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A study of Coxsackie B virus infections, 1972–1983

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SUMMARY

The results of a twelve-year study of Coxsackie B virus (CBV) infections in patients with a variety of acute and chronic illnesses are reported. CBVs were isolated from only 123 patients most of whom were children with respiratory illness. Virus diagnosis in adults was based mainly on the detection of significant rising or static high neutralizing antibody titres. Between 1972 and 1979 most investigations centred on patients with suspected viral heart disease, 12% of whom were found to have diagnostically significant CBV titres. In studies on patients with definite myo-pericarditis the number positive increased to 33%. In 1980 clinical interest switched to the possible role of CBV in myalgic encephalomyelitis (ME), an illness of diverse symptomatology. Investigation of suspected cases of ME in 1983 showed that 16% were serologically positive compared to 4% of normal adults in the West of Scotland. In patients with well-documented ME this figure rose to 41%.

The demand by clinicians for CBV neutralizing antibody tests has increased over the past twelve years and continues to escalate annually, especially in patients with chronic relapsing illness.

INTRODUCTION

Coxsackie B viruses (CBVs) are endemic in the United Kingdom. They can cause a variety of illnesses, ranging from mild respiratory infection to fatal myocarditis in the newborn. In the early 1970s good evidence as to their causative role in adult heart disease was lacking. Controlled studies of heart disease between 1966 and 1971 (Grist & Bell, 1974) showed that CBVs were associated with at least half the cases of acute myocarditis and one third of the cases of non-bacterial pericarditis; no evidence of infection was found in chronic heart disease. Most of these conclusions were based on interpretation of static high neutralizing antibody titres since the majority of patients presented too late to enable detection of virus or significant rising antibody titres. These findings generated considerable interest amongst cardiac physicians, resulting in requests for virus diagnostic tests on their patients with suspected viral heart disease.

Recent publications (Fegan, Behan & Bell, 1983; Keighley & Bell, 1983; Calder & Warnock, 1984) indicate a growing awareness that CBVs may be implicated in other illnesses not hitherto suspected of having such an association. This paper records data, based mainly on the serological diagnosis of CBV infection, collected in this laboratory between 1972 and 1983. The virus isolation techniques and the

micrometabolic inhibition test used for the estimation of CBV 1–6 neutralizing antibodies have remained virtually unchanged during this time thus allowing comparison of results.

PATIENTS AND METHODS

The majority of patients investigated were from the West of Scotland, others from East Scotland or occasionally from various parts of England. It must be emphasized that our clinical classification of patients was based entirely on information provided by many different clinicians when submitting specimens; because the numbers were too great and the sources so varied no routine follow-up enquiry was pursued.

The virus isolation techniques and the micrometabolic inhibition method used for the estimation of Coxsackie B neutralizing antibodies are described elsewhere (Grist *et al.* 1979). In CBV infections involving mainly adults, patients often present too late to detect virus and/or a significant ($\geqslant$4-fold) rise in neutralizing antibody titres. Previous studies (Grist & Bell, 1974) suggested that, in general, the higher the titre observed, the greater the probability that the infection was recent. Thus static titres of 256 were regarded as suggestive and titres of $\geqslant$512 as indicative of recent CBV infection.

RESULTS

Two relevant points of background information regarding the circulation of CBVs in our community have already been established. Firstly, there has been no major epidemic of CBV infection since 1965, when Coxsackie B5 was predominant both in Scotland and elsewhere in the U.K. Secondly, CBV antibody studies of normal population groups between 1973 and 1978 showed that 10% had titres of 256 and 4% titres of $\geqslant$512 to one or more of the group B Coxsackie viruses (Bell *et al.* 1983). Recent independent studies in 1980–1 by O'Neill *et al.* (1983) suggest that this antibody status has remained unchanged.

Table 1 lists the CBV serotypes isolated during the 12 years of our study. With two exceptions, all were from children with respiratory infections or aseptic meningitis. Although the dominant CBV type varied from year to year, B4 and B2 were the commonest viruses detected.

The number of patients tested serologically in each year and the percentage 'positive' using our diagnostic criteria are shown in Table 2. The percentage of patients 'positive' fluctuated from year to year. In the earlier years particularly this was probably a measure of both the physician's skill in selecting appropriate patients for study and the actual activity of these viruses in the community. Seventy-seven patients showed significant rising titres, most of whom had acute chest pain (pleurodynia) or acute myo-pericarditis (Table 3). A significant fall in antibody titre was detected in only three patients.

