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Respiratory Syncytial Virus

Brief Review

By

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With 2 Figures

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Respiratory syncytial virus (RSV) infection is the major cause of hospitalisation in children during the first year of life in the Western world. The virus is also recognised as an important cause of bovine respiratory disease in Europe and the United States. Despite these clear medical and veterinary needs for an effective vaccine, none is yet generally available. The virus grows poorly in cell cultures, is closely associated with cell membrane and is inherently unstable, making purification by biophysical techniques difficult. Probably for these reasons, the virus was largely ignored by molecular biologists. Hence in 1978 the Study Group on Paramyxoviridae, referring to the genus *Pneumovirus*, declared "So little is known about that genus that a future transfer to another taxon would not be surprising" (151). However, the avialability of monoclonal antibodies together with new immunological techniques and recent advances in recombinant DNA technology have overcome many of the previous constraints on RSV research and greatly increased our knowledge. In this review recent advances will be described and their relevance to the enigma of RSV pathogenesis and to the control of RSV infection will be discussed.

The Virus

History

The virus was first isolated in 1956 from a chimpanzee with mild upper respiratory signs and was named "Chimpanzee Coryza Agent" (193). The following year antigenically identical viruses were isolated from two infants both with lower respiratory disease (42). The virus was renamed Respiratory

Syncytial Virus in recognition of its characteristic cytopathogenic effect in tissue culture (38). A series of epidemiological studies in the early 1960's established that RSV was associated with bronchiolitis and pneumonia in children more frequently than any other agent and particularly in the first year of life (9, 41, 119).

In 1968, Doggett and colleagues (63) found antibodies to RSV in cattle sera and suggested the virus frequently infected cattle. Two years later bovine RSV was isolated (200) and subsequent studies have established the importance of RSV in lower respiratory disease of cattle (120, 129, 234, 257). Respiratory syncytial virus has also been isolated from a naturally infected pygmy goat with respiratory disease (160) and antibodies found in 5 of 10 goat sera (230) but little is known about the natural incidence of RSV in this species. Reports that antibodies to RSV are widespread in sheep (19) and cats (216, 230) have not been confirmed by isolation of virus from naturally infected animals. However, bovine RSV will infect lambs experimentally (159). Twenty kittens inoculated intranasally with 10^5 PFU of the Long strain of RSV failed to shed virus or show an antibody response (G. L. Toms, personal communication).

Taxonomy

Human and bovine RSV, together with pneumonia virus of mice, comprise the genus *Pneumovirus* within the family Paramyxoviridae. The family is defined as having enveloped pleomorphic virions containing helical, elongated nucleocapsids. The genome is linear, single-stranded RNA complementary in base sequence to mono-cistronic messenger RNA species. Replication occurs in the cytoplasm (151, 179).

The genus *Pneumovirus* differs from *Paramyxovirus* in lacking neuraminidase and from both *Paramyxovirus* and *Morbillivirus* genera in certain dimensions of the surface projections and nucleocapsid of the virion (Table 1). RSV also lacks a haemagglutinin which is possessed by all members of the paramyxovirus group, measles virus and pneumonia virus of mice.

Table 1. *Dimensions within the family Paramyxoviridae*

Genus	Virion		Nucleocapsid helix		Surface projections	
	Round and pleomorphic forms	Filamentous forms	Diameter	Pitch	Length	Spacing
Pneumovirus	80—500	60—110 × 5000[a]	13.5	6.5	12	6—10
Paramyxovirus	120—300	120 × 5000[a]	17.5	5.0	8	8—10
Morbillivirus	100—250	100 × 5000[a]	17.5	5.0	8	8—10

[a] Length variable up to several μm
Data from references 20, 151

Properties, Purification and Structure of the Virion

Morphologically, the virion may appear as round or pleomorphic forms measuring 80—500 nm across or as filamentous forms up to several μm in length. The outer membrane is studded with projections 12 nm in length and 10 nm apart. A majority of particles contain no internal structure and are incomplete and, presumably, non-infectious. The complete particles contain a nucleocapsid helix 13.5 nm in diameter with a pitch of 6.5 nm (5, 20, 130, 134, 299).

Virus infectivity is destroyed by ether, chloroform, 0.25 per cent trypsin or 0.1 per cent sodium deoxycholate (42, 128). The virus is rapidly inactivated at pH 3 but relatively stable above pH 4. Infectivity is also thermolabile; its half life at 56° C is 0.5—2.8 minutes and at 37° C is initially 1—7 hours. Virus is stable below —50° C for many months (112, 128, 136). Inorganic salts, particularly divalent cations such as magnesium and calcium, glucose and sucrose protect virus against inactivation (77, 162, 225, 278). The buoyant density of RSV in caesium chloride is between 1.21 and 1.24 g/ml (23, 47, 128) and sucrose between 1.16 and 1.26 g/ml (77, 157, 162, 296).

The concentration and purification of RSV has been considerably improved by the addition of 20 per cent sucrose or magnesium ions. Concentration of RSV with over 90 per cent recovery of infectivity has been achieved by precipitation with ammonium sulphate or polyethylene glycol, by ultrafiltration or by pelleting onto a sucrose cushion in the presence of 1 M $MgSO_4$ (77, 244, 271). Further purification of virus has been obtained by gel filtration and isopycnic centrifugation in discontinuous and continuous sucrose gradients, or metrizamide gradients. Under these conditions the ratio of infectivity to protein content was increased 250—3000 fold and 18—33 per cent of the infectivity was recovered (77, 157, 278, 296).

Purified virions contain a unique species of single-stranded RNA and 8 or 9 polypeptides (Table 2). The RNA has a sedimentation value of 50S and a molecular weight of approximately 5×10^6 daltons. At least 93 per cent of virion RNA is negative (non-message) polarity (125, 157, 295). Although there is now general agreement about the size and number of polypeptides in the virion, minor discrepancies still exist and may, in part, be due to differences between the various virus strains used. The smallest polypeptide, with a molecular weight between 10,000 and 13,000 daltons has been detected by only three groups (35, 78, 272). Its function and RNA origin are unknown. A second non-glycosylated protein between 19,000 and 25,000 daltons in size is regularly found but again has no known function. The M protein (27,000—28,000 daltons) can be removed from the virion by non-ionic detergent and isolated on metrizamide gradients. By analogy with other negative strand viruses this is believed to be the matrix or membrane (M) protein (205, 296). The sequence of the M protein has been deduced from a cDNA insert in a recombinant plasmid. The protein has 256 amino acids

Table 2. *Messenger RNAs of RSV and their polypeptide products*

Messenger RNA[a]		Polypeptides (molecular weight $\times 10^3$ daltons)			
No.	Molecular weight $\times 10^6$ daltons	*In vitro* translation[b]	Cell extract[c]	Purified virion[d]	Functional name
1a	0.24	9.5			
1b	0.26	11	VP 10	VP 10—13	
1c	0.26	14			
2a	0.38	34	VP 34	VP 32—38	P
2b	0.39	36	GP 92—95	GP 79—90	GP
3a	0.40	26	VP 27—28	VP 27—28	M
3b	0.40	24	VP 21—25	VP 19—25	
4	0.47	42	VP 40—44	VP 40—44	NP
5	0.74	59	GP 66—75	GP 66—68	F
			[GP 48—50+ GP 17—20]	[GP 43—56+ GP 19—22]	
7	2.5	200	VP 200	VP 200	L

Data derived from
[a] Reference 50
[b] References 34, 50, 126
[c] References 18, 35, 67, 219, 284, 296
[d] References 35, 78, 156, 162, 205, 272, 278, 296

and a molecular weight of 28,717 daltons. It is relatively basic and has two hydrophobic regions in the C-terminal third of the protein which could potentially interact with membranes of the infected cell (235). The P protein (32,000—38,000 daltons) was shown to be phosphorylated by Cash and colleagues (34) and may be associated with the nucleocapsid (296). Nucleocapsids isolated from infected cells or purified virus by detergent disruption in high salt contain predominantly NP protein (40,000—44,000 daltons) in association with RNA (205, 296). The amino acid sequence of this protein has also been deduced from the DNA sequence of a recombinant plasmid. The protein has 467 amino acids and a molecular weight of 51,540 daltons. It is rich in basic amino acids (71). The F protein (66,000—68,000 daltons) and large GP protein (79,000—90,000 daltons) are both glycoproteins believed to be located on the surface of the virion because they are released by detergent in low salt and are removed by trypsin (162, 205, 277). However, Wunner and Pringle (296) found the F protein insensitive to trypsin and this discrepancy may be due to differences between the two strains of RSV studied. Ueba (277) purified the glycoproteins and observed their club- or rod-shaped structure by electron microscopy, suggesting they were the spikes of RSV. After removal of detergent the structures aggregated to form oligomers or polymers. The F protein is composed of two smaller glycoproteins (43,000—56,000 and 19,000—22,000 daltons) linked by disulphide bonds. This led to the suggestion that, by analogy with other

paramyxoviruses, this was the fusion protein of RSV (78, 156). The hypothesis was confirmed when monoclonal antibody to F protein was found to inhibit cell fusion (281). The large GP protein in purified form will bind rapidly to cells and is probably the attachment protein of the virion, analogous to the haemagglutinin-neuraminidase of other paramyxoviruses (78, 282). Monoclonal antibodies to either of the two glycoproteins will neutralise infectivity and this is further evidence for their surface location in the virion (75, 266, 281). Little is known about L protein (160,000—200,000 daltons) but its large size and its association with the nucleocapsid suggest that it is the RNA polymerase of the virion (35, 205). The polypeptide composition of 11 human and 2 bovine strains of RSV is essentially similar, although there are small differences in the molecular weights of some proteins (35).

Replication

The growth characteristics of RSV have been studied in human and monkey cell lines. Approximately 60—90 per cent of virus adsorbed to susceptible cells in 30 minutes at 37° C and over 95 per cent by 10—12 hours (16, 164, 231). Attachment probably occurs through the large glycoprotein of the virion but the cell receptor is undefined, although it is unlikely to be sialic acid and is resistant to trypsin (164, 282). Adsorbed virus penetrates the cell within 45 minutes, presumably by membrane fusion (164). After a latent period of 12—16 hours progeny virus is produced reaching a peak of 45—150 PFU/cell after 36—48 hours post-infection but over 90 per cent of infectious virus remains cell associated (16, 157, 164, 231, 295).

