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Figure 1

mBD-6 transgene expression. A: Schematic description of the mBD-6 transgene fragment used to generate the transgenic mice. A human cytomegalovirus immediate-early (CMV-IE) enhancer is linked to the chicken β-actin promoter, followed by its first exon and intron. In addition, a rabbit β-globin poly(A) sequence is located downstream from the mBD-6 cDNA. The black bar indicates the probe of 2nd exon of mBD-6 for the southern blot analysis in panel B. The arrows indicate the primers for the RT-PCR of mBD-6 transgene. **B:** Southern blot analysis of the Bgl II -digested genomic DNA from Tg(CAGmBD6)1 mice (Tg1) and Tg(CAGmBD6)2 mice (Tg2). The wild-type genomic DNA showed two copies of 1.4-kb intrinsic mBD-6 gene and more faint 3.2-kb band maybe composed of mBD-6 pseudogene. Tg1 showed multiple extra-bands including 2.1-kB DNA fragment conrresponding to the full-length transgene size while Tg2 showed a single 3.4-kb extra-band. C: RT-PCR of mBD-6 transgene mRNA. The transgene-specific RT-PCR indicated the transgene expression in the skeletal muscle of Tg(CAGmBD6)1 mice (Tg1) and Tg(CAGmBD6)2 mice (Tg2). **D:** Western blot analysis of mBD-6 peptide extracted from skeletal muscle. 280 ng of synthetic mBD-6 peptide composed of 40 N-terminal residues was used as standard. mBD-6 peptide was detected in the extracts from Tg1 and Tg2 skeletal muscle, but not

from the wild-type mice (WT).

Figure 2

Poor growth and progressive kyphosis of Tg1 mice. A: Comparison of the body weights between the Tg mice and the wild-type littermates (WT). Each circular point (●) indicates the measured individual body weight. The mean values (diamond)± SD are also shown. Both the male and female body weights of Tg mice were significantly lower than those of WT. B: Photograph of the 6-month-old Tg1 mouse. The arrow indicates the kyphosis.

Figure 3

The evaluation of muscle strength of Tg1 mice. We evaluated muscle strength by measuring the time during which mice could hang down from a stainless lattice. The graph shows the percentage of mice which could hang down for the indicated time. While most of the wild-type littermates hang down for more than 120 seconds, many of Tg1 mice dropped before 60 seconds.

Figure 4

Progressive myofiber degeneration of Tg1 mice. H&E staining of the gastrocnemius muscles of Tg1 mice at the age of 20 days, 1 month and 6 months. At the age of 1 month, faint-stained degenerative myofibers (arrow) and cnetronucleated myofibers appeared in the Tg1 mouse, contrasting with the wild-type littermate. The arrowheads inicate the infiltration of mononuclear cells. At the age of 6 months, centronucleated myofibers were more predominant with prominent difference in size. The fiber splitting (arrowhead) is also indicated. No histological abnormalities were noted at the age of 20 days. *Scale Bars*: 40 μm

Figure 5

Evaluation of membrane permeability of Tg1 skeletal muscle. A: Serum creatine kinase activity of Tg1 mice at the age of 3 months. The Tg1 mice showed significantly higher creatine kinase activity than the wild-type littermates (WT) ($p<0.01$). **B**: After Evans Blue Dye injections, some myofibers of Tg1 mice and 1-year-old Tg2 mice accumulated the dye in cytoplasm, showing the increased membrane permeability. *Scale Bars*: 40 μm.

