MEDICAL GRAND ROUNDS

PARKLAND MEMORIAL HOSPITAL

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INFECTIOUS MONONUCLEOSIS

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DEFINITION

Infectious mononucleosis (IM) is a common clinical syndrome that has its highest incidence in adolescence and young adult life. It is characterized by fever, pharyngeal inflammation, lymphadenopathy and splenomegaly. Relative and absolute lymphocytosis with atypical lymphocytes are present and, in the usual case, hepatic function abnormalities are seen. Complications are infrequent and the course almost invariably benign. The majority of cases are heterophile antibody positive (HA+), and these are associated with evidence of infection with Epstein-Barr virus (EBV). A smaller percentage of cases are heterophile antibody negative (HA-), and these are related either to EBV infection or else to infection with a variety of agents, the most important numerically being cytomegalovirus (CMV).

ETIOLOGY

Overwhelming evidence is now being accumulated to indicate that HA+ IM is etiologically related to infection with EBV. Before citing that evidence, basic information on the nature of EBV will be presented. In 1964, Epstein, Achong and Barr reported the presence of a herpesvirus, detected by electron microscopy, in cultured lymphoblasts prepared from Burkitt's lymphoma *(34)*. Subsequently, it has been learned that EBV is antigenically distinct and is a new human virus. In common with other herpesviruses, EBV is an enveloped double-stranded DNA virus whose capsid possesses icosahedral symmetry. The capsid consists of 162 hollow tubular capsomeres and the diameter of the fully enveloped particle measures approximately 120 nanometers. *In vitro*, the virus is able to replicate only in lymphoblastoid cells derived from human and some other primate sources. EBV has the unique property of being able to transform short-lived human lymphocyte cultures into lymphoblastoid cell lines (LCL) where the cells lose contact inhibition, are capable of being passaged indefinitely, and show aggressive and invasive growth when transplanted into immunosuppressed newborn mice. The capacity to produce LCL permits the detection of mature infectious viral particles and forms the basis of an *in vitro* neutralization test. It has been determined by sensitive DNA and messenger RNA hybridization techniques that all of the cells in LCL carry the EBV genome. Only a few cells, however, in the population are actually producing fully mature infectious particles. In certain LCL, only abortive infection occurs and viral capsid proteins are not produced.

A number of EBV related antigens can be detected in LCL. The presence of these antigens permits the dissection of individual antibody responses occurring in the natural course of EBV human infections. The antigens associated with EBV can be listed as follows: (1) viral capsid antigen (VCA), (2) membrane antigen (MA), (3) early antigen (EA) complex. It has been determined that the EA complex consists of two parts: a diffuse (D) and a restricted (R) component. (4) Complement fixation (CF) antigens, and (5) immunoprecipitation antigens. Antibodies to VCA are detected by the indirect immunofluorescent test of Henle *(52)*. This is the standard and most widely used test for the detection of antibodies to EBV and uses an acetone fixed preparation of cells derived from certain LCL ((EB3 or the HR1K cell line). MA is coded for by the virus and appears on the membrane of infected cells. Antibody to MA is determined by a blocking technique. Live cells containing MA are first exposed to the sera which may contain antibody and then later to a fluorescein conjugated standard anti-MA serum. The EA complex appears in certain abortively infected LCL. Antibody to the two components of the EA complex can be determined by indirect immunofluorescent tests. The presence of complement fixing antigens in LCL enables the performance of standard complement fixation antibody test procedures.

The evidence relating HA+ IM etiologically with EBV can be summarized as follows. (1) In at least four population groups studied, the presence of EBV antibody has been found to be protective against the subsequent development of IM *(Table 1) (48)*.

