

Lactate Dehydrogenase-elevating Virus Infection at the Sensitization and Challenge Phases Reduces the Development of Delayed Eosinophilic Allergic Rhinitis in BALB/c Mice

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Abstract

The present study was conducted to determine whether lactate dehydrogenase-elevating virus (LDV) infection at the sensitization and challenge phases affect the development of delayed allergic eosinophilic rhinitis induced by ovalbumin (OVA) in BALB/c mice (DAR group). Compared to the DAR group, LDV infection at the priming (DAR/LDV_p group) and immunizing (DAR/LDV_i group) phases reduced the induction of eosinophils in the bone marrow (BM) and/or blood. However, the number of eosinophils in the BM was not affected in the DAR/LDV_i group. In addition, total blood IgE values were reduced in the DAR/LDV_p but not the DAR/LDV_i groups. Compared to the production of cytokines from splenic cells and blood IgE values in the DAR group, OVA-specific IL-4 and IFN- γ productions and IgE values were reduced in the DAR/LDV_p, whereas OVA-specific IFN- γ and IL-4 productions were increased and decreased, respectively in the DAR/LDV_i, but not the DAR/LDV_p groups. Both DAR/LDV_p and DAR/LDV_i groups reduced the development of eosinophilic rhinitis associated with reduced VCAM-1 expression on endothelium in blood vessels and ICAM-1 expression on nasal respiratory epithelium at inflamed areas. The present study suggests that LDV infection at the sensitization phase may reduce the development of T helper (Th) 1 and Th2 responses, whereas LDV infection at the challenge phase may inhibit the development of Th2 response by shifting to Th1 response. These may be responsible for the reduction of the development of DAR by LDV infection.

Introduction

Allergic airway diseases may consist of an immediate phase, mediated mainly by IgE and mast cells [1], and a late phase, mediated mainly by CD4⁺ T-helper (Th)2 cells and eosinophils in human and animal models [1–4]. Late allergic rhinitis (AR) is a chronic disease associated with nasal inflammation, oedema of the submucosa in nasal and sinus passages. Those changes lead to nasal congestion, rhinorrhoea, nasal blockage and reduced olfactory function in human [5, 6]. In addition, histologically, chronic AR is characterized by eosinophil and lymphocyte recruitment into nasal mucosae [7]. There are general agreements that Th2 cytokines, such as IL-4 [8], IL-5 [9] and IL-13 [10] and granulocyte-macrophage colony-

stimulating factor [6], correlate with eosinophil infiltration and disease severity. Multiple lines of evidences suggest that allergen-specific CD4⁺ Th2 cells may play an essential role in initiating and generating in allergic rhinitis (AR) in human.

There are several factors, including lifestyle, air pollution and infectious agents, which affect the development of AR [11–17]. Among several factors, it has been pointed out that many viruses (e.g. influenza virus, rhinovirus and respiratory syncytial virus; RSV) may exacerbate the development of AR by destruction of respiratory epithelium [18–20]. These include damages to respiratory and olfactory epithelium by infection, leading to easy access of allergens to immune systems and/or sympathetic nerve. In addition, RSV infection induces virus

specific (G-protein) CD4⁺ Th2 cells, and these Th2 cells may induce severe eosinophilic airway inflammation other than direct multiplication in airway epithelium in mice [21, 22]. Contrary, some viruses with persistent infection (e.g. Epstein–Barr virus; EBV, cytomegalovirus; CMV, hepatitis A virus and measles virus) may inhibit the development of AR [15, 23–25] without respiratory epithelial damages, suggesting that those viruses may modulate and/or affect the host immune system. For example, CMV may inhibit the development of airway allergy by altering Th1/Th2 balance [26], and a prospective epidemiological study suggests that attenuated measles virus may protect the development of AR by alteration of immunological reactions [25]. However, the effects of viruses on host immune reactions, especially altered Th1/Th2 balance and antigen presenting activity in the development of AR remain largely unknown, as most reports were carried out by epidemiological studies in human [15, 23–25].

Lactate dehydrogenase-elevating virus (LDV), a genus arterivirus, only infects the subpopulation of macrophages (about 5–10%) in mice and infected mice show a life-long viraemia without clinicopathological changes, except for a five- to 10-fold increase in enzymes such as lactate dehydrogenase (LDH) [27, 28]. Moreover, it has been reported that LDV infection impairs the antigen-presenting cell (APC) activity including a reduction in the number of Ia-positive macrophages and induction of suppressor T cells in acute (within 1 week) and relatively chronic (approximately 3 weeks after infection) phases [29–33], although production of IFN- γ from Th1 cells [34] and IL-12 from macrophage [35] increased transiently (approximately 1 day post-infection). Thus, LDV infection is considered to be a useful tool to analyse how viruses affect the development of AR without epithelial cell damage.

The aim of the present study is to clarify the effects of LDV infection during the priming and immunizing phases on the development of delayed eosinophilic AR [6, 14] in a mouse model.

Materials and methods

Mice. Eight- to 9-week-old specific-pathogen free female BALB/c AnNCrj mice (Japan Charles River Co., Yokohama, Kanagawa, Japan) were used ($n = 43$; total number of mice). The number of mice used is given in each set of experiments and paragraph (parenthesis) of figure legends. They were kept at 24 ± 2 °C room temperature, $55 \pm 10\%$ humidity and a 12-h light/dark cycle (lighting time 08:00–20:00 hours) and given autoclaved pellets (CE-2; Clea Japan Inc., Tokyo, Japan) and water freely. The animal experiments were approved by the Animal Research Ethics Board of the Faculty of Agriculture, Yamaguchi University.