Figure 6

Immunohistochemical analyses of dystrophin, α-dystroglycan, laminin and calpain 3 distributions in Tg1 skeletal muscle. The distribution of these molecules in Tg1 mice showed no difference from the wild-type mice (WT). Dystrophin is absent in dystrophin-deficient muscle (mdx). *Scale Bars*: 20 μm

Figure 7

Immunohistochemical analyses of neural cell adhesion molecule (NCAM) and IκBα distributions in young Tg1 mice and aged Tg2 mice. A: Many myofibers showed high-level expression of NCAM in 1-month-old Tg1 mice and 12-month-old Tg2 mice, contrasting with the wild-type littermates (WT). B: Many myofibers showed the accumulation of IκBα in 1-month-old Tg1 mice and 12-month-old Tg2 mice, contrasting with the wild-type littermates (WT). *Scale Bars*: 40 μm

Figure 8

H&E staining of the spinal cords of Tg1 mouse and the wild-type littermate. The number and morphology of motor neurons showed no abnormality causative of myofiber degeneration in Tg1 mouse.

Figure 9

Apoptotic feature of Tg2 skeletal muscle. Immunohistochemical analysis of serial sections about IκBα and cleaved caspase 3 indicated some myofibers (arrow) showed both accumulation of IκBα and apoptotic features. *Scale Bars*: 40 μm

Figure 1

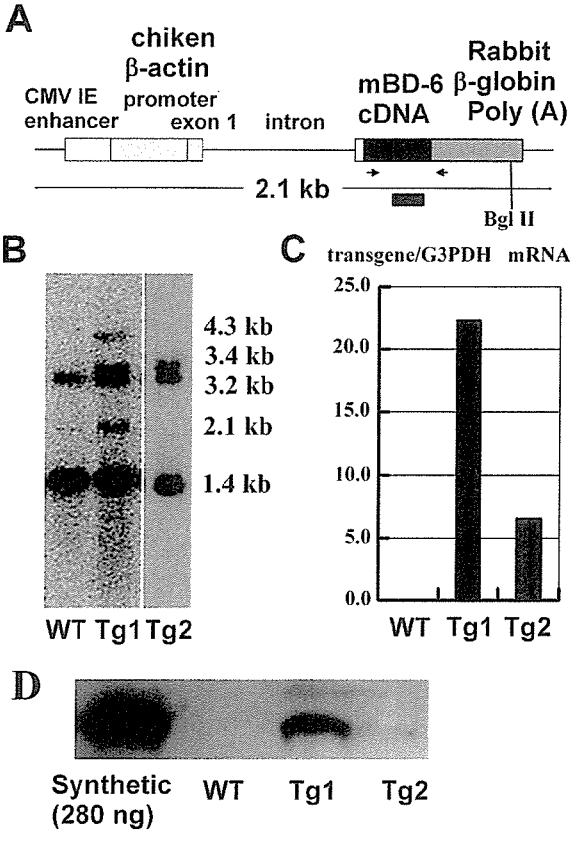

A
chiken
β-actin
promoter
CMV IE
enhancer
exon 1
intron
mBD-6
cDNA
Rabbit
β-globin
Poly (A)
2.1 kb
Bgl II
B
4.3 kb
3.4 kb
3.2 kb
2.1 kb
1.4 kb
WT Tg1 Tg2
C
transgene/G3PDH mRNA
25.0
20.0
15.0
10.0
5.0
0.0
WT Tg1 Tg2
D
Synthetic
(280 ng)
WT
Tg1
Tg2

