Human Pathology (2007) **38**, 1239–1247

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Human PATHOLOGY

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Original contribution

Lung involvement in childhood measles: severe immune dysfunction revealed by quantitative immunohistochemistry☆

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Received 14 July 2006; revised 8 January 2007; accepted 8 January 2007

Keywords:
Measles;
BALT;
Cytokines;
Lymphocytes;
Immune system;
Pneumonia

Summary Measles, accounting for nearly 1 million deaths each year, presents intense involvement of lymphoid organs and the lungs. The immune response in situ in the lungs was determined in blocks recovered from 42 necropsies of children who died from measles determined by immune cell phenotype (CD4, CD8, CD20, CD45RO, CD68, natural killer [NK], and antigen S-100 B [S100]) and cytokine production (interferon, tumor necrosis factor, interleukin [IL]-1, IL-2, IL-4, IL-10, and IL-12). Compared with the lungs of age-paired controls, patients with measles presented severe depletion of CD4+, CD20+, CD68+, NK+, and S100+ cells in alveolus- and bronchus-associated lymphoid tissue without depletion of CD8+ cells. Most of these features were similar in both forms of measles lung involvement, Hecht giant cell, or interstitial pneumonia, but S100+ cells were depleted in bronchus-associated lymphoid tissue from patients with Hecht pneumonia, which also occurs more frequently in malnourished children. IL-10– and IL-12–producing cells were depleted in patients with measles, whereas IL-1–, interferon-, and IL-4–producing cells were more frequently seen in the alveolus of patients with measles compared with controls. Quantitative in situ immune cell phenotype and function in the lung in measles demonstrated severe immune dysfunction, with loss of key cells, such as dendritic, CD4+, and NK+ cells, and deficient cytokine production, which allows for a better comprehension of local reactions in this process.

☆ This work was supported by Conselho Nacional de Pesquisas and Instituto dos Laboratórios de Investigação Médica do Hospital das Clínicas da Faculdade de Medicina da USP.
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doi:10.1016/j.humpath.2007.01.015

1. Introduction

Measles, or rubeola, is the oldest virus-induced disease known to cause serious damage to immune function [1]. Despite the efficacy of its vaccine, measles continues to cause approximately 770,000 deaths around the world each year, especially in third world countries and with isolated outbreaks in developed nations [2]. Measles is accompanied by high morbidity and mortality, mainly in malnourished children [1] because of lethal complications such as secondary infections, especially pneumonia and upper respiratory tract involvement, due to either direct measles virus (MV) effect or its indirect disabling effects on the immune system [3].

The immune system is affected by MV in both innate and adaptive immune responses. MV attacks natural killer (NK) cells leading to the down-regulation of their lytic activity [4]. Heat shock proteins are also subverted by MV to induce viral product transcription, which, in turn, induces more heat shock proteins to be synthesized, causing a positive feedback loop where the virus multiplies and raises its numbers inside the cell [5].

Infection of dendritic cells leads to fusion of these cells with macrophages and with each other, forming giant cells that are unable to exert antigen-presenting function and constitute an ideal place for virus multiplication [6]. Infection of dendritic cells also causes apoptosis, which diminishes their numbers in the lung tissue, affecting the transition from the innate phase to the adaptive phase of the immune response [7].

MV damages the adaptive immune responses, but interestingly, the virus infection induces a life-long protective immunity, although the acute disease causes severe impairment of immune responses to other microorganisms [4]. During this acute phase, Th1 type of immune response occurs with a drastic antiviral effect, clearing the virus, as seen in the skin by cytotoxic CD8+ cell infiltration [8]. Interferon γ (IFNγ) is present in the acute phase of measles in the prerash period, followed by interleukin (IL)-2, characteristic Th1 cytokines. During and after the exanthema, the immune system turns its response to the Th2 profile. IL-4 begins to predominate and acts as a Th2 immunity trigger [7]. Specific IgG subtype production changes from IgG1 to IgG4, as well as high IgE plasma levels [9].

Measles, or rubeola, presents 2 main lung involvements. The classic giant cell pneumonia, or Hecht pneumonia, is more severe and frequently associated with respiratory failure and death [1]. Unfortunately, patients often die with histological lung involvement that is indistinct from interstitial pneumonia, except for detection by additional diagnostic immunohistochemistry or electron microscopy for viral detection. The pathogenic mechanisms of lung involvement are not well understood, but they are attributed to both direct MV action and indirect effect causing generalized immune disruption [4]. The lungs are directly exposed to the external environment, being protected by cilia, mucus, alveolar macrophages, and a powerful bronchus-associated lymphoid tissue (BALT) [10]. This lymphoid lung tissue has been reported to mature and change with age, but few, if any, studies exist on pulmonary infections [10,11].

In the present study, we investigate the in situ immune response caused by MV in the lungs from children who died due to measles or rubeola, grouped according to main histological lung involvement. We quantify the numbers of immune cells and their activation evidenced by cytokine production, by quantitative immunohistochemistry in areas of lung structures such as the alveolus, septum, and BALT, and by the response to viral infection.