Sensitization and challenge. The sensitization and challenge procedures were performed by a simple modification of methods described previously [36]. As shown in Fig. 1, mice were sensitized two times (on days 0 and 10) by intraperitoneal injection of 10 μ g suspension of ovalbumin (OVA) (Grade V; Sigma Chem. Co., St Louis, MO, USA) mixed with 1.2 mg aluminum hydroxide gel (SERVA, Heidelberg, Germany) in 100 μ l of phosphate-buffered saline (PBS; pH7.2). On day 14, intranasal challenge in right and left nasal cavities was performed with 500 μ g of OVA in 20 μ l of PBS. Mice were divided into four groups as follows; DAR reaction without LDV infection (DAR group), DAR reaction with LDV infection by i.p. injection of $10^{4.5}$ median infectious doses (ID₅₀) of LDV (a gift from Dr Notkins, LOM, NIDR, NIH, USA) [37] on day 0 (sensitization phase; DAR/LDV_s group), DAR reaction with LDV infection on day 14 (challenge phase; DAR/LDV_c group), and non-sensitized and non-infected mice challenged with only OVA on day 14 (control group). Infection of LDV in DAR/LDV_s and DAR/LDV_c groups ($n = 7$ in each group) was confirmed by a significant rise of plasma LDH 4 days after LDV infection (LDV-infected mice versus non-infected mice: 6211 ± 1163 U/l versus 409 ± 112 U/l, mean $\pm$ standard error; SE, $P < 0.01$). In a separated experiment, LDV antigen localization in spleen and nasal epithelium was examined by the method described previously [38]. LDV antigens were detected in splenic macrophages, but not in nasal epithelium, in BALB/c mice ($n = 3$) 4 and 18 days after infection ($n = 3$ respectively).

Four days after challenge, mice were anaesthetized by i.p. injection of ketamine (45 mg/kg body weight; Sankyo Co., Tokyo, Japan) and xylazine (8 mg/kg body weight; Bayer Co., Tokyo, Japan), and then blood samples (plasma) were obtained from the retro-orbital sinus from each mouse by using heparinized tubes (Terumo, Tokyo, Japan). Also, bone marrow (BM) cells from femurs in each mice were suspended in phosphate-buffered balanced salt solution (PBBS; pH=7.4). The number of leucocyte was counted by haemocytometer

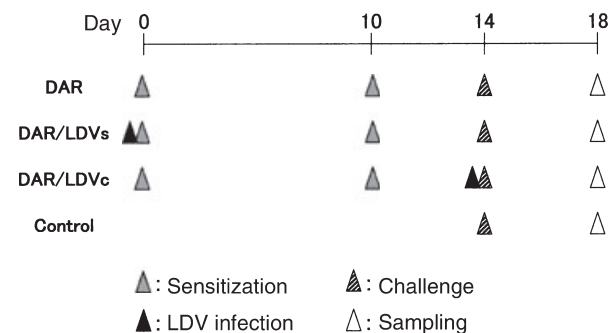


Figure 1 Experimental design. See details in Materials and methods.

after staining with Turk's solution. Cells in blood smear stained with May–Grünwald–Giemsa were differentiated, and the total number of each cell type was determined. Also blood samples (sera) obtained from the heart puncture in anaesthetized mice were used for IgE assay. The number of eosinophils in BM cells was counted and determined as described above except for erythrocytic series and megakaryocytes.

Isolation of splenic cells. Splenic cells were homogenized in a single-cell suspension in PBBS using by sterilized slide glasses. The number of cells was counted using a haemocytometer and cells were diluted in Eagle's minimal essential medium (MEM; Nissui, Tokyo, Japan) containing 5% fetal calf serum to 1×10^6 cells/ml. Splenocytes (2×10^5 cells in 200 μ l MEM) were cultured in the presence (100 μ g/ml) of OVA in flat-bottom 96-well plates for 48 h at 37 °C in a 5% CO₂ atmosphere. Supernatants were used for cytokine assay.

Cytokine assay. IFN- γ and IL-4 productions in supernatants from splenocytes or serum IgE in each mouse were measured by commercial cytokine ELISA kits for mouse IFN- γ and IL-4 (TECHNE Co., Minneapolis, MN, USA) or IgE ELISA kit for mouse (Morinaga Co., Yokohama, Kanagawa, Japan) according to the manufacturer's instructions. Approximately 500 ng/ml (IgE) and 2 pg/ml (IL-4 and IFN- γ) are minimum detectable amounts. In case of cytokines could not be detected, their concentration was estimated as 0.

Histology. Heads were fixed in 10% neutral buffered formalin (pH 7.0) for 2 days, and those were decalcified in 1 mM Tris–HCl (pH 7.4) with 20% EDTA for 5 days. Nasal region was sliced at the anterior margin of orbit transversely, as it has been reported that allergic inflammation was localized in respiratory epithelium of these parts (bold lines) in a murine AR model [39]. Paraffin sections (4 μ m thickness) were stained with haematoxylin and eosin (H&E) and alcian blue-periodic acid-Schiff. For semiquantitative analysis, the severity and the range of pathological changes (distribution of inflammatory cell infiltration in whole nasal cavity, and severity of congestion, haemorrhage and degeneration of respiratory epithelium in respiratory areas) were estimated and scored on 0–4 scale as follows: none (0), slight (1), weak (2), moderate (3) and severe (4). The number of each inflammatory cell type (eosinophil, neutrophil, lymphocyte and mast cell/mm² of nasal mucosa) at the lateral respiratory areas stained with May–Grünwald–Giemsa was counted by an eyepiece with a grid.

