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Research article

Immune cell profile in infants' lung tissue

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SUMMARY

Little is known about the normal immune cell profile in the lungs of infants without pulmonary disease.

Normal lung samples obtained at autopsy of 10 infants that died either due to incidental or inflicted causes or non-pulmonary diseases were stained for antibodies against B and T lymphocytes, macrophages, NK cells, cytotoxic cells, dendritic cells and mast cells. Cells were quantified in the airway epithelial layer, inner layer (between the epithelium and the outer smooth muscle border), outer layer (between the outer smooth muscle border and the external limits of the airway) and alveolar septa. Basement membrane or alveolar septa lengths were assessed by image analysis. Results were expressed as cells/mm.

The median age of patients was 6.8 months, ranging from 11 to 840 days. The inner layer of the airways was the region with the smallest density of cells. There was a predominance of cells related to the innate immunity such as CD56+, Granzyme B+ and CD68+ cells in the epithelial layer and alveolar parenchyma. The outer layer and the lung parenchyma presented the highest cellular density. There were very few CD4+ T cells or dendritic cells in most of the lung compartments. The numbers of CD3+ T and granzyme B+ cells correlated positively with age.

There was a compartmentalization of immune cells along airways and parenchyma, which may be related to the development of innate and acquired lung defense mechanisms.

1. Introduction

The concept that lung events occurring early in life may have a permanent impact on adult health has drawn many efforts to better understand the mechanisms regulating early life responses to pulmonary insults. In particular, there is great interest in the interactions among genetic susceptibility, environmental exposures and infections that occur in early infancy and how these can affect adult health (Henderson and Warner, 2012; Sly, 2011; Svanes et al., 2010).

Viral infections in genetically susceptible children significantly increase the risk of developing asthma (Regamey and Kaiser, 2008) and the age at first viral infection seems to determine the pattern of T cell-mediated disease during re-infection in adulthood (Culley et al., 2002). Other lung diseases may have their origins very early in life: the damage of the lung in cystic fibrosis seems to begin very early in childhood, even before lung symptoms appear (Khan et al., 1995).

At birth, the mucosal immune system is rapidly stimulated by bacterial colonization of the mucosal and external body surfaces, and the airways are seeded with inflammatory cells (Gleeson and Cripps, 2004). During the first 12 months of life, the maturation of the mucosal immune system is dependent upon the timing and type of antigenic exposure. The induction and expression of immunity at mucosal surfaces is to a large extent under regional control. Developmentally related deficiencies, especially in T cell maturation and the capacity to release some cytokines, are related to increased susceptibility of infants to infections (Holt, 1995).

What do we know about the immune cellular profile in the lungs of infants? Available data come from bronchoalveolar lavage (BAL)

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studies that have been performed in children of different life-spans (Midulla et al., 1995; Ratjen et al., 1995). Previous studies on infants that died of accidental or inflicted causes (median age 7 months) demonstrated that histologically normal lungs exhibit a range of leukocytes in the whole respiratory tree (Krous et al., 2003). However, very little is known about the immunophenotype of these cells.

Better knowledge of the cellular composition of the lung in infants would be valuable in various settings: (1) it could contribute to our understanding of cellular immune development in the lung and the responses to infectious or environmental agents; (2) it could facilitate the interpretation of BAL analysis in small children; and (3) as tissue studies are being performed more frequently in young children, especially in children with asthma (Payne et al., 2001), comparison with normal data is of clear importance.

The lack of knowledge in this field is largely related to the impossibility of studying lung tissue in healthy children. Despite all known several limitations in the use of autopsy for such studies, this is one of the only possible means to examine different lung compartments in children. Therefore, in this study, we have quantified the density of B and T lymphocytes, macrophages, NK cells, dendritic cells and mast cells in airways and parenchymal lung tissues of 10 infants that died due to non-pulmonary diseases or of accidental causes with normal lungs at autopsy.

2. Methods

This study was approved by the institutional review boards of the Sao Paulo University Medical School (Sao Paulo, Brazil) and of the Medical School of the Hannover University (Hannover, Germany).

2.1. Study population

We retrospectively analyzed lung tissue from 10 infants that were autopsied at the Department of Pathology of São Paulo University Medical School and from the Institute of Legal Medicine of the Medical School of the Hannover University between 1995 and 2004. Infants died of natural causes and had normal lungs upon macroscopic and histological analysis. Two of the infants autopsied in Hannover died of accidental or inflicted causes, and had also normal lungs.

