



Lack of both α_2 -antiplasmin and plasminogen activator inhibitor type-1 induces high IgE production

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ARTICLE INFO

Article history:

Received 8 November 2012

Accepted 28 May 2013

Keywords:

α_2 -AP

PAI-1

IgE regulation

Bone marrow cells

Gene deficient mice

ABSTRACT

Aims: We investigated the pathophysiological changes in mice lacking α_2 -antiplasmin (α_2 -AP) and plasminogen activator inhibitor type-1 (PAI-1) genes, and elucidated the involvement of these inhibitors for fibrinolysis in immune response.

Main methods: The pathophysiological changes induced by a lack of both α_2 -AP and PAI-1 were investigated using double knockout (KO) mice. The lung, liver, kidney and spleen tissues from α_2 -AP/PAI-1-double KO mice were compared with those from wild-type (WT) mice. Furthermore, the bone marrow cells from α_2 -AP/PAI-1-double KO mice were transplanted into 10-Gy X ray irradiated WT mice, and then the effects of the transplantation were studied.

Key findings: Plasma IgE levels in the α_2 -AP/PAI-1-double KO mice increased with age and exceeded 1000 ng/mL after 6 months of age. The plasma cells that produced IgE were detected in perivascular assembled lymphocytes. In the α_2 -AP/PAI-1-double KO mice, perivascular lymphocyte infiltration was observed in the lung, liver, and kidneys and peribronchial lymphocyte infiltration was present in the lung. When the bone marrow cells from α_2 -AP/PAI-1-double KO mice were transplanted into 10-Gy X ray irradiated WT mice, the phenotypes of the recipients were similar to those of α_2 -AP/PAI-1-double KO mice.

Significance: The simultaneous expression of both the α_2 -AP and PAI-1 genes contributes to the maintenance of immunological functions that are related to IgE. Moreover, it is suggested that both α_2 -AP and PAI-1 are involved in the recruitment of lymphocytes in the peripheral tissues.

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Introduction

The fibrinolytic system consists of a potential enzyme system, in which plasminogen is activated by plasminogen activators (PAs), such as tissue-type PA (t-PA) and urokinase-type PA (u-PA). Inhibitors of PAs and plasmin, the PA inhibitor (PAI) and α_2 -antiplasmin (α_2 -AP), respectively, regulate PA and plasmin enzymatic activity (Collen and Lijnen, 1991). Recently, it was found that the receptors and binding sites for these fibrinolytic factors are present on the surface of cells (Flood and Hajjar, 2011), with the cell-bound fibrinolytic factors expressing proteolytic activity at the cell's periphery causing intracellular signal transduction (Bhat et al., 1999; Smith and Marshall, 2010). The

function system of the fibrinolytic factors around cells was suggested to be a "cellular fibrinolytic system," and we distinguished it from the "blood fibrinolytic system" that was the conventional and intravascular function of fibrinolytic factors (Fukao et al., 1997). Studies of this cellular fibrinolytic system were drastically advanced by using gene-deficient mice (Carmeliet and Collen, 1998). The cellular fibrinolytic system contributes to various physiological and pathophysiological phenomena such as neovascularization, tissue repair, and tumor invasion and metastasis.

α_2 -AP is a physiological inhibitor of plasmin in plasma (Collen, 1976). Our previous studies show that α_2 -AP is associated with wound healing and fibrosis (Kanno et al., 2006, 2010). α_2 -AP is involved in tissue fibrosis through the inhibition of plasmin which triggers the cellular fibrinolytic system. PAI-1 is a physiological inhibitor of t-PA and u-PA in plasma (Dupont et al., 2009). The plasma level of PAI-1 remarkably increases in several thromboembolic diseases (Meltzer et al., 2010).

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In allergic diseases, tissue remodeling such as subepithelial fibrosis or extracellular matrix (ECM) deposition in the airways is observed. Plasmin degrades fibrin and converts the inactive pro-matrix metalloproteinase (MMP) into the active MMP which degrades the ECM proteins. Therefore, the involvement of fibrinolytic factors in allergic diseases has been studied. Namely, the expression of u-PA and PAI-1 mRNA was enhanced in the nasal tissues of patients with allergies in contrast to normal nasal tissues (Sejima et al., 2004). In ovalbumin (OVA)-challenged mice, an animal model of asthma, PAI-1 production was increased in the lungs and the increased PAI-1 promoted ECM deposition in the airways by suppressing the generation of plasmin (Oh et al., 2002). These reports suggest that fibrinolytic components, including PAI-1, contribute to the development of allergic disease.