Figure 2

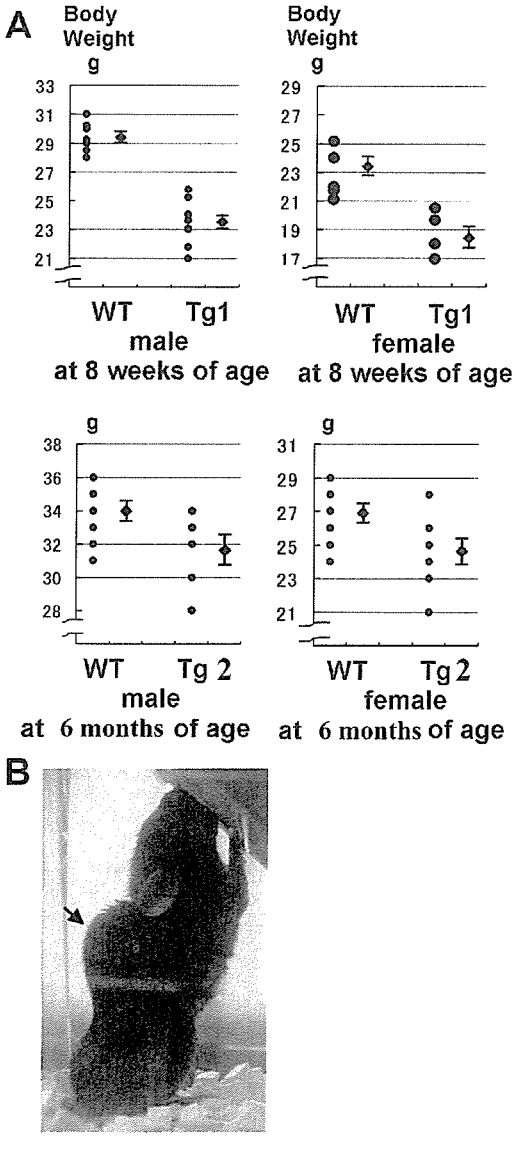

A
Body Weight
g
33
31
29
27
25
23
21
WT
Tg1
male
at 8 weeks of age
Body Weight
g
29
27
25
23
21
19
17
WT
Tg1
female
at 8 weeks of age
g
38
36
34
32
30
28
WT
Tg 2
male
at 6 months of age
g
31
29
27
25
23
21
WT
Tg 2
female
at 6 months of age
B

Figure 3

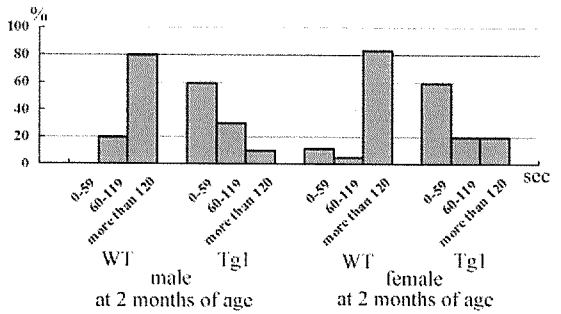
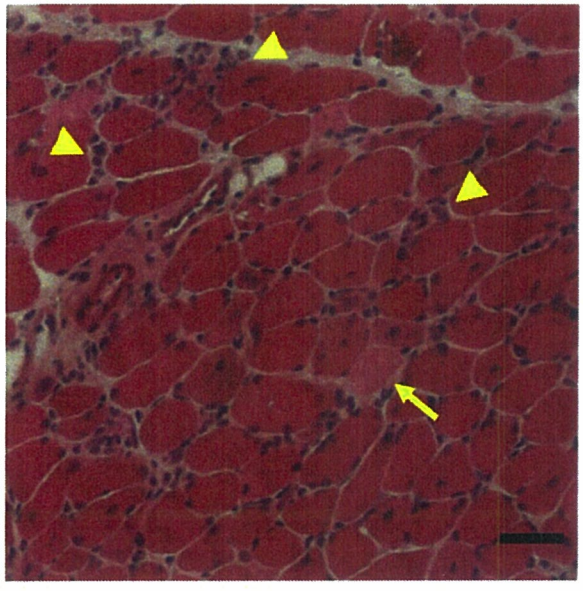
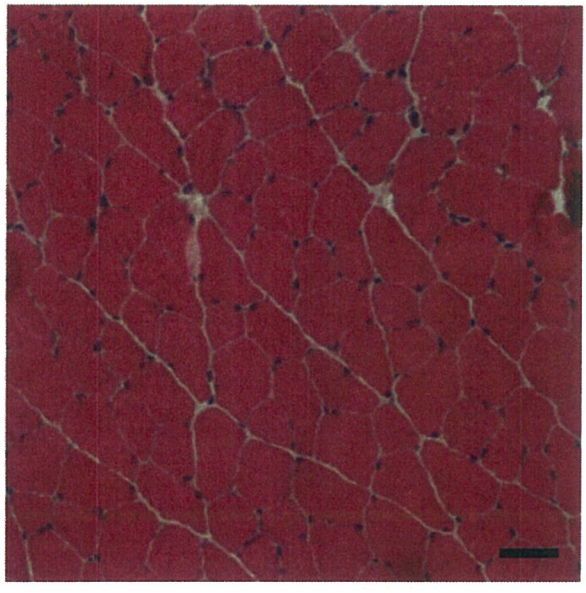