2. Methods

2.1. Cases and controls

The necropsies from patients diagnosed as having measles or rubeola were recovered from the files of Instituto Emilio Ribas in São Paulo (Brazil) and Hospital Infantil Nossa Senhora da Glória in Vitória (Brazil). We excluded cases with concomitant diagnosis of bacterial pneumonia and patients more than 20 years of age. We recovered the paraffin blocks and rechecked lung histology, excluding cases that had evidence of tissue autolysis, inappropriate fixation with artifacts, or typical bacterial infection in lung histology. This selection resulted in adequate lung tissue from 42 patients diagnosed as having isolated measles, with well-defined clinical and histological lung involvement. Their mean age was 29 months, ranging from 4 to 228 months. There was no bias caused by sex (23/42 females) or ethnicity. For noninfected controls, we recovered paraffin blocks from the lungs of age-paired children with congenital heart disease without significant lung involvement who died during surgery from the files of the Heart Institute (InCor), University of São Paulo Medical School, Brazil, and whose data were also described elsewhere [11]. All controls had no lung infection at necropsy and Heath-Edwards' first degree or no pulmonary artery hypertrophy, with the least intense pulmonary involvement as possible. A total of 18 controls were selected, 11 females (61%) and 7 males (39%), with a mean age of 12 months (range, 5-31 months). This careful approach for selecting blocks with adequate controls was performed to minimize variation to block conservation, storage, fixation, and autolysis, but one can only hope that these precautions would suffice for subsequent analysis, which is always presented as individual data.

2.2. Immunohistochemistry and electron microscopy

The paraffin-embedded lungs were resectioned, and 5-μm sections adhered to sylane-treated slides. Histology was analyzed in hematoxylin-eosin slides by 2 independent observers for diagnostic histopathology. Sections were also

Table 1 Demographic and histological findings in patients with measles and controls

Event	Noninfected controls (n = 18)	Measles (n = 40)			Statistical significance
		Total	Interstitial pneumonitis (n = 27)	Hecht pneumonia (n = 13)	
Age, mean ± SD, mo	12.7 ± 8.04	31.5 ± 40.5	33.5 ± 48.7	27.5 ± 14.7	NS
Sex (% males)	38.9	42.5	35.7	50	NS
Alveoli septum enlargement and edema (%)	0	80	70	93	PI and H > C
Presence of pneumocyte I necrosis (%)	0	NA	18	100	H > PI and C
Giant cell presence at each ×20 field (%)	NA	NA	0	100	H > PI
Presence of bronchial squamous metaplasia (%)	0	50	43	78.5	H > PI > C

Comparison between groups was tested using the Kruskal-Wallis test with Dunn post test for quantitative data or χ^2 test for frequencies.
Abbreviations: C, controls; H, Hecht pneumonia; PI, interstitial pneumonia; NA, not applicable.

submitted to immunohistochemistry, with monoclonal antibodies (mabs) revealed via the streptavidin-biotin peroxidase method using an endogenous biotin blocking system (DAKO, Carpinteria, CA, USA) [12] with modifications described elsewhere [11]. The following mabs for cellular phenotypes and cytokines were used in this process: CD4 (M834/DAKO), CD8 (M7103/DAKO), CD20 (M755/DAKO), CD68 (M786/DAKO), CD45RO (M742/DAKO), NK (M1014/DAKO), S100 (Z311/DAKO), IL-1β (LP-712/Genzyme; Cambridge, MA, USA), IL-2r (M731/DAKO), IL-4 (AB204/R&D Systems; Minneapolis, MN, USA), IL-10 (MAB 217/ R&D Systems), IL-12 (MAB 219/R&D Systems), tumor necrosis factor (TNF; IP300/Genzyme), and IFNγ (IP 500/Genzyme). Viral antigens were detected using a direct monoclonal antibody against N protein, a cytoplasmic antigen from MV (MAB8906/Chemicon), in the same protocol of streptavidin-biotin peroxidase revealing system. Ultrastructural analysis was performed in araldite-embedded glutaraldehyde fixed lungs recovered from paraffin blocks counterstained with osmium tetroxide as described elsewhere [13]. Ultrathin sections were analyzed in ZEISS electronic microscopy, targeting intranuclear nucleocapsid viral inclusions.

2.3. Lung histology and immunohistochemistry

The hematoxylin & eosin-stained sections were analyzed by 2 independent observers in blind sections using semiquantitative scores: 0—absence, 1—mild, 2—moderate, and 3—intense. We scored bronchial epithelial hyperplasia, squamous metaplasia, necrosis, erosion, ulcer, and infiltrate. In the alveoli wall and lumen, we scored necrosis, regeneration of pneumocytes, giant cells, edema, hemorrhage, and hyaline membrane. We also scored the septum thickness, edema, capillary congestion, and fibrosis. We defined the MV-associated pneumonias as Hecht pneumonia, when alveoli damage with intense presence of giant cells and type I pneumocyte necrosis predominated despite the concomitant interstitial involvement; and as interstitial pneumonia, when these giant cells and pneumocyte necrosis were scarce with main septal involvement and little disturbances in alveoli epithelium.

2.4. Quantitative in situ immune response analysis

The quantitative analysis of immunohistochemistry stained cells was performed using an ocular square grid, which marked an area of 0.0625 mm^2 by field, under ×40 objective. We analyzed at least 10 areas for alveoli and 5 fields for BALT [13], all were chosen randomly after morphological verification of the presence of available structure.