Replication occurs in enucleated cells, is resistant to mitomycin C and enhanced by actinomycin D, indicating that RSV multiplies in the cytoplasm and is not dependent on nuclear DNA synthesis (135, 157, 295). Optimal virus growth is dependent on an adequate supply of exogenous arginine (163, 295). RSV does not specifically inhibit host cell metabolism (165). Nucleic acid synthesis is unaltered 11 hours after infection but by 18 hours DNA synthesis is reduced to 50 per cent and RNA synthesis to 35 per cent of normal. Protein synthesis is unaltered throughout the virus growth cycle.

Eleven species of viral RNA and at least 9 viral polypeptides are produced in RSV-infected cells (Table 2). The largest RNA (5×10^6 daltons) equivalent in size to the genome contains both negative and positive strands and is only produced in the presence of concomitant protein synthesis and hence is the product of RNA replication. The positive strand is therefore likely to be the template for replicating the negative strand genome (125). The remaining 10 RNA species are polyadenylated, produced in the absence of protein synthesis and therefore represent viral messenger RNA's (50, 125). The mRNA's vary in molecular weight from 0.24×10^6 to 2.5×10^6 daltons and code for proteins between 9500 and 200,000 daltons (Table 2), although some of the small proteins have not yet been identified in the cytoplasm of

virus-infected cells. After translation some polypeptides undergo further modification; the P protein is phosphorylated and the GP48 portion of F protein is sulphated (34). Two of the proteins translated *in vitro*, one of 59,000 and one of 36,000 daltons, are thought to be precursors of the fusion and large glycoproteins respectively (50). Their glycosylation is inhibited by 2-deoxy-D-glucose which is incorporated in place of mannose and terminates the carbohydrate chain because it is not recognized by glycosyl transferases. Consequently the yield of infectious virus is reduced and virions with greatly reduced surface projections are produced (118, 206). Tunicamycin, a specific inhibitor of N-acetyl-glycosamine-lipid intermediates and of glycosylation of newly synthesised proteins also reduces the yield of infectious virions and inhibits cell fusion (156). The effects of both these drugs are reversible and indicate that glycosylation is necessary for the biological activities of the glycoproteins, namely infectivity and cell fusion. The fusion protein precursor of most paramyxoviruses requires proteolytic cleavage before it becomes active; but this has not yet been shown for RSV. The proteases thrombin, plasmin and trypsin enhance RSV-induced cell fusion and Bis (5-amidino-2-benzimidazolyl) methane, a powerful protease inhibitor, inhibits such fusion (68, 69, 70). However, there is no effect on the production or infectivity of progeny virions indicating that the proteases are probably acting on a cellular target rather than a component of the virions themselves.

Morphological examination of RSV-infected cells by light and electron microscopy reveals further features of virus replication. Viral antigen, detected by immunofluorescence, appears in the cytoplasm 8—9 hours after infection (16, 20). Eosinophilic inclusion bodies staining yellow-green with acridine orange appear near the nucleus. These pleomorphic electron dense inclusions have a granular or thread like appearance and the dots or threads have a diameter of 12 nm (4, 20, 137, 197). Monoclonal antibodies to nucleoprotein or phosphoprotein bind to these inclusions indicating that they contain the nucleocapsids of the virus (54, 256, 281). Virions mature by budding off from areas of the cytoplasmic membrane studded with projections 12 nm long. The budding structures were round and 80—150 nm in diameter or filamentous and up to 10 μm in length. In cross-section filaments contain 6—12 electron dense circular structures or dots 12 nm in diameter and in longitudinal section several threads of the same dimension are seen. These are probably nucleocapsids because their appearance and dimensions are similar to those in inclusions (5, 20, 137, 197). In certain cell types, the production of surface filaments is extensive, and dramatically alters the appearance of cells in the scanning electron microscope. This property is apparently characteristic of pneumoviruses and not shared by other paramyxoviruses, herpesviruses, rhabdoviruses or bunyaviruses (203). The presence of RSV antigen in these filaments has been demonstrated by electron

microscopy using *Staphylococcus aureus* as marker (217) and by immunofluorescence using antisera and monoclonal antibody to the large glycoprotein (203, 281).

Although the usual outcome of RSV infection is syncytium formation and ultimately cell destruction persistent infection of Hep-2, BS-C-1, Balb/C and bovine nasal mucosa cells has been established (6, 76, 218, 258). The mechanisms which maintain these infections without cytolysis are not fully understood. Although ts mutants are involved in BS-C-1 cells, defective interfering particles may also be involved. Passage of RSV at multiplicities of infection of 1 to 5 PFU/cell generates defective interfering particles which reduce the infectious yield by ten- to hundred-fold and may be assayed colorimetrically. Defective interfering particles are not generated if virus is passaged at a multiplicity of 0.1 PFU/cell (273, 274).

Antigenicity

Antigenic variation among isolates of RSV has been demonstrated by neutralisation tests using immune sera from ferrets, rabbits, monkeys and guinea pigs but differences were usually small and not confirmed when human sera were used, even if collected from infants after a primary infection (45, 48, 62, 263, 294). However, in 1977 strains of RSV were isolated which were unrelated to the prototype Long strain in reciprocal neutralisation tests; but they have not been studied further (116). BEEM (8) examined paired isolates from successive infections of the same patient and concluded that antigenic variation was not the cause of reinfection. Although bovine and human strains of RSV differ in plaque reduction tests using bovine sera, cattle are equally protected from bovine RSV infection by either strain (258). Any differences detected by neutralisation presumably reflect changes in epitopes on either the fusion or larger glycoproteins. Two monoclonal antibodies to the fusion protein appear to react with bovine RSV isolates but not human isolates (256). The only exception so far is the 8/60 isolate from a child in Sweden which differed markedly from 8 other human isolates in kinetic neutralisation tests with polyclonal sera (62). Thirteen other monoclonal antibodies to at least 4 different epitopes on the F protein react with both human and bovine strains of RSV indicating marked antigenic conservation in this protein. In contrast, none of 6 monoclonal antibodies to the large glycoprotein of human RSV reacted with bovine RSV strains (266, unpublished observations).

By complement fixation and immunofluorescence tests all strains of RSV cross-react. Early attempts to define the antigenic composition of RSV identified 2 complement-fixing antigens only one of which, the A antigen, induced neutralizing antibodies in guinea pigs (47, 80, 81, 244). However, radioimmunoprecipitation now shows that, as a result of infection,

human sera contain antibodies to at least the 7 largest polypeptides listed in Table 2 (284). Thus, virtually every component of the virus may play a role in either pathogenesis or protection.

Epidemiology

Geographical Distribution

The distribution of RSV is world wide. Virus has been isolated from men and cattle in the continents of Europe (200, 204), America (42, 252) and Asia (129, 263). Antibody to RSV occurs in over 40 per cent of the adult population in every continent of the world (61) and is found even in remote isolated populations (40).

Seasonal Distribution

In several centres in the United States and in England and Scotland RSV occurs in regular annual epidemics during late autumn, winter and early spring (51, 94, 96, 144, 194). The peak of the epidemic alternates between midwinter and early spring in Washington and Chapel Hill but this pattern of short (7—12 month) and long (13—16 month) intervals between epidemics is not found in Chicago, Glasgow or Newcastle (94, 96, 144, 194, 248). The midwinter epidemics following short intervals tend to be more severe than spring epidemics which follow longer intervals (93). This consistent seasonal behaviour by RSV is quite distinct from that of parainfluenza and other respiratory viruses (41, 121, 194). Similar regular annual winter outbreaks of RSV infections also occur in cattle (196, 257, Table 3).

Incidence and Prevalence of Infection

Approximately half of all infants probably become infected with RSV in the first year of life and the remainder during their second year (144). Sixty per cent of infants under one year of age exposed to RSV in the family environment become infected (52). Over 98 per cent of previously uninfected children became infected on their first exposure to RSV in a day care centre (114) and reinfection occurred in 74 and 65 per cent of these children on their second and third exposures respectively. This high incidence of reinfection also occurs in the family environment and declines only slightly in adulthood; thus 42 per cent of adults exposed to RSV become infected and the overall infection rate is 17 per 100 person-years (52).

Over 90 per cent of seronegative cattle are infected on primary exposure to RSV (Table 3) but although high levels of maternal antibody may prevent infection in younger animals 70 per cent of cattle have been infected by 9 months of age (257).

Table 3. *RSV outbreaks in cattle*

Winter	Group	No. of calves	Age[a] (months)	RSV outbreak		Respiratory disease	
				Duration[b]	% calves infected[c]	No. of cases (deaths)	%
1979—80	1	51	8	Oct-*Nov*	98	0	0
	2	41	4.5	Oct-*Nov*	95	6 (1)	15 (2)
	3	53	2	*Nov*-Dec-Jan	64	17 (2)	32 (4)
1981—82	1	51	4.5	Jan-*Feb*-Mar	94	10 (2)	20 (4)
	2	49	3	Jan-*Feb*-Mar	69	10 (3)	20 (6)
	3	48	0.5	*Feb*-Mar	21	9 (0)	19 (0)
1983—84	1	33	6	*Dec*-Jan	90	1 (0)	3 (0)
	2	32	3	*Dec*-Jan	50	15 (2)	47 (6)

[a] At start of RSV outbreak

[b] Peak month of infection in italics

[c] Infections diagnosed by single radial haemolysis tests on sera

Effects of Age, Sex and Race

In children admitted to hospital the peak incidence of RSV bronchiolitis is at 2 months of age, and declines to about 20 per cent of peak incidence by 12 months, and less than 5 per cent by 2 years of age (201, 226). Studies of families in the general population have produced conflicting results on the age incidence of RSV infection. In Tecumseh, serological evidence of infection was found in 14 per cent of children in their first year of life, 20 per cent of children aged 4—9 years and in about 4 per cent of adults over 20 years (191). In Seattle, infections were most frequently detected in children less than 1 year old and gradually declined from 24 per cent in this age group to 8.5 per cent in adults over 20 years (52). In children followed from birth to five years of age in Houston 75 per cent became infected or reinfected in each of the first two years of life and 40 per cent per year of those between three and five years of age (276).

Although sex does not affect the frequency of isolation of RSV, significantly more males than females are admitted to hospital with RSV infection in the ratio 1.3 or 1.4 to 1.0 (201, 226).

Race and ethnic origin do not appear to influence the frequency or severity of RSV infection. Although a preponderance of RSV infections were found in white patients or in the infants of Hispanic origin, socioeconomic factors could not be readily distinguished from racial or ethnic influences (95, 201).