%
100
80
60
40
20
0
0-59
60-119
more than 120
sec
WT
Tg1
male
at 2 months of age
female
at 2 months of age

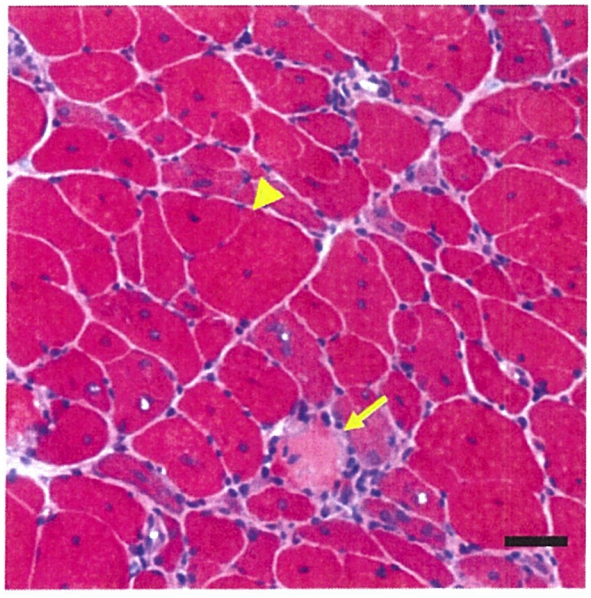
Figure 4



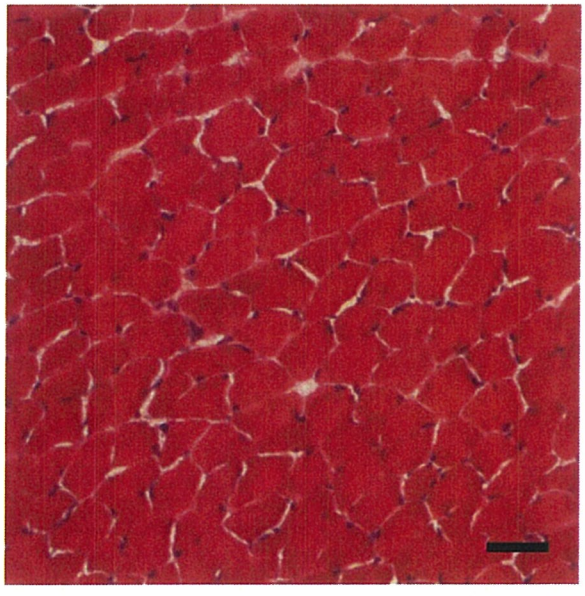
Tg1, 1 month



WT, 1 month



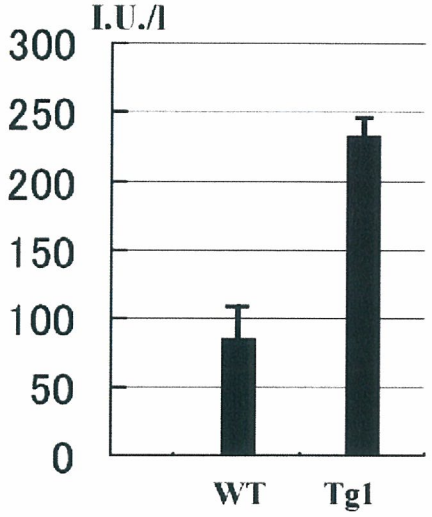