2.5. Statistical analysis

Analysis of differences in frequencies was performed using the χ^2 test. Comparison among means was tested by the Kruskal-Wallis test and Dunn post test. We compared all areas in the noninfected control and patients with measles, with subsequent subgrouping according to lung involvement [14]. In all tests, power of 0.90 (type II error) was used. We considered every difference in which the probability of similarity less than .05 ($P < .05$) was significant.

3. Results

Demographic and histological parameters of our sample are described in Table 1, with comparison to an age-matched control group from children with left ventricle congenital disease without significant lung disease. Clear histological parameters of measles with lung involvement allow classification of Hecht pneumonia (14/42) or interstitial pneumonia (28/42) based on giant cell presence and pneumocyte I necrosis. Most patients classified with Hecht pneumonia were considered malnourished at necropsy, which differs from those who died with interstitial pneumonia.

In histopathology, as shown in Fig. 1, both types of measles lung involvement had an important interstitial presence, but Hecht pneumonia was characterized by more aggressive alveoli damage and giant cells than interstitial

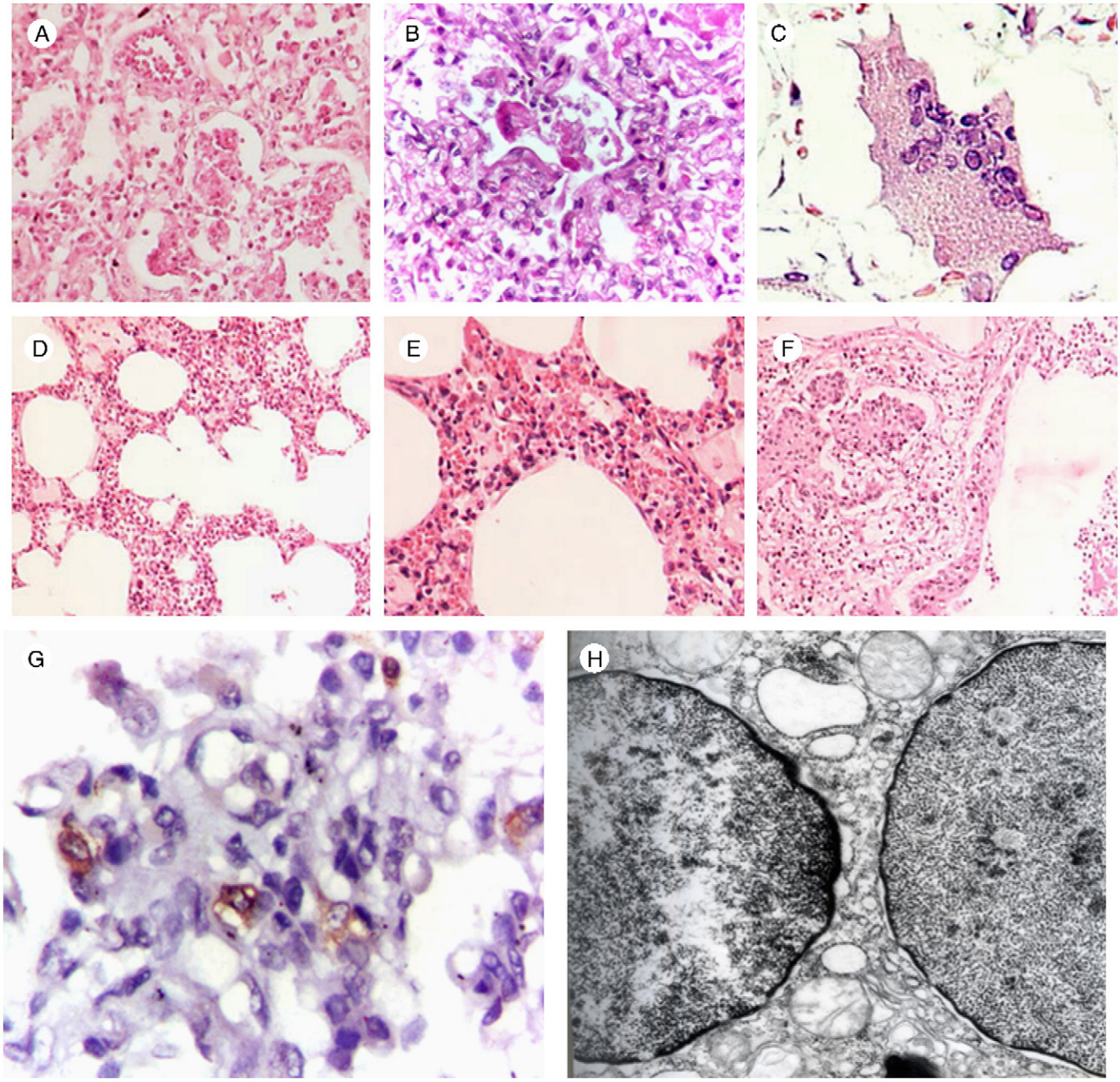

Fig. 1 Histological and diagnostic features of measles lung involvement. A-C, Hecht pneumonia with intense alveolar epithelial desquamation and septal thickening with giant cells (C) with viral inclusions in alveolar lumen (A, ×100; B, ×200; and C, ×400). D and E, Typical interstitial pneumonia with septa with inflammatory infiltrate and congestion, with minor alterations in alveolar epithelium (D, ×100; E, ×200). F, Typical squamous metaplasia in measles (×200). G, Immunohistochemistry for viral antigen showing positive staining of cytoplasm in infected cells. H, Electron microscopy showing typical intranuclear RNP inclusions (×27,000).