Transmission of RSV

Adult human volunteers carrying serum neutralizing antibody are readily reinfected by RSV inoculated by the nasal, nasopharyngeal or ocular routes

but oral administration is ineffective (103, 132, 153, 186). Virus is shed between 3 and 8 days after inoculation and may reach a titre of 10^4 $TCID_{50}$ per ml of nasal washing. A similar duration and degree of virus shedding has been observed in seronegative cattle infected with bovine RSV (269). The 50 per cent human infectious dose for RSV after 20 passages in tissue culture was about 10^4 $TCID_{50}$ when given by nose or eye (103). However, $10^{2.7}$ PFU of similar passaged virus infected 100 per cent of volunteers and $10^{5.0}$ PFU infected only 53 per cent in the study of MILLS and colleagues (186). The reasons for these discrepancies are not clear. Only 160—640 $TCID_{50}$ of second passage virus were required to infect 83 per cent of volunteers (153) suggesting that wild virus, unpassaged in tissue culture, may have greater infectivity for the natural host. This is true for bovine RSV. All four calves given 500 PFU of unpassaged RSV as lung washings from an infected gnotobiotic calf became infected but the same dose of the same strain of virus after three passages in calf kidney or calf testis cells failed to infect 8 calves (unpublished observations).

Under natural conditions RSV spreads readily causing a high secondary attack rate of 39 per cent in families (52) and frequent cross-infection in hospital paediatric wards (86, 102, 249). Forty-five per cent of children entering hospital with non-respiratory conditions and remaining for at least 7 days became infected with RSV. The modes of transmission of RSV in a paediatric ward were thoroughly and elegantly studied by HALL (98). Infants hospitalised with RSV infection shed virus in high titre (up to $10^{5.5}$ $TCID_{50}$) and for as long as 21 days (100). Nasal secretions from such infants remain infectious on countertops for up to 6 hours and infectious virus is recoverable from hands touching these surfaces (101). By allowing adult volunteers various limited degrees of contact with infected infants the relative importance of transmission by large particle aerosol, fomites or small particle aerosol was determined (99). Five of 7 volunteers exposed for 2—4 hours to an infected infant by caring for the baby in the usual manner became infected. Four of 10 volunteers who touched surfaces contaminated with the baby's secretions but who had no contact with the infant, became infected. Fourteen volunteers who sat at least 6 feet from the infant but touched nothing in the room remained uninfected. These results suggest that RSV spreads by direct contact with large particles or droplets and by fomites and self inoculation, but not by fine particle aerosols.

Association with Disease

The close association between infection with RSV and serious lower respiratory disease in children is strikingly illustrated by the correlation between peaks of RSV epidemics and of admissions to paediatric wards (96, 144, 194). In industrial areas of Britain the annual rate of admission to hospital with RSV infection is 24.5 per 1000 children between 1 and 3 months

old. For children in a rural environment the figure is halved (226). Among low income families in Houston the annual risk of hospitalisation with RSV infection during the first year of life is 4.7 per 1000 live births (95). Although infections occur in the first month of life, they tend to be less severe (107). The highest incidence of severe lower respiratory tract disease is in infants experiencing their first RSV infection between one and six months of age (201). The most common clinical diagnoses are bronchitis, bronchiolitis or pneumonia (144). The risk of severe RSV infection is increased by overcrowding and poor housing and possibly reduced by breast feeding (82, 218, 222). Although the incidence of severe disease decreases with repeated RSV infections, 13 per cent of children experiencing their third RSV infection still displayed lower respiratory tract signs (114). Following acute RSV bronchiolitis in infancy between 42 and 75 per cent of children experience subsequent episodes of wheezing which apparently cease by the age of 8 years (115, 221, 232, 247). Although 96 per cent of RSV infections in asthmatic children resulted in a wheezing attack (171) the exact relation between RSV bronchiolitis and asthma is unclear. Some surveys suggest a link between atopy and bronchiolitis (155, 232) others refute such an association (221, 247, 250, 275).

Mortality due to RSV has been estimated as 0.5 per cent in infected children (226) but this may rise to 23 per cent in immunocompromised infants (109) and 37 per cent of those with congenital heart disease (169). Thus, RSV is probably a significant cause of mortality in the first year of life. Virus was found in 8 of 19 infants who died with respiratory disease in Newcastle (90). Infection with RSV is associated with 3.5 per cent to 18 of cases of Sudden Infant Death Syndrome (64, 223, 279) but BRANDT and colleagues (25) do not believe that RSV plays a major role in this syndrome.

In adults, RSV infection is less serious but rarely symptomless. Although symptoms are similar to those of the common cold, RSV infections cause more severe and prolonged illness (105, 190). Young adult hospital staff working in an infants ward had impaired pulmonary function for 8 weeks after RSV infection (106). The virus has been isolated from the sputum of 2 per cent adults admitted to hospital with pneumonia (150). In immunocompromised adults RSV causes severe pneumonia or pneumonitis (139, 182, 254, 255) which is occasionally fatal (58). Fatal haemorrhagic pneumonia has been reported in a pregnant woman infected with RSV and *Staphylococcus aureus* (31). In institutions for the elderly and on geriatric wards RSV may cause outbreaks of respiratory disease which is clinically indistinguishable from influenza (178). A high proportion of patients develop bronchopneumonia (83) and there is significant mortality (91, 192, 220).

Reports that RSV antigen is present in osteoclasts cultured from Paget's diseased bone and in sections of affected tissue have led to suggestions that RSV is a cause of this chronic degenerative bone disease (183, 184).

However, other reports describe measles antigen in diseased bone (185, 224) and ultrastructural studies show that the distribution of microtubules in pagetic osteoclasts resembles that of measles nucleocapsids, but their dimensions are indistinguishable from those of RSV nucleocapsids (122). Thus, the inclusions may be those of "an aberrant or previously unidentified paramyxovirus" (251).

In British cattle, RSV infection is more significantly associated with respiratory disease than any other virus (257). Furthermore, although the data from cattle are less comprehensive than those from man, there is an indication that the highest incidence of severe disease is in animals between 2 and 4.5 months of age (Table 3).

Experimental Inoculation of Animals

A variety of different animal species may be experimentally infected with RSV (Table 4), however, clinical symptoms of respiratory disease have only been observed in large animals and primates, e.g. chimpanzees, lambs, calves, owl monkeys and cebus monkeys (11, 27, 159, 193, 227, 228). Of these, histopathological changes have been reported only for lambs, calves and cebus monkeys. In a small study in which there were no control animals, lesions in lambs, 7 to 11 days after inoculation with bovine RSV were composed of numerous small focal areas of consolidation with necrosis of

Table 4. *Experimental infections with respiratory syncytial virus*

Animal	Infection	Clinical disease	Microscopic lesions	Reference
Sheep	+	+	+	59, 159
Cattle	+	+	+	27, 269
Chimpanzee	+	+	ND	11, 193
Cebus monkey	+	+	+	11, 228
Owl monkey	+	+	ND	227
Squirrel monkey	+	−	ND	11
Rhesus monkey	+	−	ND	11
White-lipped marmoset	+	−	ND	46
Ferret	+	−	+	214
Mink	+	−	ND	46
Chinchilla	−	−	ND	46
African rat	−	−	ND	46
Guinea-pig	+[a]	−	ND	17, 46, 113
Hamster	+	−	+[b]	49, 293
Cotton rat	+	−	+	66, 213
Mouse	+	−	+	46, 210, 267

[a] Serological response and virus isolated from middle-ear

[b] Reports conflicting

pulmonary epithelial cells and accumulation of cell debris and mononuclear cells (59). In a controlled study of cebus monkeys inoculated with 10^8 PFU of human RSV, pulmonary changes consisted of interstial pneumonia with no significant bronchiolar pathology, despite the presence of viral antigen in bronchiolar epithelium as well as in alveolar cells (228). Necrosis of the bronchiolar epithelium with extensive peribronchiolar infiltration and in-

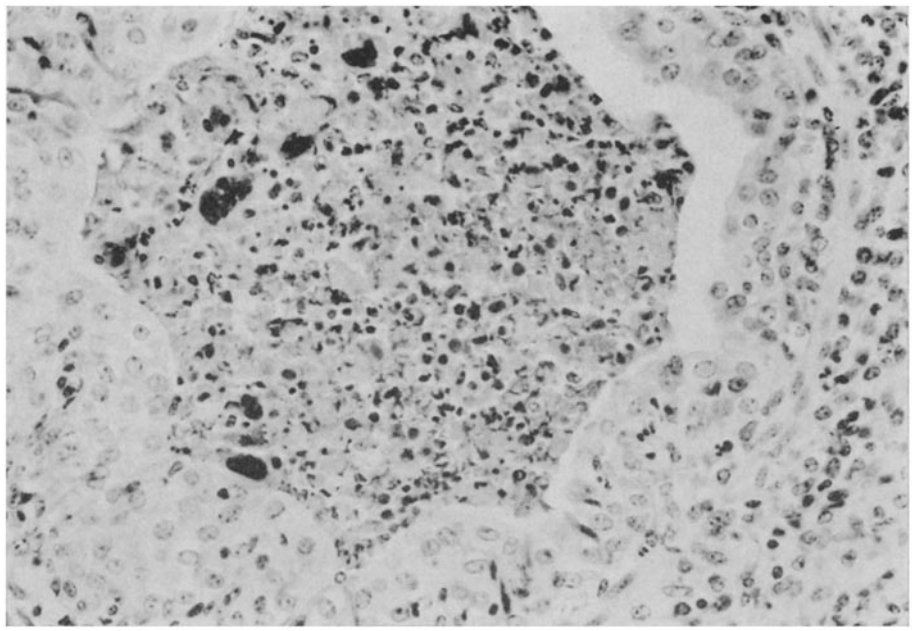

Fig. 1. Section of lung from a gnotobiotic calf inoculated 7 days previously with 4×10^5 PFU bovine RSV, showing bronchiolitis. H & E $\times 500$ (by kind permission of L. H. THOMAS)

stitial pneumonia were observed in calves inoculated with bovine RSV. In contrast, control calves had a little or no evidence of pulmonary lesions (27). With the exception of bronchiolar epithelial necrosis, similar changes were observed in the lungs of gnotobiotic calves inoculated with bovine RSV, as shown in Fig. 1 (269). In both these studies, viral antigen was detected by immunofluorescence in cells of the alveolar walls and the bronchiolar epithelium (177, 269). The histological lesion and distribution of viral antigen in these experimentally infected calves closely resembles that seen in infants with fatal RSV disease, as shown in Fig. 2 (2, 89, 259). Calves are also susceptible to infection with a human isolate of RSV (268), however, such animals failed to develop either clinical signs of respiratory disease or macroscopic lung lesions. Nevertheless, viral antigen could be detected in the lungs and there were microscopic lesions composed of

infiltration of the peribronchiolar tissue and alveolar walls by mononuclear cells (268). It is not clear if the differences in severity of pulmonary disease seen in calves given bovine and human isolates of RSV are due to the virus strain or were the result of the passage level of the virus. Thus, the human isolate of RSV (A2 strain) which produced only mild lesions in calves and lesions in cebus monkeys only after intratracheal inoculation of large