TABLE 1

EBV INFECTION AND INFECTIOUS MONONUCLEOSIS IN COLLEGE AND UNIVERSITY STUDENTS

Study Group	*EBV Antibody on Entry*			*Yearly Per Cent Developing*	
		No.	*%*	*HA+ IM*	*EBV Infection**
Yale University Freshmen	+	180	51	0	
	-	175	49	9.7	13.1
U.S. Military Academy	+	890	63.5	0	
	-	511	36.5	3.4	12.4
Freshmen in 5 English Colleges	+	835	57	0	
	-	622	43	7.1	12.0
Cornell University Medical Students	+	600	75	0	
	-	200	25	5.0	No Data
Total	+	2505	62.4	0	
	-	1508	37.6	5.8	12.3

* Infection-Appearance of Antibody-Seroconversion
Only 411 of 511 U.S. Acad. freshmen available for second bleeding

Of 2,505 EBV antibody positive entrants into the studies, none developed HA+ IM. In contrast, of 1,508 EBV negative antibody entrants, 12.3% per year had serological evidence of infection with EBV and 5.8% per year developed HA+ IM. (2) Prospective studies demonstrate conversion from negative to positive EBV antibody status when preillness sera are compared to sera collected during the course of illness. This statement pertains to anti-VCA antibody, complement fixing antibody, and specific virus neutralizing antibody. (3) Persons convalescing with HA+ IM universally show evidence of EBV antibody. (4) Early in the course of HA+ IM, IgM antibody directed against EBV can be demonstrated. This IgM antibody response is transient and by analogy with other infections can be assumed to indicate a primary infection. (5) Lymphoblastoid cell lines are easily established from persons with IM. These LCL regularly show the presence of EBV. (6) Three separate studies have shown that EBV is present in oropharyngeal secretions in IM and that the carrier state can persist $\geq$ 16 months after the onset of the disease. (7) IM has been associated with transfusion and could be specifically related back to the EBV antibody status of the donor. (8) Primates injected with EBV transformed autologous cells developed heterophile antibody as well as antibody to EBV. (9) The epidemiology of IM fits the known epidemiology of EBV. (10) Finally, despite intensive search with newer techniques for virus detection, no other etiological candidate has emerged. These lines of evidence strongly support the concept that primary infection with EBV, particularly in susceptible adolescents and young adults, has as its primary clinical manifestation the syndrome of IM.

To satisfy completely Koch's postulates, it would be necessary to reproduce the syndrome in volunteers upon inoculation of the virus. This has already been accomplished to a certain extent in experiments with non-human primates. Due to the prime candidacy of EBV as an oncogenic virus, human volunteer experiments at the present time would appear unethical. The cited lines of evidence, in total, point against EBV merely being a passenger virus in a disease known to induce lymphoproliferation. One other suggested explanation for the relationship of EBV to IM seems unlikely. It has been suggested that EBV is vertically transmitted, i.e., from mother to fetus, and that IM subsequently results with reactivation of the virus in a manner similar to herpes labialis or herpes zoster. Although given that reactivation can occur, this explanation does not account for many of the epidemiologic features of IM and, in addition, preliminary studies with fetal lymphoid tissues have failed to reveal the presence of the EBV genome by sensitive hybridization techniques.

EPIDEMIOLOGY

HA+ IM has its peak incidence in adolescence and young adult life. It has an increased incidence in upper socioeconomic class population groups and is a significant cause of morbidity at colleges and universities. During the academic year 1969-1970, the overall incidence of IM at 19 colleges and universities was 1,112 cases per 100,000 population *(Table 2) (13)*.

TABLE 2

INCIDENCE OF IM AT 19 COLLEGES AND UNIVERSITIES DURING THE ACADEMIC YEAR 1969-1970

School	*Undergraduate Population*	*No. of Cases*	*Rate per 100,000 per School Year*
Alaska	1,520	9	599
Bryn Mawr	743	6	808
California	13,848	255	1,841
Clemson	4,503	40	888
Emory	2,321	50	2,154
Harvard	6,012	107	1,780
Hawaii	13,613	15	110
Illinois	23,064	303	1,314
Indiana	21,752	386	1,775
Minnesota	33,277	248	745
Nebraska	16,463	209	1,270
Oklahoma State	14,951	154	1,030
Oregon	11,037	140	1,268
Princeton	3,400	76	2,235
Purdue	19,016	251	1,320
San Diego	17,858	126	706
Texas	25,705	217	844
Washington	22,908	185	808
Yale	4,490	74	1,722
Total	256,463	2,851	1,112

The sex incidence is probably equal, but it has been determined that the peak incidence in women probably antedates by 1 or 2 years the peak incidence in men *(Fig. 1) (50)*.