Dewerchin et al. reported that mice deficient in both α_2 -AP and PAI-1 (α_2 -AP/PAI-1-double KO mice) were viable, developed normally and were fertile (Dewerchin et al., 2001). Although the endogenous fibrinolytic capacity derived from the lack of inhibitors for the fibrinolytic system was enhanced in the double KO mice, no features of allergic disorders including high plasma IgE levels induced by an immediate type 1 allergy were reported. In the present study, the pathophysiological changes induced by a lack of both α_2 -AP and PAI-1 were analyzed. The α_2 -AP/PAI-1-double KO mice exhibited high plasma IgE levels in an age-dependent manner. This was confirmed after transplantation of α_2 -AP/PAI-1-double KO bone marrow cells into wild-type (WT) mice.

Materials and methods

Reagents

The following materials were obtained from the commercial sources indicated: anti-mouse CD3, CD4, CD8, CD19, and CD24 IgG (BD Pharmingen, San Diego, CA, USA), Fluorescein streptavidin (Vector Lab., Burlingame, CA, USA), rat monoclonal anti-mouse IgE antibody (MorphoSys UK, Oxford, UK), 3,3'-diaminobenzidine (DAB) tetra-HCl (WAKO, Tokyo, Japan), Nembutal (Abbott Laboratories, North Chicago, IL, USA). All other reagents and chemicals were of the highest grade available.

Animals

Mice lacking the α_2 -AP or PAI-1 gene were intercrossed to yield WT or α_2 -AP/PAI-1-double KO mice. The genomic background of the α_2 -AP-deficient (α_2 -AP KO) mouse was the same as that of the PAI-1-deficient (PAI-1 KO) mouse. Genotyping of the offspring was performed as described elsewhere (Carmeliet et al., 1993; Lijnen et

al., 1999). All mice were bred under pathogen-free conditions. All animal experiments were planned according to the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences. The Institutional Animal Care and Use Committee of the Kinki University School of Medicine approved the animal experiments.

At the indicated age, mice were anesthetized with 50 mg/kg body weight of Nembutal and killed. The lungs, liver, kidneys, and spleen were used for histochemical analysis.

Hematological analysis

The blood samples were collected from the inferior vena cava into a test tube with a one-tenth volume of 0.129 M sodium citrate. Blood cell counts were performed using the SFVU-1 automated analyzer (Sysmex, Kobe, Japan).

ELISA assay

Concentrations of IgE in the plasma were measured by sandwich ELISA using a mouse IgE-ELISA kit (Shibayagi, Shibukawa, Japan). IgG₁, IgG_{2b}, or IgM levels in the plasma were measured using mouse ELISA kits (Cygnus Technologies, Southport, NC, USA). IgG₃ levels in the plasma were measured using a mouse ELISA kit (Assay Designs, Ann Arbor, MI, USA).

The concentrations of cytokines in plasma were measured by using mouse IL-2, IL-4, IL-5, IL-10, IL-13, and IFN- γ ELISA Quantikine® kits (R&D Systems, Minneapolis, MN, USA).

Histochemical studies

Formalin-fixed and paraffin-embedded sections of lung, liver, kidney or spleen were stained with hematoxylin and eosin (HE).

For immunostaining of CD3, CD4, CD8, CD19, and CD24, standard immunofluorescence techniques were performed on free-floating sections. The sections were incubated with anti-mouse CD3, CD4, CD8, CD19, and CD24 IgG (dilution 1:20) in PBS containing 0.3% Triton X-100 (PBST) and 5% normal serum for 2 days at 4 °C. The sections were then incubated with biotinylated anti-hamster IgG (2.5 mg/mL) for 2 h followed by fluorescein streptavidin (dilution 1:100) for 2 h in PBST. The sections were further incubated with propidium iodide (0.1 mg/mL) in PBST for 2 h. The sections were mounted on a glass slide and sealed with Vectashield. Observation was made using a LSM510 laser-scanning confocal microscope (Carl Zeiss, Oberkochen, Germany).

Immunostaining for IgE was performed as described elsewhere (Chvatchko et al., 1996). The sections were incubated with rat monoclonal anti-mouse IgE antibody. The sections were then incubated

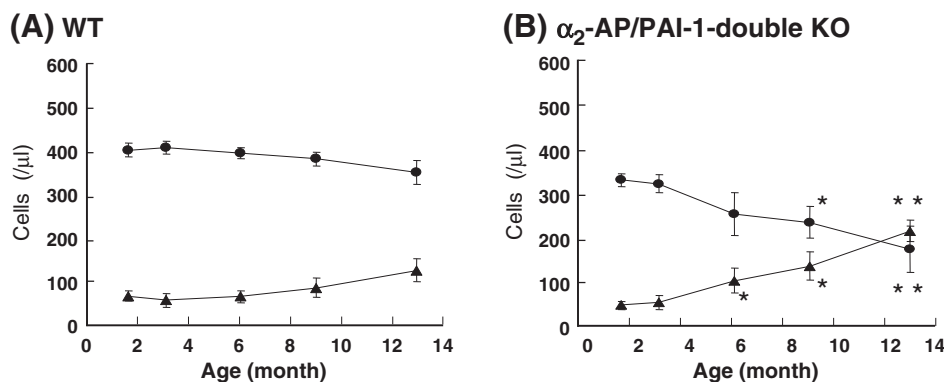


Fig. 1. Age-dependent changes in neutrophil or lymphocyte number in WT (A) and α_2 -AP/PAI-1-double KO (B) mice. Neutrophil (▲) or lymphocyte (●) counts were performed using the SFVU-1 automated hematology analyzer. Each value represents the mean $\pm$ SD, $n = 6$. * $p < 0.05$, values in 1.5 months of age vs. values in 6 or 9 months of age. ** $p < 0.001$, values in 1.5 months of age vs. values in 13 months of age.