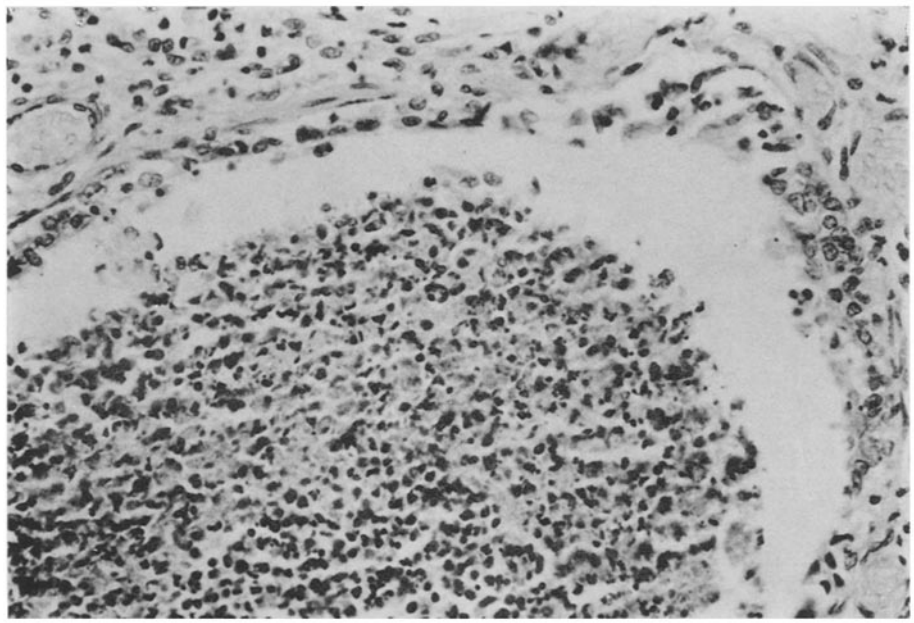

Fig. 2. Section of lung from an infant showing RSV bronchiolitis. H & E $\times 500$ (by kind permission of W. Aherne and L. H. Thomas)

quantities of virus, had undergo numerous passages in tissue culture, whereas the bovine strains of RSV which produced severe disease in calves had undergone very few passages. As already mentioned passage of bovine RSV in cell culture results in attenuation of the virus for calves (unpublished observations).

Although clinical symptoms of respiratory disease do not occur in any of the small laboratory animals examined so far, histopathological changes have been observed. After infection of ferrets with human RSV, animals developed a mild desquamative rhinitis and small foci of atelectasis were present in the lungs (214). Viral antigen was detected in nasal epithelial cells and in small foci in alveolar cells. Although ferrets do not appear to be a particularly good model for the study of the pathogenesis of RSV pulmonary disease, they do display an interesting age dependence for RSV replication in the lungs, with viral replication progressively decreasing as a function of age. The high virus titres in the lungs of infant ferrets correlates

with the severe clinical disease of early infancy in humans. The reasons for this age-related susceptibility of the lung, but not of nasal passages, in ferrets, are not known. However, a similar age dependence of RSV growth occurs in organ and monolayer cultures of ferret lung (208).

Unlike the ferrets, the cotton rat displays no age-related susceptibility to infection (213). After infection with human RSV, the histopathological changes include a moderate desquamative, exudative rhinitis, a mild proliferative bronchiolitis and some atelectasis. Immunofluorescence studies demonstrated viral antigen in the nasal epithelium and the bronchiolar epithelium, but not in the alveolar cells. Treatment of cotton rats by the immunosuppressive drug, cyclophosphamide, resulted in a prolonged infection with enhanced lung lesions (133). Pulmonary pathology in cyclophosphamide-treated animals consisted of lesions similar to that described above as well as interstitial thickening and peribronchiolar and perivascular infiltration by lymphoid cells.

Mice are also susceptible to RSV infection, and there are genetically determined differences in susceptibility which do not correlate with H-2 haplotype (210). Animals of all ages appear to be susceptible to infection (267). Following infection of BALB/C mice with human RSV, mice develop histopathological changes which include mild peribronchiolar and perivascular infiltration with lymphoid cells, some interstial thickening and formation of multinucleated giant cells (267). Viral antigen was demonstrated only in alveolar cells in small scattered foci. In contrast with the ability to recover virus from the respiratory tract of mice given human RSV, virus could not be recovered from mice inoculated with bovine isolates of RSV.

Non-respiratory infections of experimental animals by RSV have also been reported. These include encephalitis and death in suckling mice inoculated with brain-passaged RSV (36, 37), hydrocephalus in suckling hamsters and mice inoculated intra-cerebrally with RSV (154), and middle-ear inflammation in guinea-pigs (17).

Of the experimental infections of animals described, RSV in cattle appears to be the most relevant animal model for studying the pathogenesis of RSV infection. However, the need for large animal care facilities limits their widespread study. The mouse offers numerous advantages for the study of the immunology of RSV infection, since it is inbred and specific immunological reagents are readily available.

Immunity to RSV Infection

As described above, epidemiological studies have shown that although there is no solid protection against reinfection with RSV, there is an accumulative acquisition of resistance to lower respiratory tract disease. However,

RSV can be an important cause of lower respiratory tract disease in the elderly. This picture of only partial immunity to RSV infection, at least in the upper respiratory tract has also been demonstrated experimentally. Thus, adults with pre-existing serum antibody can be infected experimentally (132, 153, 186) and cattle can be reinfected within three weeks after a primary RSV infection (258). However, the duration and quantity of virus shedding is significantly less than that occurring during the first infection. Cotton rats infected with RSV developed complete resistance to pulmonary reinfection which lasted at least 18 months, whereas the nose became susceptible to reinfection by 8 months (211). This finding demonstrated that immunity to RSV infection was more durable in the lungs than in the upper respiratory tract and is consistent with epidemiological observations in man.

The relative importance of the various components of the immune response that account for this epidemiological picture of RSV infection, are not well understood.

Serum Antibody

Serum antibody to RSV can be measured by a variety of techniques, e.g. complement fixation (81), neutralization (45), enzyme-linked immunosorbent assay (229), indirect immunofluorescence (289), antibody-dependent cell-mediated cytotoxicity (241), radioimmunoassay (258), radioimmunoprecipitation assay (284), single radial haemolysis (258) and complement-dependent cell lysis (266). Using these tests, it is apparent that the serum antibody response to infection in infants less than 6 months old is often only slight (201, 202, 229, 233). This has been ascribed either to the presence of pre-existing maternal antibodies which suppress the immune response or to the immunological immaturity of the infant. Passively acquired serum antibody certainly suppresses the immune response of infants, cottons rats and calves to a parenterally administered live RSV vaccine (14, 15, 212, 300), but the effect against intranasally administered virus is not so marked (212). Further, titres of serum IgG were maintained in infants from whom virus was isolated, and these were often associated with a specific serum IgA response (199). The contribution of immunological immaturity to the poor response in infants is unclear. There are no obvious differences in the serum antibody response to RSV infection of 1 to 4 week-old gnotobiotic calves who lack maternal antibody and 6 to 7 month-old calves, in whom maternal antibody has declined to undetectable levels (unpublished observations). However, Ward and co-workers (284) failed to find antibody responses to the large GP in infants less than 12 months of age. They suggested that this was due to the poor immunogenicity of this viral antigen in infants and could be explained if the antibody response was restricted to the IgG2 and IgG4 subclasses since these isotypes respond poorly in infancy. However, IgG4 antibody to RSV has been detected in sera from children hospitalised

with RSV infection but the number of children studied was small and their ages not reported (28). It is also possible that this polypeptide is poorly immunogenic, irrespective of age, and multiple exposures are required to induce antibody. Alternatively, the large GP of the RSV strain used in their assay may be antigenically distinct from that of the strain which infected the infants.

Following a primary infection, antibody declines to low or undetectable levels within a year of infection (289). After a second infection, higher titres of antibody are produced and the antibody persists longer than after a primary infection (114, 289). Thus, there is a pattern of gradually increasing levels of serum antibody with successive infections which parallels the accumulative acquisition of resistance to illness.

There is conflicting epidemiological evidence on the role of serum antibody in resistance. Thus, most severe disease frequently occurs within the first few months of life when infants possess moderate levels of maternal antibody (26, 201). In such children, neither complement-fixing (CF) nor neutralising serum antibody appear to correlate with either resistance to infection or severity of disease. Further, significant CF and neutralizing antibodies induced by vaccination failed to protect against either infection or disease (85, 138, 148). On the other hand, there is evidence that maternal antibody may provide some protection. Thus, there is a relative sparing from bronchiolitis and pneumonia in infants less than 3 weeks old, in whom maternal antibody levels are highest, and symptoms are mainly confined to the upper respiratory tract (107, 195, 201). Furthermore, such infants shed less virus than older children (107). Even in older babies, the possession maternal antibody appears to reduce the quantity and duration of virus shedding (138). Other studies have shown that there is a correlation between the level of maternal neutralising antibody at birth and the age at the time of infection. In addition, the severity of RSV pneumonia was inversely related to the level of pre-existing neutralizing antibody (95, 158) and the mean level of maternal antibody was lower in RSV-infected infants than in symptomless or uninfected cohorts (39, 95, 199, 284). In a study of non-hospitalised children, over a number of years, there was further evidence of a relationship between serum neutralising antibody and resistance to RSV infection (74).

Although these observations suggest that maternal antibody can protect against RSV infection, the correlations between levels of maternal antibody and protection are imperfect and severe disease does occur in the presence of moderate levels of antibody. Furthermore, differences in mean maternal antibody levels in protected and infected infants are usually only 2 to 4 fold. It may be that serum antibody is not responsible for this protection and other maternal immune factors are important. For example, if the mother herself is protected from reinfection then a major source of infection for

the baby is removed; immunity may be transferred via breast-feeding or the transplancental transfer of lymphocyte sensitisation. However, if serum antibody is protective, then the protective antibody may not be detected using the available serological techniques or a critical level may be required to provide protection. Attempts to correlate levels of RSV antibody in cord sera by techniques other than neutralisation e.g. membrane fluorescence (199) or radioimmunoprecipitation of RSV polypeptides (284) have yielded similar results to that seen using neutralisation tests and there is still overlap of antibody levels in patients with and without RSV illness.

