Respiratory syncytial virus RNA in cells from the peripheral blood during acute infection

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It is not known whether respiratory syncytial virus spreads beyond the respiratory tract. With the use of reverse transcriptase polymerase chain reaction, we found viral and transcribed RNA in cells from the arterial blood of four children with bronchiolitis but none in serum or cerebrospinal fluid. Respiratory syncytial virus might therefore spread outside the respiratory tract. (J Pediatr 1998;133:272-4)

Respiratory syncytial virus causes most cases of infantile bronchiolitis, the most common cause of hospital admission for children younger than 1 year of age. Reinfections occur despite the presence of serum antibody. Viremia is an important feature of acute measles, another closely related paramyxovirus.[1] Domurat et al.[2] showed that some peripheral blood mononuclear cells bear RSV antigens during acute infection and that monocytes and lymphocytes can be infected in vitro, although these results are unconfirmed. Reverse transcriptase polymerase chain reaction has shown that RSV infection spreads to the middle ear[3] and conjunctiva,[4] but this technique has not previously demonstrated systemic spread.

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Patients and Methods

During two consecutive winters 35 children younger than 18 months of age were hospitalized with tachypnea, respiratory distress, lung crackles, apnea, or acute collapse. Blood samples were taken when routine clinical blood tests were ordered with the informed consent of parents or guardians, in accordance with approved research protocols. Nasopharyngeal samples were subjected to direct immunofluorescent antibody testing and virus culture.

During the first winter serum was obtained from 13 children and packed blood cells from 12 children. In the second winter PBMC (monocytes and lymphocytes) were isolated from blood samples (n = 22) by density centrifugation. Samples of one million PBMC in 200 μl phosphate-buffered saline solution were stored at –80° C. Ten CSF specimens were obtained; 50 μl aliquots were tested by RT-PCR.

RNA was extracted from blood, PBMC, or serum with the QIAamp blood kit (QIAGEN). RNA was extracted from CSF with proteinase K and sodium dodecylsulfate, 10 μl of extracted RNA was mixed with 4 μmol/L of first strand primer (22k1 for viral vRNA, 22k2 for mRNA) and cDNA synthesis performed according to established methods.[5] One tenth of this cDNA was used for 40 first round and 30 second round cycles of nested PCR (Perkin Elmer 9600, Applied Biosystems) with Stoffel fragment of Amplitaq (Applied Biosystems). Conditions for first and second rounds were denaturation for 2 minutes at 94° C, 40 cycles at 94° C/30 sec, 53° C/30 sec, and 72° C/30 sec. Negative controls were always included. RNA detection, cloning, and sequencing were performed by an operator who was blinded to the origins of the samples. Sensitivity was greater than 1 plaque forming unit of A2 RSV, and no false-positive results were obtained.

CSF	Cerebrospinal fluid
PBMC	Peripheral blood mononuclear cells
RT-PCR	Reverse transcriptase polymerase chain reaction

PCR products were cloned (TA cloning kit, Invitrogen) and sequenced (Sequenase version 2, Amersham International). Primers for M2 were 1. ATG TCA CGA AGG AAT CCT TGC; 2. TAG CTC TTC ATT GTC CCT CAG C (outer); 3. GA GGT CAT TGC TTA AAT GG; and 4. GC AAC ACA TGC TGA TTG T (inner). The primers for N were 1. G ATG GCT CTT AGC AAA GTC; 2. C ATG CCT GTA TTC TGG AG (outer); 3. CTG TCA TCC AGC AAA TAC AC; and 4. GTA GGA TTT TCT AGA TTC TAT C (inner).

RESULTS

The clinical features of the study group are summarized in the Table. Seventy percent of the children were positive for RSV by direct immunofluorescent antibody testing or virus culture of nasopharyngeal samples. The clinical features of children with RSV were similar to those without.

No serum sample was positive for RSV by PCR. Whole blood or separated PBMC from four children were positive for RSV M2 mRNA (Figure) and for negative strand viral RNA (not shown). Three of these were also positive for nucleoprotein RNA. Three of the four children positive by PCR had positive nasopharyngeal samples by direct immunofluorescence or virus culture. The fourth was negative for all other pathogens. All PCR products were sequenced and proved closer to A strain than B strain RSV. Although highly conserved regions were chosen for amplification, sequencing showed unique variations in two isolates compared with laboratory strain A2, thus showing that these results could not be due to laboratory contamination. CSF samples from 10 children with apneic spells (7 with RSV infection) were tested. Two of these seven were positive for RSV by PCR of blood, but in no case was RSV detected in the CSF.