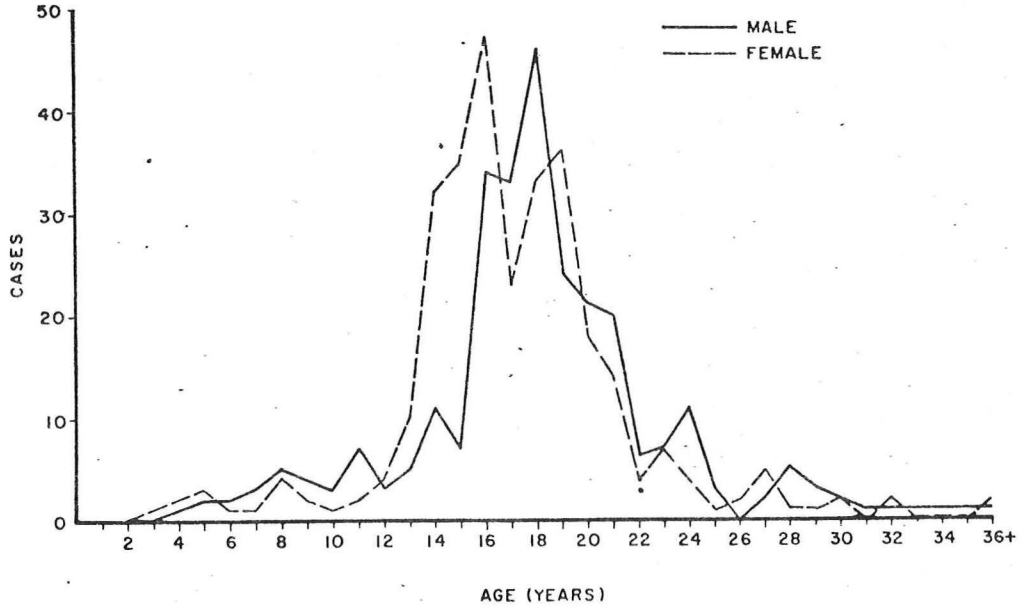

FIG. 1
Cases of infectious mononucleosis, by age and sex, metropolitan Atlanta, 1968

IM is rare in black persons and is infrequent in married persons and those above 35 years of age. It was also found to be rare in military populations stationed in the South Pacific during the Second World War, where duties prevented heterosexual contacts for long periods of time. A history of contact with an active case of IM is only infrequently observed. This lack of communicability of the disease is also manifested by a rarity of cases occurring in roommates at colleges. There is probably no true seasonal peak in incidence. However, in West Point cadets an increased incidence of cases could be related to vacations and furloughs.

From individual epidemiological case histories, Hoagland made the suggestion that the causative agent of IM is transmitted by oral-oral spread *(63)*. This connotes in the usual case "kissing of more than filial intensity", but also infers transmission by mutual sharing of cups, glasses, and other such objects. This investigator also suggested the accepted incubation period of 33 to 49 days. The association of IM with intimate oral contact is analyzed in Table 3, where it can be seen that a statistically significant relationship does occur *(38)*.

TABLE 3

COMPARISON OF THE HISTORY OF INTIMATE ORAL CONTACT IN PATIENTS WITH INFECTIOUS MONONUCLEOSIS WITH PATIENTS WITH OTHER ILLNESSES IN THE 0 TO 30 AND 31 TO 60 DAY PERIOD PRIOR TO ILLNESS

Day Prior to Illness	*Group*	*History of Intimate Oral Contact*				*P* of Difference (inf. mono. vs other)*
		+	-	*Total*	*% Pos.*	
0-30	Inf. mono.	32	20	52	61.5	P = 0.01-0.02
	Other†	158	205	363	43.5	
31-60	Inf. mono.	34	16	50	68.0	P = < 0.001
	Other†	140	199	339	41.3	

* The probability values have been calculated both by chi square and the method of difference in proportions.

† Other includes: acute respiratory disease 105, other infections 55, noninfectious 117.