Immunohistochemistry. Heads were fixed in 4% paraformaldehyde for 2 days at 4 °C, and then decalcified in 10% EDTA and 7.5% polyvinylpyrrolidone (Sigma-Aldrich, Inc., St Louis, MO, USA) in 1 mM Tris–HCl (pH 7.4) for 7 days at 4 °C. Sliced nasal tissues were embedded in OCT compound, and rapidly

frozen in n-hexane at –80 °C, and frozen sections (8 μ m thickness) were made by a cryostat. Endogenous peroxidase activity was inactivated in 3% H₂O₂ in methanol for 10 min at room temperature. Sections were incubated with rat anti-mouse intercellular adhesion molecule (ICAM)-1 monoclonal antibody (mAb) (20 μ g/ml) (Seikagaku Co., Tokyo, Japan) for 1 day at 4 °C. Thereafter, tissues were treated with 50-fold diluted horseradish peroxidase (HRP)-conjugated goat anti-rat IgG antibody (ICN Biomedicals, Inc., Aurora, OH, USA) for 30 min at room temperature, and then incubated with 3,3'-diaminobenzidine substrate (Roche Diagnostics GmbH, Mannheim, Germany) for 10 min. Sections were counterstained with Mayer's haematoxylin.

To identify the expression of vascular cell adhesion molecule (VCAM)-1, frozen sections were incubated with rat anti-mouse VCAM-1 mAb (50 μ g/ml) (Antigenox America, Inc., Franklin Square, NY, USA) for 1 day at 4 °C, and then reacted with 50-fold diluted FITC-labelled goat anti-rat IgG antibody (Biosource Int., Camarillo, CA, USA) for 30 min at room temperature. To test the specificity, the reaction without primary antibody served as a negative control. To evaluate the intensity and the distribution, the expression of adhesion molecules was estimated and scored on 0–4 scale as follows: none (0), weak and partial (1), weak and diffuse (2), moderate and diffuse (3) or strong and diffuse (4).

Statistics. Data are expressed as the mean $\pm$ SE. Mann–Whitney *U*-test and Student's *t*-test (two-tailed) used to evaluate the differences (comparisons between groups). *P*-values <0.05 was considered significant.

Results

Effects of LDV infection on the number of eosinophils, IgE and cytokine (IL-4 and IFN- γ) production

The number of blood eosinophils in the DAR/LDVs and DAR/LDVc groups was significantly lower than that of the DAR group (*P* < 0.05; Fig. 2A). The number of BM eosinophils (Fig. 2B) and serum IgE (Fig. 2C) in the DAR/LDVs group, but not DAR/LDVc, group, was significantly lower than that of the DAR group (*P* < 0.05).

Ovalbumin-specific IL-4 production from splenocytes was reduced in both groups (the DAR/LDVs and DAR/LDVc groups) compared to the DAR group (Fig. 2D), although there was no statistical difference. However, the IL-4 value in the both groups was reduced to the level of that in the control group. OVA-specific IFN- γ production from splenocytes in the DAR/LDVc group was increased compared to the DAR and DAR/LDVs groups (*P* < 0.05) (Fig. 2E).