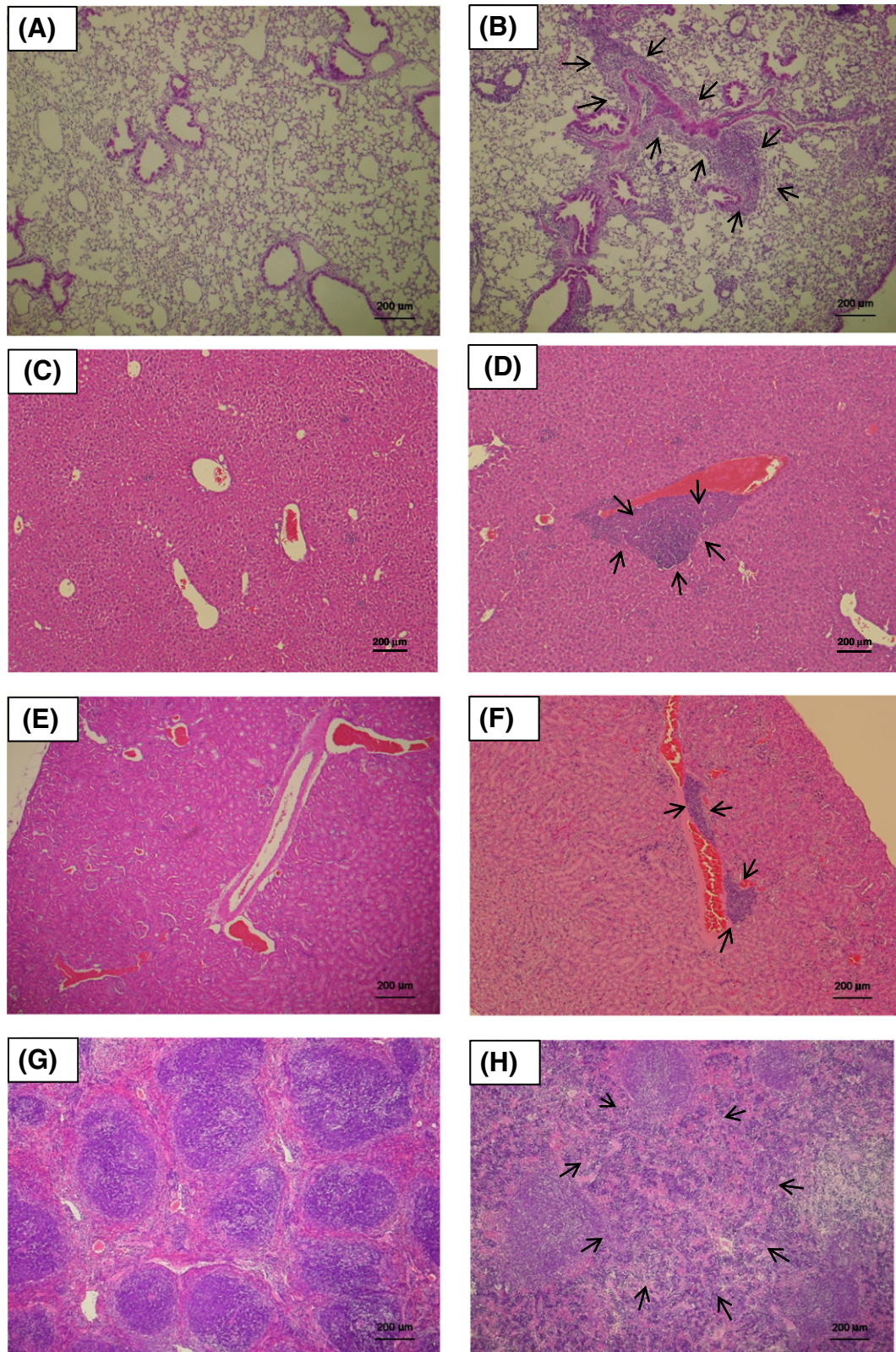


Fig. 2. Histological findings in WT mice and α_2 -AP/PAI-1-double KO mice. Sections of lung (A), liver (C), kidney (E), or spleen (G) of WT mice, and those of lung (B), liver (D), kidney (F), or spleen (H) of α_2 -AP/PAI-1-double KO mice of 5 months of age were stained with HE. The arrows in panels B, D and F indicate perivascular lymphocyte infiltration. The arrows in panel H indicate the augmented proliferation of lymphocytes.

with the appropriate secondary antibodies conjugated with horse-radish peroxidase. Positive signals were visualized with a 3,3'-diaminobenzidine(DAB)tetra-HCl solution. The sections were counterstained with HE and photographed.

