

Respiratory syncytial virus immunology

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Respiratory syncytial virus (RSV) is the most important cause of bronchiolitis in children. Since its discovery in 1956 RSV has been the subject of extensive research because of its peculiar epidemiological and immunological aspects (1, 2). First, the virus infects small infants in the presence of neutralizing antibodies of maternal origin. Second, no long-term or complete immunity is induced by natural infection, although subsequent infection with the virus leads to progressively less severe disease. Third, there is a definite relation between RSV bronchiolitis and childhood asthma. Epidemiological evidence has shown that RSV bronchiolitis is followed by recurrent wheezing in the first years of life in almost half of the cases. In addition, the majority of acute asthma exacerbations in children are probably triggered by viral upper respiratory infections. Especially in young children (under the age of 2–3 years) RSV is often isolated from the respiratory tract during acute wheezing episodes (3). Fourth, a formalin-inactivated experimental vaccine resulted in enhanced disease in vaccinated children upon subsequent infection, as compared to controls immunized with placebo. Finally, an intense inflammatory infiltrate in the lungs accompanies RSV infection in animal and human experimental models.

In summary, these data indicate that RSV bronchiolitis can be considered an immune-mediated disease. The present paper will discuss the immunology of RSV with special emphasis on hypothetical models integrating the scattered experimental data describing the effect of RSV on isolated cell populations.

Single-cell versus network hypothesis

All cells both of the specific and nonspecific arms of the immune system have been studied for their role in RSV immunology and immunopathology,

under various experimental conditions (4) (Table 1). Of special interest are the findings by Welliver et al. of RSV-specific IgE, leukotriene C₄ and histamine in the airways of children with RSV bronchiolitis, in relation to disease severity (5–7), the recognition of both CD4- and CD8-positive T cells as mediators of disease enhancement (8), the unexpected demonstration by Faden et al. of neutrophil activation by RSV (9), the possible role of alveolar macrophages in the modulation of the immune response to RSV (10), and the recent clinical and *in vitro* demonstration of eosinophil activation by RSV (11, 12).

It is nearly impossible that all these cell types act independently after infection or activation by RSV. This single-cell model does not hold in the present view of the immune system as a complex network of cells and soluble factors as cytokines and other mediators. More logical is the hypothesis that RSV is merely the trigger of a well-orchestrated immune response. Among cell types that are possible candidates for a central role in this immune response network are infected epithelial cells, alveolar macrophages and RSV-specific CD4-positive T cells (Fig 1). All these cell types have been shown to be activated by RSV and have theoretically the potential, by means of intercellular interactions and local and systemic cytokine release, for initiating a cascade of events leading to the inflammatory response in the airways of RSV-infected individuals. Criteria to be fulfilled by cells with a supposed modulating role in RSV immunology and pathogenesis are summarized in Table 2.

Epithelial cells

Respiratory epithelial cells are the primary target for RSV. The virus infects these cells after attachment to and fusion with the cell membrane by means of the G and F glycoproteins on the viral surface. Viral progeny bud from the cellular membrane and spread

Table 1. Experimental settings in which interaction of RSV with immune cells has been studied

	<i>In vitro</i> data	Animal data	Clinical evidence
Polymorphonuclear cells			
neutrophils	+	–	–
basophils	–	–	+
eosinophils	+	+	+
Lymphocytes			
NK cells	–	+	–
T cells	+	+	+
B cells	+	+	+
Monocytes/macrophages	+	+	+

to adjacent and distant cells. In the course of these events the epithelial cells are lysed and sloughed in the lumen of the airways. Being the cell type initially targeted by the virus, epithelial cell-derived cytokines, might theoretically set off the immune response following infection (13).

To my knowledge Noah et al. were the first to demonstrate cytokine production and release in the airways of children with viral upper respiratory tract infection (14). Although RSV-negative and RSV-positive patients were analysed as one group, it was shown that acute viral infection induced a rise in interleukin (IL)-1 β , IL-8, IL-6 and tumor necrosis factor- α (TNF- α) in nasopharyngeal lavage fluid. IL-1 β , IL-8 and IL-6 were traced to the epithelium using PCR probes on nasal biopsies in a subgroup of patients, suggesting these cells as the primary expressors of these cytokines.

