JACC Vol. 23, No. 3
March 1, 1994:593–8

A Prospective Case-Control Study of Antibodies to Coxsackie B Virus in Idiopathic Dilated Cardiomyopathy

PHILIP J. KEELING, MRCP,* ANDREW LUKASZYK, MB,* JAN POLONIECKI, PHD,*
ALIDA L. P. CAFORIO, MD, PHD,*† MICHAEL J. DAVIES, MD, FACC,*
JAMES C. BOOTH, PHD,* WILLIAM J. MCKENNA, MD, FACC*

London, England, United Kingdom and Padua, Italy

Objectives. **This study was conducted to determine the frequency and significance of Coxsackie B virus–specific immunoglobulin-M (IgM) in patients with idiopathic dilated cardiomyopathy and compare them with the frequency in both unmatched and matched control subjects.**

Background. **The principal evidence supporting a pathoetiologic role for Coxsackie B viruses in human dilated cardiomyopathy is derived from retrospective serologic studies. These studies have evaluated patients with end-stage disease and have failed to recognize the importance of assessing both matched and unmatched control subjects.**

Methods. **In this prospective case-control study, we assessed sera for Coxsackie B virus-specific IgM (serotypes B1 to B5) from 114 patients with dilated cardiomyopathy at diagnosis or referral to our center, 94 healthy unmatched control subjects, 41 healthy matched control subjects from the same general practitioner and 32 members of the patients' own households.**

Results. **A higher frequency of positive Coxsackie B virus IgM was observed in patients with dilated cardiomyopathy than in unmatched control subjects (33% vs. 5%; $p = 3 \times 10^{-7}$). In patients with dilated cardiomyopathy, the response was monotypic (84%), commonly against serotypes B2 and B5, and was not associated with any clinical or histologic feature. The frequency of positive virus-specific IgM was similar in patients with dilated cardiomyopathy and their 41 matched community control subjects (46% vs. 27%; $p = 0.11$) and 32 household contacts (37% vs. 28%; $p = 0.59$). Control subjects who tested positive for virus-specific IgM tended more commonly to be seropositive than did control seronegative subjects (community control subjects 37% vs. 18%, $p = 0.32$; household contacts 42% vs. 20%; $p = 0.36$) and had an identical serotypic response in 4 (33%) of 12 cases.**

Conclusions. **The frequency of Coxsackie B virus IgM was higher in patients with dilated cardiomyopathy than in unmatched control subjects but was similar in patients and control subjects who shared the same environment, indicating local spread of infection. The reason for the association between Coxsackie B virus IgM and dilated cardiomyopathy and its relevance to pathogenesis remain to be established.**

(J Am Coll Cardiol 1994;23:593–8)

Enteroviruses and particularly Coxsackie B viruses are believed to be important in the pathogenesis of idiopathic dilated cardiomyopathy (1). Although there are considerable experimental data supporting this hypothesis (2–4), the evidence in humans is controversial (5–7). The principal supporting evidence remains the finding of raised titers of neutralizing antibodies against Coxsackie B virus in patients with dilated cardiomyopathy compared with control subjects (8–11). Enteroviruses are ubiquitous, easily spread from person to person and by adulthood most people have developed neutralizing antibodies against a range of different enteroviruses (12), making interpretation of these results problematic. In an attempt to identify recent Coxsackie B virus infection, Muir et al. (13) assessed Coxsackie B virus-specific immunoglobulin-M (IgM) and showed that there was a higher frequency of virus-specific IgM in patients receiving a cardiac transplant for end-stage dilated cardiomyopathy compared with healthy unmatched blood donors (28 [33%] of 86 vs. 10 [12%] of 84, respectively, $p < 0.005$). The aim of the present study was to assess the frequency of IgM antibodies against Coxsackie B virus in a consecutive series of patients with dilated cardiomyopathy at diagnosis or presentation to our center and compare this with the frequency in healthy unmatched and matched control subjects taken from patients' own environment.