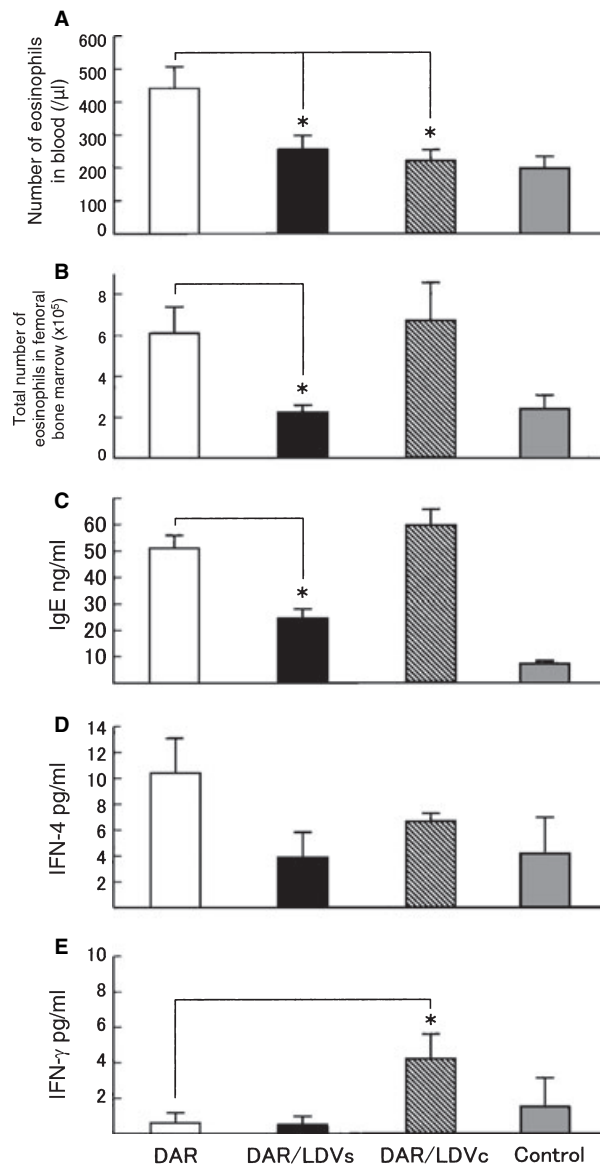


Figure 2 Numbers of eosinophils in blood (A) (the DAR, DAR/LDV_s and the DAR/LDV_c groups; $n = 12$ in each group, the control group; $n = 7$) and bone marrow (B) (the DAR, DAR/LDV_s and DAR/LDV_c groups; $n = 7$ in each group, the control group; $n = 4$), and IgE values in serum (C) (the DAR, DAR/LDV_s and DAR/LDV_c groups; $n = 12$ in each group, the control group; $n = 7$). Productions of IL-4 (D) and IFN- γ (E) from splenocytes in the presence of OVA stimulation (the DAR, DAR/LDV_s and DAR/LDV_c groups; $n = 7$ in each group, the control group; $n = 4$). Each value represents the mean $\pm$ SE. * $P < 0.05$ compared to the DAR group.

Effects of LDV infection on histology

In the DAR, DAR/LDV_s and DAR/LDV_c groups, inflammatory lesions were located mainly in nasal mucosa near by nasal-associated lymphoid tissues, lateral (Fig. 3A), septum and floor of respiratory epithelium, and no inflammatory lesions were observed in olfactory epithelium of nasal mucosa. The degree of infiltration of

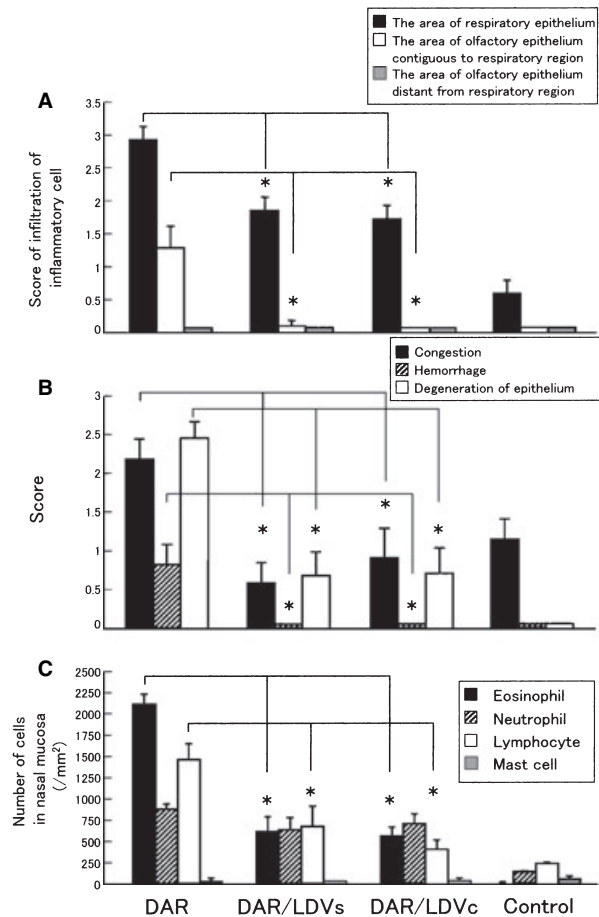


Figure 3 Distribution of inflammation (Score; A), and histopathological changes (B), and differential counts of inflammatory cells in respiratory regions of nasal mucosa (C) (the DAR, DAR/LDV_s and DAR/LDV_c groups; $n = 5$ in each group, the control group; $n = 3$). Each value represents the mean $\pm$ SE. * $P < 0.05$ compared to the DAR group.

inflammatory cells (Score: Fig. 3A), the score of congestion, haemorrhage, degeneration and/or necrosis of respiratory epithelium (Fig. 3B), and the number of infiltrated eosinophils and lymphocytes, but not neutrophils, (Fig. 3C) were statistically lower in the DAR/LDV_s and the DAR/LDV_c groups compared to the DAR group ($P < 0.05$). The number of mast cells did not increase and there was no significant difference among four groups (Fig. 3C).

The DAR group showed moderate to severe congestion in whole regions of nasal mucosa, and there were severe infiltration of eosinophils, slight to mild infiltration of neutrophils and lymphocytes in the lamina propria of respiratory areas (Fig. 4A). Adhesion of inflammatory cells on the endothelial cells of dilated sinus venous was often found in the area of respiratory epithelium. In severely inflamed lesions, some eosinophils (Fig. 4A, an arrow) and a few neutrophils infiltrated into the epithelial layer, leading to the destruction of

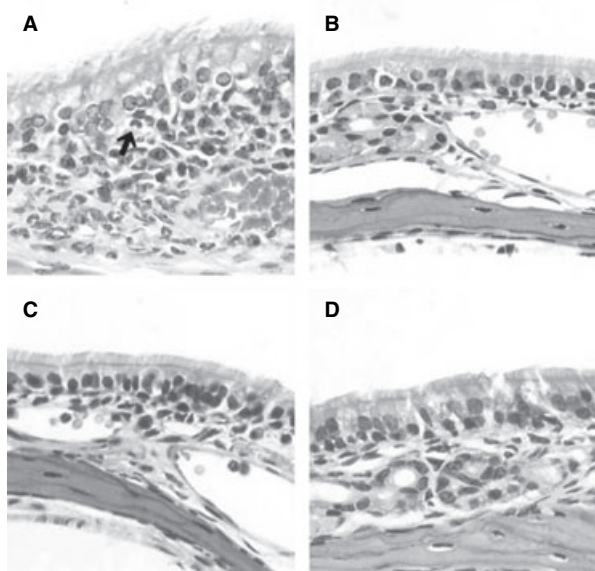


Figure 4 Representative histological changes of nasal mucosa at the lateral site. Respiratory areas from the DAR (A), the DAR/LDVs (B), DAR/LDVc (C) and control (D) groups. Severe infiltration of lymphocytes and eosinophils with degeneration of epithelial cells is visible (A). Representative histology was shown in H&E stain (original magnification: $\times 400$).

