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Original articles

Detection of antibodies to human parvovirus in erythema infectiosum (fifth disease)

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SUMMARY Two Japanese outbreaks of erythema infectiosum were investigated for evidence of human parvovirus infection by a solid phase antibody capture radioimmunoassay based on a monoclonal antibody to human parvovirus. Specific IgM and high concentrations of specific IgG were detected in 37 sera from 27 children with erythema infectiosum. No anti human parvovirus IgM was detected in a remaining case of erythema infectiosum, in five patients with Kawasaki disease, or in the 17 control children. Seven of the controls were also anti human parvovirus IgG negative, and the 10 who were seropositive had lower concentrations of anti human parvovirus IgG than the patients with erythema infectiosum. These data indicate that human parvovirus is a cause of erythema infectiosum.

Erythema infectiosum ('fifth disease', 'slapped cheek disease') is an acute exanthem, usually occurring in localised outbreaks in young school children. The disease was reported in Tokyo in 1974, 1975, and 1977,[1] and was widespread in Japan in 1980 and 1981. Epidemiological evidence indicates that it is an infectious disease, and rubella,[2] echo 12,[2] and respiratory synctial viruses[3] have been suggested, but not confirmed, as aetiological agents.

Recently, Anderson *et al* investigated cases of erythema infectiosum in London. They detected IgM and IgG antibody to the human parvovirus B19 in the patients' sera, and proposed that this virus was the cause of the outbreak.[4] The virus had previously been found in the serum of blood donors,[5] of subjects with minor febrile illness,[6 7] and of patients with chronic haemolytic anaemia undergoing an aplastic crisis.[8 9] No common disease of childhood, however, had been associated with it.

We have now tested for anti human parvovirus IgM and IgG in sera collected from children with erythema infectiosum during the Japanese outbreaks of 1977 and 1981, and we report our findings which provide further evidence that human parvovirus is the hitherto elusive agent of erythema infectiosum.

Materials and methods

Specimens.

Patients with erythema infectiosum

Throat swabs and sera were taken from 28 children who had the typical slapped cheek appearance and a rash on the limbs. Seventeen were part of a group of 21 children observed in an outbreak in 1977 and 11 were a group of children observed in an outbreak in 1981 (Table 1). The children were aged 6 to 10 years in the first and 5 to 11 in the second outbreak. Thirteen were boys and 15 girls. The specimens were examined by routine virological methods and the remaining volumes of serum were stored at −20°C until the human parvovirus study.

Controls and patients with Kawasaki disease

Seventeen age matched control sera and five sera from young children with Kawasaki disease were collected in 1982 and 1983 at the National Children's Hospital, Tokyo.

Virological Tests. Throat swabs were inoculated into human embryonic lung, human embryonic kidney, WI–38, vero and RK–13 cells. Haemadsorption tests

Table 1 *IgM and IgG class antibody to human parvovirus (HPV) in 28 cases of erythema infectiosum*

	Case No	*Days after onset of rash*	*Anti HPV (units)*		*Remarks*
			IgM	*IgG*	
M Elementary school, 1977	1	3	1·8	47·0	Paired sera
		40	1·3	62·0	
	2	4	4·7	36·0	Paired sera
		13	1·6	52·0	
	3	4	19·0	33·5	Paired sera
		15	5·6	42·0	
	4	4	1·5	24·5	Paired sera
		37	0·4	26·5	
	5	6	4·7	36·0	Paired sera
		24	1·6	52·0	
	6	1	6·1	24·0	
	7	1	1·5	51·0	
	8	1	2·4	48·0	
	9	1	1·7	43·5	
	10	6	14·5	45·5	
	11	7	1·7	25·0	
	12	9	11·0	45·0	
	13	14	1·1	70·0	
	14	18	24·5	56·0	Rash relapsed
	15	20	2·5	80·0	
	16	20	3·6	33·5	
	17	25	2·3	66·0	
JF Hospital, 1981	18	Acute*	52·0	32·0	Paired sera
		+7	14·5	50·0	
	19	Acute	8·8	54·0	Paired sera
		+14	2·6	56·0	
	20	Acute	21·0	92·0	Paired sera
		+14	5·2	84·0	
	21	Acute	4·7	49·0	Paired sera
		+17	1·3	46·0	
	22	Acute	6·8	42·5	Paired sera
		+37	4·7	56·0	
	23	Acute	44·0	36·5	
	24	Acute	62·0	34·5	
	25	Acute	3·2	52·0	Sister of case 18
	26	Acute	4·1	64·0	Brother of case 19
	27	Acute	7·4	71·0	
	28	Acute	<0·3	<0·3	