Fibrinolytic activity

Fibrinolytic activities in plasma and spleen were measured using fibrin zymography and fibrin plate assays as described previously

(Matsuo et al., 1986). In the assay using fibrin zymography, the fibrin gel on which the lytic bands appeared, was analyzed by using LAS-1000 (FUJIFILM, Tokyo, Japan). The intensity of the lytic band corresponding to t-PA or u-PA was calibrated to that of human standard t-PA (0.5 IU) or u-PA (0.1 IU), and t-PA or u-PA activity was expressed as arbitrary units (AU) (Tanaka et al., 2001).

Transplantation of bone marrow cells

Bone marrow cells (2×10^6) from α_2 -AP/PAI-1-double KO mice were transplanted into 10-Gy X ray irradiated WT mice. As the control, bone marrow cells (2×10^6) from WT mice were transplanted into 10-Gy X ray irradiated WT mice. At 3–5 months after transplantation, the plasma IgE levels and histological changes in recipient mice were evaluated.

Statistical analysis

The data were presented as the mean $\pm$ SD. The Student's t-test was used to compare the different parameters between the two groups, and a p-value of 0.05 or less was considered significant.

Results

Hematologic parameters in α_2 -AP/PAI-1-double KO mice

Numbers of erythrocytes, leukocytes, and platelets in blood did not differ significantly between the α_2 -AP/PAI-1-double KO and WT mice until 13 months of age (data not shown). In WT mice, the number of lymphocytes (closed circles in Fig. 1A) gradually decreased and the number of neutrophils (closed triangles in Fig. 1A) gradually increased. However, the number of lymphocytes in α_2 -AP/PAI-1-double KO mice age-dependently decreased (closed circles in Fig. 1B) greater than those in WT mice (closed circles in Fig. 1A). In addition, the number of neutrophils in α_2 -AP/PAI-1-double KO mice age-dependently increased (closed triangles in Fig. 1B) greater than those in WT mice (closed triangles in Fig. 1A).

Histological changes in mice

Compared to the histological examination of lungs, liver, kidneys, or spleen from WT mice (Fig. 2A, C, E and G), those from α_2 -AP/PAI-1-double KO mice showed remarkable changes. In α_2 -AP/PAI-1-double KO mice, perivascular infiltrations of cells were observed in lungs, liver, or kidneys (arrows in Fig. 2B, D and F). These cells were stained by anti-CD3, CD4, CD8, CD19, and CD24 IgG (data not shown). In the

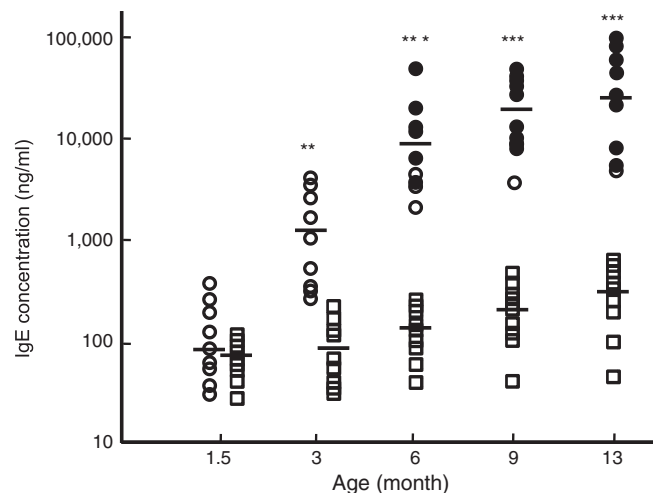


Fig. 4. Age-dependent changes of plasma IgE levels in WT or α_2 -AP/PAI-1-double KO mice. At 1.5, 3, 6, 9, or 13 months old, the IgE levels in plasma were measured by sandwich ELISA. Plasma IgE levels of α_2 -AP/PAI-1-double KO mice (circles) increased with age greater than those of WT mice (squares). The open or closed circles indicate the plasma IgE levels of α_2 -AP/PAI-1-double KO mice without or with perivascular lymphocyte infiltration in lungs, liver, or kidneys. Each mean value is represented as a small bar ($n = 9$). ** $p < 0.01$ or *** $p < 0.001$, plasma IgE levels in α_2 -AP/PAI-1-double KO mice vs. plasma IgE levels in WT at the same age (month).

spleen, the original structure shown in Fig. 2G was lost and the augmented proliferation of lymphocytes was observed (arrows in Fig. 2H).

