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The late detection of respiratory syncytial virus in cells of respiratory tract by immunofluorescence

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SUMMARY

Paired nasopharyngeal secretions were studied in 27 infants infected with respiratory syncytial virus, one taken at onset of illness and one about 7 days later. Both specimens were examined by immunofluorescence and tissue culture for respiratory syncytial virus. In 25 out of 27 (93%) specific fluorescence was still present in cells of the convalescent specimen but was much duller. Virus was difficult to isolate in convalescent specimens; only 8 out of 27 (26%) proved to be positive. Eight single secretions which were taken late in a respiratory illness were also shown to have this altered fluorescence with absence of virus isolation. Preliminary experiments using antihuman globulin suggest that the findings may be due to the attachment of local secretory antibody to the cells causing 'blocking' of staining reaction.

INTRODUCTION

Cells aspirated in nasopharyngeal secretions from the respiratory tracts of young infants were first used for the detection of respiratory syncytial (R.S.) virus by immunofluorescence in 1968 and the technique has since become a standard method for the identification of the virus in this laboratory (McQuillin & Gardner, 1968; Gardner & McQuillin, 1968). The secretions taken for the rapid diagnosis of R.S. virus infections were also used for the investigation of local neutralizing antibodies to R.S. virus in the respiratory tract; it is believed that in many acute respiratory virus infections, local antibodies bear a closer relationship to protection than do humoral antibodies (Smith, Purcell, Bellanti & Chanock, 1966; Kim *et al.* 1969). For the study of the production of local antibody in the respiratory tract, two specimens of secretion were taken at approximately 7-day intervals during the course of an acute illness. It was decided to investigate whether the development of local antibody produced any change in the fluorescent staining of virus antigen in R.S. virus-infected respiratory cells. Among other aims of this investigation were: the estimation of the length of time that virus antigen could still be detected in nasopharyngeal secretions; the determination as far as possible of the length of time that these secretions remain infective; and the study of the relationship between infectivity and fluorescence. This approach was used as a result of previous observations that R.S. virus-infected cells in nasopharyngeal

secretions of children examined late in their illness showed a duller fluorescence; this appearance often coincided with a delay in the time taken to isolate the causal virus on tissue culture.

MATERIALS AND METHODS

Paired nasopharyngeal secretions were taken from 27 infants; the first specimen, called the acute specimen, was taken within 48 hr. of onset of illness and the second specimen, called the convalescent specimen, approximately 7 days later. The exact interval between the two secretions is shown in Table 1, which also shows the age of the children. The method for collection of secretions has been described in previous publications (Gardner & McQuillin, 1968; Sturdy, McQuillin & Gardner, 1969). The 27 children were all known to be infected with R.S. virus by the previous examination of their acute secretions by immunofluorescence and virus culture; the convalescent specimens of secretion were examined by the same methods for R.S. virus.

In addition, during the course of a 2-year survey of acute respiratory infections of childhood, in which the fluorescent antibody technique was used, there were eight children from whom no virus was isolated, but specific fluorescence for R.S. virus was observed in cells of their nasopharyngeal secretions. Secretions from these children were further investigated and formed part of this study.

The tissue culture and immunofluorescent techniques employed were identical with those described elsewhere (Gardner & McQuillin, 1968; Sturdy, McQuillin & Gardner, 1969). In addition, a number of nasopharyngeal secretions were stained with fluorescein-labelled antihuman globulin by methods which have also been described (Gardner, McQuillin & Court, 1970).

Extra slides were always prepared from every specimen of nasopharyngeal secretion which came to the laboratory; all slides were fixed in acetone and stored at $-40°$ C. These stored preparations could be used for various purposes, including research investigations such as described here.

RESULTS

Table 1 gives the detailed results of the examination of the paired secretions for both immunofluorescence and isolation of virus. The relationship between the isolation of R.S. virus and the detection of its presence by immunofluorescence in the 27 second secretions is shown in Table 2. In 18 out of 27 secretions, the results for the fluorescent antibody technique were positive, but R.S. virus was not isolated, and in only seven infants did isolation of virus confirm the positive fluorescence. The acute secretions of all these patients, when stained by the fluorescent antibody technique, showed the classical appearance of strongly fluorescent intracellular R.S. virus and this is illustrated in Pl. 1, fig. 1. Fig. 2 shows the much duller, but nevertheless specific, fluorescence observed in 25 out of the 27 convalescent secretions.