Methods

Patients with dilated cardiomyopathy. Serum was available from 114 patients' with dilated cardiomyopathy (mean age 44 years, range 12 to 74, 87 male). Sera were obtained either at the time of diagnosis or when patients were referred

From the *Departments of Cardiological Sciences, Histopathology and Medical Microbiology, St. George's Hospital Medical School, London, England, United Kingdom and †Institute of Clinical Medicine, Department of Cardiology, University of Padua, Italy. Dr. Keeling was supported by a grant from the British Heart Foundation, London. Dr. Caforio was supported by a grant from the European Society of Cardiology, Padua, Italy.

Manuscript received June 17, 1993; revised manuscript received October 7, 1993, accepted October 26, 1993.

Address for correspondence: Dr. Philip J. Keeling, Department of Cardiological Sciences, St. George's Hospital Medical School, Cranmer Terrace, London SW17 ORE, England, United Kingdom.

0735-1097/94/$7.00

to our center and were collected in every month of the year from 1990 to 1992. The clinical diagnosis of dilated cardiomyopathy was made according to World Health Organization criteria (14). All patients underwent selective coronary arteriography and right ventricular endomyocardial biopsy. Histologic assessment was performed by an experienced histopathologist (M.J.D.) using the Dallas criteria (15). Patients with features of myocarditis on endomyocardial histologic examination were excluded from the study. Classification using the New York Heart Association functional class showed 47 patients were in class I, 20 in class II, 32 in class III and 15 in class IV. Patients had been symptomatic for 35 ± 46 months, 66 (58%) gave a history of clinical deterioration and 31 (27%) of an acute viral illness within 6 months of the onset of symptoms.

Control subjects. Sera were obtained from 94 unmatched control subjects (mean age 39 years, range 13 to 69, 48 male). Subjects were contacted as part of our screening program for dilated cardiomyopathy but were not geographically or temporally matched to patients with dilated cardiomyopathy. All unmatched control subjects were asymptomatic, had normal screening investigations and provided sera from every month between 1990 and 1992. To assess the importance of the patients' local environment on the frequency of positive enteroviral serology, sera were also obtained from two matched control groups and were collected within 2 weeks of the patients' sera. These groups consisted of 41 "general practitioner control subjects" (healthy individuals obtained by selecting for all patients the next patient on their general practitioner's list who was of the same gender and within 5 years of the patients' age) and 32 "household contacts" (members of the patients' own households; 27 spouses, 4 parents and 1 child).

Coxsackie B virus–specific IgM. Sera were assessed for Coxsackie B virus IgM without knowledge of clinical information using a reverse radioimmunoassay procedure similar to that described by Torfason et al. (16). Briefly, polystyrene beads were coated with rabbit anti-human IgM (specific for heavy chains) then incubated with patient serum. After washing, the presence of Coxsackie B virus IgM on the beads was indicated by their ability to bind viral antigen that had been metabolically labeled with ^{35}S-methionine. The radioactivity bound to the beads was counted in a beta counter and the results obtained compared with those of known control sera.

Preparation of labeled antigen. Coxsackievirus types B1 to B5 were prepared in Vero cells and stored at −70°C. Vero cell monolayers were inoculated with 0.5 ml of stock Coxsackie B virus. After 1.5 h absorption, the cells were washed 3 times with methionine-free Eagle's MEM supplemented with 500 units per ml of penicillin and 200 μg per ml of streptomycin, and re-fed with the same medium containing 75 μCi/ml of sulfur-35 (^{35}S)-methionine (Amersham). Gross viral cytopathology developed within 24 h, but the cultures were not harvested until 48 h; they were subjected to three cycles of freezing and thawing and the cell debris was removed by centrifuging for 10 m at 2,600 rpm (MSE Centaur centrifuge). The labeled virus supernatant was layered onto 1.8 ml of ultrapure (Gibco BRL) cesium chloride dissolved in Tris buffer (0.005 mol/liter) with a density 1.4 g/ml and centrifuged for 3 h at 40,000 rpm (Beckman SW50 rotor). Nine 0.5-ml fractions were collected and tested for trichloroacetic acid-precipitable radioactivity, the highest number of counts per minute usually being found in fractions 3 and 4. These fractions were pooled and dialyzed overnight against phosphate buffered saline solution then heated for 30 min at 57°C to destroy infectivity, stored at −70°C and used subsequently as antigen.