Before 5 months of age, although perivascular lymphocyte infiltration was not observed at all in WT mice, the rate of expression of perivascular lymphocyte infiltration in lungs, liver, and kidneys of α_2 -AP/PAI-1-double KO mice was 3/18, 3/16 and 2/16, respectively. After 5 months of age, only one of 15 WT mice showed perivascular lymphocyte infiltration in the lung. Even after 5 months, no perivascular lymphocyte infiltration in the liver or kidneys was observed in WT mice. However, after 5 months of age, the rate of expression of perivascular lymphocyte infiltration in the lungs, liver, and kidneys of α_2 -AP/PAI-1-double KO mice was remarkably high, 24/28, 20/25 and 14/25, respectively.

Moreover, IgE-positive cells (plasma cells) were detected within the perivascular assembled lymphocytes in α_2 -AP/PAI-1-double KO mice by the immunohistochemical analysis of the liver (Fig. 3).

Immunoglobulins and cytokines in the plasma

The plasma IgE levels in α_2 -AP/PAI-1-double KO mice of age 1.5 months were 88 ± 78 ng/mL, and were comparable to those in WT mice (75 ± 32 ng/mL). However, the plasma IgE levels of the

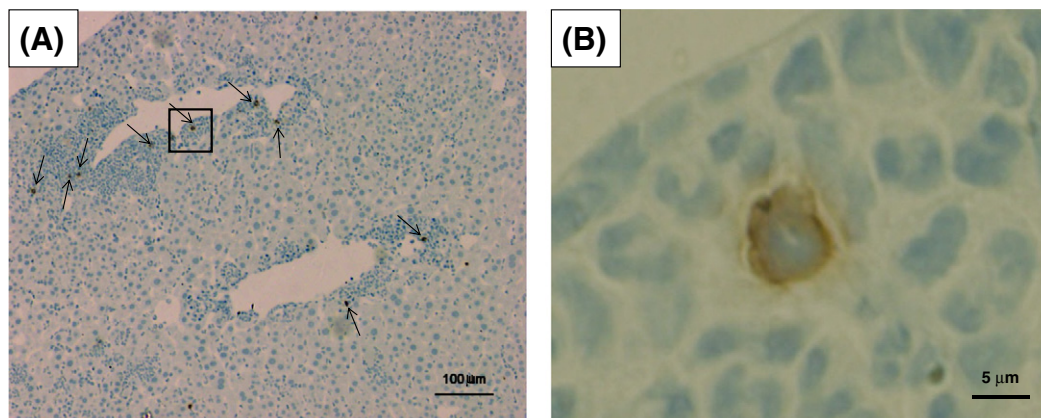


Fig. 3. Immunohistochemical evaluation of liver from α_2 -AP/PAI-1-double KO mice. Sections of liver were immunostained with anti-mouse IgE antibody. IgE-positive cells were detected within the diffuse aggregates in α_2 -AP/PAI-1-double KO mice of 5 months of age (arrows in panel A). Panel B shows a magnified view of the square in panel A.

α_2 -AP/PAI-1-double KO mice remarkably increased with age (circles in Fig. 4). The rate of increase was greater than that in WT mice (squares in Fig. 4). At 6 months, the plasma IgE levels of α_2 -AP/PAI-1-double KO mice reached 9350 ± 3471 ng/mL, significantly greater than the levels in WT mice and subsequently remained above 10,000 ng/mL. At more than 6 months of age, the α_2 -AP/PAI-1-double KO mice with perivascular lymphocyte infiltration showed higher plasma IgE levels (closed circles in Fig. 4) than those without perivascular lymphocyte infiltration (open circles in Fig. 4).

Before 5 months of age, the plasma IgG₁ or IgM levels showed no significant age-dependent changes in both WT mice and α_2 -AP/PAI-1-double KO mice (Table 1), nor were there any significant differences in IgG_{2b}, or IgG₃ (data not shown). After 5 months, the plasma IgG₁ levels in α_2 -AP/PAI-1-double KO with perivascular lymphocytes infiltration were higher than those without and significantly higher than in WT mice.

The cytokines IL-2, IL-4, IL-5, IL-10, IL-13, and IFN- γ in the plasma showed no significant differences between WT and α_2 -AP/PAI-1-double KO mice (data not shown).

Fibrinolytic activities in plasma and spleen

Fibrinolytic activity in the plasma of α_2 -AP/PAI-1-double KO mice (1.35 ± 0.37 IU/mL) was significantly higher than that in WT mice (0.31 ± 0.15 IU/mL) by the fibrin plate assay. The activities of PAs in the plasma of α_2 -AP/PAI-1-double KO mice (t-PA: 4.7 ± 0.26 AU/mL, u-PA: 6.8 ± 0.31 AU/mL) were higher than those in WT mice (t-PA: 3.3 ± 0.21 AU/mL, u-PA: 5.1 ± 0.43 AU/mL) by fibrin zymography.

