

# Failure to Demonstrate Concomitant Antibody Changes to Viral Antigens Other than Epstein-Barr Virus (EBV) During or After Infectious Mononucleosis

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A search for antibody rises to viral antigens other than to Epstein-Barr virus, the causative agent, has been carried out in serial serum samples from 82 patients with infectious mononucleosis (IM). Fourfold or greater rises in titer rarely occurred and did not cluster in time. No rises occurred to cytomegalovirus, only 1.2 percent to herpes simplex virus, and 8.5 percent to varicella zoster virus. Rises to measles antibody were found in 7.5 percent of patients and to rubella in 10.4 percent; these may represent natural infections or immunizations. A few patients also showed rises to respiratory viruses but there was no apparent connection to IM.

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Acute infectious mononucleosis (IM) is accompanied by the appearance of a wide variety of humoral antibodies in addition to those directed against the causative agent, Epstein-Barr virus (EBV). These include: (1) heterophile antibodies, (2) cold-reacting antibodies, (3) antibodies to Newcastle disease virus (NDV) treated human red cells, and (4) autoantibodies (anti-muscle, anti-nuclear, anti-lymphocyte, rheumatoid factor, etc.) [1-2]. The current study was carried out to determine if changes in antibody titers to herpes viruses other than EBV, or to other viral antigens, occurred during or after acute infectious mononucleosis (IM), such as that seen in immuno-suppressed renal transplant patients [3]. Such changes might result either from reactivation of latent agents accompanying the profound changes of immunoregulation in acute IM [4-8] or from vigorous polyclonal expansion of the B cell population [9] due in part, at least, to their infection and transformation by EBV.

To determine possible changes in humoral antibody levels, we carried out tests with 12 viral antigens on serial samples from cases of infectious mononucleosis.

## MATERIALS AND METHODS

### *Patients*

Only patients fulfilling clinical, hematological, and serologic criteria of infectious mononucleosis were studied [10]. All were heterophil antibody positive. They consisted of Yale University students or staff seen at the Yale Health Plan on whom serial specimens had been collected by one of us (JCN), stored at  $-20^{\circ}\text{C}$  for various periods, and then tested for viral antibodies.

TABLE 1  
Number of Sera Available for Testing from 82 Patients with Infectious Mononucleosis  
According to Time After Onset of Clinical Symptoms

Time after onset	Week				Month									Total
	1	2	3	4	2	3	4	5	6	7-12	13-24	>24		
No. of sera	31	63	41	32	57	31	15	7	4	10	8	3	302	

### *Antibody Tests*

The following tests were employed: (1) the complement fixation (CF) test using the 50 percent endpoint technique recommended by the National Centers for Disease Control [11] was employed to measure antibody levels to cytomegalovirus (CMV), herpes simplex (HSV), and varicella zoster (VZ) viruses; (2) the hemagglutination inhibition (HI) test for rubella was performed according to the protocol of Leibhaber [12] and that for measles after the technique of Black [13]; (3) the indirect immunofluorescence EBV-VCA antibody tests with EB3 cells followed the technique of the Henles [14]. All sera were inactivated at 56°C for 30 minutes. Positive and negative controls of known titer were included in each test. The CF and HI tests were carried out on microtiter plates. Serial dilutions and the addition of reagents were made by automated equipment produced by Cooke Engineering Co. (Alexandria, VA). The CF and HI antigens employed came from the National Centers for Disease Control (Atlanta, GA), Flow Laboratories (McLean, VA), and M.A. Bioproducts (Walkersville, MD).

Three hundred two sera from 82 IM patients were available as indicated in Table 1. The requirements for testing were that a serum sample identified by day after onset of clinical symptoms had been collected on the *first* visit to the physician for infectious mononucleosis and that one or more follow-up sera were also available for determination of rises or falls in antibody titers. Available sera from these patients were tested for antibody to herpes viruses, measles virus, and rubella virus. One hundred forty-one sera from 36 of the same patients were tested for antibody titers to adenovirus, parainfluenza 1, 2, and 3, and respiratory syncytial viruses. A small sample of 29 sera from 11 IM cases was tested for antibody to two influenza antigens. In some instances there was insufficient sera to carry out all tests, and in others technical problems (anticomplementary sera) prevented satisfactory tests. All serial sera from a patient were tested against any one antigen at the same time to provide comparability of test results.

### RESULTS

The presence or absence of antibody in sera taken on the initial examination of the patient for IM are shown in Table 2.

About one-third had serologic evidence of antibody to CMV, half to HSV, and 85 percent to VZ. Almost all patients had antibody to measles (90 percent) and most to rubella (76.9 percent). Antibodies to respiratory viruses were variously prevalent: over 80 percent had had prior infection to parainfluenza types 1-3, 70 percent to one or more common adenoviruses, and 90 percent to influenza virus (A/Victoria), either from natural infection or from immunization. Antibody to RSV and to A/New Jersey was found in only one-third of those tested.