The cells in the single secretions of eight children from whom virus was not isolated showed specific fluorescence for R.S. virus. During this period, there were 224 additional R.S. virus infections diagnosed by immunofluorescence, all con-

Table 1. *Summary of examination of paired secretions from 27 patients with R.S. virus infection*

Case no.	Name	Age	Specimen of secretion	Date received	No. of days between specimen	F.A. result for R.S.V.	Isolation of R.S. virus
1	S.S.	5 months	Acute	30. x. 68	7	+	+
			Convalescent	6. xi. 68		+	−
2	S.C.	10 weeks	Acute	4. xi. 68	7	+	+
			Convalescent	11. xi. 68		+	−
3	C.W.	7 weeks	Acute	4. i. 69	6	+	+
			Convalescent	10. i. 69		+	−
4	R.S.	10 weeks	Acute	21. x. 69	6	+	+
			Convalescent	27. x. 69		+	−
5	S.W.	10 months	Acute	3. xi. 69	4	+	+
			Convalescent	7. xi. 69		+	−
6	H.S.	3 months	Acute	11. xi. 69	6	+	+
			Convalescent	17. xi. 69		+	−
7	W.I.	12 weeks	Acute	2. xii. 69	7	+	+
			Convalescent	9. xii. 69		+	+
8	A.L.	6 weeks	Acute	9. xii. 69	7	+	+
			Convalescent	16. xii. 69		+	−
9	D.H.	13 months	Acute	11. xii. 69	7	+	+
			Convalescent	18. xii. 69		+	−
10	C.T.	6 months	Acute	12. xii. 69	7	+	+
			Convalescent	19. xii. 69		+	−
11	K.R.	6 weeks	Acute	13. xii. 69	9	+	+
			Convalescent	22. xii. 69		+	+
12	J.B.	7 weeks	Acute	15. xii. 69	8	+	+
			Convalescent	23. xii. 69		+	+
13	D.B.	11 weeks	Acute	22. xii. 69	7	+	+
			Convalescent	29. xii. 69		+	−
14	M.E.	5 months	Acute	24. xii. 69	9	+	+
			Convalescent	2. i. 70		−	−
15	A.L.	9 months	Acute	9. i. 70	7	+	+
			Convalescent	16. i. 70		+	−
16	M.H.	7 weeks	Acute	9. i. 70	7	+	+
			Convalescent	16. i. 70		+	−
17	L.G.	2 months	Acute	10. i. 70	6	+	+
			Convalescent	16. i. 70		+	+
18	G.H.	8 months	Acute	19. i. 70	7	+	+
			Convalescent	26. i. 70		+	−
19	R.D.	8 months	Acute	22. i. 70	7	+	+
			Convalescent	29. i. 70		+	−
20	G.W.	10 weeks	Acute	22. x. 69	5	+	+
			Convalescent	27. x. 69		+	+
21	S.B.	10 weeks	Acute	22. i. 70	7	+	+
			Convalescent	29. i. 70		−	−
22	S.A.	2 months	Acute	22. i. 70	6	+	+
			Convalescent	28. i. 70		+	−
23	D.C.	2 weeks	Acute	6. ii. 70	6	+	+
			Convalescent	12. ii. 70		+	+
24	K.H.	4 months	Acute	9. ii. 70	7	+	+
			Convalescent	16. ii. 70		+	+
25	M.C.	4 months	Acute	13. ii. 70	6	+	+
			Convalescent	19. ii. 70		+	−
26	S.S.	4 months	Acute	23. ii. 70	7	+	+
			Convalescent	2. iii. 70		+	−
27	C.A.	5 weeks	Acute	15. iii. 70	11	+	+
			Convalescent	26. iii. 70		+	−

firmed by isolation of virus. The positive cells in all eight secretions showed a duller fluorescence identical with that observed in those 27 secretions taken in the convalescent phase of the illness. The picture they presented is illustrated in Pl. 1, fig. 3. The study of the clinical histories of these children showed that they had all been ill for a minimum of 5 days and four of them for a fortnight before a secretion was taken for examination. These details are shown in Table 3.