The activities of PAs determined using fibrin zymography in the spleen of α_2 -AP/PAI-1-double KO (t-PA: 12 ± 0.6 AU/mL, u-PA: 19 ± 0.8 AU/mL) were higher than those in WT mice (t-PA: 9.0 ± 1.1 AU/mL, u-PA: 15 ± 1.0 AU/mL).

Transplantation of bone marrow cells

After transplantation of bone marrow cells from WT mice to irradiated WT mice, the plasma IgE levels (334 ± 103 ng/mL) were similar to those of untreated WT mice (380 ± 43 ng/mL) (Table 2). However, the plasma IgE levels of WT mice in which bone marrow cells from α_2 -AP/PAI-1-double KO mice were transplanted (4535 ± 2845 ng/mL), were significantly higher than those of untreated WT mice (380 ± 43 ng/mL, $p < 0.01$) (Table 2).

WT mice in which bone marrow cells from α_2 -AP/PAI-1-double KO mice were transplanted, showed remarkable histological changes (Fig. 5). The histological findings in the recipient mice were similar to those of α_2 -AP/PAI-1-double KO mice (Fig. 2B, D, F and H). The rates of expression of perivascular lymphocyte infiltration in the lungs, liver, and kidneys of these recipient mice were 9/28, 6/28 or 4/28, respectively (Table 3). On the other hand, when bone marrow cells from WT mice were transplanted to irradiated WT mice, no perivascular lymphocyte infiltrations were observed in the lungs, liver, and kidneys (Table 3).

Table 2
Comparison of plasma IgE levels.

		IgE (ng/ml)	
Untreated WT (n = 15)		380 ± 43	
Transplantation of bone marrow cells			
Donor	Recipient		
α_2 -AP/PAI-1-double KO	→ WT (n = 28)	4535 ± 2845	** N.S.
WT	→ WT (n = 10)	334 ± 103	

Bone marrow cells (2×10^6) from α_2 -AP/PAI-1-double KO mice or WT mice were transplanted into 10-Gy X ray irradiated WT mice. At 3–5 months after transplantation, the plasma IgE levels were measured by sandwich ELISA. Also, the plasma IgE level of untreated WT mice was measured. **, $p < 0.01$, N.S.; no significant.

Discussion

α_2 -AP and PAI-1 are inhibitors for proteases in the fibrinolytic system. These inhibitors regulate fibrinolysis by inhibiting plasmin and plasminogen activator activity. Besides the removal of fibrin, the fibrinolytic system may also play important roles in ovulation, embryogenesis, atherosclerosis and tissue repair as the cellular fibrinolytic system (Carmeliet and Collen, 1998). Our previous studies showed that α_2 -AP is associated with wound healing and fibrosis (Kanno et al., 2006, 2010). We earlier showed that PAI-1 was involved in the progression of allergic responses (Sejima et al., 2005).

Either α_2 -AP deficiency or PAI-1 deficiency in human shows several events of major hemorrhage or rebleeding after trauma or surgery (Aoki et al., 1979; Mehta and Shapiro, 2008). However, the phenotype in the absence of both inhibitors is not clear. Therefore, mice lacking the α_2 -AP or PAI-1 gene were intercrossed to yield α_2 -AP/PAI-1-double KO mice and the resulting phenotypes were investigated.

Dewerchin et al. reported that α_2 -AP/PAI-1-double KO mice showed no features of allergic disorders within their experimental period (Dewerchin et al., 2001). However, when we analyzed the α_2 -AP/PAI-1-double KO mice for a much longer time, higher plasma IgE levels could be clearly detected. Due to the limited period of the observation, they could not find the allergic disorders and high plasma IgE levels which were expressed in an age-dependent manner (Fig. 4).

As mentioned earlier, this study showed that α_2 -AP/PAI-1-double KO mice exhibited a remarkable increase in plasma IgE levels. To elucidate whether this increase was caused by a T_H2 immune response, concentrations of several cytokines (IL-4, IL-5, IL-10 and IL-13) in the plasma were measured. However, no significant differences of these cytokines levels between the WT and α_2 -AP/PAI-1-double KO mice were seen, suggesting that the high plasma IgE levels in α_2 -AP/PAI-1-double KO mice were not due to a T_H2 immune response. Furthermore, since the plasma IL-2 and INF- γ levels were comparable with those in WT mice, it is possible that the T_H1-dominant immune response is not associated with the high plasma IgE levels in α_2 -AP/PAI-1-double KO mice.

Perivascular lymphocyte infiltration was observed in neither the α_2 -AP KO nor the PAI-1 KO mice (data not shown). However, the

Table 1
Age-dependent changes of plasma IgG₁ or IgM levels in α_2 -AP/PAI-1-double KO or WT mice.