2.2. Immunohistochemistry

Formalin-fixed, paraffin embedded lung tissue from the right and left lungs was retrieved from archive files. Lungs had not been submitted to inflation. Five-micron tissue sections were stained with H&E and prepared for immunohistochemistry.

Sections were stained for antibodies against B and T lymphocytes (CD3+, CD4+, CD8+ and CD20+), macrophages (CD68+), NK cells (CD56+), cytotoxic cells (granzyme B+), dendritic cells (DC-SIGN and CD1a+) and mast cells (tryptase). For details on antibodies sources, see Table 1S in the online supplement.

Sections were deparaffinized, and a 0.3% hydrogen peroxide solution was applied for 35 min to inhibit endogenous peroxidase activity. Antigen retrieval was performed using a citrate solution for 45 min. Sections were incubated with the primary antibody overnight at 4 °C. A LSAB® Plus-HRP kit (DAKO Corporation, Carpinteria, USA) was used for the secondary antibodies, and 3,3 Diaminobenzidine (DAB) (Sigma Chemical Co., St Louis, MO) was used as a chromogen. For Granzyme B and DC-SIGN, Envision-HRP (DAKO Corporation, Carpinteria, CA) was used as secondary antibody and Vector Nova Red (Vector Laboratories, Burlingame, CA) as chromogen. The sections were counterstained with Harris hematoxylin. For negative controls, the primary antibody was replaced with PBS.

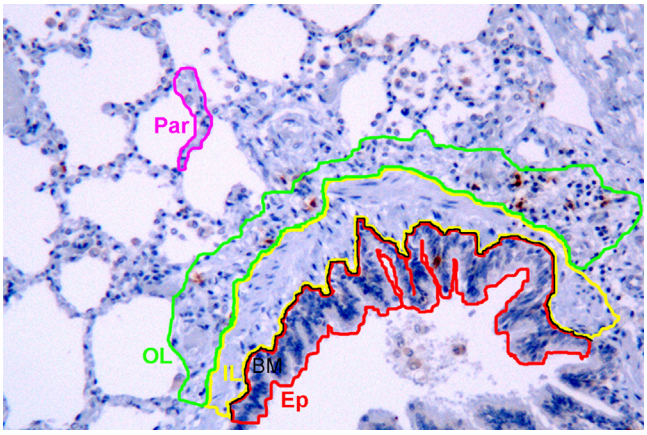

Fig. 1. The microphotograph shows how the cells were quantified in the different lung compartments. After delineating the areas of interest with the aid of the image analysis system, the cells were interactively counted. Results were expressed as cells normalized by basement membrane/alveolar septum length. Ep = Epithelium layer, BM = basement membrane, IL = inner layer, OL = outer layer, and Par = alveolar parenchyma.

2.3. Quantification

Data were obtained by image analysis using the Image-Pro® Plus 4.1 for Windows® (Media Cybernetics–Silver Spring, MD, USA) on a compatible microcomputer connected to a digital camera and coupled to a light microscope (Leica DMR, Leica Microsystems Wetzlar GmbH, Germany).

We quantified the cell densities in the epithelial layer (EL), the inner layer (IL) and outer layer (OL) of airways and the alveolar septa. For the EL, cells were quantified in all intact epithelium present in each analyzed airway. The IL was located between the epithelium and the outer smooth muscle (SM) border, including the BM and lamina propria. The OL was located between the outer SM border and the external limits of the airway (alveolar parenchyma).

We analyzed the whole circumference of all transversally cut airways present in the samples (Mauad et al., 2004a) and 20 fields containing alveolar septa per case. Basement membrane (BM) and alveolar lengths were assessed by image analysis. All fragments available in each case were analyzed. Results are expressed as cells/mm of BM or alveolar septum (Fig. 1).

2.4. Statistical analysis

Data are presented as median/IQR or mean/ranges. Comparison among cell medians was performed using the Kruskall–Wallis test followed by the Dunn post-hoc test. Correlations were performed using Spearman test. Differences at p values smaller than 5% were considered significant.

3. Results

3.1. Study population

Infants' median age was 6.8 months, ranging from 11 to 840 days. Demographic data, causes of death and available clinical data are shown in Table 1.