Between 1972 and 1979 interest was centred on patients with suspected viral heart disease, most of whom were aged 40–60 years and were predominantly male. The results of our studies on these patients, classified clinically on their admission diagnosis, are given in Table 4. Although the percentages 'positive' (using the more

Table 1. *Coxsackie B virus isolations*, 1972–1983

Year	Number of isolations of coxsackievirus						
	B1	B2	B3	B4	B5	B6	Total
1972	0	0	2	1	2	0	5
1973	0	1	8	0	0	0	9
1974	0	0	1	0	2	0	3
1975	0	7	0	16	1	0	24
1976	3	0	0	0	7	0	10
1977	2	0	0	0	4	0	6
1978	0	6	1	0	0	0	7
1979	0	2	0	8	0	0	10
1980	0	0	0	0	4	0	4
1981	0	17	1	4	2	0	24
1982	1	0	1	6	0	0	8
1983	10	0	1	2	0	0	13
Total	16	33	15	37	22	0	123

Table 2. *Results of Coxsackie B*1–6 *neutralization tests*, 1972–1983

Year	Total number patients tested	CBV antibody titres			Total 'positive' (%)
		RT/FT* (%)	⩾512 (%)	256 (%)	
1972	156	3	10	11	24
1973	134	3	4	4	11
1974	148	1	11	7	19
1975	177	4	11	9	24
1976	259	1	13	13	27
1977	241	2	9	11	22
1978	495	1	12	14	28
1979	693	1	14	17	32
1980	1023	1	23	19	41
1981	1440	1	16	16	33
1982	1485	0·5	16	15	32
1983	2226	0·3	16	17	34
Total	8477	0·9	13	13	27

* RT/FT = significant ⩾4-fold rising/falling titres respectively.

rigorous threshold of rising/falling titres or titres ⩾512) in each clinical group were three times higher than that observed in our normal adult population, it was disappointing that the results were not closer to those observed in our 1966–71 study, where the corresponding percentages for chest pain, myocarditis and pericarditis were 42, 33 and 13 respectively. These disparate results are almost certainly due to overlap in the clinical classification used by us. Specific studies on patients with well-documented myo-pericarditis in a general medical unit between 1979 and 1980 showed that 33% had titres ⩾512 (Bell *et al.* 1983), a figure similar to that of the 1966–71 study.

In 1980 there was a revival of interest in the possible viral aetiology of myalgic

Table 3. *Patients with significant rising/falling Coxsackie B antibody titres, 1972–1983*

Diagnostic group	Number of patients with rising/falling CBV antibody titres in each year												
	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	Total
'Chest pain'	0	1	0	3	0	3†	1	3	3	9	3	3*	29
'Myocarditis'	0	3	0	1	1	1	2	1	1	3	1†	2	16
'Pericarditis'	2	0	0	3	2	1	1	1	2	2	1	2	17
Other cardiac disease	2	0	0	0	0	0	0	0	0	0	0	0	3
Non-cardiac disease	0	0	2	0	0	1*	1	4*	2	3	3	0	15
Total	4	4	2	7	3	6	5	9	8	17	8	7	80

* Virus isolated: B1, 26F chest pain (1983); B2, 13M respiratory infection (1979); B5, 25M meningitis (1977).

† Three patients with significant (⩾4-fold) fall in titre.

Table 4. *Coxsackie B antibody in patients with chest pain or suspected cardiac disease*, 1972–1979

Disease category (no. patients)	Percentage with antibody rising/falling or ⩾512 in each year								Total (%)
	1972	1973	1974	1975	1976	1977	1978	1979	
'Chest pain' (557)	8	31	9	39	11	11	7	18	14
'Myocarditis' (624)	5	6	10	11	15	10	23	9	12
'Pericarditis' (513)	12	6	12	14	15	12	9	16	12
'Cardiac illness' (unspecified) (246)	12	0	20	13	15	18	7	18	12

Table 5. *Clinical diagnosis and results of Coxsackie B tests on 2226 patients studied in 1983*

Diagnostic group	Number of patients tested	CBV antibody titres			Total 'positive' (%)
		RT/FT* (%)	⩾512 (%)	256 (%)	
'Chest pain'	597	0·5	16	17	33
'Myocarditis'	139	1	15	16	33
'Pericarditis'	174	1	19	13	34
Palpitations/tachycardia	105	0	21	13	34
Myalgic enceph. (ME)	54	0	41	18	59
?Myalgic enceph. (ME)	335	0	16	24	40
Myalgia	254	0	22	19	41
Neurological	104	0	8	17	25
Diabetes	13	0	8	23	31
Miscellaneous†	451	0	12	14	26
Total	2226	0·3	16	17	34

* RT/FT = significant 4-fold rising/falling titres respectively.

† Includes patients with cardiac symptoms (diagnosis unspecified); lassitude after virus infection; arthralgia; known positive Coxsackie B patients of 1982.

Table 6. *Coxsackie B antibody responses detected* 1972–1983

	Number of patients with neutralizing antibody titre ⩾512						Dominant CBV isolations* from the community
	B1	B2	B3	B4	B5	B6	
1972	5	4	9	3	1	0	B3, B5
1973	0	2	3	2	0	0	B3
1974	3	5	5	4	1	0	B5
1975	2	7	2	9	0	0	B4
1976	2	13	3	15	3	0	B5
1977	6	3	8	4	6	0	B5
1978	16	6	22	25	4	1	B2
1979	11	21	21	59	8	0	B4
1980	35	59	36	131	10	0	B5
1981	36	61	24	135	10	0	B2
1982	29	52	13	159	1	0	B4
1983	18	134	17	255	3	0	B1
Total	165	367	163	801	47	1	—

* Mainly from children with respiratory infections or aseptic meningitis.