All four RT-PCR positive samples were taken on the first or second day after admission of children with severe disease, and all from arterial lines. Three were from children requiring ventilation; two had been born at less than 36 weeks' gestation, but none had needed neonatal ventilation. Two of the four children positive for RT-PCR had had previous respiratory illnesses, one had recurrent wheeze, and the other had previous bronchiolitis. No child was immunocompromised.

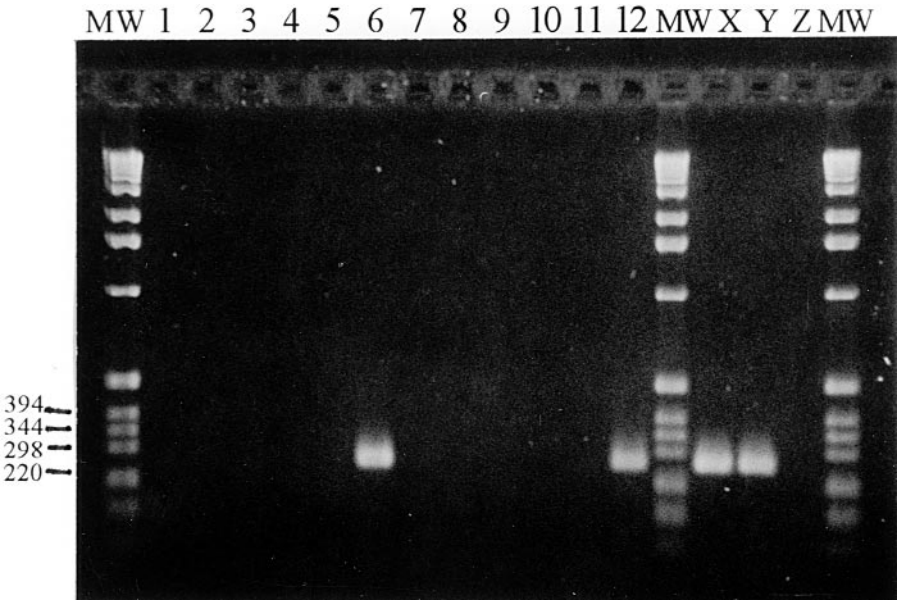

Figure. Two percent agarose gel stained with ethidium bromide showing PCR amplification of RSV genomic RNA. Lane 6 and 12 show amplification product from peripheral blood pellet of two children with RSV infections. Similar samples from other children (1-5, 7-11) are negative. Laboratory standard virus was detectable at 0.01 pfu (X) and 0.001 pfu (Y). Expected PCR product is 260 bp.

Table. Clinical features of patients in the study

	RSV (n = 24)* n (%)	No RSV (n = 10) n (%)
Male	14 (58)	6 (60)
Premature birth (≤36 wk)	9 (38)	2 (20)
Neonatal ventilation	6 (25)	1 (10)
Previous disease (respiratory or cardiac)	7 (29)	4 (40)
Age (median, range)	13 wk (3-60)	8 wk (2.5-43)
Duration of illness before admission (median, range)	2 days (0-8)	2 days (1-10)
Ventilated during hospitalization (other than oxygen alone)	15 (63)	6 (60)
Apneas	8 (33)	3 (30)

*RSV infection was documented by positive direct flourescent antibody test or isolation from nasopharyngeal aspirate. There was no statistically significant difference between the characteristics of patients with and without RSV infection ($p > 0.05$, chi-square test).

DISCUSSION

These studies establish that cells in the arterial blood of some RSV-infected children can carry genomic RSV. RSV is highly unstable ex vivo and quickly loses infectivity[6]; we and others have not been able to recover replicating RSV from peripheral blood by culture (unpublished). We are therefore unable to determine whether RSV can replicate in PBMC, but the presence of both negative strand (genomic) and messenger RNA suggests ongoing viral replication.

It is not known whether RSV can spread to tissues outside of the respiratory tract. The equivalent of the slow virus forms of measles have not been demonstrated for RSV, although Paget's disease of bone has been suggested to be paramyxoviral disease.[7,8] No unequivocal evidence exists for delayed effects of RSV on the lung or extrapulmonary sites, but our results suggest the potential for extrapulmonary dissemination. Sendai virus (also a paramyxovirus) has been

shown to infect and persist in neural tissue in mice.[9] We failed to detect RSV RNA in the CSF of any child but have not been able to test cells from the CNS.

In summary, our data show that arterial blood mononuclear cells sometimes contain RSV viral and messenger RNA during acute primary infection, but that viral RNA is not detectable in the CSF or serum. This is direct evidence that RSV might spread outside the respiratory tract.

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