In addition to these clinical data, the same group demonstrated the sequential *in vitro* production of cytokines by respiratory epithelial cells infected with RSV (15). After 4 h in culture with RSV IL-8 mRNA could be detected followed by an accumulation of this cytokine in the supernatant at 24 h, preceding the production and release of IL-6 and granulocyte macrophage colony-stimulating factor (GM-CSF) after 96 h. Arnold et al. reached comparable conclusions demonstrating IL-6 and IL-8 production by a pulmonary cell line infected with RSV (16). In addition, they showed an increase of the soluble form of TNF-receptor 1 in the infected cultures and a priming of the IL-8 release by various other cytokines and mediators, suggesting intricate cell-cell and paracrine and autocrine networks. Finally, Patel et al. demonstrated secretion of IL-1 α and - β as well as TNF- α by the same cell line as used by Arnold et al. (17). These proinflammatory cytokines could participate in the modulation of the immune response in the airways after RSV infection, leading both to recovery and pathogenesis. IL-

Table 2. Criteria of orchestrating cells in the immune cascade during RSV infection

1. Presence in the lungs or airway lumen
(RSV is a mucosal infection without viremia)
2. Proven interaction with RSV *in vivo* and *in vitro*
3. Potential for secretion of immunomodulating or inflammatory cytokines
4. Hypothetical inducers or modulators of:
 - IgE production
 - eosinophil recruitment or activation
 - basophil/mast cell recruitment or activation

8 (previously known as NAP-1 or neutrophil activating protein-1) is chemotactic for neutrophils and selected populations of T lymphocytes which have been shown to infiltrate the airways of laboratory animals early after infection (18). Moreover TNF- α recruits eosinophils and neutrophils during airway inflammation (19). Homing of leucocytes to the place of infection is facilitated by activation-dependent adhesion mechanisms. IL-1, IL-8 and TNF- α can upregulate cellular expression of CD18, one of the subunits of the CD11a/CD18 complex (LFA-1) on leucocytes (20). In addition IL-1 α is a potent inducer of the counterreceptor for LFA-1, intercellular adhesion molecule-1 (ICAM-1) (17).

In conclusion, epithelial cells fulfill most of the criteria described in Table 2 and are likely candidates to play at least a partial orchestrating role in the immune cascade during RSV infection.

Alveolar macrophages

Alveolar macrophages (AM) have a dual role in the immune system. After phagocytosis of infectious particles, partial or extensive intracellular killing takes place. Next to this first-line defense against invading micro-organisms, macrophages are the most important antigen-processing cells. After intracellular processing, microbial antigens are presented at the cell membrane of the macrophage in association with MHC Class II molecules which are recognized by antigen-specific T lymphocytes. Both the process of phagocytosis and antigen processing is accompanied by activation of the macrophages, leading to cytokine production and release. These cytokines have typically chemotactic and inflammatory effects, and thus enhance the immune response.

Although studies with peripheral blood monocytes/macrophages and RSV were carried out in the mid-eighties, Panuska et al. demonstrated for the first time productive infection of AM with RSV (21, 22). The same group showed that AM secreted TNF- α after infection with RSV (23). Becker et al. reproduced these data and showed additionally that RSV induced also effectively IL-6 and IL-8 mRNA expression albeit less vigorously than TNF- α

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Possible orchestrating cells in the immune response to RSV.