Prior to the discovery of EBV and based upon similarities in attack rates as determined by socioeconomic class between IM and poliomyelitis, it was believed that IM was caused by a virus that was widespread in early childhood among lower socioeconomic class populations and that infection in this age group was usually inapparent. Persons from upper socioeconomic class population groups escaped infection at an early age with the virus, but were susceptible to it when exposed at college. In line with the increasing affluence of most population groups in this country and probable decreased exposure to the virus causing IM in childhood, it has now been found that the incidence of IM is increasing. This is illustrated by studies in Connecticut *(Fig. 2)*, where

IM is a reportable disease *(37)*. Studies in Rochester, Minnesota, over the last 20 years also support an increasing incidence of IM *(51)*.

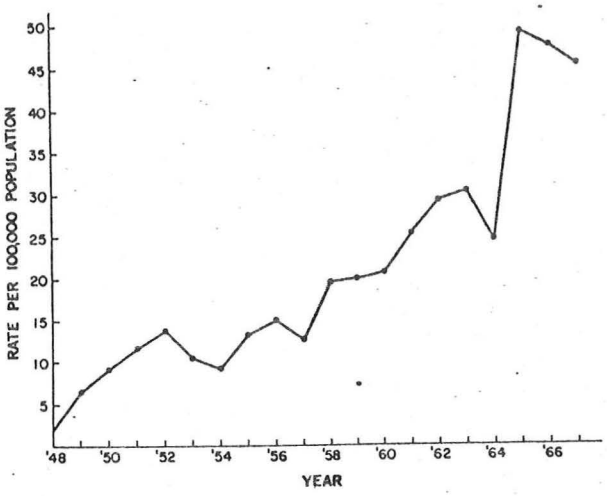

FIG. 2

Infectious Mononucleosis in Connecticut, 1948-1967

The epidemiology of IM fits the known epidemiology of EBV in population groups. The Henles initially became aware of the possibility that EBV could cause a clinical syndrome similar to IM by their serological investigation of persons included in the longitudinal Cleveland Family study *(53)*. Later, a technician in their laboratory developed HA+ IM and preillness and convalescent serum specimens demonstrated seroconversion to EBV.

EBV has now been found to be a ubiquitous virus that is widely distributed in most population groups throughout the world. Antibody prevalence is increased in persons from lower socioeconomic classes. Infection in childhood is usually inapparent. The prime age of acquisition of EBV infection is in the years 0 to 4, with lesser chances of infection in the 5 to 14 year age group. An expected rise in antibody prevalence occurs during adolescence and young adult life. This is illustrated in Table 4, where it can be seen that the antibody prevalence during the years 5 to 14 may actually decrease as compared to 0 to 4 year old children *(99)*.

TABLE 4

OVERALL RESULTS OF EB VIRUS ANTIBODY TESTS

Age Group (Years)	*No. of Sera Tested*			*% Positive*		
	Male	*Female*	*Both*	*Male*	*Female*	*Both*
0-4	81	51	132	53	49	52
5-14	86	69	155	43	41	42
15-24	47	129	176	72	70	70
25-34	31	61	92	77	82	80
35-44	21	42	63	90	83	86

In upper socioeconomic class population groups, the spread of EBV in families occurs, but may be limited. Figure 3 illustrates that the spread of EBV in middle to upper socioeconomic class families appears to be limited and quite unlike rubeola or varicella (*54*).