Reverse radioimmunoassay technique. The beads (6.5-mm etched polystyrene beads, Northumbria Biologicals, Ltd., UK) were coated overnight with rabbit anti-human IgM antibody that was specific for heavy chains (Dako A426). About 200 of these beads were added to a solution containing 112 μl of the antibody in 30 ml of 1 M hydrochloride and left at 4°C overnight. They were then "blocked" by rinsing three times with phosphate buffered saline 1% bovine serum albumin and soaked overnight in 100 ml of the same buffer. The serum specimens to be tested were diluted (1:40) in phosphate buffered saline solution containing 0.5% bovine serum albumin, 0.1% Tween 20 and 0.02% sodium azide; 200 μl was then incubated at 37°C for 4 h with one of the dried coated beads. These beads were then washed three times using the Abbot Qwik-Wash machine and incubated overnight at room temperature with 200 μl of the labeled virus antigen after this had been diluted in the same solution as just described but using 20% fetal bovine serum. The next day, the beads were washed three times, dried at 37°C and then placed into clean scintillation tubes to which was added the 1.5-ml liquid scintillator NE 260 (Nuclear Enterprises) before counting. Positive sera were retested against an antigen prepared from uninfected cells and were all found to be negative.

Interpretation of results. The specificity of the Coxsackie B virus IgM test was evaluated for each coxsackievirus antigen using known positive and negative sera supplied by the Enterovirus Reference Laboratory, Public Health Laboratory, West Park Hospital, Epsom, England. The results obtained for known positive sera for Coxsackie B2 and B5 virus (each reacting more strongly with one serotype) and five known negative sera is shown in Figure 1A. For each antigen, stronger reactions were detected with positive sera known to show a degree of specificity for the homologous serotype and weaker reactions (cross reactions) with heterologous serotypes. No reactivity was detected with any of the negative sera. Because of variability in the radiolabeling process, large batches of sera (≥150) were assayed at a time using each of the five prepared Coxsackie B virus antigens. The cutoff level between a positive and negative result was determined for each antigen by two experienced independent observers who constructed a frequency histogram of the number of specimens falling within each of a series of ranges of progressively increasing radioactivity counts (Fig.

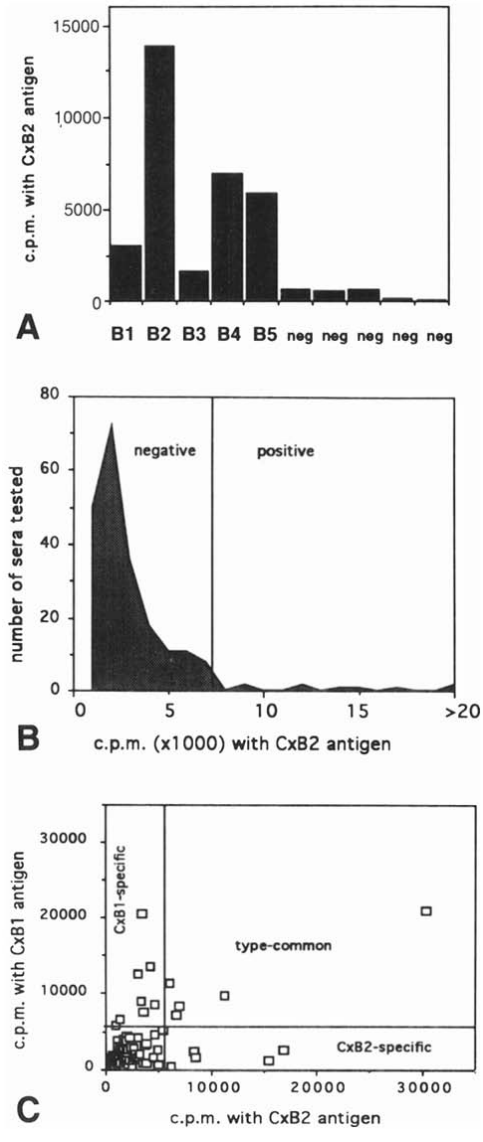


Figure 1. Reverse radioimmunoassay for the detection of Coxsackie B virus–specific immunoglobulin-M (see text). c.p.m. = counts per minute; CxB1 and CxB2 = Coxsackie virus B1 and B2, respectively; neg = negative.