pneumonia (Fig. 1A-C). Quantitative analysis of Hecht pneumonia showed greater aggressiveness ($P < .001$, χ^2 test) when compared with interstitial pneumonia with intra-alveolar multinucleate giant cells with intranuclear viral inclusions and increased frequency of pneumocyte I necrosis and bronchial squamous metaplasia (Fig. 1F), as shown in Table 1. Interstitial pneumonia is shown in Fig. 1D and E, with less prominent direct viral effects despite large septum infiltrate. In all cases, we observed viral antigens by immunohistochemistry, mainly in the cytoplasm of infected cells, mostly mononuclear (Fig. 1G). Via electron microscopy, most cases showed paramixovirus ribonucleoprotein structures occupying most of the nucleus of the pneumocyte, an aspect of viral inclusion at electron microscopy (Fig. 1H).

The quantitative distribution of immune cells in the main lung areas as compared with the controls can be seen in

Table 2 Measles: quantitative analysis of lung cell surface phenotype in selected areas of the lung involved in measles and controls

Surface marker	Lung area	Noninfected controls	Measles			Significance ($P < .05$)
			Total	Hecht pneumonia	Interstitial pneumonitis	
CD4	Alveoli	2.50 ± 1.14 (18)	0.18 ± 0.30 (42)	0.22 ± 0.39 (14)	0.15 ± 0.25 (28)	C > M (PI = H)
	BALT	24.5 ± 25.1 (18)	2.73 ± 7.5 (39)	6.10 ± 12.3 (13)	1.03 ± 1.80 (26)	C > M (PI = H)
CD8	Alveoli	3.10 ± 2.76 (18)	2.57 ± 3.7 (42)	3.74 ± 5.63 (14)	1.98 ± 2.05 (28)	NS
	BALT	6.43 ± 4.07 (18)	6.27 ± 8.2 (38)	8.34 ± 10.8 (13)	5.19 ± 6.38 (25)	NS
CD20	Alveoli	0.65 ± 0.56 (17)	0.07 ± 0.21 (42)	0.07 ± 0.18 (14)	0.07 ± 0.23 (28)	C > M (PI = H)
	BALT	17.1 ± 28.3 (18)	0.74 ± 3.7 (38)	2.2 ± 6.43 (13)	0.01 ± 0.05 (26)	C > M (PI = H)
CD68	Alveoli	2.33 ± 1.72 (18)	1.6 ± 2.9 (41)	1.31 ± 1.76 (13)	1.76 ± 3.30 (28)	NS
	BALT	3.24 ± 2.54 (18)	0.8 ± 2.4 (38)	1.33 ± 4.12 (12)	0.53 ± 0.87 (26)	C > M (PI = H)
NK	Alveoli	0.60 ± 1.02 (18)	0.5 ± 0.9 (42)	0.42 ± 0.46 (14)	0.54 ± 1.02 (28)	NS
	BALT	1.96 ± 2.09 (18)	0.8 ± 1.3 (38)	0.71 ± 0.56 (12)	0.83 ± 1.54 (26)	C > M (PI = H)
S100	Alveoli	0.77 ± 1.26 (18)	0.08 ± 0.2 (42)	0.05 ± 0.13 (14)	0.08 ± 0.23 (28)	C > M (PI = H)
	BALT	4.52 ± 3.45 (18)	0.92 ± 0.91 (39)	0.56 ± 0.95 (13)	1.09 ± 0.85 (26)	C > M (PI > H)
CD45RO	Alveoli	1.61 ± 1.32 (18)	1.15 ± 2.1 (42)	1.67 ± 2.31 (14)	0.88 ± 1.95 (28)	NS
	BALT	14.3 ± 13.1 (18)	4.17 ± 5.9 (39)	6.30 ± 8.97 (13)	3.10 ± 3.16 (26)	C > M (PI = H)

Data are expressed as mean of number of positive cell ± SEM (analyzed sample size) by area. Comparison between groups is tested using Kruskal-Wallis test, with Dunn post test.
Abbreviation: M, measles.

Table 2, whereas representative fields of main aspects are shown in Fig. 2. The distribution of values of main immune cells from individual patients is shown in Fig. 3.

BALT showed the greatest involvement of the 3 compartments analyzed in lungs involved in measles demonstrating significant depletions of CD4+ (Fig. 2A), as well as CD20+ (Fig. 2I), CD68+, NK (Fig. 2G), S100+, TCD45RO+ cells in both types of pneumonias as compared with controls. The alveoli of the lungs from patients with measles had severe depletion of TCD4+ (Fig. 2B), S100+, and CD20+ as compared with controls, but with similar numbers of CD8+ cells (Fig. 2E) and other immune cells tested. Similar findings of depletion of immune cells were also observed in the epithelium, which was also associated with depletion of CD68+ and NK+ cells.