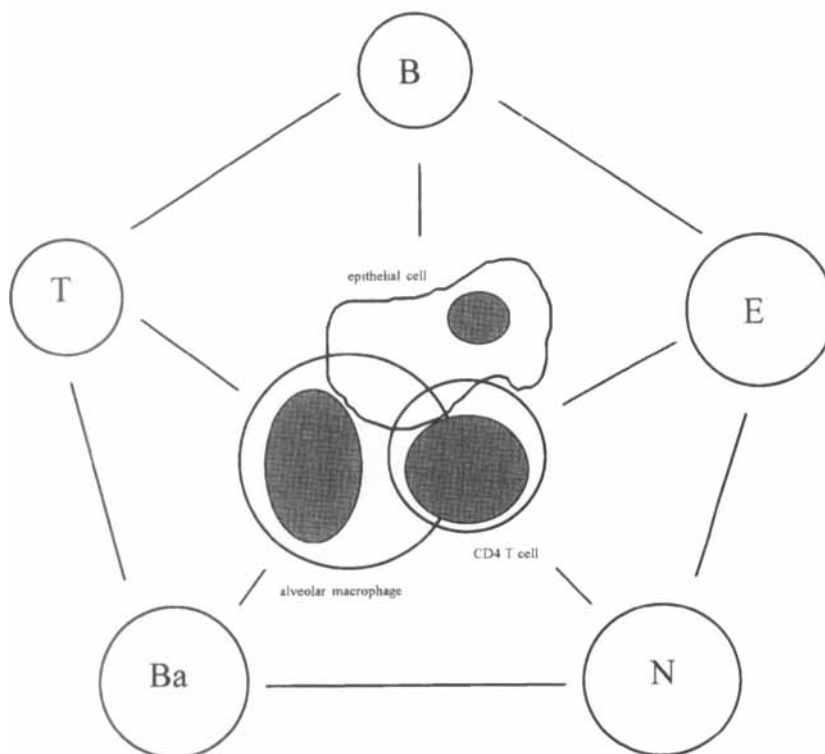
B = B lymphocyte

T = T lymphocyte

N = neutrophil

E = eosinophil

Ba = basophil



mRNA expression (24). Subsequent to these experiments with healthy, non-smoking adult volunteers, Midulla et al. performed bronchoalveolar lavage (BAL) on 6 children with severe symptoms of RSV bronchiolitis. Associated with the presence of RSV, increased expression of HLA-DR, IL-1 β and TNF- α was found in AM recovered by BAL (25). Moreover, AM were quantitatively the most important cell type recovered by BAL, which is consistent with findings in experimental infections of mice with RSV (Kimpen, unpublished results). Finally, Panuska et al. showed induction of IL-10 by AM infected with RSV but found less effect on IL-1 β expression (26).

Taken together these data suggest that AM might play a regulatory role in the immune response during infection with RSV, although their exact function in this regard remains to be elucidated. Both immunostimulatory and pro-inflammatory cytokines as IL-1 β and TNF- α are being induced, the last one having a direct antiviral effect, as well as immunosuppressing cytokines as IL-10. Adding to the confusion around this topic, it was demonstrated recently that accessory functions of AM were increased after RSV infection while microbicidal activity was diminished, hypothetically predisposing RSV-infected infants to bacterial superinfection (27).

In conclusion, it can be stated that AM possibly contribute to the regulation of immunity and patho-

genesis of RSV infection. Many data, however, are still speculative and more research in this interesting area is needed. It is interesting that at the same time in the asthma literature, increasing research efforts are dedicated to the study of macrophages, as mediators of the immune response (28).

RSV-specific CD4-positive T cells

Perhaps the most natural candidates for the orchestration of the local and systemic immune response during RSV infection are RSV-specific CD4-positive T cells. These cells have a sophisticated membrane-associated accessory molecule complex together with a highly-specific T cell receptor and advanced possibilities in the secretion of various immunoregulatory cytokines and mediators. In asthma, a disease resembling RSV bronchiolitis to a great extent, T cells are considered the most important regulating cell type (29). The recognition of a mutually exclusive post-activation differentiation into two T-helper (Th) cell types on the basis of their cytokine profile has expanded considerably our understanding of the pathogenesis of allergic disorders as asthma. Th-1 cells express IL-2 and IFN- γ while Th-2 cells secrete IL-4, IL-5 and IL-10. Both cell types produce IL-3 and GM-CSF. IFN- γ and IL-10 are inhibitory factors for the reciprocal cell population. Th-1 cells are antimicrobial and protective, are responsible for delayed-type hypersensitivity and

provide help for specific antibody production. Th-2 cells are more pathogenetic, are responsible for the IgE switch in B cells, the activation and recruitment of eosinophils and the activation of mast cells and basophils. A disequilibrium in favor of Th-1 cytokines is seen during the recovery phase of viral and mycobacterial infections, while a disbalance in favor of a Th-2 cytokine profile is seen in allergic diseases as asthma (30).