1B). The substantial peak observed at lower counts was taken to represent sera negative for Coxsackie B virus IgM, whereas higher readings falling outside this distribution were considered to be positive. Figure 1C shows a comparison of the readings obtained on a single batch of sera against two different Coxsackie B virus antigens (for example, B1 and B2). This allowed the ready identification of sera giving type-specific (monotypic) or type-common (heterotypic) results.

Statistical methods. Sample size calculations were based on a prevalence of 12% and 33%, respectively, of Coxsackie B virus IgM in control subjects and patients with dilated cardiomyopathy (13). For patients and unmatched control subjects, a standard computation showed that assessment of a total of 200 serum samples gave a power of 0.95 to detect a statistically significant difference between these groups. The sample size for a matched case-control study is more difficult to determine prospectively. Using the same assumptions and a correlation coefficient for infection of 0.1 between patients and control subjects, a sample size of 41 (general practitioner control subjects) and 32 (household contacts) had power of 0.8 to detect an odds ratio of 5.0 and 6.5, respectively. The clinical results were analyzed with the Student t test for numeric data or chi-square test for comparison of frequencies. The frequency of positive Coxsackie B virus IgM results in patients with dilated cardiomyopathy and control subjects was compared using the chi-square test. The relative risk of the association between disease and Coxsackie B virus IgM was given by the odds ratio and the confidence interval for this value calculated from the binomial distribution. Seasonal variation in the frequency of positive Coxsackie B virus IgM results throughout the year was assessed by contingency analysis of the relative proportion of positive results in each of the four seasons. A p value < 0.05 was considered statistically significant.

Results

Coxsackie B virus IgM. *Patients with dilated cardiomyopathy.* Thirty-eight (33%) of 114 patients with dilated cardiomyopathy tested positive for Coxsackie B virus IgM at diagnosis. No differences were observed between patients with and without positive virus-specific IgM with respect to age (42 ± 16 vs. 44 ± 13 years, p = 0.45), New York Heart Association functional class (class I or II in 22, class III or IV in 16 vs. class I or II in 45, class III or IV in 31, p = 0.95), duration of symptoms (31 ± 40 vs. 36 ± 48 months, p = 0.69), clinical deterioration (24 [63%] vs. 42 [55%], p = 0.55) or acute viral illness at disease onset (8 [21%] vs. 23 [30%], p = 0.41). Patients whose sera produced high radioactivity counts by radioimmunoassay (>10,000 cpm) had no distinguishing clinical characteristics. Monotypic responses predominated (n = 32 [84%]) and were most commonly against serotypes B2 and B5 (Table 1). Positive Coxsackie B virus IgM results were seen throughout the year (Fig. 2) and showed a nonsignificant increase in the proportion of positive results during summer/autumn compared with the rest of the year (38% vs. 29%, p = 0.39). During 1990 to 1992, no major outbreak of Coxsackie B virus infection was observed.