Mice	Lymphocyte infiltration	IgG ₁ (mg/mL)			IgM (mg/mL)		
		<3 M	3–5 M	<5 M	<3 M	3–5 M	5 M<
α_2 -AP/PAI-1-double KO	Without	0.67 ± 0.261 (n = 5)	0.68 ± 0.202 (n = 8)	0.41 (n = 2)	0.47 ± 0.126	0.48 ± 0.127	0.42
	With	–	1.11 ± 0.085 (n = 3)	$1.09 \pm 0.247^*$ (n = 23)	–	0.73 ± 0.071	0.66 ± 0.115
WT	Without	0.65 ± 0.226 (n = 6)	0.66 ± 0.241 (n = 12)	0.70 ± 0.305 (n = 14)	0.44 ± 0.130	0.47 ± 0.145	0.48 ± 0.118

The IgG₁ or IgM levels in plasma were measured in mice of <3, 3–5, <5 months old by sandwich ELISA. The values were measured in α_2 -AP/PAI-1-double KO mice with or without lymphocyte infiltration, and in WT mice were shown.

* $p < 0.05$, α_2 -AP/PAI-1-double KO mice with lymphocyte infiltration vs. WT mice at the same age (month).

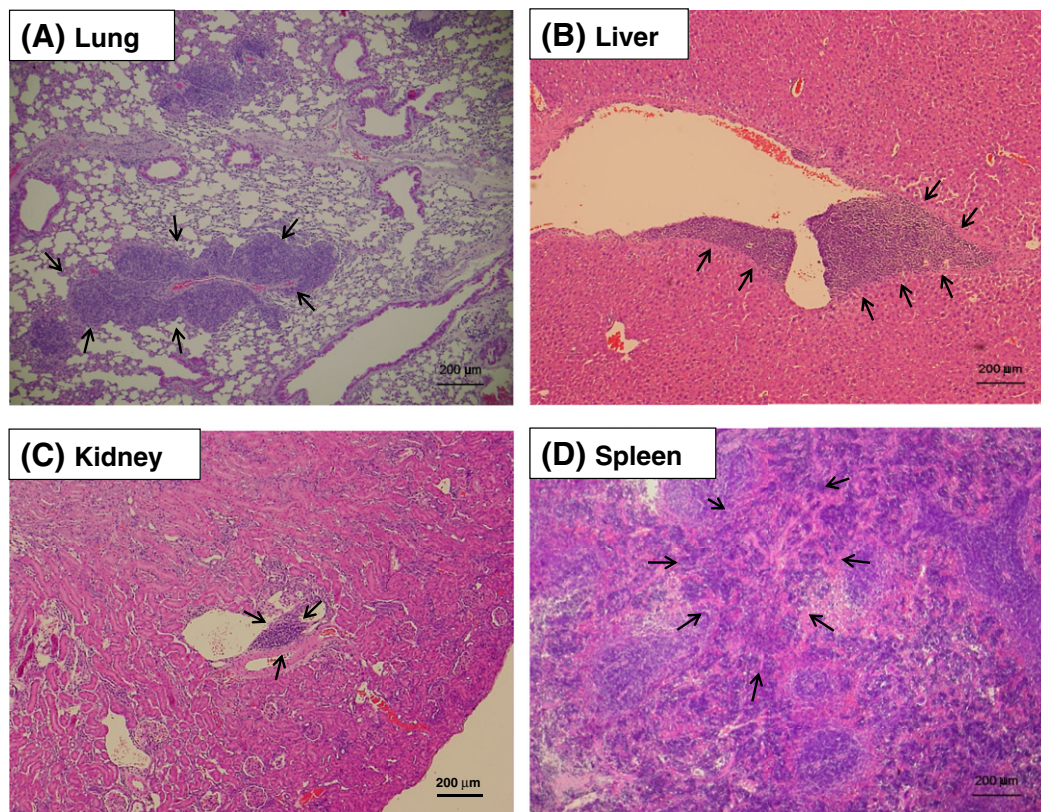


Fig. 5. Histological findings in WT mice in which the bone marrow cells obtained from α_2 -AP/PAI-1-double KO mice were transplanted. Sections of lung (A), liver (B), kidney (C), or spleen (D) in recipient mice were stained with HE. The arrows in A, B, and C indicate perivascular lymphocyte infiltration. The arrows in D indicate the augmented proliferation of lymphocytes.

recruitments of cells in lungs, liver, and kidneys were observed in α_2 -AP/PAI-1-double KO mice (Fig. 2B, D and F). Since these cells were stained by anti-CD3, CD4, CD8, CD19, and CD24 IgG (data not shown), it was suggested these cells were either T lymphocytes or B lymphocytes. To elucidate whether the lack of both α_2 -AP and PAI-1 genes in hematopoietic stem cells induced perivascular lymphocyte infiltration, the bone marrow cells in α_2 -AP/PAI-1-double KO mice were transplanted into WT mice. After 3–5 months, perivascular lymphocyte infiltration in the lungs, liver, or kidneys was observed in 9/28, 6/28 and 4/28 recipient mice, respectively (Table 3). Therefore, it is suggested that the bone marrow cells in α_2 -AP/PAI-1-double KO mice may partially cause perivascular lymphocyte infiltration.