In order to define the role of circulating antibody in RSV infection, the ability of passively transferred antibody to protect experimental animals from RSV infection has been examined. Serum containing high titres of neutralising antibody, obtained from previously infected ferrets, administered to infant ferrets by either the oral or intraperitoneal route, failed to protect them against RSV infection (260). In contrast, passively transferred serum from previously infected cotton-rats conferred almost complete protection against virus replication in the lung of cotton-rats (211). A mouse monoclonal antibody (MAB) to the large GP and another to the F glycoprotein, given intraperitoneally to cotton-rats also protected the lung against virus replication (283). In both these studies the transferred antibody had little or no effect on virus replication in the nasal passages. Other MABs to the two surface glycoproteins also protected mice from RSV infection in the lungs (266). Thus, serum antibody can protect the lung from RSV infection but probably has little protective effect in the nose. Serum antibody probably gains access to the alveolar or bronchiolar regions of the respiratory tract as a result of diffusion before infection and/or exudation after infection. However, the mechanism of protection is unclear. As discussed above, epidemiological evidence suggests that protection may not be mediated by neutralisation, and there is support for this from other studies. Thus, in a comparison of an inactivated vaccine with two live vaccines given parenterally to calves, the inactivated and one of the live vaccines stimulated similar levels of neutralising antibodies against the challenge virus, but only the inactivated vaccine provided complete protection against RSV infection (258). A similar level of protection against challenge induced by the two live vaccines occurred despite very different levels of neutralising antibody to the challenge virus. In addition, although the MABs to RSV glycoproteins, which passively protected cotton rats, had high virus neutralising titres in the presence of complement (283), a more extensive study of a number of different MABs to the 2 glycoproteins, showed that there was no correlation between virus-neutralising activity and protection (266).

Antibody may be protective by mediating complement-dependent lysis (CDCL) of virus-infected cells. Certainly the most protective MABs possessed CDCL activity. However, the correlation between protection and CDCL

activity was not complete and one MAB to the F glycoprotein which was completely protective *in vivo* and mediated CDCL *in vitro*, was also completely protective in C5-deficient mice (unpublished observations).

Another possible mechanism of action could be antibody dependent cell-mediated lysis (ADCC) of virus-infected cells and this possibility is under investigation in mice. ADCC against RSV-infected cells has been demonstrated with human peripheral blood lymphocytes (PBL) and human sera, colostra and nasal secretions (57, 143, 180, 241), and with owl monkey PBL and sera (152), but attempts to demonstrate ADCC using bovine antibody to RSV and a variety of bovine leukocytes have failed so far (unpublished observations). In man, there is no correlation between ADCC antibody and neutralising antibody. Thus, following a primary RSV infection ADCC antibody rose and fell more rapidly than neutralising antibody. Secondly, neutralising antibody could be detected in the absence of ADCC antibody in children given an attenuated vaccine. Thirdly, whereas the neutralising antibody response was enhanced after a secondary infection, the ADCC antibody response was no greater than on initial infection (180). These differences could be due to differences in the IgG sub-class or to differences in the persistence of the antigen responsible for inducing each type of antibody response. It would be interesting to know if resistance correlates better with ADCC antibody in cord or acute phase sera than it does with neutralising antibody. Presumably babies born to mothers who had undergone a recent RSV infection would receive a greater maternal gift of ADCC antibody than those born to mothers who had not had a recent infection.

The mode of action of protective antibody is dependent not only on those biological properties which are dependent on its Ig isotype, but also on its antigenic specificity. Thus, antibodies to the F protein may mediate protection by inhibiting cell fusion and release of infectious virus (181, 281), and antibodies to the large GP may act by neutralisation of virus (282). In this respect it is interesting that the two MABs to the F protein which completely protected mice against infection, were the only ones which reacted with one of the four or five epitopes identified on this viral polypeptide (256, 266). The protection conferred by these MABs was dose-related, indicating that a critical level of antibody may be important for protection (266).

It seems therefore, that serum antibody can protect the lung against RSV infection, but that neutralising antibody may not be the most important in mediating protection. The observation that MABs to one particular site on the F protein will completely protect mice against RSV challenge, suggests that serological tests may need to be devised which will detect antibodies to distinct epitopes on RSV polypeptides in order to measure levels of protective antibodies. Further, if other effector mechanisms (e.g.

complement, leukocytes) are required to interact with antibody, then knowledge of the Ig subclasses involved may also be important.

Local Antibody

As mentioned previously immunity to reinfection of the nasal passages appears to be less durable than pulmonary immunity. The reasons for this are not clear. Serum antibody may contribute to pulmonary immunity to a greater extent than to nasal immunity (211, 283) and local antibody may be of greater importance in the nasal passages. In a study of experimental infection in adult volunteers, high levels of nasal neutralizing antibody appeared to prevent extensive infection and upper respiratory tract illness, regardless of the level of serum antibody (186), suggesting an important role for locally produced neutralizing antibody in resistance. Experimental infection of previously-infected calves has also indicated a role for local antibody in resistance (187). Significant increases in the neutralizing activity of nasal secretions have been detected following natural infection (147, 238, 239) and experimental infection with a ts mutant of RSV (145, 146). However, Kim *et al.* (146) found that a large proportion of infants less than 6 months old, who were born after the last outbreak of RSV, possessed neutralizing activity in nasal secretions. Further, McIntosh *et al.* (173) observed that, following natural RSV infection, neutralizing activity in nasal secretions from infants fluctuated widely and levels bore no relationship to either onset of symptoms or cessation of virus shedding. In addition, there was no correlation between neutralizing activity and Ig-class specific RSV antibodies, as measured by indirect immunofluorescence, in nasal secretions. Although these findings suggest that non-specific inhibitors of RSV are present in nasal secretions, some neutralizing activity may be due to IgA antibody as neutralizing activity has been removed by absorption of nasal secretions with anti-human IgA (239).

As mentioned above, IgG, IgA and IgM antibodies to RSV have been detected in nasal secretions using the indirect immunofluorescence test, and there is an accelerated production of all three classes after reinfection (173, 174). Preceding their detection in secretions, immunoglobulins of all three classes have also been observed bound to exfoliated RSV-infected nasal epithelial cells, with IgA-coated cells predominating (88, 142, 174). The reasons for the predominance of IgA-coated cells at a time when the predominant antibody in the secretions was IgG is unclear. It has been suggested that the IgA antibody blocked the attachment of IgG (174) or that IgG-coated cells had been lysed by ADCC (57). However, it is possible that some of the IgA may be present on exfoliated epithelial cells merely by virtue of the secretory route for this Ig. Further, problems in measuring RSV-specific IgA antibody in bovine nasal secretions have been encountered, due to non-specific binding of IgA to RSV antigen (unpublished observa-

tions); a similar reaction may also occur in human nasal secretions. The apparance of a local antibody response correlated with the elimination of virus from the nasal secretions (173, 174). The poor correlation between Ig-class specific antibodies measured by immunofluorescence and neutralizing activity suggests that if the local antibody is mediating the clearance of virus from the nasal secretions then it is by a mechanism other than virus neutralization. An increase in antibody in nasopharyngeal secretions capable of mediating ADCC has been demonstrated (57, 143). This rise in ADCC activity in nasopharyngeal secretions correlated with the increase in Ig-class specific RSV antibody and with recovery from infection. ADCC in nasal secretions appeared to be mediated primarily by IgG. Unlike ADCC antibody in serum, that in nasal secretions increased with repeated infection (143). This discrepancy may be due to differences in the quantitation of ADCC antibody. Thus, the level in serum was determined by an endpoint titration, whereas that in nasal secretions was expressed as per cent lysis (143, 180).

Lysis of virus-infected cells by antibody and complement may be another important mechanism of resistance. Complement components have been observed bound to RSV-infected nasopharyngeal epithelial cells, generally in association with cell-bound Ig (142). The occasions when complement was detected in the absence of Ig may be due to activation of complement by RSV alone (253). Alternatively, the goat antiserum used to detect complement may contain RSV antibodies. This suspicion is increased by the observation that nasopharyngeal cells from patients with other virus infections failed to show fixation of complement, although cell-bound Igs were present (142). However, if complement is activated in RSV infection, this could lead to enhanced virus clearance not only by CDCL, promoting phagocytosis and neutrophil-mediated cytotoxicity (140), but also by lysis of RSV-infected cells directly (253, unpublished observations).

Antibody bound to infected cells may also inhibit cell fusion and release of infectious virus as described in the previous section on serum antibody.

None of these observations, however, explain the greater susceptibility to reinfection of the nasal passages compared with the lower respiratory tract. McIntosh *et al.* (173) postulated that the IgA antibody produced during an infection may have a low affinity but a high specificity. Thus, the local immune defences could be overwhelmed by strains of RSV exhibiting minor antigenic variations similar to those described by Coates *et al.* (45). Presumably, more cross-reactive immune responses prevent lower respiratory tract disease.

Cell-Mediated Immunity (CMI)

Because of the poor correlation between neutralizing antibody and resistance, attention has turned to the role of CMI in RSV infections. CMI to RSV has been detected using the lymphocyte transformation (LT) test

(149, 240), leukocyte migration inhibition test (21) and the development of cytotoxic lymphocytes (261, 262, unpublished observations).

An LT response to RSV has been demonstrated in approximately 40 per cent of healthy adults (237, 243) and in approximately 70 per cent of infants hospitalised with RSV infection, during the two months following acute illness (56, 240). The earliest time for detection of an LT response appeared to be 1 to 2 weeks following admission to hospital (242). A similar development of an LT response has been observed in experimentally infected owl monkeys (152), and in gnotobiotic calves experimentally infected with bovine RSV (unpublished observations). Attempts to demonstrate an LT response in naturally infected calves have failed so far. The LT response in children and calves was mediated by T lymphocytes (240, unpublished observations). LT responses in RSV-infected infants, appeared to be related to the age of the patient or the severity of disease (240, 287). However, this has not been confirmed in later reports (56, 242), and there are no reports of a comparison of the LT responses of hospitalised and non-hospitalised infants infected with RSV. Although there was no correlation between LT responses and seroconversions (242), an LT response was detected more frequently than a CF antibody response in infants less than 6 months old (240), suggesting that maternal antibody may not affect the LT response. There is evidence from studies in calves given an inactivated RSV vaccine, that the LT response is not greatly influenced by the level of pre-existing maternal antibody at the time of vaccination (unpublished observations).