After the disastrous vaccination trials with the formalin-inactivated RSV vaccine in the late sixties, T cells have become an important focus of interest in the study of RSV immunology. RSV-specific T cells have been demonstrated systemically and in the lungs of both laboratory animals, healthy adult volunteers and patients with RSV bronchiolitis (31–33) and T cell-stimulating RSV antigens have been characterised (34). It was soon realized that RSV-specific T cells are both necessary for viral clearance and play a role in pathogenesis of disease due to RSV (35). Both CD4-positive and CD8-positive T cells have been implicated in immunopathogenesis (8).

In view of the epidemiological and pathological similarities between RSV bronchiolitis and asthma, one could speculate on common T cell-modulated immunopathogenic mechanisms in both disease entities. It is evident from literature that T cells, more particularly Th-2, cells are the main cell type involved in the pathogenesis of asthma, leading to antigen-specific IgE production and eosinophil and basophil activation. No direct evidence indicates that T cells involved in RSV disease are of the Th-2 type. In fact the only study published to date by Anderson et al. describing cytokine responses elicited by RSV stimulation of peripheral blood mononuclear cells of RSV-seropositive and RSV-seronegative individuals showed a predominantly Th-1 cytokine profile (36). None of these patients, however, had acute RSV disease at the moment of cell sampling. On the contrary, indirect evidence suggests that, at least in a subgroup of patients, Th-2 cytokines might have been induced. Welliver et al. demonstrated RSV-specific IgE in nasopharyngeal secretions of patients with RSV bronchiolitis and showed that the height of the RSV-specific IgE titer as well as the incidence of this immunoglobulin in this patient population correlated with recurrent wheezing episodes later in childhood (37). Volovitz et al. found release of LTC-4, the main leukotriene secreted by eosinophils, in the respiratory secretions of children with acute RSV bronchiolitis (6). This eosinophil activation by RSV was confirmed by Garofalo et al., who demonstrated high concentrations of eosinophil cationic protein in nasopharyngeal secretions of children with severe RSV disease (11). Finally, basophil activation was suggested by Well-

iver et al., who measured high levels of histamine in bronchiolitis cases (7).

In addition to these studies in humans, Openshaw showed Th-2 cytokine profiles as well as eosinophil infiltration of the airways in mice infected experimentally with RSV (38).

These data suggest that CD4-positive T cells play probably an important role in the immunity and pathogenesis of RSV disease. Probably more than infected epithelial cells or AM, CD4-positive T cells can explain all the particular immune phenomena mentioned in the introduction and summarized in Table 2.

Conclusion

Despite extensive research on the immunology of RSV infection, much has still to be learned. Cell types have been studied separately or in well controlled experimental conditions. Future research will have to focus on the immune cascade initiated by RSV in the most complete but also most complex of systems, the human infant with bronchiolitis.

Clarifying the mechanisms involved in immunity to and immunopathogenesis of RSV infection will be of paramount importance for the development of treatment strategies and vaccines. Therapy aimed at immunomodulation will be the topic of future trials. Vaccines will have to be constructed incorporating the main immunogenic peptides of the RSV virion, eliminating antigens to which the immune response would be detrimental. Thus far these peptides have not been characterized to any extent.

Finally, the challenge of finding the etiological and pathological link between RSV bronchiolitis and childhood asthma puzzles scientists in the field of pediatric infectious diseases, pulmonology and allergology. Attention should be shifted from the study of single cells towards the study of immune networks, as has been done for asthma in children and adults.