Table 7. *Examples of homotypic and heterotypic antibody responses*

		Antibody titres in acute and convalescent sera					
Patient	Isolate	B1	B2	B3	B4	B5	B6
F26: chest	B1	<64	<64	<64	<64	<64	<64
pain		*256*	512	256	512	256	256
M13: sore	B2	<64	64	64	<64	<64	<64
throat		<64	⩾*1024*	⩾1024	<64	<64	<64
M25:	B5	<16	<16	<16	<16	32	<16
meningitis		<16	<16	<16	<16	*256*	<16

encephalomyelitis (ME) best known previously as 'Royal Free Disease' after the outbreak in that London Hospital in 1955 (Behan, 1980). The result was that the scope of patients investigated widened to include diverse symptomatology, e.g. general myalgia, chest pain with palpitations (no ECG changes), vertigo, labyrinthitis, paraesthesia. Laboratory requests for investigation of patients with non-cardiac disease now exceeded those with suspected cardiac illness. In contrast to the 1972–9 studies, many patients were now in the 30- to 50-year age group, with females predominating. This upsurge in interest in CBV infections in 1980 resulted in an increase in the number of patients found 'positive' serologically. Table 5 demonstrates typical clinical categories now regularly tested for the presence of Coxsackie B antibodies.

Selecting titres ⩾512 to one or more Coxsackie B viruses, the most frequent response detected was to Coxsackie B4 (Table 6), this despite the fact that incidental isolations from the community, especially from children with respiratory infections or aseptic meningitis, were not predominantly B4 virus. Various reports

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from other parts of the world have also shown a greater frequency in detection of B4 antibody, compared with the other viruses within this group. One possible explanation is that B4 infection may be acquired with or without symptoms, when young. Infection with another Coxsackie B virus later in life results in an anamnestic boost of the 'old' Coxsackie B4 antibody. This anamnestic response is common in poliovirus and influenza infections. Coxsackie B4 and B2 antibody responses were detected more frequently than B1, B3 or B5; B6 antibody was rarely found.

On the few occasions when Coxsackie B was isolated and paired sera were available for study, the titres of heterotypic antibody equalled or even exceeded those of homotypic antibody (Table 7). Thus, in the absence of virus isolation, prediction of the infecting virus type in patients with multiple rising titres is almost impossible.

DISCUSSION

Several conclusions and cautionary notes can be drawn from this 12-year study. Although very labour intensive, the micrometabolic inhibition technique permits the testing of large numbers of sera not possible by conventional tube-culture methods. These tests give reproducible results if adequate cell, serum and virus controls are included.

Antibody responses in the individual patient vary and the clinician must interpret the virological result with careful regard to the patient's illness and onset date. Some patients may only achieve maximum titres of 128 (especially in the case of B1, B3, B5 and B6 antibodies), which fall short of our minimum 'positive' criteria and yet such titres may be relevant. In contrast, detection of a high titre may be purely coincidental. In general, however, our serological criteria based on high static titres have proved useful in clarifying the role of Coxsackie B viruses in adult heart disease (Wood *et al.* 1978; Bell *et al.* 1983), and our findings have been confirmed independently by other investigators (Griffiths, Hannington & Booth, 1980; O'Neill *et al.* 1983).

In illnesses with a chronic relapsing course, e.g. atypical left chest pain, myalgic encephalomyelitis (ME), titres $\geqslant 512$ may remain static for three or more years, thus making interpretation of their significance more difficult. Detection of CBV specific IgM in such patients could infer recent infection or perhaps persisting viral antigen. Several workers have spent much time and effort in the development of a CBV specific IgM assay using the modern virological techniques of ELISA or MACRIA (Dörries & Ter Meulen, 1983; King *et al.* 1983; Morgan-Capner & McSorley, 1983). All have detected non-specific cross-reactions, some not only within the CBV group but also with other enteroviruses and hepatitis A virus. Much further work is needed but in the meantime tests for the quantitation of neutralizing antibodies will have to be retained.

The role of CBVs in certain adult heart disease is now well recognized. Other diseases are coming under closer scrutiny, e.g. ME, diabetes and pancreatitis. The pathology of many of these illnesses is seen when mice are infected with these viruses, so a similar role in human disease would not be totally unexpected.

Although serological testing for CBV neutralizing antibodies is time-consuming

and its limitations in respect of accurate virus diagnosis considerable, the demand for this service by clinicians increases annually. There is no specific antiviral therapy available but patients, often adults in the 30- to 40-year age group who are struck down with illness mimicking a heart attack or with debilitating ME, apparently derive considerable psychological benefit if a presumptive diagnosis of CBV infection is made. Clinicians report that such a virus diagnosis often prevents these patients from becoming 'cardiac' or 'psychiatric' cripples and that they accept their illness with a positive attitude.

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