Patients with dilated cardiomyopathy and control subjects. A higher frequency of positive Coxsackie B virus IgM was observed in patients with dilated cardiomyopathy compared with healthy unmatched control subjects (38 [33%] vs. 5 [5%], p < 0.001) (Fig. 3A). However, a similar frequency of positive Coxsackie B virus IgM was observed in patients with dilated cardiomyopathy and their 41 matched community control subjects (19 [46%] vs. 11 [27%], odds ratio 3.0 [95% confidence interval 0.91 to 12.76], p = 0.11) (Fig. 3B) and 32 household contacts (12 [37%] vs. 9 [28%], odds ratio 1.5 (95% confidence interval 0.44 to 8.15, p = 0.59) (Fig. 3C). Sera with high radioactivity counts (>10,000 cpm) were

Table 1. Serotypic Responses Observed in Patients With Dilated Cardiomyopathy and Control Subjects With Positive Coxsackie B Virus IgM Serology

Patient Group	No.	Serotypic Response					
		B1	B2	B3	B4	B5	Multitypic
Dilated cardiomyopathy	38	4	9	3	6	10	6
Dilated cardiomyopathy	19	2	4	2	4	3	4
GP control subjects	11	1	1	3	2	1	3
Dilated cardiomyopathy	12	1	3	1	2	2	3
Household contacts	9	3	1	1	1	1	2

GP = general practitioner; IgM = immunoglobulin M.

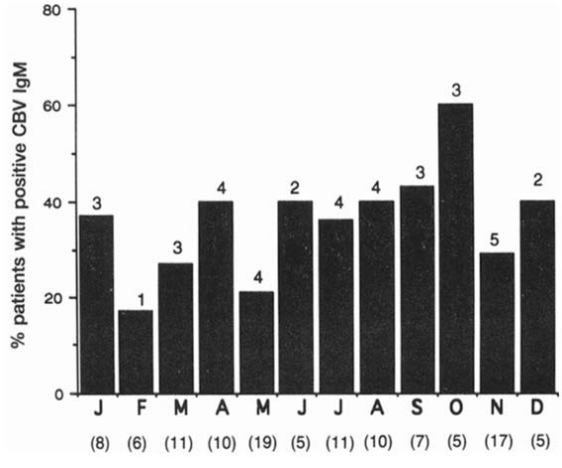
equally common in patients with dilated cardiomyopathy and control subjects. The serotypic responses in patients with dilated cardiomyopathy and their matched control subjects is shown in Table 1. Monotypic responses predominated both in general practitioner control subjects and in household contacts (73% and 78%, respectively), with serotypes B3 and B1 being the most common in general practitioner control subjects and household contacts, respectively. These findings did not differ significantly from those observed in patients with dilated cardiomyopathy. Control subjects for patients who tested positive for virus-specific IgM more commonly were seropositive than were patients who tested IgM negative (community control subjects 37% vs. 18%, p = 0.32; household contacts 42% vs. 20%, p = 0.36) and often had identical serotypic response (4 [33%] of 12).

Discussion

The pathogenesis of dilated cardiomyopathy is heterogeneous (17) and likely to include viral infection, autoimmunity and genetic factors. Although enteroviruses, particularly Coxsackie B virus, are believed to be of pathogenic importance, there has been a consistent failure to isolate virus from or detect viral antigens within the myocardium of patients with dilated cardiomyopathy. The principal supporting evidence remains the finding of high titers of neutralizing antibodies against Coxsackie B virus in patients with dilated cardiomyopathy (8–11) or a higher positive rate for virus-specific IgM at heart transplantation compared with that in healthy blood donors (13). The frequency and significance of Coxsackie B virus IgM in patients earlier in the natural history of dilated cardiomyopathy than previously thought and the importance of the patients' environment have not previously been addressed.

In this study, we confirm the increased frequency of Coxsackie B virus IgM in patients with dilated cardiomyopathy compared with that in unmatched control subjects (13). Patients in our study had recently had their condition diagnosed or were newly referred and demonstrated that the association between Coxsackie B virus IgM and dilated cardiomyopathy is present earlier in the natural history of the disease than previously assessed. Although we recognize that serum was not always available at the patients' primary diagnosis, there was no difference in the duration of symptoms between patients with a first diagnosis at our center and those referred from another center, and the serum nonavailability is unlikely to have influenced the overall results. The finding of Coxsackie B virus IgM in 5% of healthy unmatched control subjects is in agreement with previous studies (18–20) conducted in the absence of a major outbreak of Coxsackie B virus infection and probably reflects natural background infection within the community. In this study, we have shown for the first time a high and similar frequency of positive Coxsackie B virus IgM in matched control subjects taken at the same time and from the same environment as the patients. This finding together with the trend toward an increased frequency of positive virus-specific IgM in control subjects of seropositive compared with seronegative patients (often with an identical serotypic response) is highly suggestive of cross infection within the environment of the patient.