epithelium and dilation of interepithelial space. Respiratory epithelial cells in inflamed areas were hypertrophic and hyperplastic with elongated and disorganized cilia showing vacuolar degeneration and necrosis. In some cases of the DAR group, exudation of neutrophils and mucus secretion into nasal cavity and haemorrhage in submucosa were found. On the other hand, in the DAR/LDVs and DAR/LDVc groups, those changes were less severe compared to the DAR group (Fig. 4B,C). The control group showed no remarkable changes except for infiltration of a few neutrophils and lymphocytes in nasal mucosa (Fig. 4D). Goblet cells with much mucus in the DAR/LDVs and DAR/LDVc, but not DAR, groups were recognized. Slight goblet cell hyperplasia and metaplasia were seen in the DAR/LDVs and DAR/LDVc groups.

Effects of LDV infection on expression of ICAM-1 and VCAM-1

There was no difference in ICAM-1 expression on vascular endothelium among four groups (Fig. 5). The score of ICAM-1 expression on respiratory epithelium in the three groups was increased compared to the control group, but its expression in the DAR/LDVs and DAR/LDVc groups was lower than that in the DAR group. The expression of ICAM-1 on olfactory epithelium was not detected in four groups (data not shown). The score of VCAM-1 expression on endothelial cells increased at inflamed areas in three groups compared to the control group, but its

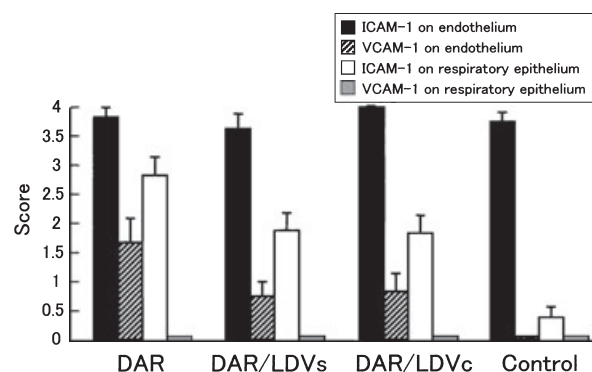


Figure 5 Intensity of expression (Score) of adhesion molecules (the DAR, DAR/LDVs and DAR/LDVc groups; $n = 7$ in each group, the control group; $n = 4$). Each value represents the mean $\pm$ SE.

expression in the DAR/LDVs and DAR/LDVc groups was reduced compared to the DAR group (Fig. 5). There was a faint or no expression of VCAM-1 on respiratory (Fig. 5) and olfactory epithelial cells (data not shown) in four groups.

As shown in Fig. 6, intense ICAM-1 expressions on inflammatory cells outside of vessels and respiratory epithelium were recognized in the DAR group (Fig. 6A) compared to the DAR/LDVs (Fig. 6C) and DAR/LDVc (Fig. 6E) groups. Respiratory epithelium in the control group did not show any ICAM-1 expression (Fig. 6G).

Intense VCAM-1 expression on vascular endothelium in inflamed areas was recognized in the DAR group (Fig. 6B), whereas its expression on those was weak in the DAR/LDVs (Fig. 6D) and DAR/LDVc (Fig. 6F) groups respectively. There was no expression of VCAM-1 on vascular endothelium in the control group (Fig. 6H).

Discussion

The present study clearly demonstrated that LDV infection at the sensitization and challenge phases reduced the development of Th2 type delayed allergic eosinophilic rhinitis. The mechanisms of reduced DAR reaction, however, may be different by the timing of LDV infection as follows. The present study suggests that LDV infection at the sensitization phase may reduce not only Th2 (IL-4) but also Th1 (IFN- γ) responses including IgE value systemically. It is possible, as decreased APC activity may contribute to reduction in general immune reactions [29–33], leading to the inhibition of the differentiation from naïve Th0 cells to Th1 and Th2 cells, although productions of IFN- γ and Th1-related IL-12 were temporally increased shortly after LDV infection [34, 35]. On the other hand, LDV infection at the challenge phase increased IFN- γ and IL-12 productions transiently after infection as described above may suppress systemic IL-4 production by mutual inhibitory effects between Th1 and

