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Laboratory diagnosis and clinical significance of rubella in children with cancer

D. J. MORRIS[1], P. MORGAN-CAPNER[2], D. J. WOOD[1]*, M. DALTON[3]†, J. WRIGHT[2], H. I. J. THOMAS[2] AND R. F. STEVENS[3]

[1] *North Manchester Regional Virus Laboratory, Booth Hall Children's Hospital, Manchester M9 2AA, UK,* [2] *Department of Virology, Preston Infirmary, Preston PR1 6PS, UK,* [3] *Department of Paediatric Oncology, Royal Manchester Children's Hospital, Manchester M27 1HA, UK*

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SUMMARY

Virus-specific antibody responses were studied in 12 children with cancer in whom rubella was diagnosed by seroconversion or a rising titre ($\geqslant$ fourfold) of haemagglutination inhibiting (HI) antibody. Our results confirmed the difficulties of making a diagnosis of rubella infection in immunocompromised children using criteria for interpreting antibody assays established in immunocompetent patients. Specific IgM antibody persisted for more than 2 months in 7 of 10 children with probable primary rubella, 3 of whom had high concentrations of such antibody 6, 7 and 11 months after the rash. Radial haemolysis and specific IgG_1 and IgG_3 antibody responses were low in 4, 2, and 4 patients, respectively. One child apparently had a rubella reinfection and, in another, rubella antibody passively acquired from blood transfusions was probably responsible for the HI seroconversion. Nonetheless, the benign clinical course of rubella in immunocompromised children was confirmed.

INTRODUCTION

Most laboratories currently rely on antibody detection rather than virus isolation for the diagnosis of rubella. In our recent study of virus infections in children with cancer, a laboratory diagnosis of rubella was made in children with rubelliform rashes by detection of seroconversion or a $\geqslant$ fourfold rise in the titre of rubella-specific haemagglutination inhibiting (HI) antibody (1), an established strategy for the diagnosis of recent rubella (2). In the present study we have investigated detection of specific IgM antibody as a method for the laboratory diagnosis of rubella in children with cancer. We have also evaluated radial haemolysis (RH) (2) and IgG subclass-specific enzyme-linked immunosorbent assay (ELISA) (3) as alternative methods for the confirmation of such a diagnosis.

Current address:
* National Institute for Biological Standards and Control, South Mimms, Potter's Bar, UK.
† Royal Preston Hospital, Sharoe Green Lane, Preston, UK.

Our results reveal the difficulties of serodiagnosis in children with cancer and of applying to these patients methods for rubella diagnosis established in immunocompetent patients.

Rubella is perceived to be of little clinical significance in children with cancer (4–6), encephalitis being the only reported complication (7). The data available are sparse, however, and we have therefore attempted to assess the clinical significance of rubella in our study population.

MATERIALS AND METHODS

Patients and sera

Twelve children with cancer were selected retrospectively for this study on the basis of a rubelliform rash and a laboratory diagnosis of rubella established by seroconversion or rising titres ($\geqslant$ fourfold) of HI antibody at the North Manchester Regional Virus Laboratory. Sera collected prior to, at the time of, and subsequent to the rash were stored at -20 °C for up to 8 years.

Serological assays

All sera were tested further at the Department of Virology, Preston. IgM capture radioimmunoassay (MACRIA), RH, and rubella-specific IgG subclass ELISAs were performed as described (3, 8, 9). Samples of some sera were too small for examination by all the tests. Total IgM levels were determined in selected sera by laser nephelometry.

RESULTS

Antibody responses

The results of assays for rubella-specific antibodies are given in the Table. Rubella specific-IgG_2 and IgG_4 were not detected in any sera.

A pre-illness serum was available for 11 children and all cases were diagnosed as rubella by demonstrating HI antibody seroconversion. A pre-illness serum was not available from patient 9 but a rising HI titre was found. Only this patient and patients 3, 5, and 7 developed a high concentration (titre $\geqslant 160$) of HI antibody.