Table 2. *The relationship between isolation technique and fluorescent antibody technique in identifying R.S. virus in 27 second secretions*

R.S. virus isolated. F.A. positive	R.S. virus not isolated. F.A. positive	R.S. virus isolated. F.A. negative	R.S. virus not isolated. F.A. negative
7	18	0	2

Table 3. *Findings in single secretions from children: immunofluorescence positive, culture negative for R.S. virus*

Case no.	Name	Age when specimen taken	Length of illness before specimen taken (days)
28	S.T.	6 weeks	14
29	A.B.	4 months	14
30	C.P.	22 months	7
31	P.K.	4 months	5
32	D.M.	15 months	14
33	B.K.	18 days	6
34	D.F.	28 days	14
35	D.W.	8 weeks	7

The changes observed by immunofluorescence in the staining properties of the cells in the convalescent secretions coincided in a number of instances with an increase of local neutralizing antibody which would account for the increased difficulty of isolation of virus. It was therefore decided to examine three sets of paired secretions by using fluorescein-labelled antihuman globulin in parallel with the examination by the original method of indirect fluorescent antibody staining for R.S. virus. Two single specimens of secretion from which we failed to isolate R.S. virus were treated in the same way. In both these specimens a large number of cells were observed to have been stained with the fluorescein-labelled antihuman globulin. An example of such a cell is illustrated in Pl. 1, fig. 4. In the three paired secretions, there was an increase in fluorescence in the convalescent secretion as compared with the acute, but it was difficult to put this observation on a firm quantitative basis. The number of cells fluorescing with antihuman globulin did not always appear equal to the number of cells showing abnormal staining with R.S. virus antiserum and anti-rabbit globulin; there was also some variation in the nature of the fluorescence.

DISCUSSION

The results showed that specific fluorescence for R.S. virus remained in the cells of the nasopharyngeal secretion for 25 out of 27 patients (93%) for at least 7 days after the onset of acute symptoms, but in the second specimen the fluorescence was much duller. Isolation in the convalescent specimen was only effective in seven out of 27 (26%). The identical picture seen in eight specimens taken late in the respiratory illness from which virus was not isolated suggests that the failure to isolate virus in these cases was not due to false positive fluorescence but to the late stage in the illness when the specimen was taken. There are three possible explanations for this failure. One explanation may be that the 'blocking' of virus and decrease of infectivity may result from the development of the patient's own neutralizing antibody; another, that the change in fluorescent appearance, coupled with the failure to isolate, is due to antigen persisting in the cells which, although it can still be detected by immunofluorescence, has undergone some deterioration and is no longer infective. A third simple explanation could be faulty isolation techniques, but the successful isolation of virus from all acute specimens of the 27 paired secretions, in addition to the failure to isolate from the eight other patients, with single secretions, where there was delay between onset of illness and collection of specimen, appears to rule out this possibility.

Scott & Gardner (1970) have shown that a number of these paired secretions developed neutralizing antibody which coincided with the change in fluorescent appearance and loss of infectivity. Kim and her colleagues in 1969 have also shown that neutralizing antibodies to R.S. virus develop in nasal secretions during the course of a respiratory infection. The result of the preliminary and limited investigation performed on these secretions using antihuman globulin suggests, too, that during the course of the illness human globulin becomes attached to nasopharyngeal cells. We have no direct evidence, however, that the antibody which may be coating cells in the nasopharynx is R.S. virus antibody. We believe that it could be because of the coinciding rise of neutralizing antibody in a number of paired secretions and the demonstration of human globulin on cells in convalescent specimens. The development of this altered fluorescence, if due to 'blocking' by human globulin and the coinciding rise of neutralizing antibody, gives support to this hypothesis. The attachment of antibody *in vivo* to virus-infected cells might occur, provided that the membranes of the infected cells have been altered by the invading virus or virus protruding through the cell membrane. R.S. virus has been classified with the myxoviruses which have an intimate connexion with the surface of their host cell.

Scott & Gardner (1970) have also suggested, as a result of some hitherto unpublished experiments, that IgA is the predominant fraction of globulin present in the nasal secretions of all these infants. The examination of cells from more secretions with specific fluorescein-labelled anti-IgA will demonstrate whether it is this antibody which is attaching itself to infected cells.

If it can be shown that this 'blocking' of fluorescence observed in cells of convalescent secretions is due to development of neutralizing antibodies binding to

infected cells, it will provide evidence that this mechanism is a factor in limiting the length of time that a patient remains infective. The possibility of deterioration of antigen must be explored further should it not be proven that antibody attaches itself to the cells. This seems less likely, as the cells appear to be intact and in as good condition as those observed in the acute specimens of secretion.