Figure 2. Frequency of positive Coxsackie B virus (CBV) immunoglobulin-M (IgM) in patients with dilated cardiomyopathy from January (J) to December (D). **Numbers above the bars** indicate number of positive samples; **numbers in parentheses below the bars** indicate total number of samples assessed in each month.



Matching control subjects. The higher frequency of Coxsackie B virus IgM in patients with dilated cardiomyopathy than in healthy unmatched control subjects demonstrates a clear association between Coxsackie B virus IgM and dilated cardiomyopathy and is not invalidated by the similar fre-

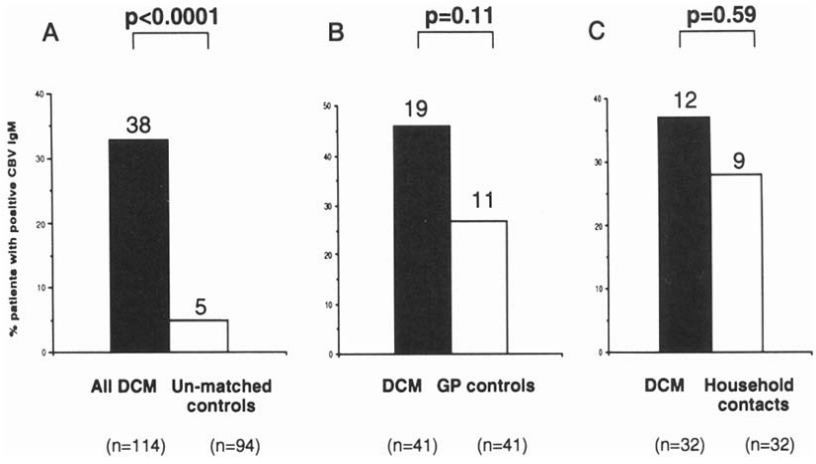


Figure 3. Frequency of positive Coxsackie B virus (CBV) immunoglobulin-M (IgM) in patients with dilated cardiomyopathy (DCM), unmatched control subjects and two groups of matched control subjects. GP = general practitioner.

quency of virus-specific IgM in patients and environmental control subjects. Failure to appreciate the importance of the matching of control groups has led to major difficulties in interpreting the results of serologic studies not only in dilated cardiomyopathy, but also in other conditions in which enteroviral pathogenesis is suspected. Initial reports of a higher positive rate of Coxsackie B virus IgM in patients with insulin-dependent diabetes mellitus (19) and postviral fatigue syndrome (21) than in unmatched control subjects supported a pathogenic role for enteroviruses in these conditions. However, subsequent studies (20,22) in which matched environmental control subjects were assessed demonstrated a similar high frequency of virus-specific IgM in patients and control subjects and has been interpreted as not supporting enteroviral pathogenesis.

Pathogenic importance. The demonstration of an association between Coxsackie B virus IgM and dilated cardiomyopathy does not prove causation but simply reflects exposure to the virus. For instance, such an association may arise because of coincidental acute Coxsackie B virus infection if it were to cause a patient with dilated cardiomyopathy and stable symptoms to show deterioration and to present to the hospital, thereby biasing the patient cohort in toward positive Coxsackie B virus IgM. This is unlikely in the present study because a similar proportion of patients with and without serum for positive Coxsackie B virus IgM experienced worsening of symptoms before inclusion in the study. One causal explanation for the association between Coxsackie B virus IgM and dilated cardiomyopathy concerns recurrent acute Coxsackie B virus infection. Experimental models have recently shown that acute Coxsackie B virus infection may trigger cardiac autoimmunity resulting in chronic myocarditis (23,24) and that recurrent infection promotes this immunopathology (25). Although several markers of autoimmunity have been identified in human dilated cardiomyopathy (26,27), the relation between autoimmunity and Coxsackie B virus infection is unknown and at present we can only speculate as to why some individuals but not others develop virus-induced myocarditis and dilated cardiomyopathy.