Quantitative data on cytokine-producing cells can be seen in Table 3, with representative fields of immunohistochemistry peroxidase reaction (IH) stains presented in Fig. 2K to N. Lungs from patients with measles showed low numbers of IL-12+ cells, unrelated to histological type, as compared with controls. The distribution of individual values for IL-4– and IL-10–labeled cells can be seen in Fig. 4. In the alveoli, IL-1β+, IFN+, and IL4+ cells were found more frequently in patients with measles. In BALT, there were low numbers of IL-10 cells in patients with measles compared with controls. No difference was found in any level between controls and measles in the numbers of IL-2– and TNF-producing cells.

When the 2 types of measles pneumonias, Hecht pneumonia and interstitial pneumonia, were compared, more intense depletion of CD4+ (Fig. 3B) and CD68+ was observed in interstitial pneumonia as revealed by low numbers of these cells in the BALT of these lungs, but a relative depletion of dendritic S100+ cells was found in Hecht pneumonia. No differences in cytokine-produced cells were observed in these subgroups of measles lung involvement.

4. Discussion

We observed that the main subtypes of cells detected in situ and involved in measles lung immune dysfunction are T helper lymphocytes (CD4+), B lymphocytes (CD20+), and pulmonary dendritic cells (S100+), which were depleted in all pulmonary tissues studied such as in alveoli and BALT. Both measles forms of pneumonia, Hecht giant cell and interstitial pneumonia, showed this pattern of involvement of immune cells in the lung, confirmed by viral etiology performed by immunohistochemistry and electron microscopy. The severe clinical signs and symptoms of these patients reflect the fact that these cells are cornerstones of the immune system, which are playing important roles in antigen presentation and cellular and humoral immunity [7]. CD8+ cells are less affected, probably because they are preformed and not related to the acute immune response. The effect on dendritic cells in the lungs probably disables the phase of transition from the innate to an adaptive immune response, especially harming the antigen-presenting function. We could observe, in situ, the decrease of dendritic cell amount in the lungs induced by this infection, probably also affecting their function and quality. The reported inhibitory effect of MV upon IL-12 production [8], observed in peripheral blood, correlates with the findings observed in situ in measles-affected lungs, where depletion of IL-12+–labeled cells could be demonstrated in BALT and alveoli, probably affecting the cellular immune response, due to the crucial role of this cytokine in this response [15].

