Preliminary Communications

A Serological Method for Demonstrating Recent Infection by Rubella Virus

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Serological evidence of infection by rubella virus may be obtained by neutralization (Parkman *et al.*, 1964), complement-fixation (Sever *et al.*, 1965), or haemagglutination-inhibition tests (Steward *et al.*, 1967). As rubella often presents atypically or even inapparently, diagnosis on clinical grounds alone is liable to error. Because of the risk of subsequent congenital malformations (Gregg, 1941), serological tests are particularly valuable in the assessment of mothers exposed to or acquiring rubella early in pregnancy. Both neutralizing and haemagglutination-inhibiting (H.I.) antibodies often rise sharply to a high level early in the disease, a significant level of H.I. antibody sometimes being present on the first day of rash (Stewart *et al.*, 1967 ; A. Field, personal communication). In our experience many patients with rubella, particularly those who are pregnant, may be five to seven days or more from the onset of symptoms by the time they are referred for investigation. In these circumstances there may not be a significant rise in antibody titre between an acute phase sample of serum and a subsequent one taken two to three weeks later, both having equally elevated titres. This makes it impossible to say whether their antibody results from recent or from past infection. Although a rising or high complement-fixing antibody titre is useful, since it suggests relatively recent infection (Sever *et al.*, 1965 ; Schmidt and Lennette, 1966), a significant rise in antibody titre may not become detectable until four to six weeks after onset of symptoms (Sever *et al.*, 1966 ; Banatvala, unpublished observations), peak titres sometimes being achieved as late as three to six months after infection (Veronelli and Eckert, 1966).

The first immunoglobulins (IgM) to be detected after primary antigenic stimulus are of high molecular weight (Smith, 1960 ; Uhr and Finkelstein, 1963 ; Svehag and Mandel, 1964). These are almost entirely replaced within a few weeks by those of lower molecular weight (IgG and IgA). This paper describes a method of detecting the presence of recently acquired rubella antibody by H.I. tests, titrating early and late convalescent sera in parallel before and after treatment with 2-mercaptoethanol (2ME) ; this being a sulphydryl-reducing compound which breaks down IgM immunoglobulins.

Materials and Methods

Serial serum samples were obtained from patients with rubella at variable intervals from the day of onset (day 1), to 14 months after illness. Sera showing elevated H.I. titres in acute (day 1–5), early convalescent (day 7–30), or late convalescent (6 weeks to 14 months) were pretreated with 25% kaolin and 50% day-old chick red blood cells according to the method described by Stewart *et al.*, 1967. Sulphydryl reduction was demonstrated by treating sera with 1/10 volume 0.5 *M* 2ME for one hour at 37° C. An equal volume of phosphate-buffered saline was added to untreated sera. Antibody titres were measured by H.I. tests (Stewart *et al.*, 1967), employing 8 HA units of ether-tween split virus antigen with a micro-titre apparatus and plastic disposable " V " plates (Linbro Ltd.). Treated and untreated sera were titrated in parallel. The results of most of these tests were confirmed with a double blind technique. Complement-fixing tests were performed with microtitre apparatus, employing overnight fixation at 4° C. with 1 unit of antigen (Flow Laboratories Ltd.) and 1.5 units of complement.

Results

Eighteen (85.7%) of 21 sera obtained during the acute or early convalescent phase of illness showed consistent reductions in antibody titre after treatment with 2ME (Table I). The most marked reductions occurred in five sera (Cases 1–5) obtained during the first few days of illness (4 to 32-fold reductions) ; these included two sera obtained on the first day of rash. This suggests that even at this early stage true antibody consisting principally of IgM immunoglobulin is present. Thirteen (76.5%) of the 17 sera obtained in the early convalescent phase of illness showed consistent reductions in antibody titre, mostly twofold, but on two occasions fourfold in magnitude. However, of nine sera containing antibody to rubella virus collected during the first 14 days after the onset of illness, all showed reductions in antibody titre. The four early convalescent sera which were not reduced were obtained later, on days 16, 21, 23, and 27 respectively. A fourfold or greater rise in complement-fixing antibody between acute and early convalescent sera occurred in only 7 of 16 (43.7%) sera tested (Table I), although a twofold or greater rise was present in 14. Repeated tests on 14 late convalescent sera obtained from 11 patients failed to show reduction in antibody titre between treated and untreated sera (Table II), although complement-fixing antibody titres persisted or increased. Thus reductions in antibody titre after treatment with 2ME indicated that infec-

TABLE I

Case	Acute Sera					Early Convalescent Sera				
	*Day	H.I.			C.F.	Day	H.I.			C.F.
		Untreated	2ME	Fold Redn.			Untreated	2ME	Fold Redn.	
1	1	256	8	32	<4	17	2,048	512	2	8
2	1	16	<4	4	4	22	2,048	1,024	2	32
3	2	16	<4	>4	<4	16	1,024	1,024	—	8
4	3	32	<4	>8	8	23	512	512	—	16
5	5	256	64	4	NT	16	4,096	2,048	2	NT
6	1	<4	—	—	<4	7	4,096	2,048	2	16
7	1	<8	—	—	<8	14	512	256	2	16
8	2	<4	—	—	<4	14	8,192	4,096	2	32
9	1	4	—	—	4	14	2,048	512	4	16
10	1	<4	—	—	4	16	1,024	512	2	16
11	2	4	—	—	4	18	2,048	1,024	2	8
12	2	<4	—	—	<4	19	16,384	8,192	2	16
13	1	<4	—	—	4	19	1,024	512	2	8
14	2	4	—	—	<4	23	4,096	1,024	4	4
15	1	<4	—	—	<4	30	16,384	8,192	2	32
16	2	32	NT	NT	4	21	8,192	8,192	—	4
17	3	<4	—	—	<4	27	1,024	1,024	—	8

* Onset of rash = day 1. NT = not tested.

