PAPERS AND ORIGINALS

Infectious Mononucleosis and its Relationship to EB Virus Antibody

A joint investigation by University Health Physicians and P.H.L.S. Laboratories*

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Summary

In October 1969 tests made on 1,457 students entering English universities and colleges showed that 57% already possessed antibody to EB virus. The students without antibody in these initial tests were retested seven months later and by then 12% had acquired antibody. In about one-third of them the acquisition of antibody was not associated with any illness. In about 20% respiratory and other illness had occurred, but these symptoms were almost equally frequent in students who had not acquired antibody. Nearly half had developed infectious mononucleosis. In students in whom the acquisition of EB virus antibody was associated with the clinical and haematological manifestations of infectious mononucleosis the Paul-Bunnell test was almost invariably positive. In contrast, when these manifestations were not associated with the acquisition of EB virus antibody the Paul-Bunnell test was always negative.

Tests for cytomegalovirus antibody were also made on the students at entry. The proportion of students with this antibody was much less (30%) and only a small proportion (1·4%) of those without antibody had acquired cytomegalovirus antibody seven months later. In the only patient in whom the acquisition of cytomegalovirus antibody alone was associated with the clinical and haematological features of infectious mononucleosis the Paul-Bunnell test was negative.

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The reagents were prepared and standardized by Dr. C. M. Patricia Bradstreet, Dr. Joan M. Edwards, and Miss E. Margaret Bailey, of the Standards Laboratory, Colindale. The fluorescein conjugated antihuman globulins were provided by Hyland Laboratories and the Wellcome Research Laboratories.

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Introduction

It has been shown that the acquisition of antibody to Epstein-Barr (EB) virus is often associated with the development of infectious mononucleosis (Henle *et al.*, 1968; Niederman *et al.*, 1970; Sawyer *et al.*, 1971). The present inquiry was designed to investigate the frequency with which EB antibody is acquired in adolescence by college students in England and the clinical manifestations associated with its acquisition.

General Plan

The investigation began in October 1969 in the Universities of Nottingham, Keele, and Leicester and in Colleges of Education in Bedford and Gloucester. Cases of infectious mononucleosis were also reported from Birmingham University. The present report concerns the findings in the students' first academic year, and is based on the following observations:

(1) A blood sample—the entry sample—was taken from student entrants and tested for presence of antibodies to EB virus and cytomegalovirus.

(2) From students lacking antibody to either virus a second blood sample was taken and tested about seven months later. If conversion had apparently occurred these samples were retested in parallel with the entry specimens.

(3) On attending to give the second sample, students were asked whether they had suffered any illness since the preceding sample was taken.

(4) Any student, irrespective of whether an entry sample had been given, who reported to the university physician with symptoms suggestive of infectious mononucleosis was tested for antibody to EB virus, cytomegalovirus, and rubella virus (and at Leicester to herpes simplex virus as well). In almost all cases a haematological examination, a Paul-Bunnell/Davidsohn test, and the antibody titrations were made when the case was first seen and again four to six weeks later. (The haematological findings were based on reports from the local laboratory, but the tests were repeated in almost every case in a single laboratory, a standard technique being used throughout; differences between the findings from the local laboratories and reference laboratory were rare.) The clinical symptoms and signs were abstracted from the routine medical records.

Materials and Methods

All sera awaiting test were stored at temperatures between −20 and −40°C. Standard procedures and reagents were used in the collaborating laboratories and suitable positive and negative controls were included.

EB Virus.—EB3 cells were grown and used for the estimation of antibody by the indirect immunofluorescence test as described by Henle and Henle (1966).

Cytomegalovirus and herpes simplex.—Complement-fixing antibody was titrated by a method based on that described by Bradstreet and Taylor (1962).

Rubella.—Antibody was titrated by haemagglutination inhibition (H.A.I.).

Heterophil antibody test.—The method used was that described by Davidsohn and Henry (1969). A serum was considered positive when the agglutinating titre after absorption with guinea-pig kidney suspension was more than four times the titre after absorption with ox cell suspension.

Results

VIRAL ANTIBODIES AT ENTRY

The initial serum samples from 1,457 students showed that 835 (57%) already had antibody to EB virus and 431 (30%) already had antibody to cytomegalovirus. Antibody to EB virus was more often present among the 96 students from overseas (81, or 84%) than among the 1,361 students domiciled in Britain (754, or 55%). In contrast, foreign and home students did not differ appreciably in relation to antibody to cytomegalovirus—32% and 29% respectively.

VIRAL ANTIBODIES AFTER ENTRY

Of the 622 students without antibody to EB virus on entry 496 (80%) had a second serum sample examined about seven months later and 60 (12%) were found to have acquired antibody (Table I). Of the 1,026 students without antibody to cytomegalovirus initially second tests were made on 713; only 10 (1·4%) had developed antibody.