The results of antibody tests during illness with IM are summarized in Table 3, the results on individual patients in Table 4, and multiple antibody titers in the same pa-

TABLE 2  
Results of Antibody Tests at Time of First Visit to Physician for Infectious Mononucleosis

Test	No. Pts. Tested	Antibody Present		
		No.	%	
<b>Viruses of Herpes Group</b>				
Cytomegalovirus	CF	65	22	33.8
Herpes Simplex	CF	81	38	46.9
Varicella Zoster	CF	82	70	85.4
<b>Exanthem Viruses</b>				
Measles	HI	80	72	90.0
Rubella	HI	77	60	76.9
<b>Respiratory Viruses</b>				
Adenovirus	CF	36	25	69.4
Parainfluenza 1	HI	36	33	91.6
2	HI	36	29	80.5
3	HI	36	36	100.0
Respiratory Syncytial	CF	36	12	33.0
Influenza A/New Jersey	HI	11	4	36.4
A/Victoria	HI	11	10	90.1

tient in Table 5. The timing of the rises and falls in titer in relation to the onset of frank clinical symptoms of IM is depicted in Fig. 1.

Among herpes group antibodies other than EBV, there was remarkably little change in titer in serial sera tested for cytomegalovirus (CMV) and herpes simplex virus (HSV): only one patient in each group showed a rise or fall in titer. Somewhat more common changes were found for VZ in the 82 patients tested: seven showed a rise in titer (8.5 percent) and two a drop (2.4 percent). However, these changes in

TABLE 3  
Significant<sup>a</sup> Rises or Falls in Antibody Titer to Viruses Other than EBV  
During the Course of Infectious Mononucleosis

Antibody Tested	Test Used	Number Tested		No. and % of Patients With			
		Patients	Sera	Rise in Titer No.	%	Fall in Titer No.	%
<b>Herpes Group</b>							
Cytomegalovirus	CF	65	276	0	0	1	1.5
Herpes Simplex	CF	81	285	1	1.2	1	1.2
Varicella Zoster	CF	82	287	7	8.5	2	2.4
<b>Exanthem</b>							
Measles	HI	80	281	6	7.5	1	1.25
Rubella	HI	77	280	8	10.4	8	10.4
<b>Respiratory</b>							
Adenovirus	CF	36	141	0	0	1	2.8
Parainfluenza 1	HI	36	141	1	2.8	0	0
2	HI	36	141	1	2.8	0	0
3	HI	36	141	2	5.5	1	2.8
Respiratory Syncytial	CF	36	141	2	5.5	1	2.8
Influenza A/New Jers.	HI	11	29	0	0	0	0
Influenza A/Victoria	HI	11	29	0	0	0	0

<sup>a</sup>Significant fourfold or greater rise or fall in titer compared with first sera

CF = Complement Fixation

HI = Hemagglutination Inhibition

## ANTIBODY TO:

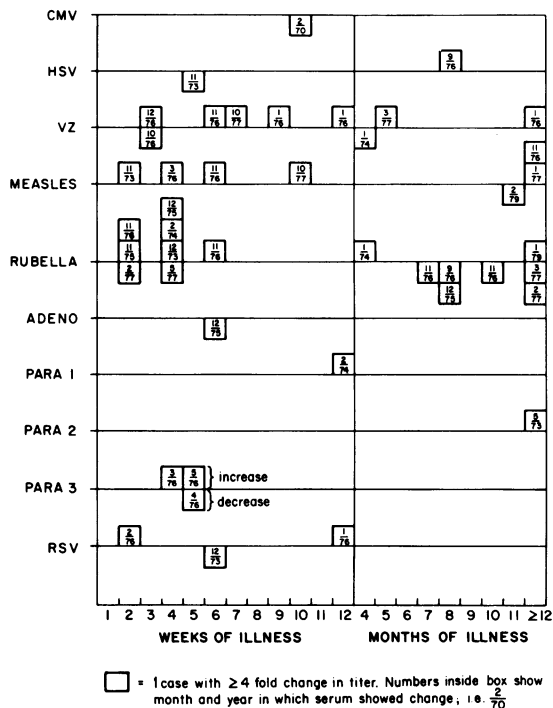


FIG. 1. Individual rises or falls of antibody titers to 10 viruses according to weeks of infectious mononucleosis.

titer were widely spread out after onset of acute IM symptoms (Fig. 1), ranging from three weeks to over a year later.

Measles and rubella infections are not uncommon in the student population and some may have received immunizations during this period. Six patients with IM showed a rise and one a fall in titer to measles antibody; these were spread out at various points of illness but tended to cluster in the fall months of various years (Fig. 1). Of eight patients with an antibody rise to rubella virus, six occurred in the first six weeks of IM illness but were in different months or years. They may possibly be related to immunoregulatory changes in IM.

Among seven respiratory antibodies tested, only one or two patients with IM showed a rise or fall in titer (Table 3); there was no consistent temporal pattern (Fig. 1). Infections with these viruses are not uncommon in a student population and the results are probably unrelated to IM.