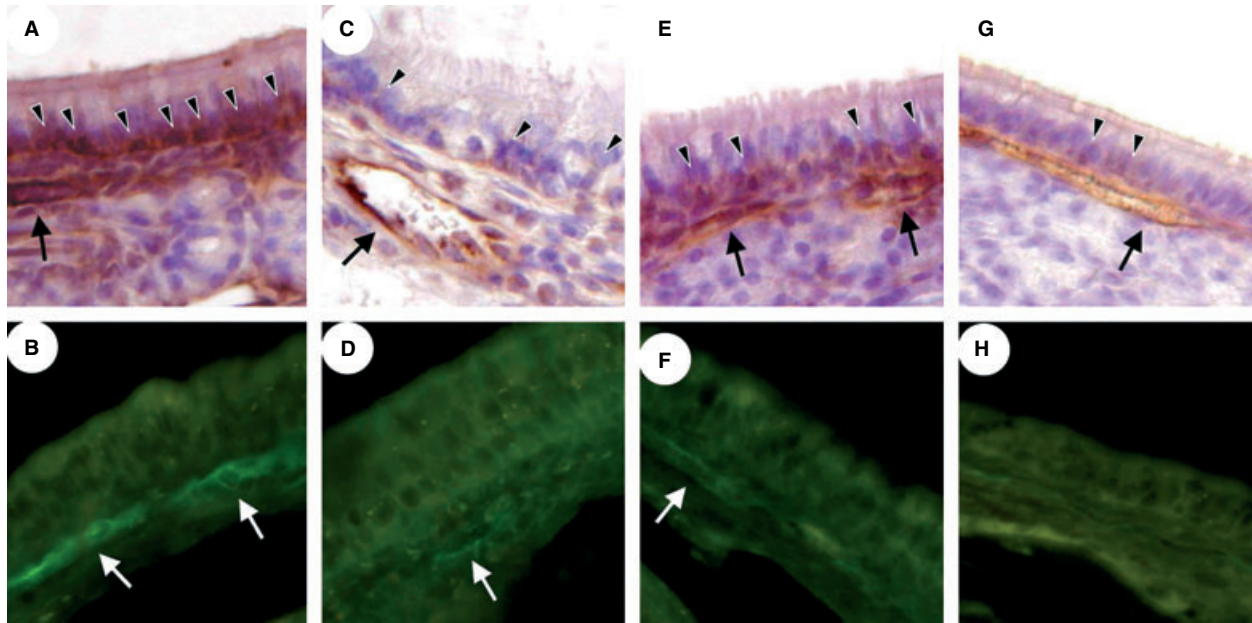


Figure 6 ICAM-1 expression (immunoperoxidase; arrowheads: epithelium; arrows: endothelium) of the DAR (A), DAR/LDV (C), the DAR/LDVc (E) and the control groups (G), and VCAM-1 expression (immunofluorescence; arrows) of the DAR (B), DAR/LDV (D), DAR/LDVc (F) and the control groups (H). Representative photograph was shown (original magnification: $\times 400$).

Th2 cells [40] rather than decreased APC activity [41]. However, increased eosinophils in BM and blood IgE values cannot be inhibited by LDV infection at the immunizing phase, since those may be due to the development of Th2 response against OVA at that time.

The two groups infected with LDV compared to the DAR group reduced production of IL-4 resulting in a weak induction of VCAM-1 expression on endothelium, which is induced by IL-4 [42] in inflamed nasal areas. This suggests a low concentration may not be enough to induce VCAM-1 expression, leading to decreased infiltration of lymphocytes and eosinophils, both of which have the $\beta 1$ -integrin very-late activation antigen (VLA)-4, a ligand for VCAM-1. In addition, an increased ICAM-1 expression on respiratory epithelial cells may, at least in part, be correlated with infiltration of eosinophils in respiratory area of the DAR group as reported previously in a mouse model of rhinitis and human patients with rhinitis [43, 44]. It seems likely, as there was no infiltration of eosinophils and lymphocytes in areas of olfactory epithelium, lacking ICAM-1 expression, and also reduced ICAM-1 expression in the two groups with LDV infection may result in less severe eosinophil infiltration compared to the DAR group. Moreover, production of eotaxin released from epithelial cells by IL-4 stimulation may also play a role in infiltration of eosinophils [45]. It seems likely, as reduced IL-4 production and eosinophil infiltration in the two groups with LDV infection suggests this possibility. Alternatively, destructive changes of respiratory

epithelium may be induced by cytotoxic protein (e.g. major basic protein and eosinophil peroxidase) released from infiltrated eosinophils [14] in DAR reaction. It seems likely, as those were reduced in the two groups infected with LDV. On the other hand, there was no difference in ICAM-1 expression on endothelium among four groups. This may, at least in part, be responsible for no difference in infiltration of neutrophils, which have lymphocyte-function associated antigen-1, the counter receptor for ICAM-1 [46].

There are non-respiratory tropic viruses (e.g. EBV, CMV, hepatitis A and measles, etc) which show persistent infection and modulate immune reaction including allergic respiratory diseases [15, 23–26]. It has been suggested that reduction of allergic airway inflammation in human by those viruses may be induced by decreased Th2 reaction by mutual Th1/Th2 inhibitory mechanism. In addition, it has been suggested that AR might be reduced by disruption of major histocompatibility complex class II in CMV infection [24, 47]. However, the mechanisms including time of exposure during the disease process such as immediate or delayed phase are not well understood. Thus, the present results will give some clues in immune modulation especially in the development of DAR by those viruses described above. Furthermore, Anderson *et al.* suggested that the events (e.g. continuous cycles of viral replication in a renewable population of cells, escape from antiviral humoral and cellular immune responses, accumulation of virus in germinal centres concomitant with a polyclonal

activation of B cells, and a potential sexual transmission of the infection as well as a transplacental infection of the fetus from an infected mother) associated with a persistent LDV infection of mice strongly resemble those which are observed to occur in humans during the asymptomatic persistent phase of infection with human immunodeficiency virus (HIV)[48]. And it has been reported recently that large numbers of patients with HIV who present with allergic respiratory symptoms including rhinitis [49]. Taken together, LDV is unique as a model of some viruses which modulate allergic respiratory disease including delayed eosinophilic rhinitis in human. We are now investigating the effects of chronic LDV infection (more than 3 weeks after infection) on the development of delayed eosinophilic allergic rhinitis..