Rubella-specific IgG_1 and IgG_3 antibody and IgG antibody assayed by RH developed or increased in concentration at the time of the rash or was present after the rash in virtually all patients. However, the rubella antibody response in patient 11 comprised RH and specific IgG_1 but not specific IgG_3 antibody during the brief period of observation. The maximum concentrations of IgG antibody were low in some patients: the maximum zone size by RH was less than 10 mm in patients 3, 5, 7, 8, and 12; the IgG_1 response was only of low level (< 20 arbitrary units (a.u.)) in patients 5 and 8; and the IgG_3 response was only of low level (< 20 a.u.) in patients 3, 5, 7, 8, 9, 10 and 12. Rubella-specific IgM antibody developed at the time of, or was present after, the rash in 9 of 12 patients. The order of appearance of different classes of rubella antibody at the time of the exanthem could be discerned in four patients, three of whom (patients 2, 4 and 8) developed specific IgM before specific IgG_1 and RH antibodies. In patient 4 specific IgG_3 and IgM antibodies appeared before the rash, and in patient 7 the RH antibody response was delayed long after the rash.

Table 1. *Rubella-specific antibody responses in children with cancer*

Patient and disease*	Days before (−) or after (+) rash	HI antibody† (titre)	RH antibody† (zone in mm)	Specific IgG (a.u.)†		Specific IgM† (a.u.)
				IgG_1	IgG_3	
1 (ALL)	−119	< 10	NS	NS	NS	< 1
	0	80	8	52	⩾ 100	> 30
	+21	ND	10	⩾ 100	⩾ 100	13
	+138	ND	8	⩾ 100	4	< 1
2 (ALL)	−34	< 10	No zone	< 3	< 3	< 1
	0	20	No zone	< 3	< 3	22
	+56	80	10	⩾ 100	33	7
	+185	ND	10	⩾ 100	12	5
	+372	ND	10	⩾ 100	7	< 1
	+470	ND	9	⩾ 100	6	< 1
3 (ALL)	−35	< 10	NS	NS	NS	NS
	+7	640	NS	NS	NS	NS
	+77	ND	9	50	17	7
	+933	ND	ND	⩾ 100	8	3
4 (ALL)	−28	< 10	No zone	< 3	< 3	< 1
	−8	< 10	No zone	< 3	14	15
	+2	80	6	12	⩾ 100	> 30
	+186	ND	12	⩾ 100	⩾ 100	14
	+201	ND	12	⩾ 100	53	9
5 (NHL)	−395	< 10	No zone	< 3	< 3	< 1
	+15	160	NS	NS	NS	> 30
	+275	ND	8	14	3	14
	+369	ND	8	15	3	7
6 (ALL)	−301	< 10	No zone	NS	NS	< 1
	+1	80	5	13	< 3	> 30
	+219	ND	12	100	95	> 30
7 (ALL)	−142	< 10	No zone	< 3	< 3	< 1
	+5	< 10	No zone	< 3	< 3	< 1
	+89	160	No zone	27	< 3	> 30
	+156	ND	9	47	5	> 30
	+184	ND	9	55	3	> 30
8 (ALL)	−109	< 10	No zone	< 3	< 3	< 1
	+3	20	No zone	< 3	3	> 30
	+31	20	No zone	5	13	> 30
	+59	20	4	7	17	> 30
	+123	10	4	5	19	> 30
	+324	ND	6	14	12	> 30
9 (ALL)	+1	160	9	25	< 3	< 1
	+8	80	NS	29	< 3	< 1
	+86	⩾ 2560	13	⩾ 100	4	3
	+156	ND	11	⩾ 100	4	< 1
	+252	ND	11	⩾ 100	3	< 1
10 (AML)	−7	< 10	NS	< 3	< 3	< 1
	+8	40	8	20	5	< 1
	+12	40	NS	NS	NS	< 1
	+29	40	7	NS	NS	< 1
	+1056	ND	12	⩾ 100	15	< 1
	+1198	ND	10	⩾ 100	12	< 1