Among the lymphocytes assembled around the vasculature in α_2 -AP/PAI-1-double KO mice, plasma cells that produce IgE were observed as shown in Fig. 3. A large amount of IgE secreted by the plasma cells was thought to be associated with the high plasma IgE levels in α_2 -AP/PAI-1-double KO mice. In general, IL-4 that is produced by helper T cells induces immunoglobulin class switching and promotes

plasma cells to secrete IgE. Therefore, although the plasma IL-4 level was not significantly high in α_2 -AP/PAI-1-double KO mice, it is possible that the helper T cells that produce IL-4 may exist in the assembled lymphocytes or near the IgE-producing plasma cells.

α_2 -AP/PAI-1-double KO mice are genetically deficient in both an inhibitor for plasmin, α_2 -AP, and that for PA, PAI-1, resulting in enhancement of fibrinolytic or proteolytic activity. Indeed, the fibrinolytic activity in α_2 -AP/PAI-1-double KO mice was 4 times higher than that in WT mice as determined by the fibrin plate method. The activities of PAs in the spleen of α_2 -AP/PAI-1-double KO mice were higher than those in WT mice by fibrin zymography were. It was suggested that the higher activities of PAs induced higher proteolytic activities in the spleen and caused the destruction of the histological structure of spleen as shown in Fig. 2H.

It is reported that high plasmin activity around cells is involved in degradation of extracellular matrix and migration of the cell (Gong et al., 2008). Since the plasmin activity in α_2 -AP/PAI-1-double KO mice is higher than that in WT mice, it is thought that lymphocytes in blood may easily migrate outside of vessels and in fact, perivascular lymphocyte infiltrations did occur in α_2 -AP/PAI-1-double KO mice as shown in Fig. 2B, D and F. The migration of lymphocytes from the bloodstream to perivascular lesions may cause the remarkable decrease in lymphocyte numbers in blood as indicated in Fig. 1B.

Lymphoid organs can be divided broadly into primary lymphoid organs, where lymphocytes are generated, and secondary lymphoid organs, where mature naïve lymphocytes are maintained and adaptive immune responses are initiated. The primary lymphoid organs are the bone marrow and the thymus. The secondary lymphoid organs include the lymph nodes, the spleen and the other mucosal lymphoid tissues. The lymphoid aggregations as shown in lungs, liver, or kidneys of α_2 -AP/PAI-1-double KO mice (Fig. 2B, D and F), are not thought to be primary or secondary lymphoid organs. Since the third type of lymphoid organs consist of large numbers of lymphocytes and play an

Table 3

The rate of expression of perivascular lymphocyte infiltration in lung, liver or kidney of transplanted mice.

Donor	Recipient	Lung	Liver	Kidney
		Positive mice/ total mice	Positive mice/ total mice	Positive mice/ total mice
α_2 -AP/PAI-1-double KO	WT	9/28	6/28	4/28
WT	WT	0/10	0/10	0/10

Bone marrow cells (2×10^6) from α_2 -AP/PAI-1-double KO or WT mice were transplanted into 10-Gy X ray irradiated WT mice. At 3–5 months after transplantation, recipient mice were sacrificed and the lung, liver or kidney was removed. Sections of lung, liver or kidney were stained with HE and the recipient mice, in which perivascular lymphocyte infiltration was detected, were estimated as positive mice.

immunological role only when they are only exposed to antigens (Goldsby et al., 2003), the lymphoid aggregations observed in the present study seem to be the third lymphoid organs.

Conclusion

We investigated the pathophysiological changes in mice lacking α_2 -AP and PAI-1 genes, and elucidated the involvement of these inhibitors for fibrinolysis in immune response. Plasma IgE levels in the α_2 -AP/PAI-1-double KO mice increased with age. When the bone marrow cells from α_2 -AP/PAI-1-double KO mice were transplanted into 10-Gy X ray irradiated WT mice, the phenotypes of the recipients were similar to those of α_2 -AP/PAI-1-double KO mice. A lack of both α_2 -AP and PAI-1 causes alterations in the biofunctional characteristics of bone marrow cells, resulting in high IgE production and pathophysiological changes. Namely, it is suggested that both α_2 -AP and PAI-1 are simultaneously involved in regulation of the immune response.

Disclosure of conflict of interests

The authors state that they have no conflict of interest.

Acknowledgments

This work was supported in part by Grants-in Aid for Scientific Research (C) (19590217, 20590222, 22590219 and 23590268) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), the “High-Tech Research Center” Project for Private Universities: a matching fund subsidy from MEXT, 2002–2009, Medical and Engineering Link at Kinki University, Graduate School of Medicine from MEXT, 2006–2010. The authors thank S. Kurashima and Y. Tatsumi (Kinki University Faculty of Medicine) for help with the transplantation of bone marrow cells. The authors thank T. Chikugo, M. Kashima and Y. Shigeyoshi (Kinki University Faculty of Medicine) for help with the histochemical studies.

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