Antibody coating cells in the respiratory tract is unlikely to be IgG. If maternal IgG penetrated to the respiratory tract in acute infections, the cells in the acute specimen of secretion should show this 'blocking' effect. The use of antisera to the individual fractions of globulin will throw light on this very important topic.

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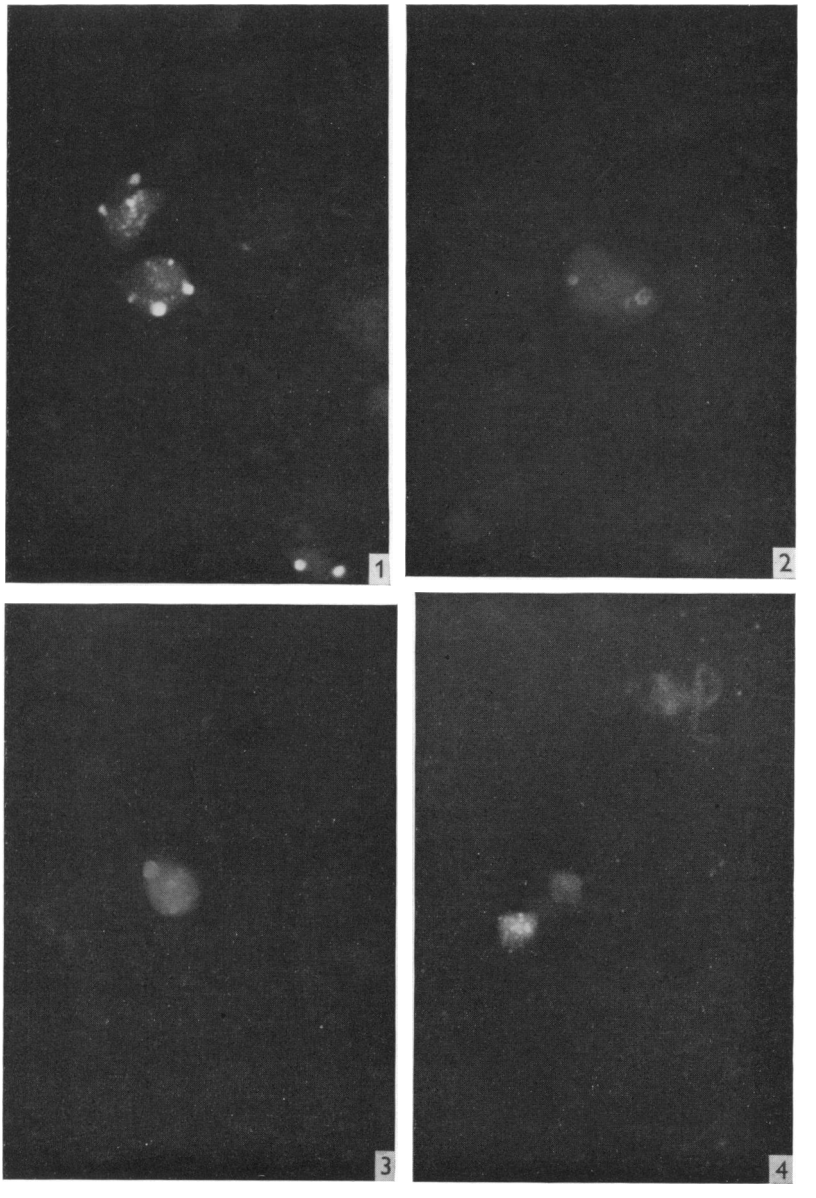
EXPLANATION OF PLATE

Fig. 1. Cells in a nasopharyngeal secretion taken at the onset of bronchiolitis and stained by the indirect fluorescent antibody technique for R.S. virus. Magnification × 1200.

Fig. 2. A cell in nasopharyngeal secretion taken 7 days after first secretion and stained by the indirect fluorescent antibody technique for R.S. virus; the duller fluorescence is demonstrated. Magnification × 1200.

Fig. 3. A cell in a nasopharyngeal secretion taken late in bronchiolitis and stained by the indirect fluorescent antibody technique for R.S. virus; the duller fluorescence is demonstrated. Magnification × 1200.

Fig. 4. A cell in convalescent nasopharyngeal secretion stained by fluorescein-labelled antihuman globulin; the attachment of human globulin to the cell is demonstrated. Magnification × 1200.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