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Table 1 (*cont.*)

Patient and disease*	Days before (−) or after (+) rash	HI antibody† (titre)	RH antibody† (zone in mm)	Specific IgG (a.u.)†		Specific IgM† (a.u.)
				IgG_1	IgG_3	
11 (NHL)	−124	< 10	No zone	< 3	< 3	< 1
	+1	20	5	NS	NS	< 1
	+9	20	7	20	< 3	< 1
12 (AML)	−19	< 10	NS	NS	NS	NS
	0	40	7	⩾ 100	10	< 1
	+73	ND	6	16	6	< 1
	+84	ND	6	50	4	< 1
	+98	ND	No zone	39	4	< 1
	+160	ND	No zone	< 3	< 3	< 1

* ALL, acute lymphoblastic leukaemia; NHL, Non-Hodgkin's lymphoma; AML, acute myeloid leukaemia.

† HI, haemagglutination inhibition; RH, radial haemolysis; MACRIA, M-antibody capture radioimmunoassay; a.u., arbitrary units; NS, no serum left; ND, not done.

Persistence of antibodies

The persistence of rubella HI antibody after the exanthem was not assessed. Rubella IgG antibody persisted throughout the observation period in all patients except patient 12. The end of the rubella-specific IgM antibody response was identified in three patients: in patients 1 and 2 specific IgM was detected 2 months and 7 months after the rash, respectively; patient 9 had a low level of specific IgM antibody in one serum taken 3 months after the rash. In the other six patients, the end of this response was not identified with specific IgM antibody being detected at 6–30 months after the rash. Sera from the three patients (numbers 6–8) with persistent high-level rubella-specific IgM responses (> 30 a.u.) were assayed for total IgM; patients 7 and 8 had normal or elevated total IgM levels (normal range 0·4–2·0 g/l), whereas patient 6 had an initially low total IgM level (0·08 g/l) which later became normal.

IgM antibody negative patients

Three patients did not develop a rubella-specific IgM antibody response. In only one of these patients (patient 10) was isolation of rubella virus attempted by innoculation of RK 13 cells; a positive result, confirmed by indirect immunofluorescence using a rabbit anti-rubella virus antiserum, was obtained from a urine specimen collected 32 days after the rash. The transfusion histories of the other two patients revealed that patient 11 had received 2 units of packed red cells 11 days before the rash and patient 12 had received 8 units of packed red cells and 24 units of platelets between 3 days before and 100 days after the rash. Circumstantial evidence supporting the diagnosis of rubella in patient 11 was provided by the detection of rubella-specific IgM (> 30 a.u.) in a serum collected from the patient's sister 2 days before the rash and three days after the sister had developed a rubelliform rash.

Clinical presentation

The illness at the time of the rubelliform rash was clinically mild in all 12 patients, with minor cervical lymphadenopathy, fever, arthralgia, or arthritis. None of the patients had encephalitis.

DISCUSSION

Seven of our 12 children with cancer had serological or virological evidence of primary rubella. They lacked rubella-specific IgG antibody detected by RH or IgG_1-ELISA in sera taken before the rash, and either subsequently shed rubella virus in urine (patient 10) or seroconverted and developed a rubella-specific IgM antibody response (patients 2 and 4–8). Two further children (patients 1 and 3) who developed a specific IgM antibody response also probably had primary rubella, though the absence of specific IgG antibody before the rash was shown only by HI, a less sensitive assay for specific IgG antibody than RH or IgG_1-ELISA (2, 3). In none of these nine children could passive acquisition of specific IgG antibody from transfused blood explain the serological results.

The rubella-specific antibody response in the 9 patients with probable primary rubella generally shared three features with that seen in similarly infected immunocompetent patients (2, 3,8–10): IgM and HI antibodies appeared within a few days of the rash; RH and specific IgG_1 and IgG_3 antibodies appeared after the rash, and persisted throughout the observation period; and IgM and HI antibodies appeared before IgG_1, IgG_3, and RH antibodies. The rubella antibody response in children with cancer differed, however, in four respects from that seen in immunocompetent patients.