There is little information on the relationship of the LT response to resistance to infection. Infants vaccinated with a formalin-inactivated RSV vaccine developed an LT response, significantly greater than that observed in infants infected naturally with RSV, but they were still susceptible to RSV infection (149). Further, FERNALD *et al.* (74) found that although there was a higher infection rate in children with low levels of LT, than in those with high levels of LT, the differences were not significant and there was no enhanced effect of CMI on protection associated with serum antibody. In contrast, the LT response does appear to correlate with resistance induced by an inactivated RSV vaccine in calves (unpublished observations). However, the relative contribution of CMI and antibody to resistance in these studies has not yet been defined, although resistance does not correlate with neutralizing antibody (258).

Leukocyte migration inhibition factor has been demonstrated in serum from 17/25 children during the acute phase of RSV-bronchiolitis but in only 5/25 of the children 2 weeks later (21). The significance of this observation is unclear as there was no information on the specificity for RSV, or whether the serum factor was a T-cell product.

Leukocytes capable of lysing RSV-infected cells, but not uninfected cells, have been demonstrated in the lung and, to a lesser extent, in the

mediastinal lymph node of RSV-infected cotton rats (262). The cytotoxic activity peaked 5 days after RSV inoculation, was neither virus-specific nor MHC restricted and was associated with both adherent and non-adherent lung cells. There was a good correlation between the appearance of these cytotoxic leukocytes in the lung of normal and irradiated cotton rats, and clearance of RSV, suggesting a role for these cells in recovery from RSV infection (261, 262). The characteristics of the cytotoxic response were similar to those reported to be mediated by macrophages and natural killer (NK) cells in other animal models (22, 43, 297). However, the demonstration that RSV is a poor inducer of interferon in the respiratory tract (104, 170) and that interferon is a potent inducer of NK activity *in vitro*, may mean that NK-mediated lysis of RSV-infected cells is not an important mechanism of resistance. Nevertheless, NK activity can be induced by viral glycoproteins without the need for interferon (32, 33). There is evidence that NK cells may not contribute to recovery from RSV infection in mouse lungs, to any great extent. Thus, despite the fact that nude mice have high levels of NK activity, even in the lung (168), they were unable to clear RSV from the lungs (unpublished observations). Thus, RSV was cleared from the lungs of normal BALB/C mice by day 11 (267), but virus was still present in high titres in the lungs of nude mice 21 days after inoculation. This contrasts with the observations of WYDE *et al.* (298), who found that RSV had been cleared from the lungs of nude C3H mice by day 7 of infection. However, virus not replicate in the lungs of immunologically normal C3H mice. These discrepancies may be due to differences in levels of NK activity in C3H and BALB/C mice (7), so that although defence mechanisms in nude mice were initially inadequate to prevent RSV infection, virus infection induced immune responses, perhaps NK cells, in C3H mice more readily than in BALB/C mice. Extrapulmonary dissemination was not observed in either mouse strain. NK activity could have prevented spread of virus from the lungs, or, alternatively, RSV may be unable to replicate in mouse tissues outside the respiratory tract.

Persistence of RSV in the lungs of nude, BALB/C mice suggests that cytotoxic T cells may be important in recovery from infection. Preliminary studies, demonstrated that the lungs of RSV-infected mice developed cytotoxic lymphocytes capable of lysing RSV-infected, but not uninfected cells (unpublished observations). The cytotoxic activity was maximal 7 to 9 days after infection, was virus specific and MHC-restricted, suggesting that the cells were cytotoxic T lymphocytes (Tc). Furthermore, the increase in Tc activity in the lungs occurred in the absence of any marked increase in NK activity, as measured by lysis of YAC-1 cells. There is much evidence which shows the importance of Tc in recovery from a number of virus infections, in particular influenza in mice (1, 167). Additional studies in man on Tc memory to influenza virus have shown that in individuals with

no serum antibody, those with high Tc responses subsequently shed little or no virus from the nasal passages after challenge with live influenza A. In contrast, volunteers with low levels of Tc immunity all shed virus (176). These findings suggested that Tc could protect against infection by contributing to early recovery. Following influenza infection in man Tc memory is not life-long, but declines with an approximate half-life of 2 to 3 years (175). If, as seems likely from the mouse studies, Tc to RSV are important in man, then the decline in Tc memory may contribute to risk of reinfection. Such a phenomenon could contribute to the high incidence of bronchopneumonia seen in some outbreaks of RSV infection amongst the elderly, especially if they have been isolated from the rest of the community for some time.

Evidence that Tc are involved in recovery from RSV infection in man is limited. A peribronchiolar and perivascular infiltration of lymphocytes has been observed in the lungs of infants with fatal RSV disease. Children with immunodeficiency diseases or on immunosuppressive therapy suffer severe symptoms, prolonged RSV excretion and occasional extrapulmonary dissemination of virus (55, 79, 109). However, circulating and local antibody responses are suppressed as well as CMI in such children. Prolonged virus shedding and more severe lung lesions have also been observed in calves and cotton rats treated with immunosuppressive drugs (133, 268). Studies in immunosuppressed, experimentally infected animals together with a selective restoration of their immune responses may help to elucidate the relative importance of the various components of the immune response in recovery from RSV infection.

Role of Breast-Feeding

Some epidemiologic studies have suggested a beneficial effect of breast feeding in respiratory syncytial virus infections (65, 94, 222), but the evidence is conflicting (264). Studies on the effects of suckling on RSV infection in ferrets and cotton-rats also indicate a beneficial effect (211, 260). However, in contrast to man, infant ferrets and cotton rats acquire the majority of antibody via colostrum and milk. Nevertheless, both these animal models have revealed the presence of a protective factor in the products of lactation which appears to be absent or deficient in serum. Thus, although protection in suckled infant ferrets correlated with maternal serum neutralizing titre, passive administration of immune ferret serum failed to protect (260). Resistance in infant cotton-rats also generally correlated with serum neutralizing antibody levels, however some infants were identified who displayed complete pulmonary immunity despite a very low or undetectable level of serum neutralizing antibody, and others who were susceptible but had high levels of antibody (211). It has been suggested that protection by the products of lactation are due to secretory antibody or to a transfer of lymphocyte

sensitization to RSV. Neutralizing activity and specific IgA and IgG antibody to RSV have been detected in the colostrum of the majority of women (65, 270). However, specific antibody was usually undetectable in milk after the first week of lactation. Although high neutralzing titres correlated with high levels of IgA antibody, some of the neutralizing activity was due to non-specific inhibitors of RSV (270). Because of the low levels of antibody after the first week of lactation, it is difficult to explain how colostral antibody alone is responsible for the protection against RSV illness afforded by breast-feeding. A lymphocyte transformation response to RSV has been detected in about one third of colostral samples and this response did not correlate with levels of specific IgA or IgG antibody in either colostrum or serum (243). It is possible, therefore, that this RSV-specific cellular reactivity is transferred to the infant, where it could play a role in protection against RSV-infection. Further work is required to determine if breast-feeding in man is as important in protection as it is in ferrets and cotton-rats, and further, what the mechanisms of such protection are.

Pathogenesis of Disease

Any theories on the pathogenesis of RSV infection have to explain the rather unique age-distribution of the severe disease. Several observations have led to the suggestion that RSV disease is the result of an immunopathological reaction. Thus, most severe disease occurs at a time when specific antibody, of maternal origin, is present. Further, recipients of a formalin-inactivated RSV vaccine, which induced high levels of serum antibody, were not protected from infection, and in fact, developed more severe disease than did their unvaccinated counterparts (85, 138, 148). The disease exhibited by the vaccinees was no different from severe natural disease, except that it occurred in children who were older than usual. In the light of this experience with inactivated RSV vaccine, a common mechanism of pathogenesis which explains the age distribution of naturally occurring severe RSV disease and the vaccine associated disease has been sought. Although certain aspects of pathogenesis may be similar there is no firm evidence for a common pathway. Therefore, theories of pathogenesis relating to the inactivated vaccine will be dealt with in the section on Vaccines and Chemotherapy.

Several theories of the pathogenesis of RSV disease have been proposed, based on the immunological immaturity of the infant, a role for serum antibody, a delayed hypersensitivity-type reaction, an IgE-mediated reaction and non-immunological mechanisms. These theories have been reviewed in detail elsewhere (172) and, therefore, only recent findings will be discussed here.

Immunological Immaturity

The observation that an IgG response to the large glycoprotein could not be detected in infants between the age of 6 and 12 months contrasts with their ability to produce antibodies to the nucleoprotein and the fusion glycoprotein (284). As mentioned previously, this could be due to the poor ability of infants to mount IgG2 and IgG4 antibody responses which tend to be induced by antigens with carbohydrate moieties. However, how this relates to the pathogenesis of RSV is unclear, especially as antibodies to the fusion glycoprotein are probably more important in limiting virus infection than are antibodies to the large glycoprotein (181). Furthermore, in order to explain the unique age distribution of RSV disease, RSV would have to be different from paramyxoviruses and myxoviruses in the contribution which carbohydrate moieties make to the antigenicity of the glycoproteins.

Role of Serum Antibody in RSV Pathogenesis

CHANOCK and colleagues (39) suggested that disease in young infants could be a type III hypersensitivity reaction resulting from the interaction of serum antibody with virus. However, neither the levels of serum antibody nor of circulating haemolytic complement correlated with the severity of disease in naturally infected infants (3, 26, 158, 201). Although small amounts of Ig were demonstrable in sections of lungs from infants who died with RSV bronchiolitis (89) there was no deposition of C3 (39). Furthermore, the relative frequency of Ig and complement attached to nasopharyngeal cells in various forms of clinical disease or age groups was no different (142).

Another possible explanation is that ADCC might cause the observed inflammation and tissue damage without complement activation. However, neither ADCC antibody in serum or secretions correlated with the form of clinical illness (143, 180).

Experiments in animal models also failed to demonstrate a pathogenic potential for serum antibody to RSV. Thus, passive immunisation of cotton rats and mice with various MABs to RSV did not enhance the severity of the lung lesions (266, 283).

RSV immune complexes interact with neutrophils, *in vitro*, and induce superoxide, thromboxane B_2 and other factors which are capable of causing bronchoconstriction (73, 141). Further, neutrophils from children with RSV infections are metabolically hyperactive *in vitro* (246). It is possible, therefore, that local antibody, rather than serum antibody, may lead to immune complex formation in the respiratory tract. The interaction of such immune complexes with neutrophils could then contribute to the pathogenesis of RSV disease.

Antibody could also contribute to the pathogenesis of disease by enhancing virus shedding. Thus, passively acquired antibody could block the

induction of Tc, which may be important in recovery from infection, as seen in influenza virus infections (161). RSV infectivity may be enhanced by antibody as demonstrated for dengue virus and other Flaviviruses (111, 207). Such a reaction has, in fact, been demonstrated for RSV using mouse MABs and mouse macrophage cell lines (unpublished observations). However, there was no evidence that any of the MABs enhanced virus shedding or disease in passively immunised mice (267).