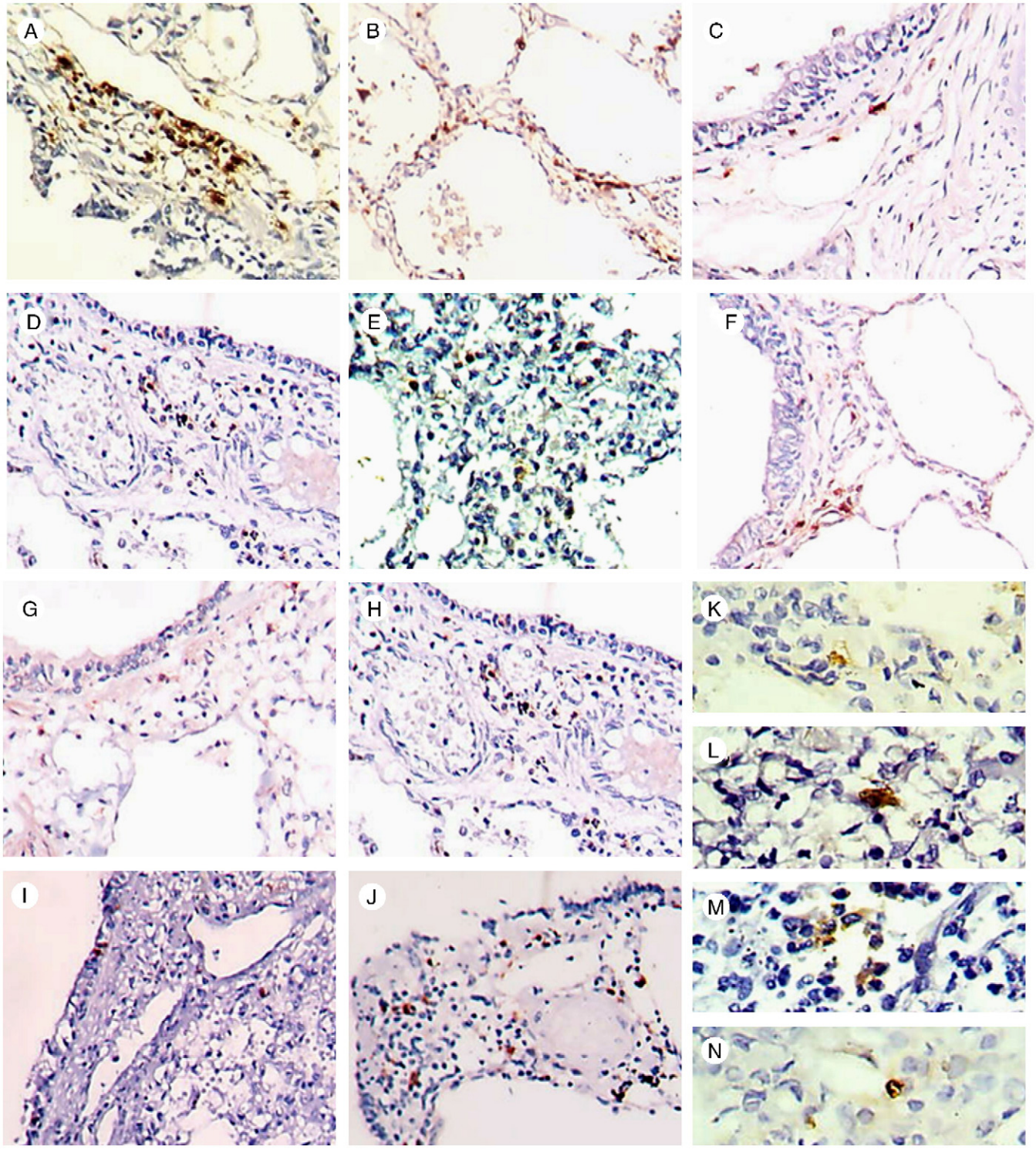

Fig. 2 In situ detection of immune cells in the lungs from measles and noninfected children. A-C, CD4 IH; D-F, CD8 IH; A and D, BALT from patients with measles; B and E, alveoli from patients with measles; C and F, BALT from noninfected controls; G and H, NK IH; I and J, CD20 IH; G and I, BALT from patients with measles; H and J, BALT from noninfected controls; K-N, lung from patients with measles IH for cytokines; K, TNF; L, IFN IH; M, IL-10; N, IL-4 (A-I, ×200; K-N, ×400). Dark brown–labeled positive cells are seen in Harris hematoxylin counterstain.

This is also supported by the depletion of DC cells in our patients with measles, similar to those found in IL-12+ cells, which are also produced by dendritic cells [16].

CD4+ T helper lymphocytes were diminished in the lungs, similar to dendritic cells, and significantly reduced in alveoli, bronchial epithelium, and BALT, showing the

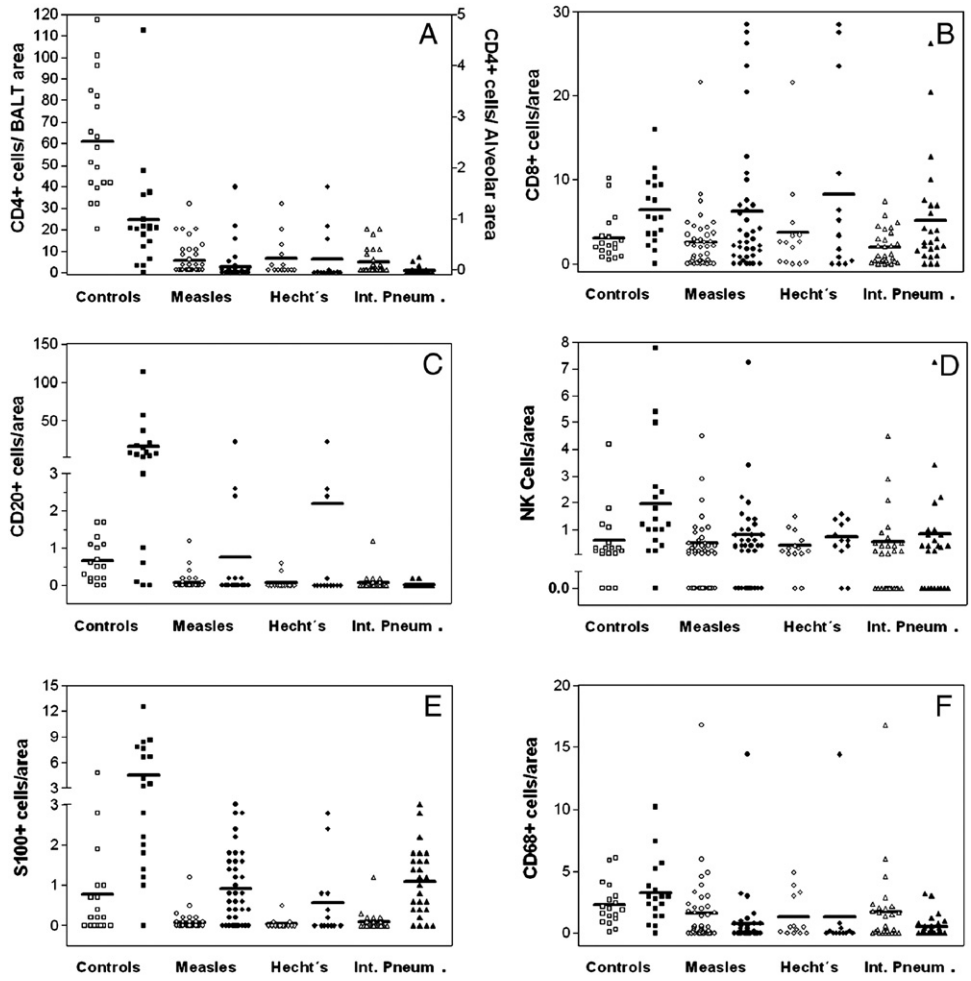

Fig. 3 Individual distribution of relevant immune cells as CD4+ lymphocytes (A), CD8+ lymphocytes (B), CD20 B lymphocytes (C), NK cells (D), S100+ dendritic cells (E), and CD68+ macrophages (F) in alveolus (open symbols) or BALT (closed symbols) from the lungs of patients with measles (dots), with Hecht's pneumonia (diamonds), or interstitial pneumonia (triangles) as compared with controls (squares). Bars represent mean values. Statistical significance is shown in Table 2.