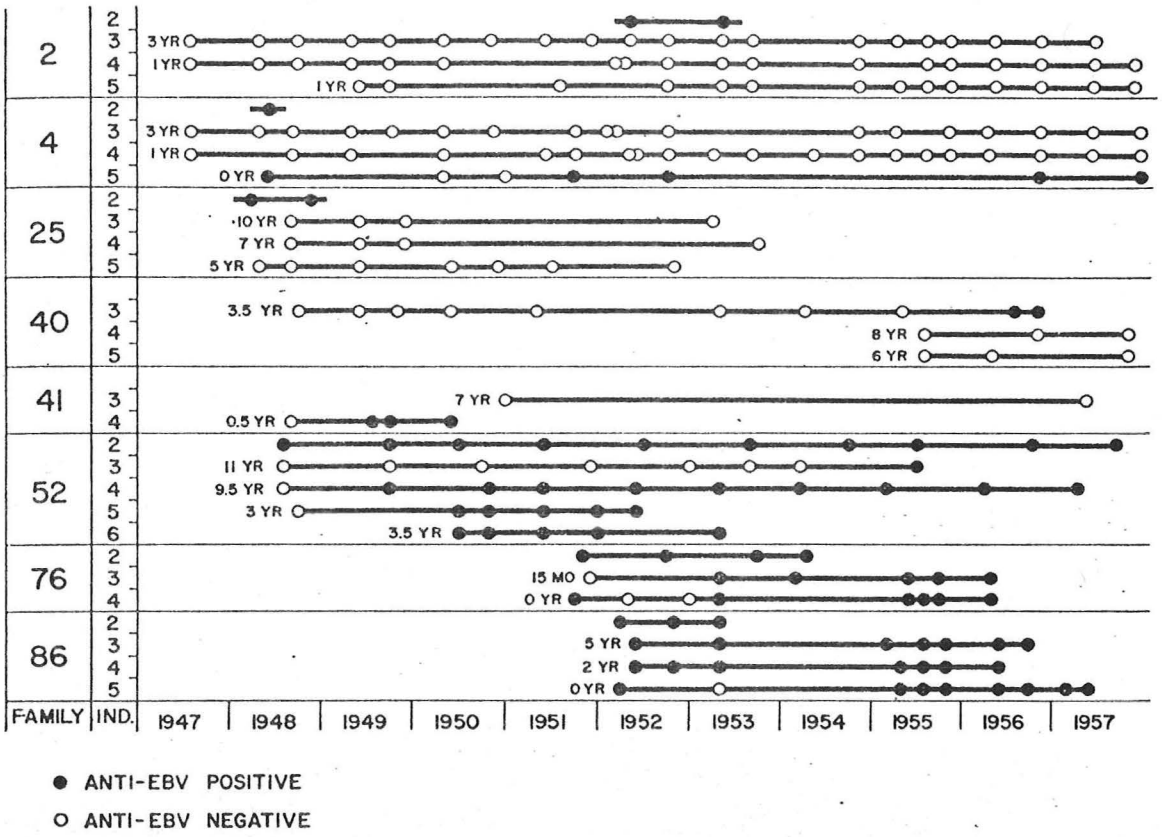

FIG. 3

Examples of intrafamily spread of EBV or lack of it. Individual 2 is the mother; the age of each child (individuals 3-6) at the start of the observation period precedes the first symbol.

The limited spread in such population groups finally results in persons coming to colleges and universities who have never been exposed to the virus (*Table 5*) (*92*).

TABLE 5

PREVALENCE OF EBV ANTIBODIES IN YOUNG ADULT POPULATIONS

Population	*Age (Years)*	*Years Tested*	*No. Studied*	*% Antibody Positive**
United States:				
Yale University Freshmen	17-18	1958-63	424	26.0
Smith College Freshmen	17-18	1960	87	38.0
University of Wisconsin undergraduates†	17-22	1966-68	48	48.0
Yale University graduates	21-26	1968	150	64.0
Peace Corps volunteers§	20-34	1964-65	164	76.0
Philippines:				
University of Philippines undergraduates†	17-22	1966-68	49	75.0
University of Philippines 1st year medical students	20-21	1967-68	53	77.0
Colombia:				
Military recruits	18-20	1965	109	87.0
Totals			1,084	51.5

* At 1:5 or 1:10 serum dilution

† Infirmary admission for illness other than infectious mononucleosis

§ Volunteers having assignments in Colombia

During 1958-1963, only 26.0% of Yale University freshmen were EBV antibody positive at the beginning of college. However, 64.0% of Yale University graduates were EBV antibody positive. These figures can be contrasted with an EBV antibody prevalence of 75% for University of Philippines undergraduates, where it can be assumed that the likelihood of acquisition of antibody to EBV would be increased in early childhood. It has now been found that EBV is present in the oropharyngeal secretions of patients with IM and that the carrier state can persist for as long as 16 months. The virus is usually in low titer, and this may well be the counterpart to Hoagland's suggestion that oral-oral contact of a non-casual nature is important in getting the disease (*Table 6) (88)*.