Role of Delayed Hypersensitivity in RSV Pathogenesis

WELLIVER *et al.* (287) proposed that a CMI reaction may contribute to the pathogenesis of certain clinical forms of naturally occurring RSV disease. This was based on their observations that children with RSV bronchiolitis and asthma had an increased LT response to RSV in the acute phase of illness. In addition, patients who later developed a high degree of LT were more prone to subsequent episodes of wheezing than were infants who developed only minimal LT responses. This exaggerated LT response may contribute to bronchospasm in RSV infections and even in response to other antigens subsequent to infection since it has been shown that lymphocytes stimulated with viral antigens release soluble factors which enhance antigen-stimulated basophil histamine release (127).

The reasons for the exaggerated LT response in some patients with RSV infection is unclear. A number of explanations have been proposed. Thus, it may be due to prior sensitisation by either a previous infection, prenatal exposure of foetal lymphocytes to RSV antigen, transplacental transfer of reactivity or acquired from products of lactation. Alternatively, it could be the result of an imbalance in immuno-regulatory mechanisms modulating T-cell responses. Prior sensitization is unlikely to be due to a previous infection since the temporal occurrence of bronchiolitis during an RSV epidemic does not corroborate this suggestion. Furthermore, most infants with bronchiolitis do not show an anamnestic antibody response to RSV (24, 233, 240). An LT response to RSV has been demonstrated in cord blood (242, 243, 245) and in colostrum (243). Further work is required to determine whether cellular reactivity to RSV in cord blood or colostral samples is either acquired by, or persists for any length of time in, the infant, and if it affects the outcome of subsequent RSV infection. There are other studies, in fact, which have failed to demonstrate any relationship between LT responses to RSV and either the age of the patient or the severity of illness (56, 242).

Role of IgE in RSV Pathogenesis

The distribution of RSV antigen was patchy and not very extensive in the lungs of infants with RSV bronchiolitis (89, 259), contrasting with the widespread distribution of viral antigen in the lungs of infants with pneu-

monia. This finding led to the suggestion that bronchiolitis may result from an IgE-mediated (type I) hypersensitivity reaction (89). Thus, a prior RSV infection may have led to the production of IgE antibody and little or no immunity and on reinfection, severe disease occurs. In support of this theory was the demonstration of neutralizing activity in acute nasal secretions of infants with RSV bronchiolitis (238, 239). It has been shown, however, that such activity is probably due to non-specific inhibitors of RSV (173). Furthermore, as mentioned previously epidemiological evidence, does not support the theory that disease only results after a prior sensitizing RSV infection.

Nevertheless, there is evidence that IgE-mediated release of pharmocologically active mediators occurs in RSV infections. Welliver and colleagues (288, 290) detected IgE bound to RSV-infected nasopharyngeal cells and measured RSV-specific IgE antibody in nasal secretions. They demonstrated that the frequency of appearance of RSV — specific IgE, and peak titres reached, were statistically significantly greater in patients with RSV disease associated with wheezing than in those RSV infections free from wheezing. This finding also corresponded with levels of histamine in nasopharyngeal secretions. It was suggested that an exaggerated IgE response to RSV may occur in some individuals due to an abnormal immunoregulatory mechanism. These findings may also explain the development of recurrent episodes of wheezing seen after RSV bronchiolitis in infancy (171, 232). Elevated levels of IgG4 antibodies to RSV have been demonstrated in acute phase sera from infants hospitalised with RSV infection (28). This class of antibody is also capable of mediating anaphylaxis, and it has been suggested that the swift IgG4 antibody response seen in these patients could contribute to the bronchospasm of RSV infection.

Role of Non-Immunological Mechanisms

A further theory for the pathogenesis of RSV bronchiolitis may be based on purely physical and mechanical considerations. The diameter of the airways in a baby's lung is half that in an adult and conductance reaches its lowest level at one month of age (236) when bronchiolitis becomes a major problem. Into these narrowed airways there enters a virus which characteristically generates on the surface of infected cells, filaments which may extend up to 10 μm into the lumen, a property not shared by other respiratory viruses of man (203). Such a theory would account for the unique ability of RSV to cause bronchiolitis primarily in 2 to 6 month-old children and cattle, but filamentous obstruction of bronchioles has not yet been seen in post-mortem material.

Vaccines and Chemotherapy

Any successful vaccine against RSV must overcome two unique problems presented by the epidemiology of this virus. First protection must be

provided for infants and cattle between 2 and 6 months of age and therefore vaccine must be administered to neonatal subjects who usually possess high levels of maternal antibody or to mothers to boost the passive transfer of antibody. Secondly, since natural infection does not completely protect against reinfection, any successful vaccine must provide immunity which is superior to that resulting from infection. Similarly, successful chemotherapy must carry low toxicity because the highest risk group is so young.

Vaccines

Three types of vaccine against RSV have been tested. Inactivated antigen combined with adjuvant has been given intramuscularly. Live attenuated viruses have been inoculated intranasally and finally, live modified virus has been injected intramuscularly. Each of these approaches has particular advantages and problems but none has yet provided generally acceptable immunization against RSV.

The earliest RSV vaccine consisted of the MK5 human strain grown in grivet monkey kidney cells, inactivated with formalin and concentrated 5- to 25-fold by adsorption to alum adjuvant. When this material was injected intramuscularly into 5 to 11 year old children their geometric mean neutralizing antibody titre increased two-fold and four seronegative children showed significant rises in titre (209). A field trial of this antigen combined into a heptavalent vaccine was conducted in 407 children aged between 3 and 5 years. Of the 199 children who received these doses of vaccine 35 per cent were initially seronegative and 20 per cent of these showed a significant response to vaccine. During the first ten weeks of the study RSV was isolated from 28 children and there was a 36 per cent reduction in severe respiratory disease (285). However, it is not possible to conclude that protection was due to the RSV antigen because of the additional 6 respiratory viral and mycoplasmal antigens in the vaccine.

A similar vaccine derived from the Bernett strain of RSV and grown in vervet monkey kidney cells was also formalin-inactivated and combined with alum adjuvant after one hundred fold concentration. This vaccine was given intramuscularly to children between 2 months and 10 years of age in four independent trials (44, 85, 138, 148). The vaccine was highly antigenic inducing significant rises in complement fixing antibodies in 96 to 100 per cent of seronegative recipients and in 68 to 91 per cent of all children who received three doses of vaccine. Rises in neutralizing antibody to the vaccine strain of RSV were detected in 43 per cent of vaccinees (148) and to local RSV isolate in 98 per cent of children (138). Despite these impressive antibody responses there was no evidence that the vaccine protected against RSV infection. In three tirals RSV attack rates per 100 children studied were 65, 45 and 8 respectively after RSV vaccine and 53, 29 and 5 respectively after parainfluenza virus vaccine (44, 85, 148). However, the most alarming

finding in all four trials was that the RSV vaccine significantly enhanced the frequency and severity of lower respiratory disease during subsequent infection in infants between 6 and 23 months old, but not in older children. One vaccinee required prolonged assisted ventilation and two vaccinated infants died with RSV in their lungs (85, 148).

Formalin-inactivated RSV combined with either Freund's incomplete adjuvant or aluminium hydroxide induced high levels of neutralizing antibody in seronegative calves, but not all vaccinated animals were protected against challenge (131, 188). Bovine nasal mucosa cells persistently infected with RSV, fixed with glutaraldehyde and administered intramuscularly combined with Freund's incomplete adjuvant completely protected eleven of twelve calves against intranasal challenge with RSV (258). Nasal antibody was not detected in any of these calves but there was RSV antibody of the G_1 subclass in lung washings. Field trials of this antigen in over 250 calves have shown a significant reduction in RSV-associated disease and no evidence of undesirable side effects (unpublished observations).

The absence of vaccine-associated disease in early trials with formalin-inactivated RSV may be due to low antigenicity of the vaccine and the use of older children. At the time of the tirals three hypotheses were advanced to explain vaccine enhanced disease. First, serum antibody in the absence of nasal antibody was immunopathogenic (44, 138, 148). Secondly, the parallelism with results of inactivated measles vaccination suggested altered reactivity to killed virus (85, 138, 148). Thirdly, delayed hypersensitivity induced by the vaccine caused untoward reactions.

The first hypothesis was attractive because it could also explain the epidemiology of RSV if maternal antibody was found to contribute to RSV bronchiolitis. However, as discussed above, there is no firm evidence which implicates maternal or vaccine-induced serum antibody in the pathogenesis of RSV.

The second hypothesis has been considerably developed for inactivated measles vaccine where an effect on fusion protein is demonstrable. Antibodies to the HN glycoprotein of another paramyxovirus, SV-5, prevent spread of infection by neutralizing released virus particles, but do not prevent spread by cell fusion. In contrast, antibodies to the fusion glycoprotein prevent spread of infection by both cell fusion and released infectious virus (181). These findings together with the observation that formalin appears to denature the fusion glycoprotein of measles virus, so that following vaccination with formalin-inactivated measles virus little or no antibody to the fusion glycoprotein was induced, offered an explanation for the severe complications that sometimes occurred in vaccinees subsequently infected with measles virus (198). Thus, in the absence of antibodies to the fusion glycoprotein, measles virus still spreads by cell fusion in a vaccinated

individual. The viral antigen produced would stimulate an anamnestic response to those antigens which provided the primary response. This could lead to the formation of immune complexes which activate complement and cause tissue damage and inflammation. A similar sequence of events, could explain the severe illness seen in children given the formalin-inactivated RSV vaccine. However, there is no evidence that formalin-inactivation of RSV denatures the fusion glycoprotein. In fact, calves vaccinated with formalin-inactivated RSV developed a good antibody response to the fusion glycoprotein as measured by a competition binding assay against MABs (unpublished observations). Furthermore, protective MABs to fusion glycoprotein bind to RSV antigen even after fixation with formalin (unpublished observations).