Another causal explanation for the association between Coxsackie B virus–specific IgM and dilated cardiomyopathy concerns persistent infection. Persistent enteroviral infection remains a major hypothesis for the pathogenesis of dilated cardiomyopathy. The recent finding of enteroviral genome within the myocardium of a significant proportion of patients with dilated cardiomyopathy provided the first direct evidence for this (28), but other subsequent studies (5,29,30) have provided conflicting results. Persistent enteroviral-specific IgM has been reported in seven patients with dilated cardiomyopathy and has been taken to indicate chronic enteroviral infection (13). However, the small number of patients and difficulty in distinguishing between persistent and recurrent infection using the enzyme-linked immunosorbent assay (ELISA) technique (31) make interpretation of these results difficult. In the present study, the serologic findings in the environmentally matched patients and control subjects suggest that the presence of Coxsackie B virus IgM in patients with dilated cardiomyopathy was the result of local cross infection and not due to persistent infection in patients with dilated cardiomyopathy. We are currently assessing Coxsackie B virus IgM in sequential serum samples from patients with dilated cardiomyopathy.

Conclusions. In this study, we have confirmed the association between Coxsackie B virus–specific IgM and dilated cardiomyopathy. The reason for this finding and its importance with regard to pathogenesis remain to be established.

References

1. Burch GE, De Pasquale NP. Viral Myocarditis. Cardiomyopathies. Edinburgh: Ed Churchill Ltd., 1964:99–115.
2. Matsumori A, Kawai C. An animal model of congestive (dilated) cardiomyopathy: dilatation and hypertrophy of the heart in the chronic stage in DBA/2 mice with myocarditis caused by encephalomyocarditis virus. Circulation 1982;66:355–60.
3. Neu N, Craig SW, Rose NR, Alvarez F, Beisel KW. Coxsackievirus induced myocarditis in mice: cardiac myosin autoantibodies do not cross-react with the virus. Clin Exp Immunol 1987;69:566–74.