TABLE I—*Acquisition of Viral Antibody during Seven Months after Entry to College*

	No. of Students without Antibody at Entry	Second Sera	
		No. Examined	No. Positive
EB virus	622	496	60 (12·0%)
Cytomegalovirus ..	1,026	713	10 (1·4%)

ACQUISITION OF EB VIRUS ANTIBODY

Table II shows that of the 60 students known to have acquired EB virus antibody in the seven months following their entry to college, 22 (37%) had reported to their university physician and were investigated as cases of infectious mononucleosis. In a further five the family doctor had diagnosed "glandular fever" during a vacation. Other illnesses, mainly respiratory, were reported by 13, but the remaining 20 could not recall any illness at all. In contrast, of the 436 students in whom EB virus antibody was not acquired, only six reported to their student health physician and were investigated as cases of infectious mononucleosis. Four others reported that their family doctor had diagnosed "glandular fever" during a vacation. The total incidence under these headings is therefore only 2% compared with the 45% in the students who had acquired EB virus antibody. Other illnesses were equally frequent in the two groups.

TABLE II—*496 Students without EB Virus Antibody on Entry to College; Acquisition of Antibody and Incidence of Illness during the Seven Months Subsequent to Entry*

Group	Total	Investigated as Suspected Infectious Mononucleosis at University	History from Student		
			Diagnosed Glandular Fever by Family Doctor. Not Investigated at University	Other Illness	No Illness
Acquired EB virus antibody after entry	60 (100%)	22 (37%)	5 (8%)	13 (22%)	20 (33%)
Did not acquire EB virus antibody after entry	436 (100%)	6 (1%)	4 (1%)	90 (21%)	336(77%)

PATIENTS WITH INFECTIOUS MONONUCLEOSIS

During the first academic year of the inquiry 213 patients reported to their university physicians with symptoms which were investigated as infectious mononucleosis. These patients include both students for whom initial serum samples were available (as shown in Table II) and students without initial samples. Eight of the patients (all without antibody to EB virus at entry) had a serum sample taken at the onset of illness but none later, and in consequence these eight cases have been omitted from the analysis as unproved. The remaining 205 patients can be studied under two headings.

Group A. Patients for Whom it was Known whether EB Virus Antibody had been Acquired since Entry

These 94 patients (Table III) fall into two classes: A1, 45 known to have acquired antibody since entry, and A2, 49 who did not acquire EB virus antibody after entry to the university. Of the former, 22 had an entry sample which did not contain antibody, and antibody developed subsequently. The others had no entry sample, but when they became ill antibody was absent in the first specimen and present in the second (11), or present in the first and showed a fourfold rise in the second(12). Of the latter, 16 had an entry specimen which already contained antibody; 33 had no entry sample, but were without antibody when they first became ill and failed to develop EB virus antibody during the course of the illness.

Table III shows that in all but one of the 45 patients who had acquired EB virus antibody the Paul-Bunnell test was positive, whereas there was no positive test in any of the 49 who had not acquired the antibody.

The haematological findings are also very different. Atypical lymphocytes were observed in the blood of all patients who developed EB virus antibody. They were not, however, a regular feature in patients who did not develop this antibody, about 40% of whom did not have atypical lymphocytes in their blood smears. Modified differential leucocyte counts were also made, in which the cells were classified simply as "granulocytes" and "mononuclear cells." (The category of "mononuclear cells," defined in this way, includes normal lymphocytes and monocytes as well as atypical lymphocytes.) Table III shows that a large percentage of total mononuclear cells ($\geqslant 70\%$) was found only in patients who developed EB virus antibody.

In contrast, the differences in the recorded symptoms of illness were much less pronounced, though rather more patients who acquired antibody had adenitis with throat inflammation than did patients who failed to acquire antibody.

Six patients developed antibody to viruses other than the EB virus (they are included in the 49 patients who failed to acquire EB virus antibody). Antibody to herpes was found in one, to cytomegalovirus in another, and to rubella virus in the remaining four.

Group B. Patients for Whom it was Not Known whether EB Virus Antibody had been Acquired since Entry

These 111 patients had no serum samples on entry and were found to have EB virus antibody when they first became ill. In no case did a fourfold increase occur in the antibody titre during the illness; in consequence there is no indication whether or not the development of the antibody was associated with the illness. In 63 of these patients (B1) the Paul-Bunnell test was positive and in the remaining 48 (B2) it was negative (Table IV). The haematological findings and symptoms in the Paul-Bunnell positive group (Table IV) are very similar to those in the Paul-Bunnell positive group shown in Table III who were known to have acquired EB virus antibody since entry. It is also evident that the findings in the Paul-Bunnell negative group are very similar to those in the group (Table III) who are known not to have acquired EB virus antibody since entry. These findings are in keeping with the observation shown in Table III that a positive Paul-Bunnell test shows a close association with the recent acquisition of EB virus antibody.

