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Gene expression of cell surface antigens in the early phase of murine influenza pneumonia determined by a cDNA expression array technique

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***Background*: Influenza virus is a worldwide health problem with significant economic consequences. To study the gene expression pattern induced by influenza virus infection, it is useful to reveal the pathogenesis of influenza virus infection; but this has not been well examined, especially *in vivo* study.**
***Aims*: To assess the influence of influenza virus infection on gene expression in mice, mRNA levels in the lung and tracheal tissue 48 h after infection were investigated by cDNA array analysis.**
***Methods*: Four-week-old outbred, specific pathogen free strain, ICR female mice were infected by intranasal inoculation of a virus solution under ether anesthesia. The mice were sacrificed 48 h after infection and the tracheas and lungs were removed. To determine gene expression, the membrane-based microtechnique with an Atlas cDNA expression array (mouse 1.2 array II) was performed in accordance with the manual provided.**
***Results and conclusions*: We focused on the expression of 46 mRNAs for cell surface antigens. Of these 46 mRNAs that we examined, four (CD1d2 antigen, CD39 antigen-like 1, CD39 antigen-like 3, CD68 antigen) were up-regulated and one (CD36 antigen) was down-regulated. Although further studies are required, these data suggest that these molecules play an important role in influenza virus infection, especially the phase before specific immunity.**

Key words: Influenza, cDNA array, Cell surface antigen, Cytokine, Murine

Introduction

Influenza is a major epidemic viral disease in humans. Its continuing importance lies in its ability to cause pneumonitis, which occurs in previously fit individuals and in the elderly. The infection of mice with mouse-adapted influenza virus strains by aerosol inoculation results in severe disease with alveolar involvement. The pulmonary pathology in mice is similar to that seen in viral influenza pneumonia in humans.

Although 15–20 genes have been characterized as showing increased expression after infection of the respiratory tract with influenza virus, these genes probably represent only a small fraction of all the genes that are induced under these circumstances. Little is known about the genes involved in the inflammatory response to the influenza virus. In particular, little information has been gathered about the early phase of this response, before the appearance of specific cytotoxic T cells. During this phase, the influenza virus interacts with cells on the luminal side of the airways and alveoli to induce the release of immunoactive mediators, thus initiating a cascade of events resulting in the eventual elimination of the virus. However, cell surface antigens are expressed on the cell surface and play important roles in cell-cell interactions in various situations involving the site of influenza virus infection. Examining the expression of cell surface antigens in influenza virus infection contributes important information about the network among various cells.

Recently, techniques allow characterization of the mRNA expression status of many genes.[1,2] In the present study, we examined mRNA expression in murine influenza pneumonia using a cDNA array technique, especially focusing on cell surface antigens. Via this experiment, it may be understood that the molecule that until now has not seemed to take part in influenza virus infection, does actually take part in pathogenesis of the infection.

Materials and methods

Preparation of the virus

The lung-adapted strain of influenza A/PR/8/34 (PR8) virus (H1N1 subtype) was propagated in the chorioallantoic cavity of 10-day-old embryonated hen eggs for 48 h at 35°C. The chorioallantoic fluid was

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Table 1. The cDNAs for cell surface antigens tested in the present study

Bone marrow stromal cell antigen 1	CD8beta opposite strand
CD81 antigen	CD9 antigen
CD151 antigen	Coagulation factor III
CD19 antigen	Complement receptor-related protein
CD1d2 antigen	Duffy blood group
CD24a antigen	Epithelial V-like antigen
CD33 antigen	Tumor necrosis factor receptor superfamily, member 6
CD34 antigen	Integrin-β_2
CD36 antigen	Lymphocyte antigen 64
CD37 antigen	Tumor-associated calcium signal transducer 1
CD38 antigen	Lymphocyte antigen 78
Ectonucleoside triphosphate diphosphohydrolase 1	Interleukin-1 receptor-like 1
Ectonucleoside triphosphate diphosphohydrolase 3	Lymphocyte antigen 86
CD44 antigen precursor	Lymphocyte antigen 9
CD48 antigen	Lymphocyte antigen 94
CD6 antigen	Sialophorin
Cd63 antigen	Tumor necrosis factor (ligand) superfamily, member 4
CD68 antigen	Tumor necrosis factor receptor superfamily, member 5
CD72 antigen	Tumor necrosis factor receptor superfamily, member 8
Immunoglobulin-associated alpha	Tumor rejection antigen P1A
CD8 antigen, alpha chain	CD3 antigen, delta polypeptide
CD8 antigen, beta chain	CD3 antigen, epsilon polypeptide
Kangai 1 (suppression of tumorigenicity 6, prostate)	CD3 antigen, gamma polypeptide
CD83 antigen	CD3 antigen, zeta polypeptide
CD84 antigen	CD3 antigen, zeta polypeptide
CD86 antigen	

collected and stored in small portions at –80°C after centrifugation at 1,000 x *g* for 10 min. The virus titer of the chorioallantoic fluid was 1.9 x 10^8 plaque forming units as determined on Mardin-Darby canine kidney cells, as described previously.[3]

Virus infection of mice and sample preparation

Four-week-old outbred, specific pathogen free strain, ICR female mice (body weight, ~17 g) were obtained from SLC Co. Ltd (Hamamatsu, Japan) and were infected by intranasal inoculation of a virus solution containing 1.0 x 10^4 plaque forming units/25 μl (five 50% lethal doses of virus) under ether anesthesia. The mice were sacrificed 48 h after infection and the tracheas and lungs were removed. The resected trachea and lungs were frozen by liquid nitrogen immediately after resection.

cDNA expression array

To determine gene expression, the membrane-based microtechnique with an Atlas cDNA expression array (mouse 1.2 array II; Clontech Tokyo, Japan) was performed in accordance with the manual provided. The array included 1176 mouse cDNAs and nine housekeeping control cDNAs and negative controls immobilized on a nylon membrane. The cDNAs on a membrane were divided into 23 categories including 46 cDNAs for cell surface antigens (see Table 1).