Finally, although delayed eosinophilic AR in the present mouse model showed many similarities that in human cases, but thickening of basement membrane, hyperplasia of nasal gland in inflamed nasal areas and lesions in the areas of olfactory epithelium in human patients with AR [5, 50] were not found. Although, we have no explanation for the discrepancy of these histological changes between human and animal, it must be a property of the species, including anatomical and physiological differences.

In conclusion, the present study will give a suitable animal model for those viruses which modulate the DAR reaction as discussed. In addition, this is the first report that effects of persistent virus infection without damage of respiratory epithelium on the development of delayed eosinophilic rhinitis in mouse model of rhinitis as far as we know.

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References

- Galli SJ. Complexity and redundancy in the pathogenesis of asthma: reassessing the roles of mast cells and T cells. *J Exp Med* 1997;186:343–7.
- Corrigan CJ, Hamid Q, North J *et al.* Peripheral blood CD4 but not CD8 T-lymphocytes in patients with exacerbation of asthma transcribe and translate messenger RNA encoding cytokines which prolong eosinophil survival in the context of a Th2-type pattern: effect of glucocorticoid therapy. *Am J Respir Cell Mol Biol* 1995;12:567–78.
- Kosgren M, Erjefalt JS, Korsgren O, Sundler F, Persson CG. Allergic eosinophil-rich inflammation develops in lungs and airways of B cell-deficient mice. *J Exp Med* 1997;185:885–93.
- Takeda K, Hamelmann E, Joetham A *et al.* Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell-deficient mice. *J Exp Med* 1997;186:449–54.
- Klimek L, Eggers G. Olfactory dysfunction in allergic rhinitis is related to nasal eosinophilic inflammation. *J Allergy Clin Immunol* 1997;100:158–64.
- Baraniuk JN. Mechanisms of allergic rhinitis. *Curr Allergy Asthma Rep* 2001;1:207–17.
- Varney VA, Jacobson MR, Sudderick RM *et al.* Immunohistology of the nasal mucosa following allergen-induced rhinitis. Identification of activated T lymphocytes, eosinophils, and neutrophils. *Am Rev Respir Dis* 1992;146:170–6.
- Coly AJ, Le Gros G, Bertrand C *et al.* Interleukin-4 is required for the induction of lung Th2 mucosal immunity. *Am J Respir Cell Mol Biol* 1995;13:54–9.
- Kaneko M, Hitoshi Y, Takatsu K, Matsumoto S. Role of interleukin-5 in local accumulation of eosinophils in mouse allergic peritonitis. *Int Arch Allergy Appl Immunol* 1991;96:41–5.
- Zhu Z, Horner RJ, Wang Z *et al.* Pulmonary expression of interleukin-13 cause inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities and eotaxin production. *J Clin Invest* 1999;103:779–88.
- von Mutius E, Martinez FD, Fritzsche C, Nicolai T, Roell G, Thiemann HH. Prevalence of asthma and atopy in two areas of West and East Germany. *Am J Respir Crit Care Med* 1994;149:358–64.
- Huovinen E, Kaprio J, Koskenvuo M. Factors associated to lifestyle and risk of adult onset asthma. *Respir Med* 2003;97:273–80.
- D'Amato G. Urban air pollution and respiratory allergy. *Monaldi Arch Chest Dis* 2002;57:136–40.
- Salib RJ, Drake-Lee A, Howarth PH. Allergic rhinitis: past, present and the future. *Clin Otolaryngol Allied Sci* 2003;28:291–303.
- Matricardi PM, Rosmini F, Panetta V, Ferrigno L, Bonini S. Hay fever and asthma in relation to markers of infection in the United States. *J Allergy Clin Immunol* 2002;110:381–7.
- Uter W, Stock C, Pfahlberg A *et al.* Association between infections and signs and symptoms of 'atopic' hypersensitivity-results of a cross-sectional survey among first-year university students in Germany and Spain. *Allergy* 2003;58:580–4.
- Humlova Z. Bacteria and their role in allergic diseases. *Cas Lek Cesk* 2004;143:21–5.
- Greiff L, Venge P, Andersson M *et al.* Effects of rhinovirus-induced common colds on granulocyte activity in allergic rhinitis. *J Infect* 2002;45:227–32.
- Mejias A, Chavez-Bueno S, Rios AM *et al.* Asthma and respiratory syncytial virus. New opportunities for therapeutic intervention. *An Pediatr (Barc)* 2004;61:252–60.
- Marsland BJ, Scanga CB, Kopf M, Le Gros G. Allergic airway inflammation is exacerbated during acute influenza infection and correlates with increased allergen presentation and recruitment of allergen-specific T-helper type 2 cells. *Clin Exp Allergy* 2004;34:299–306.
- Schwarze J, Makela M, Cieslewicz G *et al.* Transfer of the enhancing effect of respiratory syncytial virus infection on subsequent allergic airway sensitization by T lymphocytes. *J Immunol* 1999;163:5729–34.
- Openshaw PJ, Clarke SL, Record FM. Pulmonary eosinophilic response to respiratory syncytial virus infection in mice sensitized to the major surface glycoprotein G. *Int Immunol* 1992;4:493–500.
- Nilsson C, Linde A, Montgomery SM *et al.* Does early EBV infection protect against IgE sensitization? *J Allergy Clin Immunol* 2005;116:438–44.
- Sindorchuk A, Wickman M, Pershagen G, Lagarde F, Linde A. Cytomegalovirus infection and development of allergic diseases in early childhood: interaction with EBV infection? *J Allergy Clin Immunol* 2004;114:1434–40.
- Jedrychowski W, Maugeri U, Jedrychowska-Bianchi I. Prospective epidemiologic study on respiratory diseases in children and immunization against measles. *Int J Occup Med Environ Health* 2004;17:255–61.