Tg1, 6 months



Tg1, 20 days

Figure 9

A. Serum Creatine Kinase Activity



B. Evans Blue Dye labelling

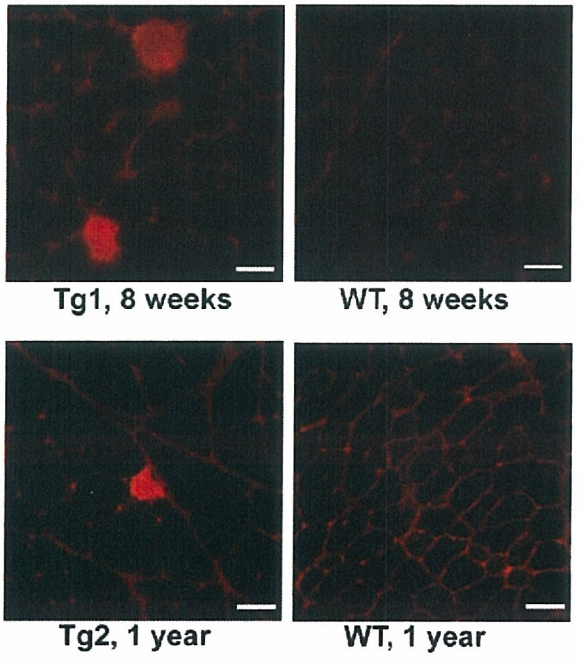


Figure 6

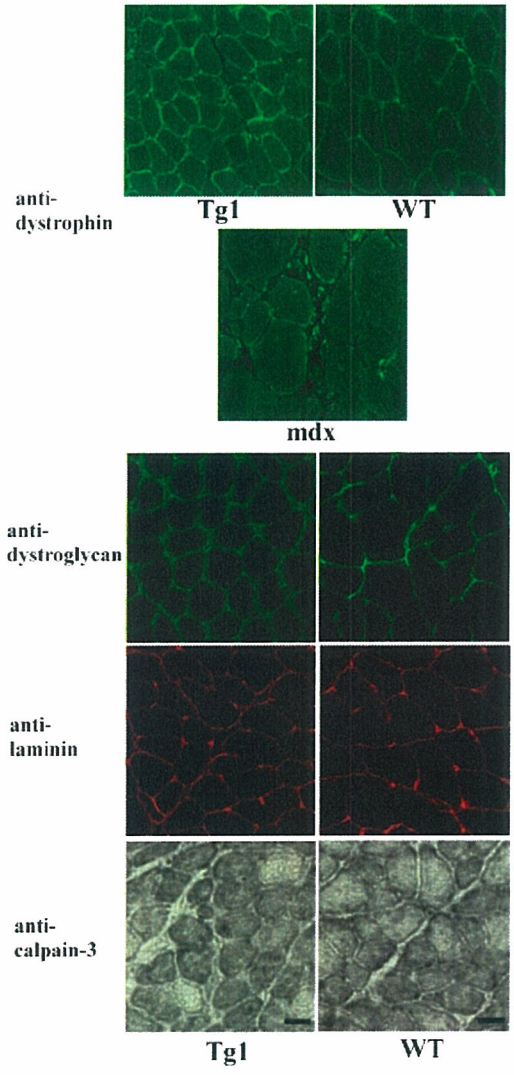
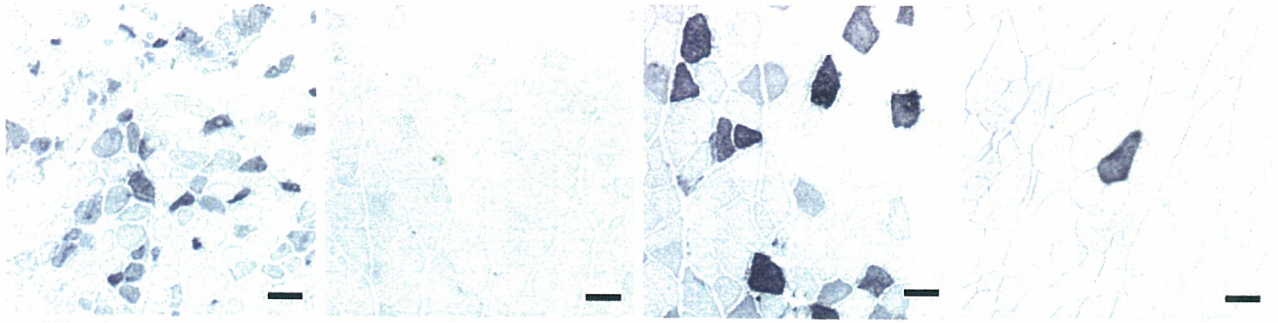