The third hypothesis that delayed hypersensitivity caused vaccine reactions was further encouraged by the finding of sensitized lymphocytes in vaccinated children (149). A mechanism by which this might operate is suggested by work on influenza virus in mice. Inactivated 'flu virus, and Sendai virus with inactive fusion protein, stimulate little or no cytotoxic T-cells (Tc) *in vivo*, although antibody and other sub-sets of T-cells are stimulated. T-cells which are stimulated include suppressor cells (Ts) which suppress Tc, and cells (T_D) which mediate IA region-restricted delayed hypersensitivity responses. Whereas passive transfer of 'flu virus specific Tc resulted in virus clearance and protection from pneumonia in mice passive transfer of IA region-restricted T_D, specific for 'flu virus, not only failed to protect against infection, but resulted in enhanced mortality due to 'flu pneumonia when the mice were challenged with homologous virus (1). The lethal effects of these T_D cells can be abrogated by a class of Ts, which do not affect the antibody response, and are only induced by infection of mice with live 'flu virus (166). Thus, the delayed-type hypersensitivity response which is apparently of little or no use to the host against virus infection and can, in fact, induce pathological lesions, is effectively suppressed following infection with live virus by the stimulation of Ts. It is possible that children whose primary exposure to RSV was inactivated virus developed an antibody response, which did not protect against infection, and a T-cell response which included T_D, which contributed to the lung lesions on subsequent infection. In contrast, those children whose primary exposure was to live virus developed protective T-cells, i.e. Tc which enhanced virus clearance and Ts to suppress the harmful T_D response. On vaccination with inactivated virus, a secondary response may occur in the Tc and Ts cells, again suppressing the development of a harmful T_D response. This could explain why severe RSV disease occurred primarily in vaccinees below 2 years of age and not in older vaccinees who were more likely to have had a natural exposure to RSV prior to vaccination. Incidentally, the presence of a large percentage of defective, fusion-negative particles may also induce

IA region-restricted T_D lymphocytes (72). Thus, the presence of defective interfering particles of RSV (274) could contribute to natural RSV disease by stimulating T_D and suppressing protective Tc in a way analogous to that described above.

Primarily as a result of the undesirable reactions associated with killed vaccines, attempts were made to develop attenuated vaccines which could be given intranasally to stimulate both local and humoral antibody. The first of these was a cold adapted virus derived by passage of the A2 strain of RSV at 26° C. This virus possessed decreased infectivity and virulence in adult volunteers (84). However, in infants who lacked prior experience of RSV, the cold-adapted strain induced mild lower respiratory tract disease (146).

A series of temperature sensitive (ts) mutants of RSV were then derived using 5 fluorouridine as mutagen. After evaluation *in vitro* and *in vivo* in hamsters, one mutant ts-1, was selected for further investigation. The ts-1 mutant was restricted in a late function at 37° C and grew to titres of 10^3 PFU/g in the nasal turbinates, but not in the lungs of hamsters (92, 293). The ts-1 mutant did not induce symptoms when given intranasally to 13 adult volunteers but did confer resistance to challenge with wild-type RSV 45 days later (292). Although the mutant was genetically stable in adults, when inoculated into 32 infants and children 0.1 per cent of the virus recovered was genetically altered and able to replicate at 37° C. Furthermore, seven previously uninfected infants developed mild rhinitis and one had otitis (145). On the basis of these results, further defective clones of the ts-1 mutant were derived by exposure to nitrosoguanidine. Two such clones, ts-1 NG-1 and ts-1 NG-16 were more sensitive to temperature and genetically stable than ts-1. These clones, together with the ts-2 mutant which had a postulated defect in the fusion protein, were assessed in chimpanzees, owl monkeys and newborn ferrets (12, 13). The ts-2 mutant appeared to be most restricted and therefore suitable for trials in man. Between 10^5 and $10^{6.3}$ $TCID_{50}$ of the ts-2 were given intranasally to 14 adults and then to 20 sero-positive and 9 seronegative children. No illness was detected but virus was recovered from only one of the seronegative children and the vaccine induced a serological response in only 2 adults and 2 of the seronegative children (291). Five of seven seronegative children followed through the RSV season became infected. Thus, the ts-2 mutant appeared to be over-attenuated and its poor infectivity suggested that defective fusion protein may be important in determining *in vivo* infectivity.

A third approach to RSV vaccination was developed independently by Buynak and colleagues (30) for children and Wellemans and co-workers (286) for cattle. The vaccine for children was derived from strain 287 passaged twice in grivet monkey kidney cells and five or ten times in WI-38 cells. Electron microscopy revealed 10^7 to 10^8 particles per ml of vaccine

but its infectivity was only $10^{3.5}$ $TCID_{50}$ per ml. A single dose of 0.5 ml was given intramuscularly to 36 children aged 6 months to four years. Nineteen of 22 seronegative children developed neutralizing antibody. The vaccine did not cause any illness and did not spread to susceptible contacts. Extension of these studies revealed neutralizing antibody responses to the vaccine in 97 per cent of 116 initially seronegative children. There was no evidence of vaccine-enhanced disease among 392 persons during the year after vaccination but protection against RSV infection was not assessed (29). This vaccine and the Long strain of RSV partially or completely protected cotton-rats against intranasal challenge, reducing the virus titres in nasal turbinates and lungs by at least one hundred fold (215). This work also showed that inactivation of the virus destroyed its antigenicity and protective efficacy and that neither live virus nor its antigens could be detected at the intramuscular site of inoculation nor in the respiratory tract, suggesting that vaccine virus underwent an abortive cycle of replication only. Maternal antibody or passively administered antiserum to RSV blocked parental immunization of cotton-rats but not immunization by the intranasal route (212, 215). A double-blind, placebo-controlled field trial of the strain 287 vaccine in 510 children aged 6 to 47 months showed no difference between vaccine and placebo groups in the frequency of upper or lower respiratory tract disease caused by RSV (14). Thus, the live intramuscular vaccine was not efficacious in children, although 68 of 98 initially seronegative vaccinees developed antibody to RSV.

Experience with live intramuscular RSV vaccines in cattle is similar, in many respects, to that in cotton rats and children. Vaccine virus does not spread to in-contact susceptible calves but UV-irradiation destroys its immunogenicity (258, 286, 301). The ts-1 mutant of human A2 virus, restricted in replication at 37° C, induced protection in cattle (where body temperature is 39° C) which was similar to that afforded by the bovine vaccine (258). This is further evidence that an abortive cycle of replication is adequate to induce immunity, and that infected cells constitute the antigen. This hypothesis is further supported by the marked dose-dependent response to live intramuscular vaccine (301). Two doses of bovine vaccine given 3 weeks apart do not prevent infection by intranasal challenge with RSV but significantly reduce the duration and amount of virus shedding (258). In field trials of this vaccine results are conflicting. When 227 calves vaccinated at 2 to 12 months of age were compared with 161 control animals there was evidence of protection against RSV-associated disease (60, 300). However, a controlled trial in 273 animals in the Netherlands showed no difference in the disease scores of vaccinated and control animals after natural RSV infection (280). In a further tiral in 295 beef calves aged 1 to 5 months respiratory disease was diagnosed in 23 of 143 vaccinated animals and in 22 of 152 control calves during a subsequent RSV outbreak (STOTT

et al. to be published). This latter trial also showed that serum neutralizing antibody titres greater than 2 reduced the proportion of calves responding to vaccine from 23 of 28 to 4 of 66.

The future of vaccination against RSV will undoubtedly be affected by the advent of recombinant DNA technology and monoclonal antibodies. Despite the dangers of formalin-inactivated RSV vaccine, the demonstration that cattle can be protected against infection and disease by glutaraldehyde-fixed antigen encourages the belief that effective inactivated RSV vaccine containing sufficient antigen of the correct specificity may still be an attainable goal. This view is supported by experiments demonstrating the protection of cotton-rats and mice by passive transfer of monoclonal antibodies to fusion and large glycoproteins of RSV (265, 266, 283). These experiments also begin to define the essential epitopes which must be incorporated in a successful vaccine. These antigens may eventually be produced by gene cloning and expression in a eukaryotic system, by peptide synthesis or by deriving anti-idiotypic monoclonal antibodies.

It is difficult to envisage how live intramuscular vaccines can overcome their inhibition by maternal antibody in the target age group. This is the major advantage of live intranasal vaccines since the upper respiratory tract contains little or no maternally derived antibody. The problems of genetic instability and over- or under-attenuation of mutants may now be solved by the construction of specific mutants using recombinant DNA. Nevertheless, it is difficult to conceive how attenuated RSV will induce immunity when that afforded by the wild virus is so poor.

Chemotherapy

Ribavirin, 2-deoxy-D-glucose, amidines and interferon all have activity against RSV *in vitro* but trials in man have so far only been conducted with ribavirin.

Ribavirin, a synthetic nucleoside, inhibits RSV plaque production in cell cultures at a concentration of 3 μg/ml of medium even when added 12 hours after infection (123). A continous aerosol of 2 mg/ml of ribavirin reduced the titre of RSV in lungs and turbinates of cotton-rats by over 90 per cent (124). When the drug was administered as a small-particle aerosol to 8 young adults two days after intranasal inoculation with RSV, the number shedding virus 6 to 9 days after infection was significantly less than in 8 placebo treated volunteers. Systemic complaints and fever were also significantly less in the drug treated group. The aerosol was administered for 12 hours a day for three days, giving an estimated dose of 660 mg per patient per 12 hours (110). A double-blind trial in 33 infants naturally infected with RSV showed that a continuous aerosol of ribavirin significantly reduced the severity of illness and amount of virus shed (108). No virus recovered from treated animals or patients had increased resistance to

ribavirin. Hence, the emergence of drug resistant mutants appears unlikely. Thus ribavirin appears to be a promising compound for the treatment of RSV disease.

The glucose analog, 2-deoxy-D-glucose inhibits RSV *in vitro* (118) but did not reduce virus shedding when given intravenously to RSV-infected calves at a daily dose-rate of 10 mg per kg of body weight (189).

HALL (97) has suggested that administration of interferon might limit an RSV infection which itself produces very little (104, 170). However, the *in vitro* sensitivity of RSV to interferon is variable (53, 87, 117). A possible explanation for this is suggested by recent experiments showing that RSV is more sensitive to α-interferon induced by RSV than to similar interferon induced by influenza (10).

The search for more potent synthetic inhibitors of RSV will no doubt continue but the potential of monoclonal antibodies as therapeutic agents should not be overlooked. Monoclonal antibody to fusion protein, inoculated intravenously into mice infected five days previously with RSV, cleared the virus from their lungs within 6 hours (unpublished observations). This observation raises the possibility of using human monoclonal antibodies to treat RSV bronchiolitis but fears that antibody has an immunopathogenic role must first be allayed.

Conclusion

The battle against RSV has been long and littered with setbacks and disappointments. Improvements in the management of infant bronchiolitis and pneumonia have reduced mortality but the virus remains a major cause of serious, distressing and, occasionally fatal, disease in the very young. Painstaking work in many hospitals and laboratories has built up a detailed picture of the virus and its epidemiology but its pathogenesis and prevention remain enigmatic. The stage is now set for dramatic advances against this important disease of man and animals in which the powerful new techniques in immunology and biotechnology must be the principal players.

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