- 26 Wu CA, Puddington L, Whiteley HE *et al.* Murine cytomegalovirus infection alters Th1/Th2 cytokine expression, decreases airway eosinophilia, and enhances mucus production in allergic airway disease. *J Immunol* 2001;167:2798–807.
- 27 Notkins AL. Lactic dehydrogenase virus. *Bacteriol Rev* 1965;29:143–60.
- 28 Rowson KE, Mahy BW. Lactate dehydrogenase-elevating virus. *J Gen Virol* 1985;66:2297–312.
- 29 Isakov N, Feldman M, Seqal S. Lactic dehydrogenase virus (LDV) impairs the antigen-presenting capacity of macrophages yet fails to affect their phagocytic activity. *Immunobiology* 1982;162:15–27.
- 30 Michaelides MC, Simms ES. Immune responses in mice infected with lactic dehydrogenase virus. III. Antibody response to a T-dependent and a T-independent antigen during acute and chronic LDV infection. *Cell Immunol* 1980;50:253–60.
- 31 Inada T, Mims CA. Pattern of infection and selective loss of Ia positive cells in suckling and adult mice inoculated with lactic dehydrogenase virus. *Arch Virol* 1985;86:151–65.
- 32 Hayashi T, Mori I, Yamamoto H. Lactic dehydrogenase virus infection prevents development of anti-nuclear antibody in (NZB x NZW)F₁ mice; role of prostaglandin E₂ and macrophage Ia antigen expression. *Int J Exp Pathol* 1992;72:593–601.
- 33 Inada T, Mims CA. Live lactate dehydrogenase-elevating virus (LDV) induces suppressor T cells that inhibit the development of delayed hypersensitivity to LDV. *J Gen Virol* 1986;67:2103–12.
- 34 Markine-Goriaynoff D, Hulhoven X, Cambiaso CL *et al.* Natural killer cell activation after infection with lactate dehydrogenase-elevating virus. *J Gen Virol* 2002;83:2709–16.
- 35 Coutelier JP, Van Broeck J, Wolf SF. Interleukin-12 gene expression after viral infection in the mouse. *J Virol* 1995;69:1955–8.
- 36 Hayashi T, Adachi Y, Hasegawa K, Morimoto M. Less sensitivity for late airway inflammation in males than females in BALB/c mice. *Scand J Immunol* 2003;57:562–7.
- 37 Hayashi T, Salata K, Kingman A, Notkins AL. Regulation of enzyme levels in the blood: influence of environmental and genetic factors on enzyme clearance. *Am J Pathol* 1988;132:503–10.
- 38 Hayashi T, Mori I, Noguchi Y, Itoh T, Saitoh M. Immunofluorescent antibody response to lactic dehydrogenase virus in different strains of mice. *J Comp Pathol* 1992;107:179–83.
- 39 Hussain I, Randolph D, Brody SL *et al.* Induction, distribution and modulation of upper airway allergic inflammation in mice. *Clin Exp Allergy* 2001;31:1048–59.
- 40 Mosmann TR, Coffman R. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989;7:145–73.
- 41 Romagnani S. Type 1 T helper and type 2 T helper cells: functions, regulation and role in protection and disease. *Int J Clin Lab Res* 1991;21:152–8.
- 42 Jahnsen FL, Brandtzaeg P, Haye R, Haraldsen G. Expression of functional VCAM-1 by cultured nasal polyp-derived microvascular endothelium. *Am J Pathol* 1997;150:2113–23.
- 43 Yamada T, Kataoka S, Ogasawara K *et al.* Mucosal immunity of nasopharynx: an experimental study in TCR-transgenic (OVA23-3) mice. *Rhinology* 2005;43:190–8.
- 44 Demoly P, Sahla M, Campbell AM, Bousquet J, Crampette L. ICAM-1 expression in upper respiratory mucosa is differentially related to eosinophil and neutrophil inflammation according to the allergic status. *Clin Exp Allergy* 1998;28:731–8.
- 45 Fujisawa T, Kato Y, Atsuta Jterada A *et al.* Chemokine production by the BEAS-2B human bronchial epithelial cells: differential regulation of eotaxin, IL-8, and RANTES by TH2- and TH1-derived cytokines. *J Allergy Clin Immunol* 2000;105:126–33.
- 46 Tonnesen MG. Neutrophil-endothelial cell interactions: mechanisms of neutrophil adherence to vascular endothelium. *J Invest Dermatol* 1989;92:53S–8.
- 47 Cebulla CM, Miller DM, Zhang Y *et al.* Human cytomegalovirus disrupts constitutive MHC class II expression. *J Immunol* 2002;169:167–76.
- 48 Anderson GW, Rowland RRR, Palmer GA *et al.* Lactic dehydrogenase-elevating virus replication persists in liver, spleen, lymph node, and testis tissues and results in accumulation of viral RNA in germinal centers, concomitant with polyclonal activation of B cells. *J Virol* 1995;69:5177–85.
- 49 English RG, Fairall LR, Bateman ED. Keeping allergy on the agenda: integrated guidelines for respiratory disease in developing countries. *Allergy* 2007;62:224–301.
- 50 Takamura H. Immunohistochemical study of inferior turbinate of nasal allergy with reference to eosinophil. *J Otolaryngol Jpn* 1994;97:61–6.