TABLE 6

RECOVERY OF TRANSFORMING AGENTS FROM THROAT WASHINGS OF PATIENTS WITH INFECTIOUS MONONUCLEOSIS

Days After Onset	*Specimens*		
	No. Studied	*No. Positive*	*% Positive*
0-14	16	13	81.3
15-28	12	10	83.3
29-150	11	11	100.0
> 150	3	2	66.7
Totals	42	36	85.7

IMMUNOLOGY

The immunological aspects of IM have always attracted attention. Following Sprunt and Evans' initial description of clinical cases of IM, Paul and Bunnell were the first to describe the occurrence of heterophile antibody in patients with IM. Later, Davidsohn discerned the typical features of this heterophile antibody, viz., absorption by beef erythrocytes but not significantly by extracts of guinea pig kidney. The Paul-Bunnell-Davidsohn test has been and is the widely used serological diagnostic test for IM. In addition to the heterophile antibody, a variety of other antibodies are produced during the course of IM. These can be divided into those related to specific infection with EBV, heterophile antibodies, and a variety of iso- and autoantibodies (*Table 7*).

One hundred per cent of patients convalescing with HA+ IM have antibodies directed against the VCA and MA of EBV. One hundred per cent of patients also have complement fixing and neutralizing antibody against EBV. Of interest is the fact that only approximately 70% of patients with IM have antibody demonstrable against the diffuse component of the EA complex. Probably only a rare person with HA+ IM has antibody directed against the restricted cytoplasmic component of the EA complex. The anti-VCA EBV antibodies have been found to be both of the IgM and the IgG class. A variety of heteroantibodies, directed against constituent antigens of other species, are produced and are largely of the IgM class. This includes the classical heterophile antibody, a false positive test for syphilis, antibodies against proteus OX-19, streptococcus MG, salmonella group D, and salmonella group E. A variety of iso- and autoantibodies are produced. These too largely are of the IgM immunoglobulin class. Cold agglutinins are found in approximately 35% of patients with IM and are largely of the anti-i variety. These anti-i antibodies have a wide thermal amplitude of activity, occasionally are active at 37°C, and represent the most important cause of the hemolytic anemia which may complicate IM. Patients not infrequently have positive rheumatoid and antinuclear factor tests. They can also have antibodies directed against smooth muscle and B and T lymphocyte populations. Lymphocytotoxins can be produced that have a narrow thermal amplitude and are inactive *in vivo*. Cryoglobulins consisting of IgM and IgG complexes can also be seen during IM.

TABLE 7

ANTIBODY FORMATION IN INFECTIOUS MONONUCLEOSIS (HA+)[*]

Category	*Specific Antibody*	*Predominant Immunoglobulin Class*	*% of Patients With Antibody (Range)*
Anti-EBV	Anti-VCA	IgM, IgG	100
	Anti-MA		100
	Anti-EA complex		
	D component		70
	R component		(1/60) 1.7
	Complement fixing		100
	Neutralizing		100
Heteroantibodies	Heterophile (sheep, horse, ox, beef RBCs)	IgM	100
	False + test for syphilis (anti-reagin)	IgM	0-7.1; best estimate 0.7-1.0
	Proteus OX-19		3
	Streptococcus MG		12
	Salmonella Group D		4
	Salmonella Group E		2
Iso- and Autoantibodies	Cold agglutinins	IgM	35
	anti-i		30
	anti-I		2
	anti-Ii		3
	+ Indirect Coombs		14
	Rheumatoid factor	IgM	14
	Antinuclear factor	IgM, IgG	2-67%; low titered
	Anti-smooth muscle	IgM	81
	Lymphocytoxins[†]		
	Anti-B lymphocyte[§]		20
	Anti-T lymphocyte[§]	IgM	25
	Cryoglobulins	IgM·IgG complexes	

[*] References, 14,15,18,23,24,28,30,43,58,68,70,71,72,122

[†] Cold antibodies with a narrow thermal amplitude, inactive *in vivo* and separate from anti-i and anti-I

[§] Cold antibodies demonstrated by immunofluorescence with living cells

The heterophile antibody appears early during the course of IM. The course of heterophile and EBV antibody formation and disappearance is outlined (*Fig. 4) (91).*