Discussion

The results of this study leave no doubt, as would be expected from the findings of Pereira *et al.* (1969), that many British students already have EB virus antibody by the time they enter university (55%). An even greater rate (84%) was observed in students from tropical countries. Probably this antibody usually develops without overt infectious mononucleosis since it is rarely diagnosed in children.

Evidently EB virus antibody is acquired by many students early in college life; indeed the findings during seven months would give an annual incidence of about 20%. If this rate were to continue about three-quarters of all students would have antibody to the virus by the time they leave university. These findings, provided they are generally applicable, would explain the low incidence of infectious mononucleosis among older persons in Britain.

The development of infectious mononucleosis in contacts of overt cases is thought to be uncommon, and in the present study only three students developed the disease after contact with known cases. The findings suggest that this characteristic is due, at least in part, to the large proportion of student contacts who already possess EB virus antibody and the relative infrequency of overt infectious mononucleosis even in those students who do acquire antibody. Thus one-third of the students who acquired antibody recollected no illness. Both these factors would tend to reduce the apparent frequency of spread to contacts.

The findings also show that the acquisition of antibody to the viruses of herpes, rubella, and cytomegalovirus may sometimes be associated with some of the features of infectious mononucleosis and, therefore, that the recorded clinical characteristics did not invariably provide a reliable indication of the specific virus involved. Similarly, the haematological findings were not always helpful in distinguishing EB virus infections from others. Atypical lymphocytes, for example, were found in all these infections though in the routine differential leucocyte counts 70% or more mononuclear cells were found only in patients who developed EB virus antibody.

In contrast to these clinical and haematological findings, a positive Paul-Bunnell test in patients with manifestations of infectious mononucleosis seemed to provide an almost specific indication of the recent acquisition of EB virus antibody. In only one patient in whom EB virus antibody was known to have been recently acquired was the Paul-Bunnell test negative,

TABLE III—*94 Patients in Whom the Acquisition or Non-acquisition of EB Virus Antibody since Entry was Established: Paul-Bunnell Tests, Haematological Results, and Recorded Incidence of Throat Inflammation and Adenitis*

Group	EB Virus Antibody	No.	Paul-Bunnell		Atypical Lymphocytes			Percentage of Total Mononuclear Cells in Differential Leucocyte Count*				Symptoms			
			Pos.	Neg.	Seen	Not Seen	Not Recorded	<50	50-69	⩾70	Not Recorded	Adenitis and Throat Inflammation	Adenitis without Throat Inflammation	Throat Inflammation but No Adenitis	Not Recorded
A1	Acquired since entry	45	44	1	43 (100%)	0	2	5 (11%)	11 (25%)	28 (64%)	1	39 (89%)	4 (9%)	1 (2%)	1
A2	Not acquired	49	0	49†	24 (59%)	17 (41%)	8	35 (74%)	12 (26%)	0	2	35 (74%)	2 (4%)	10 (21%)	2

TABLE IV—*111 Patients in Whom it was Not Known whether EB Virus Antibody was Acquired after Entry: Paul-Bunnell Tests, Haematological Findings, and Recorded Incidence of Throat Inflammation and Adenitis*

Group	EB Virus Antibody	Paul-Bunnell	No.	Atypical Lymphocytes			Percentage of Total Mononuclear Cells in Differential Leucocyte Count*				Symptoms			
				Seen	Not Seen	Not Recorded	<50	50-69	⩾70	Not Recorded	Adenitis and Throat Inflammation	Adenitis without Throat Inflammation	Throat Inflammation but No Adenitis	Not Recorded
B1	+	+	63	56 (97%)	2 (3%)	5	9 (15%)	20 (34%)	30 (51%)	4	54 (92%)	2 (3%)	3 (5%)	4
B2	+	−	48	17 (55%)	14 (45%)	17	32 (76%)	10 (24%)	0	6	31 (69%)	3 (7%)	11 (24%)	3

*Modified differential leucocyte counts in which cells were recorded either as granulocytes or as mononuclear cells—the latter group thus comprises all leucocytes other than granulocytes (see text).
†In four cases the Paul-Bunnell tests were reported as negative at Reference Laboratory, but weakly positive at local laboratory on one occasion.

whereas, with four doubtful exceptions, the test was never positive in patients in whom the antibody had not been recently acquired. In short, in this population infectious mononucleosis accompanied by a positive Paul-Bunnell test was an entity associated with the development of EB virus antibody. On the other hand, cases resembling infectious mononucleosis but with a negative Paul-Bunnell test may be caused by a variety of agents.