First, rubella-specific IgM antibody persisted in 7 children beyond the 1–2 months usually seen in immunocompetent individuals (9, 11). Following the rash, high concentrations of specific IgM antibody (> 30 a.u.) persisted in three patients for 6–11 months, and lower concentrations persisted for 6–30 months. Persistence of specific IgM antibody for more than a few months has previously been reported uncommonly, has always been at low levels, and usually in vaccinees (9, 11, 12–14). The normal concentrations of total IgM antibody in the sera showing persistent high concentrations of specific IgM antibody suggested that these aberrant antibody responses did not reflect false quantitation by MACRIA because the test measures the relative proportion of IgM antibody in a serum that is rubella-specific (9).

Second, patient 10 had primary rubella without developing a detectable specific IgM antibody response.

Third, the concentrations of rubella HI, RH, IgG_1 and IgG_3 antibodies were often lower in children with cancer than in immunocompetent patients (2, 3, 10). Indeed, the RH antibody response was occasionally delayed long after the rash.

Fourth, specific IgM and IgG_3 antibody appeared before the rash in patient 4. This unusual event (2) could reflect delay in the onset of the rash secondary to immunosuppression consequent on disease or chemotherapy. Nonetheless, the role of immunopathological events in the pathogenesis of the rash of rubella remains uncertain (2).

The rubella-specific antibody responses in patients 11 and 12 could have reflected primary rubella without an IgM response, as seen in patient 10. As virus isolation was not attempted in these patients, the results do not exclude the detection of specific IgG antibody passively acquired from transfused blood. The antibody response seen in patient 11 may have reflected primary rubella, as the patient's sister had rubella. In patient 12, rubella antibody had disappeared 5 months after its appearance; passive acquisition of specific IgG antibody therefore appeared likely.

The lack of sera collected before the rash meant that we could not be certain whether patient 9 had primary rubella or reinfection. Comparison with the antibody responses seen in immunocompetent individuals (2, 3, 15) suggested that the transient low concentration of specific IgM with a high concentration of specific IgG_1 and a low concentration of specific IgG_3 reflected a reinfection.

The clinical presentation of rubella seen in this study of children with cancer confirms the view that this disease is probably of little clinical significance in such children (4–6), though a role for rubellavirus in the causation of more severe disease in patients without a rash cannot be excluded.

Accurate laboratory diagnosis of rubella in children with cancer presents considerable difficulties. By analogy with other viruses (1), virus excretion is likely to be the most sensitive marker of rubella in such children. Our reliance on serological rather than virological diagnosis of rubella in immunocompromised children may have led to underdiagnosis. The detection of a rising titre of HI antibody cannot be taken as indicating recent primary rubella infection, as we have previously done, unless passive acquisition of rubella-specific IgG antibody from transfused blood products is excluded. For similar reasons, the appearance of rubella IgG_1, IgG_3 or RH antibody at the time of the rash must be interpreted cautiously. The detection of virus-specific IgM antibody in a single serum sample, even at high level, does not establish a diagnosis of recent rubella. The only certain methods of serologically diagnosing rubella in children with cancer are either the detection of rubella IgG seroconversion at the time of the rash and detection of specific IgM antibody after the rash, or the detection of rubella IgM seroconversion at the time of the rash. These methods are likely to allow the early diagnosis of rubella within a few days of the onset of a rubelliform rash in a child with cancer.

This report thus extends to rubella the viruses which may cause persistent specific IgM antibody responses in immunocompromised patients. Such aberrant antibody responses have been previously reported in renal transplant recipients with cytomegalovirus infection (16), and in a heart transplant recipient with coxsackie B virus infection (17). Persistent virus-specific IgM antibody responses may indeed be a general feature of the immune response to virus infections in immunocompromised patients.

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