Table 3 Measles: quantitative analysis of in situ lung cellular cytokine production in selected areas of the lung involved in measles and controls

Cellular cytokine staining	Lung area	Noninfected controls	Measles			Statistical significance ($P < .05$)
			Total	Hecht pneumonia	Interstitial pneumonitis	
IL-1β	Alveoli	0.05 ± 0.11 (18)	0.42 ± 0.75 (41)	0.36 ± 0.84 (14)	0.45 ± 0.71 (27)	C < M (PI = H)
	BALT	0.16 ± 0.30 (18)	0.08 ± 0.2 (37)	0.13 ± 0.33 (13)	0.04 ± 0.10 (24)	NS
IL-2	Alveoli	0 ± 0 (18)	0 ± 0.03 (41)	0 ± 0 (14)	0.01 ± 0.04 (27)	NS
	BALT	0.01 ± 0.04 (18)	0.02 ± 0.1 (38)	0.21 ± 0.37 (13)	0.01 ± 0.04 (24)	NS
IL-4	Alveoli	0.01 ± 0.04 (18)	0.07 ± 0.16 (42)	0.07 ± 0.21 (14)	0.06 ± 0.13 (28)	C < M (PI = H)
	BALT	0.23 ± 0.61 (18)	0.12 ± 0.26 (38)	0.21 ± 0.37 (13)	0.07 ± 0.15 (25)	NS
IL-10	Alveoli	0.04 ± 0.08 (18)	0.06 ± 0.2 (41)	0.05 ± 0.09 (14)	0.05 ± 0.21 (27)	NS
	BALT	0.53 ± 0.82 (17)	0.06 ± 0.16 (36)	0.03 ± 0.07 (12)	0.06 ± 0.18 (24)	C > M (PI = H)
IFNγ	Alveoli	0 ± 0 (18)	0.01 ± 0.9 (42)	0.01 ± 0.03 (14)	0 ± 0.02 (28)	C < M (PI = H)
	BALT	0.05 ± 0.15 (16)	0.04 ± 1.5 (38)	0.03 ± 0.07 (13)	0.04 ± 0.12 (25)	NS
TNFα	Alveoli	0.14 ± 0.28 (18)	0.44 ± 0.9 (42)	0.32 ± 0.50 (14)	0.5 ± 1.02 (28)	NS
	BALT	0.84 ± 0.92 (18)	1.04 ± 1.5 (38)	1 ± 1.25 (13)	1.06 ± 1.58 (25)	NS
IL-12	Alveoli	0.16 ± 0.40 (18)	0.05 ± 0.1 (42)	0.04 ± 0.09 (14)	0.05 ± 0.12 (28)	C > M (PI = H)
	BALT	0.11 ± 0.21 (18)	0.03 ± 0.1 (38)	0.01 ± 0.05 (13)	0.03 ± 0.11 (25)	C > M (PI = H)

Data were expressed as numbers of cells expressing cytokine ± SEM (analyzed sample size) by 0.0625 mm^2 microscopic field of selected area. Comparison between groups is tested using Kruskal-Wallis test with Dunn post test.

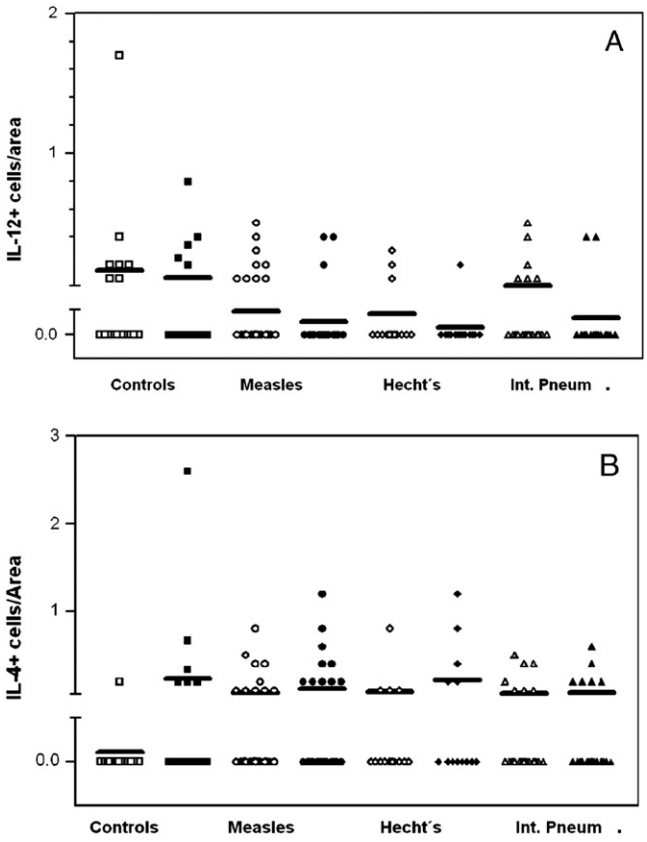

Fig. 4 Individual distribution of relevant populations of cytokine-producing cells, such as IL-12 and IL-4, in alveoli (open symbols) or BALT (closed symbols) from the lungs of patients with measles (dots), with Hecht's pneumonia (diamonds), or interstitial pneumonia (triangles) as compared with controls (squares). Bars represent mean values. Statistical significance is shown in Table 3.

harmful effect of MV to most cells from the immune system. Interestingly, the number of cytotoxic lymphocytes (CD8+) was similar in patients and controls, suggesting that the effect of the virus or the acute inflammation may be insufficient to reduce these cells or may be more intense in the CD4 cells, as the infection could more efficiently promote the death of CD4 lymphocytes by higher expression of FAS-ligand in these cells [17]. CD8+ lymphocytes are usually activated cells that were previously selectively grown elsewhere and probably less affected, which is a scenario similar to that reported in blood cells [17].