The purified RNA, which was analyzed for genomic DNA contamination by polymerase chain reaction with primers specific for β-actin, was processed with gene-specific CDS primer mix (Clontech), deoxynucleoside triphosphate, [^{32}P]dATP, and reverse transcriptase for preparation of cDNA. The ^{32}P-labeled cDNA was purified through a Chroma Spin-200 column (Clontech). The labeled cDNA in a solution of ExpressHyb (Clontech) with heat-denatured, sheared-salmon-test DNA was then hybridized overnight to the Atlas array membrane at 68°C. The membrane was washed in 2 x standard saline citrate (SSC) (1 x SSC is 0.15 M NaCl + 0.015 M sodium citrate) with 1% sodium dodecyl sulfate, in 0.1 x SSC with 0.5% sodium dodecyl sulfate, and in 2 x SSC, sequentially, and was then exposed to PhosphorImager (Storm 860; Molecular Dynamics, Sunnyvale, CA, USA). Results of the gene expression were analyzed by computer using Atlas imaging software (Clontech).

Statistic analysis

The comparison of gene expression findings between non-infected control mice and infected mice was analyzed by Student's *t* test.

Results and discussion

Table 1 presents the cDNAs for cell surface antigens that were tested in the present study. Of 46 mRNAs examined, four were up-regulated and one mRNA was down-regulated 48 h post infection (Fig. 1). Gene expression levels (spot intensity) of CD1d2 antigen, CD39 antigen-like 1, CD39 antigen-like 3 and CD68 antigen before infection were 3.7±1.86, 2.0±0, 3.0±0 and 4.0±1.63, respectively. At 48 h after infection, gene

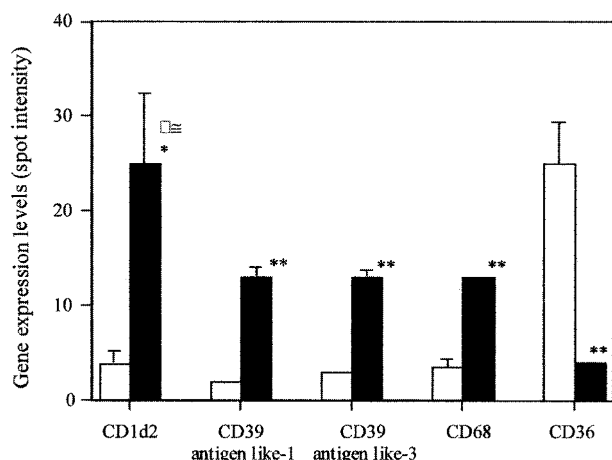

FIG. 1. The mRNA expression levels for cell surface antigen in the lung and trachea tissue of influenza-infected mice. Results are presented as the mean ± SE from three independent experiments. Open column, before infection; filled column, 48-h after infection of influenza virus. * $p < 0.05$ ** $p < 0.01$ compared with non-infected controls, analyzed by Student's *t*-test.

expression levels of CD1d2 antigen, CD39 antigen-like 1, CD39 antigen-like 3 and CD68 antigen were 25.0±7.76, 12.0±1.63, 13.0±1.23 and 13.0±0.41, respectively. Gene expression levels of CD36 antigen before infection and 48 h after infection were 24.03±6.54 and 4.0±0.82, respectively. Results are presented as the mean (SE from three independent experiments (each experiment was carried out once).

In the present study, we examined the expression of 46 cell surface antigen mRNAs. The expression of four mRNAs (CD1d2 antigen, CD39 antigen-like 1, CD39 antigen-like 3, and CD68 antigen) were up-regulated. CD36 antigen was down-regulated (Fig. 1).

CD1d demonstrated an important role in natural killer (NK) T-cell development.[4-7] NK T cells release large amounts of IL-4 within hours of activation, and it has been proposed that this population of T cells may be the earliest source of IL-4 that could shift an immune response toward a T helper cell type 2 phenotype.[5,8] However, the mechanisms of NK T-cell stimulation and the function of CD1d is not fully understood. This finding provides a further clue to understanding these mechanisms.

CD39 regulates vascular inflammation and thrombosis by hydrolyzing adenosine triphosphate and adenosine diphosphate. Recently, CD39 has been identified as responsible for Langerhans cell-associated ecto-NTPDase activities, and contradictory outcomes of CD39 deficiency have been demonstrated in skin inflammation and immune responsiveness.[9]

CD68 is expressed on macrophages, monocytes, neutrophils, basophils and lymphocytes, and is especially useful as a marker for monocytes, but its function is not well understood.[10-12]

CD36 is known as one of a Class B scavenger receptor family, a candidate for the receptor of phosphatidylserine, which plays an important role in the recognition and phagocytosis of senescent and apoptotic cells.[13-15] In the present study, expression of CD36 mRNA was down-regulated.

In the present study, we have demonstrated that the expression of some cell surface antigen mRNAs were up-regulated or down-regulated 48 h after influenza virus infection in the lung and trachea of mice. The relation to the influenza virus infection of these molecules has not been pointed out until now. Why the mRNA expression of these molecules is neither up-regulated nor down-regulated is understood only by this experiment. However, new information to elucidate the pathogenesis of influenza pneumonia might be discovered by researching these molecules and the relations to infection in the future. A cDNA array technique is a useful tool to obtain information such as the biological response to viral infections in a pilot study.

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