*ie collected within 7 days of onset of rash.

were applied to vero and RK–13 cells, and viral interference was sought by challenging the RK–13 cells with vesicular stomatitis virus. Three blind passages were done in each cell line before a negative result was recorded. The sera collected from patients with erythema infectiosum were tested by complement fixation, haemagglutination inhibition, neutralisation, fluorescence, and plaque reduction tests,[10] as appropriate, for antibody to rubella virus, respiratory syncytial virus, parainfluenza viruses (types 1 and 3), influenza viruses A and B, rotavirus, Epstein Barr Virus, adenovirus group, echovirus group (types 4,7,11), Coxsackie A virus (types 4,7,9), Coxsackie B virus (types 1,2,3,5,6), and *Mycoplasma pneumoniae*.

All the sera were tested for evidence of human parvovirus infection. A full description of the methods used to prepare antigen, to set up assays for antibodies to human parvovirus, and to quantify results has been given previously.[11] Briefly, anti human parvovirus IgM and IgG and human parvovirus antigen were detected by an antibody capture solid phase radioimmunoassay, as follows. Polystyrene beads were coated by immersion in a 1 in 3000 dilution of goat anti human μ-chain serum. They were then incubated with 1 in 300 dilutions of the sera under test and, after washing, with human parvovirus antigen. The antigen had been obtained from the plasma of a blood donor with viraemia, and purified by fractionation on a sephacryl S–300 column and centrifugation through a sucrose gradient. The beads were washed again and monoclonal anti human parvovirus was added. The monoclonal antibody was the IgG fraction of ascitic fluid collected from a mouse previously inoculated with a clone of anti human parvovirus producing hybridoma cells. After another incubation the beads were washed and ^{125}Iodine-labelled sheep anti-mouse Ig (Amersham International) was added. Finally, the beads were washed and the bound

radioactivity measured in a gamma counter. The concentrations of anti human parvovirus IgM were measured in units by comparing them with that of a standard serum of strength 100 arbitrary units. Anti human parvovirus IgG was detected by the same method, but with the antibody captured onto a solid phase coated with anti human IgG (γ chain specific). It was quantified in the same way as anti human parvovirus IgM, using a 100 unit anti human parvovirus IgG standard serum.

To detect human parvovirus antigen the IgM-capture radioimmunoassay system was modified by using a serum known to be anti human parvovirus IgM positive and inserting a 1 in 10 dilution of the specimen and under test (antigen status unknown) in place of human parvovirus antigen.

Results

Patients with erythema infectiosum. No virus was isolated in tissue culture and there were no high or rising antibody titres to any antigen other than human parvovirus. Anti human parvovirus IgM and IgG were detected in 37 (97·4%) of 38 sera taken from 28 patients (Table 1). In all 10 children from whom a second serum was collected the concentration of anti human parvovirus IgM had fallen in convalescence and in eight of them the concentration of anti human parvovirus IgG had risen. Serum from one patient diagnosed as having erythema infectiosum was anti human parvovirus IgM and IgG negative and did not contain human parvovirus antigen. The mean anti human parvovirus IgM was highest within a week of onset and fell to low values after four to five weeks (Fig. 1). The mean anti human parvovirus IgG was high in the first week of illness and continued to rise in the second and third week (Fig. 2).