4. Wilson FM, Mirander QR, Chason JL, Lerner AM. Residual pathologic changes following murine Coxsackie virus A and B myocarditis. Am J Pathol 1969;55:253–65.
5. Keeling PJ, Jeffery S, Cafcrio ALP, et al. Similar prevalence of enteroviral genome in myocardium from patients with idiopathic dilated cardiomyopathy and controls by the polymerase chain reaction. Br Heart J 1991;68:554–9.
6. Tracy S, Wiegand V, McManus B, et al. Molecular approaches to enteroviral diagnosis in idiopathic cardiomyopathy and myocarditis. J Am Coll Cardiol 1990;15:1688–94.
7. Caforio AL, McKenna WJ. Dilated cardiomyopathy: chronic viral infection or autoimmune disease? A critical view of the viral and the autoimmune hypotheses. Indian Heart J 1990;42:399–402.
8. Cambridge G, MacArthur CG, Waterson AP, Goodwin JF, Oakley CM. Antibodies to Coxsackie B viruses in congestive cardiomyopathy. Br Heart J 1979;41:692–6.
9. Kawai C. Idiopathic cardiomyopathy: a study on the infection-immune theory as a cause of the disease. Jpn Circ J 1971;35:765–70.
10. Falase AD, Fabiya A, Odegbo-Olukoya OO. Coxsackie B viruses and heart disease in Nigerian adults. Trop Geogr Med 1979;31:237–43.
11. Lau RCH. Coxsackie B virus infection in New Zealand patients with cardiac and noncardiac diseases. J Med Virol 1983;11:131–7.
12. Banatvala JE. Coxsackie B virus infections in cardiac disease. In: Waterson AP, editor. Recent Advances in Clinical Virology. Churchill Livingstone, 1983:99–115.
13. Muir P, Nicholson F, Tilzey AJ, Signy M, English TA, Banatvala JE. Chronic relapsing pericarditis and dilated cardiomyopathy: serological evidence of persistent enterovirus infection. Lancet 1989;1:804–7.
14. Brandenberg RO, Chazov E, Cherian G, et al. Report of the WHO/ISFC task force on definition and classification of the cardiomyopathies. Circulation 1981;64:1397–9.
15. Aretz HT, Billingham ME, Edwards WD, et al. Myocarditis, a histopathologic definition and classification. Am J Cardiovasc Pathol 1987; 1:3–14.
16. Torfason EG, Frisk G, Diderholm H. Indirect and reverse radioimmunoassays and their apparent specificities in the detection of antibodies to enteroviruses in human sera. J Med Virol 1984;13:13–31.
17. Caforio ALP, Stewart J, McKenna WJ. Idiopathic dilated cardiomyopathy. Br Med J 1990;300:890–1.
18. Banatvala JE, Bryant J, Schernthaner G, et al. Coxsackie B, mumps, rubella and cytomegalovirus-specific IgM responses in patients with juvenile onset insulin-dependent diabetes mellitus in Britain, Austria and Australia. Lancet 1985;1:1409–12.
19. King ML, Shaikh A, Bidwell D, Voller A, Banatvala JE. Coxsackie B virus-specific IgM responses in children with insulin-dependent diabetes mellitus. Lancet 1983;i:1397–9.
20. Tuvemo T, Dahlquist G, Frist G, et al. The Swedish childhood study III: IgM against Coxsackie B viruses in newly diagnosed type I (insulin dependent) diabetic children. No evidence of increased antibody frequency. Diabetologia 1989;32:745–7.
21. Calder BD, Warnock PJ, McCartney RA, Bell EJ. Coxsackie B viruses and post-viral syndrome: a prospective study in general practice. J R Coll Gen Pract 1987;37:11–4.
22. Miller NA, Carmichael HA, Calder BD, et al. Antibody to Coxsackie B virus in diagnosing postviral fatigue syndrome. Br Med J 1991;302:140–3.
23. Wolfgram LJ, Beisel KW, Herskowitz A, Rose NR. Variations in the susceptibility to Coxsackievirus B3-induced myocarditis among different strains of mice. J Immunol 1986;136:1846–52.
24. Van Houten N, Huber SA. Genetics of Coxsackie B3 (CVB3) myocarditis. Eur Heart J 1991;12 Suppl D:108–12.
25. Beck MA, Chapman NM, McManus BM, Mullican JC, Tracy S. Secondary enterovirus infection in the murine model of myocarditis: pathologic and immunologic aspects. Am J Pathol 1990;136:669–81.
26. Caforio A, Bonifacio E, Stewart J, et al. Novel organ-specific circulating autoantibodies in dilated cardiomyopathy. J Am Coll Cardiol 1990;15: 1527–34.
27. Caforio AL, Stewart JT, Bonifacio E, et al. Inappropriate major histocompatibility complex expression on cardiac tissue in dilated cardiomyopathy: relevance for autoimmunity? J Autoimmun 1990;3:187–200.
28. Bowles NE, Richardson PJ, Olsen EG, Archard LC. Detection of Coxsackie B virus-specific RNA sequences in myocardial biopsy samples from patients with myocarditis and dilated cardiomyopathy. Lancet 1986;1:1120–3.
29. Jin O, Sole MJ, Butany JW, et al. Detection of enterovirus RNA in myocardial biopsies from patients with myocarditis and cardiomyopathy using gene amplification by polymerase chain reaction. Circulation 1990; 82:8–16.
30. Grasso M, Arbustini E, Silini E, et al. Search for Coxsackievirus B3 RNA in idiopathic dilated cardiomyopathy using gene amplification by polymerase chain reaction. Am J Cardiol 1992;69:658–64.
31. Torfason EG, Galindo R, Keyserling HL. Comparison of five ELISA assays for IgG antibody against Coxsackievirus B1. J Med Virol 1988;25: 53–60.