The magnitude of individual antibody rises and falls (Table 4) indicates that such changes, while technically classified as fourfold or greater, actually represent changes from  $< 10$  to 20 or vice versa and may fall within technical variation.

A few patients showed multiple antibody changes (Table 5). However, there is not very strong temporal association of the changes in any one patient except for patient 244, who showed rises in VZ, measles, and rubella, all occurring during the sixth week after onset of IM.

## DISCUSSION

Profound immunoregulatory changes occur during acute infectious mononucleosis. There is a vigorous polyclonal expansion of B cells, leading to an increase

TABLE 4  
Actual Rises and Falls in Antibody Titer Occurring  
During the Course of Infectious Mononucleosis

Antibody	Patient No.	Initial Titer	Subsequent Titer
CMV	152	80	20
HSV	112	10	80
	17	20	< 10
Var	385	10	40
	307	20	160
	209	20	< 10
	235	20	> 160
	244	< 20	> 160
	112	10	80
	17	640	80
	144	< 10	20
	126	10	80
Measles	492	20	< 10
	325	< 10	20
	177	10	40
	244	20	80
	112	20	80
	17	40	160
	52	40	160
Rubella	302	20	< 10
	443	< 10	20
	11	80	320
	391	80	< 10
	165	320	40
	26	160	640
	84	< 10	20
	1	320	40
	95	160	1280
	244	< 10	40
	261	40	160
	112	640	160
	120	320	80
	187	320	80
	52	160	640
	152	320	40
Adenovirus	126	20	< 10
Parainfluenza 1	11	10	40
2	44	< 10	20
3	191	80	20
	167	20	80
	177	40	160
RSV	120	10	40
	17	20	< 10
	126	< 10	20

in immunoglobulin production and the appearance of a wide array of antibodies. These include antibodies to various EBV antigens, the heterophil antibody, and many antibodies of the auto-immune type, including connective tissue and anti-nuclear antibodies [1,2]. It would seem possible that an increase in viral antibodies, other than to EBV, might also result from this polyclonal expansion. A second source of increase in antibody titer might be due to reactivation of latent viruses,

TABLE 5  
Multiple Rises and Falls on Same Patient

Antibody	Patient No.	Initial Titer	Subsequent Titer	Time of Illness of Rise or Fall
HSV	112	10	80	8 months
V-Z		10	80	1 year
Rubella		640	160	8 months
Measles		20	80	1 year
HSV	17	20	< 10	2 weeks
V-Z		640	80	4 months
Measles		40	160	2 weeks
RSV		20	< 10	7 weeks
CMV	152	80	20	10 weeks
Rubella		320	80	1½ years
V-Z	244	< 20	> 160	6 weeks
Measles		20	80	6 weeks
Rubella		< 10	40	6 weeks
V-Z	126	10	80	12 weeks
Adenovirus		20	< 10	6 weeks
RSV		< 10	20	12 weeks
Measles	177	10	40	4 weeks
Parainfluenza 3		40	160	4 weeks
Measles	52	40	160	3 years
Rubella		160	640	3 months
Rubella	11	80	320	4 weeks
Parainfluenza 1		10	40	11 weeks

particularly of the herpes group, that results from the changing pattern of immunoregulation that occurs during infectious mononucleosis. The details of these changes are now emerging through the use of monoclonal antibodies and other immunological techniques [5-8,15]. Despite these two possible sources of antibody rises from polyclonal antibody production or viral reactivation, the current study has failed to demonstrate any concomitant antibody changes among twelve viral antigens tested in serial sera obtained during and after acute IM. The few changes seen were not clustered at any one point in illness, as one might expect if due to a common point in polyclonal B cell expansion or to changes in immunoregulation. Varicella zoster showed the most change among the herpes viruses (except for EBV), but even here only 8.5 percent of 82 patients tested showed a rise in titer. There were also 7.5 percent with antibody rises to rubella and 10.4 percent with antibody rises to measles, which may well be due to natural infection and/or immunization.

Abnormal B cell proliferation occurs in the X-linked lymphoproliferative syndrome [16], in fatal EBV infection in children [17,18] and adults [19], and in renal transplant patients [20]. The levels of antibodies other than EBV have not been studied in several of these syndromes, but in renal transplant patients there is clear evidence of reactivation of herpes viruses as indicated both by antibody rises and by viral excretion [3,21-24]. The failure to find these changes during acute IM may be due to the different nature, lower severity, or shorter duration of the alterations in the immunoregulatory process in the patient with infectious mononucleosis than those in the renal transplant recipient in whom multiple transfusions, the transplanted kidney, and prolonged drug-induced immunosuppression all may play a role.

From a practical standpoint, the current studies indicate the uniqueness of the rises of EBV and heterophile antibodies in IM which are not therefore confused with non-specific rises to other viral antibodies, albeit many other types of antibodies appear. Clinically, the evidence suggests that infectious mononucleosis is not commonly complicated by concomitant or reactivated viral infections.

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