References

1. RUUSKANEN O, OGRA PL. Respiratory syncytial virus. *Curr Probl Pediatr* 1993; 23: 50–79.
2. KIMPEN JLL, HEYMANS HSA. Respiratory syncytial virus: immunity and immune injury. *Immunol Infect Dis* 1993; 3: 281–8.
3. DUFF AL, POMERANZ ES, GELBER LE, et al. Risk factors for acute wheezing in infants and children: viruses, passive smoke, and IgE antibodies to inhalant allergens. *Pediatrics* 1993; 92: 535–40.
4. KIMPEN JLL. Experimental models for respiratory syncytial virus infections. *Rev Med Microbiol* 1996; 7: 115–122.
5. WELLIVER RC, KAUL TN, OGRA PL. The appearance of cell-bound IgE in respiratory-tract epithelium after respiratory-syncytial-virus infection. *N Engl J Med* 1980; 303: 1198–202.

6. VOLOVITZ B, WELLIVER RC, DE CASTRO G, KRSTOFIK DA, OGRA PL. The release of leukotrienes in the respiratory tract during infection with respiratory syncytial virus: role in obstructive airway disease. *Pediatr Res* 1988; 24: 504-7.
7. WELLIVER RC, WONG DT, SUN M, MIDDLETON E JR, VAUGHAN RS, OGRA PL. The development of respiratory syncytial virus-specific IgE and the release of histamine in nasopharyngeal secretions after infection. *N Engl J Med* 1981; 305: 841-6.
8. GRAHAM BS, BUNTON LA, WRIGHT PF, KARZON DT. Role of T lymphocyte subsets in the pathogenesis of primary infection and rechallenge with respiratory syncytial virus in mice. *J Clin Invest* 1991; 88: 1026-33.
9. FADEN H, HONG JJ, OGRA PL. Interaction of polymorphonuclear leukocytes and viruses in humans: adherence of polymorphonuclear leukocytes to respiratory syncytial virus-infected cells. *J Virol* 1984; 52: 16-23.
10. ROBERTS NJ JR, PRILL AH, MANN TN. Interleukin-1 and interleukin-1 inhibitor production by human macrophages exposed to influenza virus or respiratory syncytial virus. *J Exp Med* 1986; 163: 511-9.
11. GAROFALO R, KIMPEN JLL, WELLIVER RC, OGRA PL. Eosinophil degranulation in the respiratory tract during naturally acquired respiratory syncytial virus infection. *J Pediatr* 1992; 120: 28-32.
12. KIMPEN JLL, GAROFALO R, WELLIVER RC, OGRA PL. Activation of human eosinophils in vitro by respiratory syncytial virus. *Pediatr Res* 1992; 32: 160-4.
13. CROMWELL O, HAMID Q, CORRIGAN CJ, et al. Expression and generation of interleukin-8, IL-6 and granulocyte-macrophage colony-stimulating factor by bronchial epithelial cells and enhancement by IL-1 β and tumour necrosis factor- α . *Immunology* 1992; 77: 330-7.
14. NOAH TL, HENDERSON FW, WORTMAN IA, DEVLIN RB, HANDY J. Nasal cytokine production in viral acute upper respiratory infection of childhood. *J Infect Dis* 1995; 171: 584-92.
15. NOAH TL, BECKER S. Respiratory syncytial virus-induced cytokine production by a human bronchial epithelial cell line. *Am J Physiol Lung Cell Mol Physiol* 1993; 265: L472-8.
16. ARNOLD R, HUMBERT B, WERCHAU H, GALLATI H, KOENIG W. Interleukin-8, interleukin-6, and soluble tumour necrosis factor receptor type I release from a human pulmonary epithelial cell line (A549) exposed to respiratory syncytial virus. *Immunology* 1994; 82: 126-33.
17. PATEL JA, KUNIMOTO M, SIM TC, et al. Interleukin-1 mediates the enhanced expression of intercellular adhesion molecule-1 in pulmonary epithelial cells infected with respiratory syncytial virus. *Am J Respir Cell Mol Biol* 1995; 13: 602-9.
18. KIMPEN JLL, OGRA PL. T cell redistribution kinetics after secondary infection of BALB/c mice with respiratory syncytial virus. *Clin Exp Immunol* 1993; 91: 78-82.