TABLE II.—*Late Convalescent Sera*

Case	Months from Onset	HI			C.F.
		Untreated	2ME	Fold Reduction	
1	1½	1,024	1,024	—	64
2	3	4,096	4,096	—	32
	7	4,096	4,096	—	32
3	3	4,096	4,096	—	32
4	3	2,048	2,048	—	8
	9	1,024	1,024	—	8
5	4	2,048	2,048	—	32
6	5	2,048	2,048	—	8
7	6	1,024	1,024	—	>64
8	6	4,096	4,096	—	4
9	6	1,024	1,024	—	32
	11	4,096	4,096	—	8
10	12	128	128	—	NT
11	14	4,096	4,096	—	8

tion had occurred recently, whereas no reduction in antibody titre was suggestive, but was not conclusive evidence of less recent infection.

Discussion

These results are similar to those obtained by Schluederberg (1965), who found consistent twofold to fourfold reductions in antibody titre after treatment with 2ME in serum obtained at 12-day to 21-day intervals after measles vaccination and natural infection with mumps virus, but no reduction in those obtained two months or more after infection. Lindquist *et al.* (1965), employing neutralization tests, observed fourfold or greater reductions in rubella antibody titre in seven of nine infants aged 10 weeks or less with the rubella syndrome, suggesting that, in addition to maternal IgG immunoglobulins, infants were synthesizing their own antibody, consisting principally of IgM components either before birth or in the neonatal period. Although treatment with 2ME provides a considerably less precise estimate of the presence of IgM immunoglobulins than, for example, analyses of antibody components by means of sucrose density gradients or gel filtration on Sephadex, the technique is simpler and can be used more readily if many sera have to be tested routinely. Even though the action of 2ME may not be entirely selective in breaking down only IgM immunoglobulins, both this and Schluederberg's series (1965) demonstrate that reduction in antibody titre after virus infection in early but not late convalescent sera, even though sometimes of small magnitude, is consistent. Reductions in antibody titre after treatment with 2ME compared favourably with complement-fixing tests as an index of recent infection by rubella virus. However, treatment with 2ME may be especially useful in assessing the duration of antibody in single serum samples obtained in the early stages of illness, a period at which greatest reductions in antibody titres are to be expected, or in obtaining evidence of recent infection in a proportion of patients who might present in early convalescence before complement-fixing antibody has fully developed. These preliminary results suggest that this test, since it can be both simply and rapidly performed, may, in conjunction with virus isolation and other serological tests, be of additional value in the assessment of patients who have been contacts of or who gave a history of rubella during pregnancy.

Summary

Eighteen of 21 (85.7%) acute and early convalescent sera from patients with rubella showed reduction in haemagglutination-inhibiting antibody titre after treatment with 2ME, whereas all late convalescent sera showed no reduction. This test provided a method for recognizing recent infection by rubella virus that may be particularly useful in the serological assessment of mothers contracting rubella during pregnancy.

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Medical Memoranda

Guillain-Barré Syndrome Associated with Chronic Lymphatic Leukaemia

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The occurrence of the Guillain–Barré syndrome in patients with chronic lymphatic leukaemia is rare. There seem to be no previous reports of this association, and it has been seen in only two instances in our series of more than 500 patients with chronic lymphatic leukaemia.

Case 1

A man aged 60 was admitted to hospital at the end of November 1965 with a history of pain in the region of the right hip, radiating into the right thigh, accompanied by increasing weakness of the arms and legs. A diagnosis of chronic lymphatic leukaemia had been made in July 1965 after the examination of a peripheral blood smear and bone-marrow biopsy. He had been treated with chlorambucil.

On admission he was hardly able to walk. Fine movements with his hands were impossible. He was not anaemic, and there was no lymphadenopathy. His spleen was palpable 1 cm. below the left costal margin. He had generalized muscle weakness of his limbs and trunk. The muscles were flaccid ; there was no fasciculation but some tenderness, particularly of the leg muscles. All the tendon jerks were absent. The plantar reflexes were flexor. There was loss of appreciation of painful stimuli in a stocking and glove distribution. After admission he developed cranial nerve involvement, with a bilaterally symmetrical facial nerve weakness and some difficulty in swallowing.

Investigations.—The haemoglobin was 14.2 g. ; W.B.C. 15,200/cu. mm., with 11,400 lymphocytes ; E.S.R. 2 mm./hour (Westergren). The cerebrospinal fluid contained 58 mg. of protein per 100 ml. Porphyrins were not detected in the urine. Electromyography showed changes compatible with a widespread peripheral neuropathy, and a suggestion of primary muscle disease in the forearm muscles. The creatine kinase level was 0.3 unit. The serum protein contained a low level of gammaglobulin. The IgG was 720 mg./100 ml. IgM was 63% and IgA 23% of a reference normal serum.

Large doses of corticosteroid (60 mg. of prednisolone daily) brought slow but steady improvement. He was able to walk, and fine movements of his hands were possible. Electromyographic changes, still