Journal of Clinical Virology 61 (2014) 585–589

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

The roles of ADAM33, ADAM28, IL-13 and IL-4 in the development of lung injuries in children with lethal non-pandemic acute infectious pneumonia

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ARTICLE INFO

Article history:
Received 11 August 2014
Accepted 11 October 2014

Keywords:
ADAM33
ADAM28
Virus
Pneumonia
IL-4
IL-13

ABSTRACT

Background: ADAM28, ADAM33, IL-13, IL-4 and other cytokines (IL-6 and IL-10) seem to play important roles in the persistence and maintenance of acute inflammatory processes that ultimately lead to lung remodeling and pulmonary fibrosis, which may be responsible for the high morbidity and mortality rates associated with non-pandemic acute viral pneumonias in childhood.
Objectives: The aim of this study was to evaluate the roles of ADAM33, ADAM28, IL4, IL6, IL10 and IL13 in the development of inflammation and alveolar fibrosis due to lethal acute respiratory infections of the lower airway in a pediatric population, especially in those with viral etiology.
Study design: For this study, 193 cases were selected, and samples from the cases were processed for viral antigen detection by immunohistochemistry and then separated into two groups: virus-positive ($n = 68$) and virus-negative ($n = 125$). Immunohistochemistry was performed to assess the presence of metalloproteinases (ADAM33 and ADAM28) and inflammatory cytokines (IL-4, IL-13, IL-6, IL-10) in the alveolar septa.
Results: The virus-positive group showed stronger immunolabeling for ADAM33, ADAM28, IL-4 and IL-13 ($p < 0.0001$ for all variables). The staining intensities for ADAM33 and ADAM28 were directly proportional to the intensities for IL-4 and IL-13 ($p < 0.0001$).
Conclusions: The results of this study suggest that these proteins play important roles in pulmonary inflammatory reactions elicited against etiological viral agents. In addition, these mediators may affect the process of lung remodeling and the development of pulmonary fibrosis.

1. Background

Pneumonia is a generic term used to described acute inflammation of the lower respiratory tract and is a primary cause of hospitalization and death in children under 5 years of age [1–9] worldwide, and viruses and bacteria are the most common etiological agents [5–7].

The ADAMs (A Disintegrin and Metalloproteinases) appear to play a role in the pathophysiology of respiratory tract infections [10]. These metalloproteinases are expressed in many normal adult tissues, such as alveolar fibroblasts and bronchial smooth muscle cells. In addition, ADAMs are overexpressed in inflamed tissues and during recovery processes, suggesting a relationship with the inflammatory response [10,11]. ADAM28, or MDC-L, is specifically expressed on lymphocytes, suggesting an immunological function [12,13]. This protein also exists in a secreted form (ADAM28s), but some of its functions remain unknown [14].

Interleukin (IL)-4 is a cytokine that regulates the immune system by contributing to the life and growth of lymphocytes [15] and plays a role in cellular regulation and differentiation [16]. IL-13 is known to be a central mediator of the allergic asthma phenotype and has multiple effects on airway epithelial cells. IL-13 also

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http://dx.doi.org/10.1016/j.jcv.2014.10.004
1386-6532/

plays a role in mucous cell hyperplasia, stimulating the activation of matrix metalloproteinase and the expression of growth factors derived from the epithelium [15–17].

Based on the possible involvement of ADAM33, ADAM28, IL-4, IL-13, IL-6 and IL-10 in the development of inflammatory processes and tissue remodeling, we tested the hypothesis that these proteins are involved in the development of lethal non-pandemic acute pneumonias within the pediatric population.

2. Objectives

The aim of this study was to correlate expression of these proteins in lung samples obtained from children with lethal non-pandemic acute pneumonia with etiological (presence or absence of virus), demographic (sex, age, seasonality), anatomopathological (morphological features of the injury) and survival factors within the population studied [18,19].

3. Study design

From 794 pediatric cases involving necropsy (occurring between 1960 and 2004) and whose cause of death was lethal non-pandemic acute pneumonia, 193 formalin-fixed paraffin-embedded samples of lung tissue were selected (1994–2004). Samples were categorized according to age: those from children younger than 1 year and those from children over 1 year old. To account for seasonality, we categorized the samples according to when the children's death occurred: during cold or warm months. The anatomopathological patterns found were categorized as either bronchopneumonia or interstitial pneumonitis. Survival was defined as the period of time between the date of hospitalization and death. All cases had already been tested for the presence of respiratory syncytial virus (RSV), anti-adenovirus (AdV), anti-parainfluenza 1, 2 and 3 (PIV1, PIV1 and PIV1) and anti-influenza A and B (A FLU and B FLU) using immunohistochemistry, and they were divided into two groups: virus-positive and virus-negative [18,19].