Controls and patients with Kawasaki disease. None

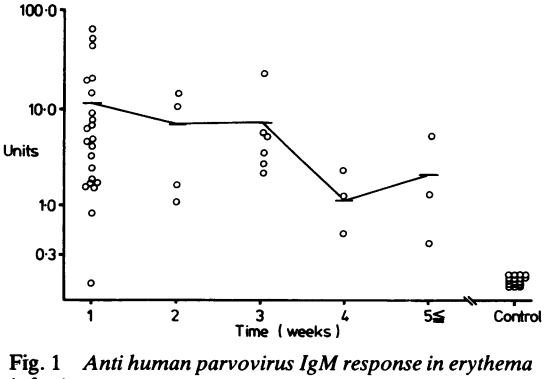

Fig. 1 *Anti human parvovirus IgM response in erythema infectiosum.*

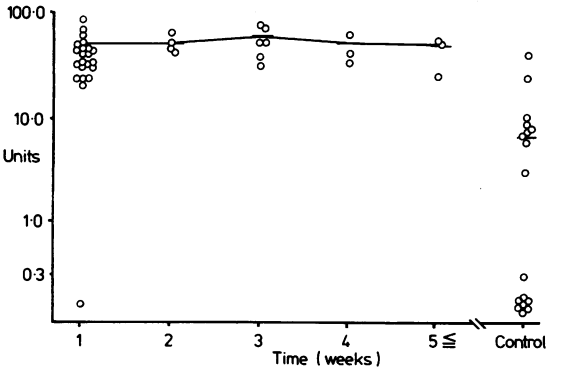

Fig. 2 *Anti human parvovirus IgG response in erythema infectiosum.*

of the 17 controls had anti human parvovirus IgM (less than 0·3 units). Anti human parvovirus IgG was detected in 10 controls (58·8%). The five patients with Kawasaki disease, who were younger than the patients with erythema infectiosum, had no anti human parvovirus of either IgM or IgG class.

Discussion

A strong specific IgM response that falls in convalescence and a rising specific IgG response are typical of recent human parvovirus infection. Both were present in children with erythema infectiosum in this study. By contrast, some of the controls and all the Kawasaki disease patients lacked antibody to human parvovirus, and the remaining controls had no IgM antibody and lower concentrations of anti human parvovirus IgG than the erythema infectiosum patients. No evidence of infection with other agents was found in the patients with erythema infectiosum in spite of extensive testing, and we conclude that human parvovirus was the cause of erythema infectiosum in the two outbreaks. It was not the cause of Kawasaki disease in the five patients studied.

Erythema infectiosum differs in several ways from the other disease closely associated with human parvovirus, the aplastic crisis of haemolytic anaemia. Several common features of erythema infectiosum—the rash itself, arthralgia, a tendency to relapse, and eosinophilia in convalescence—have scarcely been recorded in aplastic crises. Yet all these features were observed in the 1977 outbreak of erythema infectiosum studied here (Table 2). Viraemia is rarely found in human parvovirus-associated erythema infectiosum (Mortimer, unpublished observations) but is usually present at the start of an aplastic crisis. If, as is likely, it does occur

Table 2 *Features of erythema infectiosum in 21 children M Elementary School, Tokyo, 1977*

Rash	Face	21
	Arms	19
	Legs	19
	Trunk	7
Itching		10
Fever	(37–39°C)	3
	(>39°C)	0
Joint pain		3
Relapse of rash		2
Headache		1
Abdominal pain		1
Nausea		1
Lymphadenopathy		0
Duration of illness (days) Mean, range		8·2 (2–20)
Eosinophil count (acute)(/mm^3) Mean, range		250 (91–490)
Eosinophil count (convalescent) (/mm^3) Mean, range		333 (60–972)

in erythema infectiosum it must precede the rash. There is some evidence for this from blood donors who had human parvovirus antigenaemia and subsequently developed an illness consistent with erythema infectiosum.[7 12] By contrast with aplastic crisis, in which human parvovirus antibody appears during the illness, human parvovirus antibody of both IgM and IgG classes is already present in erythema infectiosum at the onset of disease. These differing clinical features and contrasting serological patterns in the two conditions suggest that the viruses of erythema infectiosum and aplastic crisis may not be identical. On the other hand, the gravity of aplastic crisis may have deflected attention from associated minor features of human parvovirus infection, and the seeming serological differences between it and erythema infectiosum could be explained by the timing of samples. No human parvovirus strain variation has been reported that might support the view that aplastic crisis and erythema infectiosum are caused by different viruses.

Several other outbreaks of erythema infectiosum have recently been investigated for evidence of human parvovirus infection (unpublished, Public Health Laboratory Service Communicable Disease Report 1984/07). Almost all the patients in these outbreaks showed serological evidence of recent infection, as was the case in this study. Our findings are also in close agreement with the 1983 report from London associating erythema infectiosum with acute human parvovirus infection. Because the Japanese outbreaks were remote in time and place from the London outbreak, they give strong support to the proposition that human parvovirus is the specific cause of erythema infectiosum. The investigation of more outbreaks of this disease is now required to prove this point.

P P Mortimer is a member of the Public Health Laboratory Service Working Party on Fifth Disease, other members of which are Drs S M Hall, M J Anderson, E O Caul, and B J Cohen.

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