anti-
dystrophin
Tg1
WT
mdx
anti-
dystroglycan
anti-
laminin
anti-
calpain-3
Tg1
WT

Figure 7

A

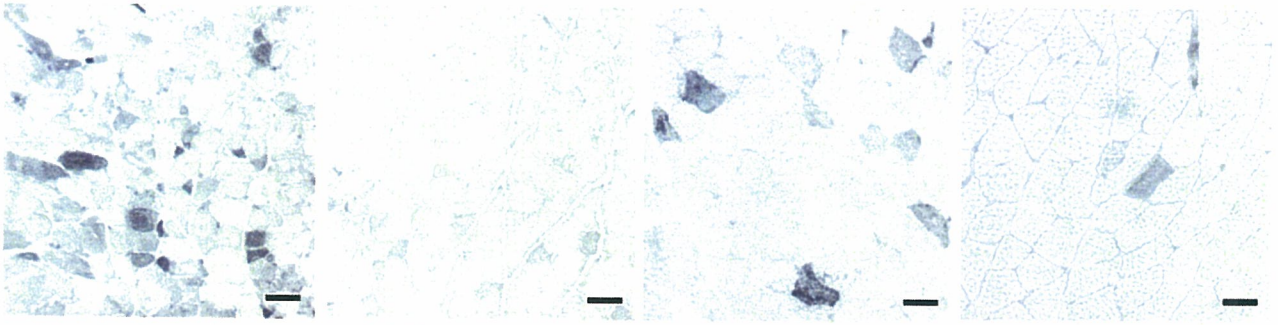
anti NCAM



Tg1, 1 month WT, 1 month Tg2. 12 months WT, 12 months

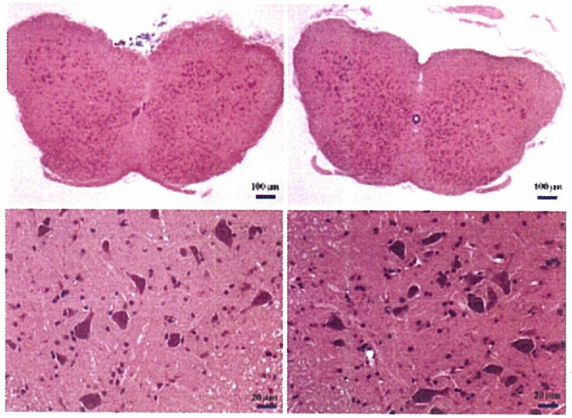
B

anti IκBα



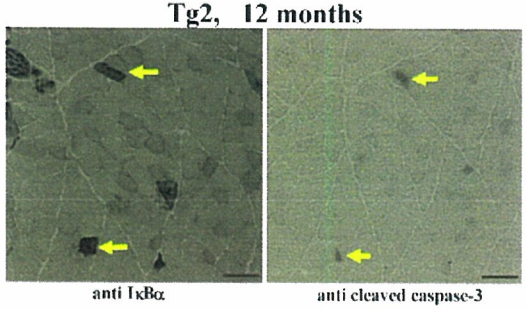
Tg1, 1 month WT, 1 month Tg2. 12 months WT, 12 months