19. MADGE P, PATON JY, MCCOLL JH, MACKIE PL. Prospective controlled study of 4 infection-control procedures to prevent nosocomial infection with respiratory syncytial virus. *Lancet* 1992; 340: 1079-83.
20. ETZIONI A. Adhesion molecules in host defense. *Clin Diagn Lab Immunol* 1994; 1: 1-4.
21. MIDULLA F, HUANG YT, GILBERT IA, CIRINO NM, McFADDEN ER JR, PANUSKA JR. Respiratory syncytial virus infection of human cord and adult blood monocytes and alveolar macrophages. *Am Rev Respir Dis* 1989; 140: 771-7.
22. PANUSKA JR, CIRINO NM, MIDULLA F, DESPOT JE, McFADDEN ER JR, HUANG YT. Productive infection of isolated human alveolar macrophages by respiratory syncytial virus. *J Clin Invest* 1990; 86: 113-9.
23. PANUSKA JR, MIDULLA F, CIRINO NM, et al. Virus-induced alterations in macrophage production of tumor necrosis factor and prostaglandin E. *Am J Physiol Lung Cell Mol Physiol* 1990; 259: L396-402.
24. BECKER S, QUAY J, SOUKUP J. Cytokine (tumor necrosis factor, IL-6, and IL-8) production by respiratory syncytial virus-infected human alveolar macrophages. *J Immunol* 1991; 147: 4307-12.
25. MIDULLA F, VILLANI A, PANUSKA JR, et al. Respiratory syncytial virus lung infection in infants: immunoregulatory role of infected alveolar macrophages. *J Infect Dis* 1993; 168: 1515-9.
26. PANUSKA JR, MEROLLA R, REBERT NA, et al. Respiratory syncytial virus induces interleukin-10 by human alveolar macrophages. *J Clin Invest* 1995; 96: 2445-53.
27. FRANKE-ULLMANN G, PFOERTNER C, WALTER P, et al. Alteration of pulmonary macrophage function by respiratory syncytial virus infection *in vitro*. *J Immunol* 1995; 154: 268-80.
28. POULTER LW, JANOSSY G, POWER C, SREENAN S, BURKE C. Immunological/physiological relationships in asthma: potential regulation by lung macrophages. *Immunol Today* 1994; 15: 258-61.
29. KAY AB. "Helper" (CD4) T cells and eosinophils in allergy and asthma. *Am Rev Respir Dis* 1992; 145: S22-6.
30. OPENSHAW PJM, O'DONNELL DR. Asthma and the common cold: can viruses imitate worms? *Thorax* 1994; 49: 101-3.
31. KIMPEN JLL, RICH GA, MOHAR CK, OGRA PL. Mucosal T cell distribution during infection with respiratory syncytial virus. *J Med Virol* 1992; 36: 172-9.
32. BANGHAM CRM, MCMICHAEL AJ. Specific human cytotoxic T cells recognize B-cell lines persistently infected with respiratory syncytial virus. *Proc Natl Acad Sci U S A* 1986; 83: 9183-7.
33. ISAACS D, BANGHAM CRM, MCMICHAEL AJ. Cell-mediated cytotoxicity response to respiratory syncytial virus in infants with bronchiolitis. *Lancet* 1987; ii: 769-71.
34. CHERRIE AH, ANDERSON K, WERTZ GW, OPENSHAW PJM. Human cytotoxic T cells stimulated by antigen on dendritic cells recognize the N, SH, F, M, 22K, and 1b proteins of respiratory syncytial virus. *J Virol* 1992; 66: 2102-10.
35. CANNON MJ, OPENSHAW PJM, ASKONAS BA. Cytotoxic T cells clear virus but augment lung pathology in mice infected with respiratory syncytial virus. *J Exp Med* 1988; 168: 1163-8.
36. ANDERSON LJ, TSOU C, POTTER C, et al. Cytokine response to respiratory syncytial virus stimulation of human peripheral blood mononuclear cells. *J Infect Dis* 1994; 170: 1201-8.
37. WELLIVER RC, SUN M, RINALDO D, OGRA PL. Predictive value of respiratory syncytial virus-specific IgE responses for recurrent wheezing following bronchiolitis. *J Pediatr* 1986; 109: 776-80.
38. OPENSHAW PJM, 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.