Those affected T helper lymphocytes, as demonstrated previously, express a disturbed production of cytokines during measles [16]. In peripheral blood, the cytokines shift from a Th1 to a Th2 pattern, being relevant IFNγ and IL-2 production in initial steps of the disease, fading thereafter, and raising the levels of IL-4 after the rash [3]. IL-12–producing cells, as described above, were severely depleted in lung involvement in measles, both in the alveoli and BALT, probably leading to an environment that favors Th2 shifting [18]. Further evidence supporting this phenomenon is provided by the fact that levels of IL-4+ cells show greater expression in patients with measles than in controls. Despite observing the same pattern with IFNγ (the main Th1 cytokine), it is known by experimental studies that Th2 cytokine effects tend to predominate those of Th1 cytokines [18]. This fact is probably related to measles-induced depletion of TCD4+ cells described above and not to the low expression of cytokines by cells in the lungs from control patients, which exhibit no inflammation.

BALT at rest has an environment favoring Th2 immune response, which induces the production of IgA to the alveoli and bronchial lumen. Incidentally, we observed that IL-10 and CD20+ lymphocytes had significant statistical differences in BALT in controls and patients with measles. Depleting B cells and IL-10 expression probably leads to poor IgA production favoring secondary infections in the lungs affected by measles [19].

Macrophages also presented important depletion in measles at BALT and bronchial epithelium, perhaps leading to an inefficient antigen presentation [20]. Similar to other cytokines, we believe that the absence of significant differences in IL-1β– and TNFα-producing cells in situ in controls and measles could be secondary to MV immune suppressor effects on the inflammatory response.

We also found a disruption of innate immunity, partially demonstrated by depletion of NK cells at bronchial epithelium and BALT. Losing memory immunity in the lungs during measles, as shown in the decreased quantities of CD45RO+ cells at BALT and bronchial epithelium, may explain reactivation of latent infections such as tuberculosis [17].

The 2 main histopathological manifestations were Hecht pneumonia and interstitial pneumonia. Septal thickness and edema, capillary congestion, alveoli macrophages, bronchial metaplasia, pneumocyte I necrosis, pneumocyte II regeneration, and giant cells with viral inclusions were all more severe in Hecht pneumonia than in interstitial pneumonia. On the other hand, in assessment of IH immune cells, there are minor differences, such as CD4+ T cells in BALT, greater in Hecht than in interstitial pneumonia. Probably, the intensity of lung injury is greater in Hecht pneumonia because of the higher viral load, as detected by the presence and numbers of giant cells and intensity of immunohistochemistry for viral antigens. CD4+ T cells are recruited from circulation in giant cell pneumonia to clear the greater viral load, but it also results in more inflammation and lung damage with unsuccessful viral clearance. Our data are isolated, and we cannot speculate on a sequential relationship between these lung histological findings, evolving from acute Hecht pneumonia to interstitial pneumonia, in which the immune response already cleared most viral infected cells.

The involvement of the lung in measles, either as Hecht or interstitial pneumonia, provokes intense disruption of lung immunity. Despite the more severe histopathological and clinical aspects of giant cell pneumonia, both patterns exhibit intense depletion of immune cells in bronchial epithelium, alveoli, and especially in BALT.

The correlation of malnutrition and Hecht pneumonia confirms the classic descriptions of a more severe scenario of measles in the face of malnutrition, probably related to the characteristic vitamin A deficit in this group of patients, which renders the epithelia more vulnerable to the aggressive behavior of the virus and also favors secondary infections. This observation also corroborates the fact that prescribing vitamin A to patients with measles reduces morbidity and mortality due to this infection [21]. Recently, an enormous effort has begun in Africa aimed at vaccine coverage and vitamin A supplementation to reduce child mortality to one third its current levels within 15 years [22].

Measles is the oldest virus-induced immune-suppressive disease known to cause human disease [2]. It continues to cause severe childhood disease, with half a million deaths mainly in developing countries [22], and pneumonias are important complications [1]. Our data demonstrate several aspects of the cells and their cytokines as inflammatory components at alveoli and bronchi, in situ, in measles pneumonias. We believe that these data could enhance knowledge on measles immune pathogenesis in the lungs, but understanding these mechanisms requires further in-depth studies to eliminate vaccine-preventable disease that continues to take lives around the world.

Acknowledgments

The authors acknowledge Dr Carlos Musso of the Hospital Infantil Nossa Senhora da Glória-Vitória ES, Brazil, and the Instituto Emílio Ribas-São Paulo for providing paraffin blocks of the lungs from measles-infected necropsies; and Dr Vera Demarchi Aiello of the Heart Institute (InCor), University of São Paulo Medical School, Brazil, for providing blocks of control cases to the present work. The authors thank James Hesson for providing the English revision of this article.

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