A differing virological origin for cases of infectious mononucleosis with a positive Paul-Bunnell test and for cases with very similar clinical and haematological features but in whom the Paul-Bunnell test is negative goes far to explain the differences that have been reported between such positive and negative cases. These differences include incubation period, the age groups mainly affected, infectivity, and the occurrence of outbreaks (Hobson *et al.*, 1958; Hoagland, 1967; Finch, 1969).

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References

Bradstreet, C. M. Patricia, and Taylor, C. E. D. (1962). *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service*, 21, 96.
Davidsohn, I., and Henry, J. B. (editors) (1969). *Todd-Sanford Clinical Diagnosis by Laboratory Methods*, 14th edn. Philadelphia, Saunders.
Finch, S. C. (1969). *Infectious Mononucleosis*. Oxford and Edinburgh, Blackwell.
Henle, G., and Henle, W. (1966). *Journal of Bacteriology*, 91, 1248.
Henle, G., Henle, W., and Diehl, V. (1968). *Proceedings of the National Academy of Sciences of the United States of America*, 59, 94.
Hobson, F. G., Lawson, B., and Wigfield, M. (1958). *British Medical Journal*, 1, 845.
Hoagland, R. J. (1967). *Infectious Mononucleosis*. New York and London, Grune and Stratton.
Niederman, J. C., Evans, A. S., Subrahmanyan, L., and McCollum, R.W. (1970). *New England Journal of Medicine*, 282, 361.
Pereira, M. S., Blake, J. M., and Macrae, A. D. (1969). *British Medical Journal*, 4, 526.
Sawyer, R. N., Evans, A. S., Niederman, J. C., and McCollum, R. W. (1971). *Journal of Infectious Diseases*, 123, 263.

Platelet Defect of Infectious Mononucleosis

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Summary

Platelet function has been studied in 16 patients with uncomplicated infectious mononucleosis. Some abnormality of platelet aggregation and/or release of platelet factor III or platelet factor IV was found in all patients. Six patients with platelet defects were retested after three to four months and were found to have normal platelet function and appreciably higher platelet counts. Abnormal platelet function may reflect a platelet defect which predisposes to premature platelet destruction. Recent viral illness should be excluded before attributing abnormal platelet function to other factors or to a congenital disorder.

Introduction

Infectious mononucleosis, as well as a number of other viral diseases, can be associated with thrombocytopenia of all grades (Ackroyd, 1949; Hudson *et al.*, 1956). Asymptomatic thrombocytopenia is common (Carter, 1965; Cantow and Kostinas, 1966), but thrombocytopenic purpura occurs only as a rare complication (Sharp, 1969). The pathogenesis of virus-associated thrombocytopenia is unknown but may involve several mechanisms.

This study shows that abnormal platelet function is common in uncomplicated infectious mononucleosis, and it is suggested that these abnormalities reflect platelet damage which may predispose to premature platelet destruction.

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Patients and Methods

Sixteen patients (10 females and 6 males) were studied in the second or third week of illness. Their ages ranged from 16 to 35 years, except for one woman who was 58. Most of them had been admitted to hospital because of the severity of symptoms, thus providing a selection bias to this series. Fifteen presented with a typical "pharyngeal" onset of sore throat, fever, malaise, and lymphadenopathy. One (Case 10) presented with hepatic involvement, and several others had abnormal liver function tests. Splenomegaly was present in six. All patients had a peripheral blood picture consistent with infectious mononucleosis, the most striking feature being an atypical lymphocytosis. The Paul-Bunnell test for heterophil antibody was positive in all patients after serum was absorbed with guinea-pig kidney. No patient had purpura. The Hess test was negative in all cases and an Ivy bleeding time was normal in the eight tested. Patients had not taken aspirin within four days of study, though some had ingested aspirin earlier in their illness. Six who had platelet defects were studied three to four months after the onset of illness; all were well at that time and had negative Paul-Bunnell tests and normal blood films, except for occasional atypical lymphocytes.

PLATELET INVESTIGATIONS

Platelet counts were performed by the method of Brecher and Cronkite (1950). Platelet factor III availability was determined by incubation of platelet-rich plasma with kaolin at 37°C, with assay of platelet coagulant activity released at 1, 5, 10, and 20 minutes, and expressed as reduction in the Stypven-calcium time after 20 minutes' incubation (Spaet and Cintron, 1965; Hardisty and Hutton, 1965). Platelet factor IV (anti-heparin factor) was determined by prolongation of the thrombin time in the presence of heparin by platelet-rich plasma (Niewiarowski and Thomas, 1969).

Platelet aggregation was studied by a modification of the method of Born (1962) and O'Brien (1962). Aggregating agents and their final concentrations were: adenosine diphosphate