The tissue microarray (TMA) paraffin blocks used to perform the immunohistochemical reactions, contained two lung samples per case.

To observe the expression of ADAM33 and ADAM28 metalloproteinases and interleukin-13, -4, -6 and -10 in the alveolar septa using immunohistochemistry, the following primary antibodies were used: Anti-ADAM33 (mouse polyclonal; dilution 1:200; produced by UFPR, Curitiba, Brazil) [20]; anti-ADAM28 (rabbit polyclonal; 1:800; from Biorbyt™); anti-IL-4 and anti-IL13 (rabbit polyclonal; both 1:600; both from Bioss™); anti-IL-6 (rabbit polyclonal; 1:200; from Imuny Biotechnology™); and anti-IL-10 (rabbit polyclonal; 1:200; from Affinity™). All TMA staining procedures included both a negative control (which was missing primary antibody) and a positive control (normal lung).

An immunoperoxidase assay with modifications was part of the immunohistochemistry, as reported by Chong and colleagues [18]. Antigen retrieval was performed using a BioSB™ ImmunoRetriever. Tissue samples were incubated with the primary antibodies (anti-ADAM33, ADAM28, IL-13, IL-4, IL-6 and IL-10) in a moist chamber at room temperature for one hour. Incubations with the secondary antibody (Dako Advance™ HRP System, DakoCytomation, Inc., CA, USA) were carried out for 30 min. Incubations with 3,3′-diaminobenzidine and hydrogen peroxide substrate (DakoCytomation, Inc., CA, USA) were performed for 3 min to visualize positive staining.

TMA slides were examined by an investigator who did not have previous knowledge of the viral testing results using a BX50 Olympus (Japan) optical microscope at a 40-fold magnification. For each case, eight high power fields (HPF) were randomly selected, and the area of immunolabeling in the alveolar septum was quantified using the image analysis program Image-Pro Plus™. The mean tissue immunoexpression of the interleukins and metalloproteinases per square micrometer (μm^2) was determined in each HPF.

The results of the study are expressed as the means, median and standard deviations, frequencies or percentages. To compare the quantitative variables between groups, the Kruskal–Wallis non-parametric test was performed. To compare the categorical variables between groups, either the Fisher exact test or the chi-squared test was performed. p values < 0.05 are considered statistically significant.

4. Results

When the mean tissue expression of ADAM28 was assessed, virus-positive cases showed higher levels of ADAM28 immunoexpression compared to virus-negative cases ($p < 0.0001$). Similar results were observed for the tissue immunoexpression of ADAM33, IL-4 and IL-13 ($p < 0.0001$) (Table 1). When the virus-positive group was divided into viral subtypes (RSV, AdV, PIV1, PIV1, PIV1, FLU A and FLU B) [21,22], we observed that virtually all viral types exhibited higher immunoexpression levels of these proteins compared to the virus-negative cases (Table 2).

There were no significant correlations between IL-4, IL-13, ADAM33 and ADAM28 expression and the demographic and anatomopathological data of the samples (sex, age, seasonality and injury pattern) (Table 3).

Likewise, the tissue immunoexpression levels of IL-6 and IL-10 were not significantly correlated with the viral positivity, viral subtype, and demographic or anatomopathological profiles of the samples (Tables 1–3).

The mean survival of the present sample was 13.3 days, and the median was 11 days (minimum survival: 2 days and maximum: 35 days); differences between the virus-positive and virus-negative groups, as well as the other demographic and anatomopathological data collected (age, seasonality and injury pattern), were not statistically significant. The tissue immunoexpression levels of the studied proteins were not correlated ($p > 0.05$) with the survival of the children.

However, the tissue immunoexpression of IL-4 and IL-13 was significantly and positively correlated with ADAM33 and ADAM28 immunoexpression ($p < 0.0001$ for IL-4 versus ADAM28, $p = 0.007$

Table 1
Tissue expression of ADAM33, ADAM28, IL-4, IL-6, IL-10 and IL-13 according to the mean area (square micrometers) per high power field, and correlations with the presence or absence of virus in the sample.

	Overall mean of sample	Overall median of sample	Virus-negative group median	Virus-positive group median	p Value
ADAM28	2547.5938	1680	965.5691	4671.459	≤0.0001
ADAM33	1296.08	224	118.486	1049.76	≤0.0001
IL-4	20,881.876	16,760	10819.14	25,807.46	≤0.0001
IL-13	1732.6817	877	607.6203	1725.213	≤0.0001
IL-6	1554.3864	424	353.0939	498.9561	0.5389
IL-10	31.245,977	31.542	31.73,796	31.32,309	0.9954

$p < 0.05$ values are underlined.