Figure 8



Tg1 WT

Figure 9



Adrenomedullin insufficiency increases allergen induced airway hyperresponsiveness in mice

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Complete List of Authors:	Yamamoto, Hiroshi; University of Tokyo, Graduate School of Medicine, Department of Geriatric Medicine Nagase, Takahide; University of Tokyo, Graduate School of Medicine, Department of Respiratory Medicine Shindo, Takayuki; Shinshu University Graduate School of Medicine, Department of Organ Regeneration Teramoto, Shinji; University of Tokyo, Graduate School of Medicine, Department of Geriatric Medicine Aoki-Nagase, Tomoko; University of Tokyo, Graduate School of Medicine, Department of Geriatric Medicine Yamaguchi, Yasuhiro; University of Tokyo, Graduate School of Medicine, Department of Geriatric Medicine Hanaoka, Yoko; University of Tokyo, Graduate School of Medicine, Department of Geriatric Medicine Kurihara, Hiroki; University of Tokyo, Graduate School of Medicine, Department of Physiological Chemistry and Metabolism Ouchi, Yasuyoshi; University of Tokyo, Graduate School of Medicine, Department of Geriatric Medicine
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Adrenomedullin insufficiency increases allergen induced airway hyperresponsiveness in mice

Hiroshi Yamamoto[1], Takahide Nagase[2], Takayuki Shindo[3], Shinji Teramoto[1], Tomoko Aoki-Nagase[1], Yasuhiro Yamaguchi[1], Yoko Hanaoka[1], Hiroki Kurihara[4], and Yasuyoshi Ouchi[1]

Department of [1]Geriatric Medicine, [2]Respiratory Medicine, and [4]Physiological Chemistry and Metabolism, Graduate School of Medicine, University of Tokyo, Tokyo, Japan
[3]Department of Organ Regeneration, Shinshu University Graduate School of Medicine, Nagano, Japan

Running head: enhanced airway responsiveness in ADM mutant mice

Address correspondence to:
Dr. Hiroshi Yamamoto,
Department of Geriatric Medicine,
Graduate School of Medicine,
University of Tokyo,
7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan, 113-8655
E-mail address: hyamamot-tky@umin.ac.jp
TEL: 81-3-5800-8652
FAX: 81-3-5800-6530

Abstract

Adrenomedullin (ADM), a newly identified vasodilating peptide, is reported to be expressed in lungs and have bronchodilating effect. We hypothesized whether ADM could be involved in the pathogenesis of bronchial asthma. We examined the role of ADM in airway responsiveness using heterozygous ADM deficient mice ($AM^{+/-}$) and their littermate control ($AM^{+/+}$). Here, we show that airway responsiveness is enhanced in ADM mutant mice after sensitization and challenge with ovalbumin (OVA). The immunoreactive ADM level in the lung tissue after methacholine challenge was significantly greater in the wild type mice than that in the mutant. However, the impairment of ADM gene function unaffected immunoglobulins (OVA-specific IgE and IgG1), Th1 and Th2 cytokines, and leukotrienes. Thus, the conventional mechanism of allergen induced airway responsiveness is not relevant to this model. Further, morphometric analysis revealed that eosinophilia and airway hypersecretion were similarly found in both the OVA-treated ADM mutant mice and the OVA-treated wild type mice. On the other hand, the area of the airway smooth muscle layer of the OVA-treated mutant mice was significantly greater than that of the OVA-treated wild type mice. These results suggest that ADM gene disruption may be associated with airway smooth muscle hyperplasia as well as enhanced airway hyperresponsiveness. ADM mutant mice might provide novel insights to study the pathophysiological role of ADM in vivo.

Key Words: asthma, airway hyperresponsiveness, remodeling, adrenomedullin, knockout mouse