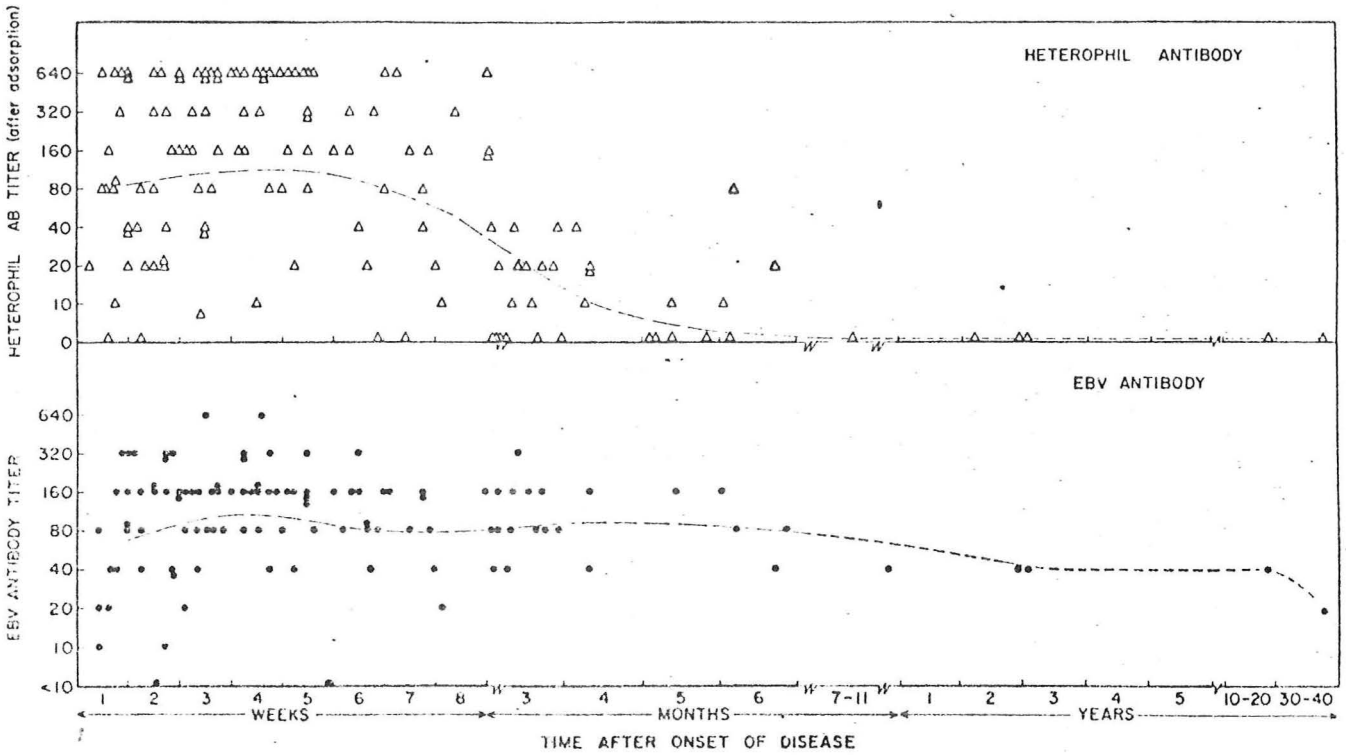

FIG. 4

Most EBV infections in childhood are inapparent, but typical IM can occur at this age group. The height of the heterophile antibody response may be influenced by age with the maximum titer reached being less in younger children (*Fig. 5) (4).*

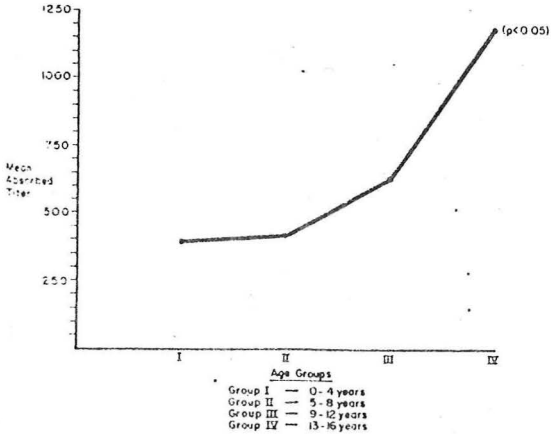

FIG. 5

Relationship of mean of guinea pig kidney's absorbed titer to ages of patients with infectious mononucleosis

