the respiratory epithelium (Ep) with cilia (C) and serous cell (Se) with secretion granules were seen. [stained with Toluidine Blue, maginification: X66 (132 for small figures)].
C (Group IV): In electron microscopic findings of babies from control mother group, epithelium (Ep) with cilia (C) and serous cell (Se) with secretion granules were seen as normal. Basement membrane (*). (stained with uranyl acetate-lead citrate, maginification: X12,500).
D (Group III): In electron microscopy of babies from asthmatic mothers, apical cytoplasm of epithelial cells (Ep) were full with secretory granules (Se). Basement membrane integrity (*) was not disrupted. Ciliary structure (C). (stained with uranyl acetate-lead citrate, maginification: X12,500).

REFERENCES

1. Gern JE, Lemanske RF Jr, Busse WW. Early life origins of asthma. J Clin Invest 1999;104:837-43.
2. Holt PG, Jones CA. The development of the immune system during pregnancy and early life. Allergy 2000;55:688-97.
3. Anlar FY. Çocukluk astmasında prognoz. Turkiye Klinikleri J Med Sci 1997;17 :357-59.
4. Wright AL. The epidemiology of the atopic child: who is at risk for what? J Allergy Clin Immunol 2004;113(1 Suppl):S2-7.
5. Sekerel BE, Kalayci O. Çocukluk çağında kronik asthma tedavisi. Turkiye Klinikleri J Med Sci 1997;17:326-37.
6. Prescott SL. Maternal allergen exposure as a risk factor for childhood asthma. Curr Allergy Asthma Rep 2006;6:75-80.

7. Holloway JW, Beghé B, Holgate ST. The genetic basis of atopic asthma. Clin Exp Allergy 1999;29:1023-32.
8. Eder W, von Mutius E. Genetics in asthma: the solution to a lasting conundrum? Allergy 2005;60:1482-4.
9. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med 1995;332:133-8.
10. Litonjua AA, Carey VJ, Burge HA, Weiss ST, Gold DR. Parental history and the risk for childhood asthma. Does mother confer more risk than father? Am J Respir Crit Care Med 1998;158:176-81.
11. Wright AL, Holberg CJ, Taussig LM, Martinez FD. Factors influencing the relation of infant feeding to asthma and recurrent wheeze in childhood. Thorax 2001;56:192-7.
12. Leme AS, Hubeau C, Xiang Y, Goldman A, Hamada K, Suzaki Y, et al. Role of breast milk in a mouse model of maternal transmission of asthma susceptibility. J Immunol 2006;176: 762-9.
13. Prescott SL, Macaubas C, Holt BJ, Smallacombe TB, Loh R, Sly PD, et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. J Immunol 1998;160:4730-7.
14. Szépfalusi Z, Nentwich I, Gerstmayr M, Jost E, Todoran L, Gratzl R, et al. Prenatal allergen contact with milk proteins. Clin Exp Allergy 1997;27:28-35.
15. Temelkovski J, Hogan SP, Shepherd DP, Foster PS, Kumar RK. An improved murine model of asthma: selective airway inflammation, epithelial lesions and increased methacholine responsiveness following chronic exposure to aerosolised allergen. Thorax 1998;53:849-56.
16. Uzuner N, Kavukçu S, Yilmaz O, Ozkal S, Işlekel H, Karaman O, et al. The role of L-carnitine in treatment of a murine model of asthma. Acta Med Okayama 2002;56:295-301.
17. Busse WW, Rosenwasser LJ. Mechanisms of asthma. J Allergy Clin Immunol 2003;111(3 Suppl):S799-804.
18. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today 1993;14:353-6.
19. Lin H, Mosmann TR, Guilbert L, Tuntipopipat S, Wegmann TG. Synthesis of T helper 2-type cytokines at the maternal-fetal interface. J Immunol 1993;151:4562-73.
20. Warner JA, Jones AC, Miles EA, Warner JO. Prenatal sensitisation. Pediatr Allergy Immunol 1996;(9 Suppl):98-101.