The meaning of heterophile antibody occurring in the course of IM has aroused speculation. This antibody is not absorbed with LCL known to contain EBV antigens. In a series of experiments with squirrel monkeys, Shope and Miller showed that autologous EBV transformed lymphoblasts when injected back into these animals elicited both EBV antibody and heterophile agglutinin. A continuing infection could not be induced in this primate species. Later, both intact cells and cell membranes, but not cell lysates, produced a rise in antibody titer to EBV and heterophile antibody. They postulated that the heterophile antigen was present on the surface of the lymphoblasts and that it had either been coded for by the EBV genome or else was uncovered by infection with the virus *(Fig. 6) (111)*.

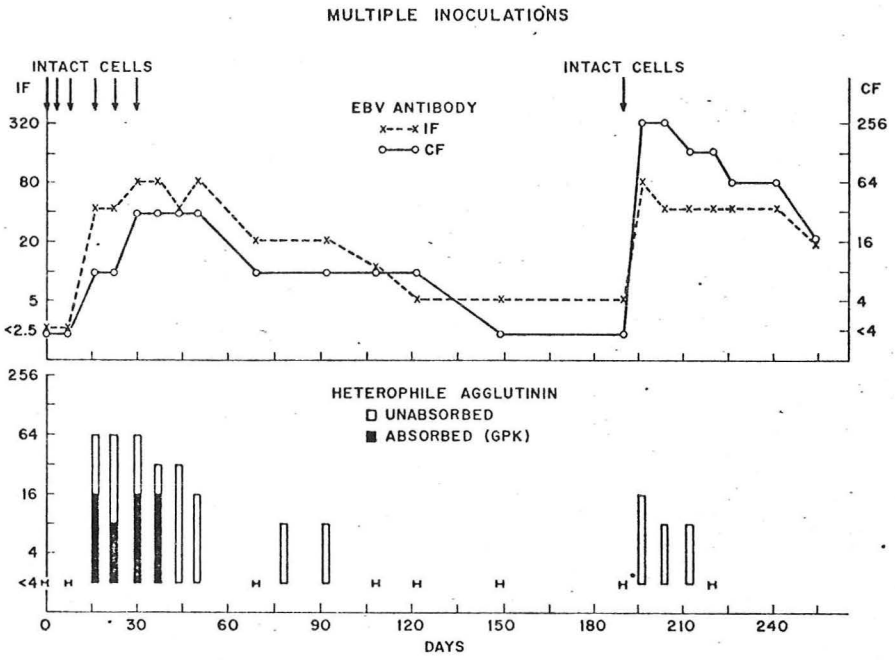

FIG. 6

Squirrel monkey. Heterophile and EBV-specific antibody responses after repeated inoculations of autologous EBV-transformed lymphoblasts; followed by challenge 7 months later with autologous EBV-transformed whole cells (H indicates a heterophile agglutinin titer less than 1:4).

The variety of antibodies formed, both nonspecific and specifically related to EBV virus infection, implies enhanced activity of the antibody forming cells. Whether this results from the presence of the virus, enhancing substances, or loss of the postulated T cell influence on B lymphocytes remains to be determined. Almost 100% of patients receiving ampicillin during the course of IM develop a skin rash. This phenomenon might conceivably be related to enhanced activity of the antibody forming system in IM.

The atypical lymphocyte is a differentiated mature cell that can be seen in a variety of conditions other than IM (other viral infections, hypersensitivity states, etc.), but rarely in the numbers that are seen in this disease. The atypical lymphocyte is in a state of increased DNA synthesis and has been likened to the lymphocyte in cell culture that has already been transformed by exposure to phytohemagglutinin or to previously experienced antigens. Recent evidence suggests that the atypical lymphocyte in IM is derived from the T lymphocyte population pool. Two studies have demonstrated spontaneous rosette formation around atypical lymphocytes. The number of cells forming spontaneous rosettes, a T cell marker, was found to coincide with the numbers of atypical lymphocytes present in the blood. Appropriate controls demonstrated that the cells forming spontaneous rosettes against sheep red blood cells lack IgM surface immunoglobulin, thereby excluding the possibility that these atypical lymphocytes were actually forming heterophile antibody *(Fig. 7) (109).*

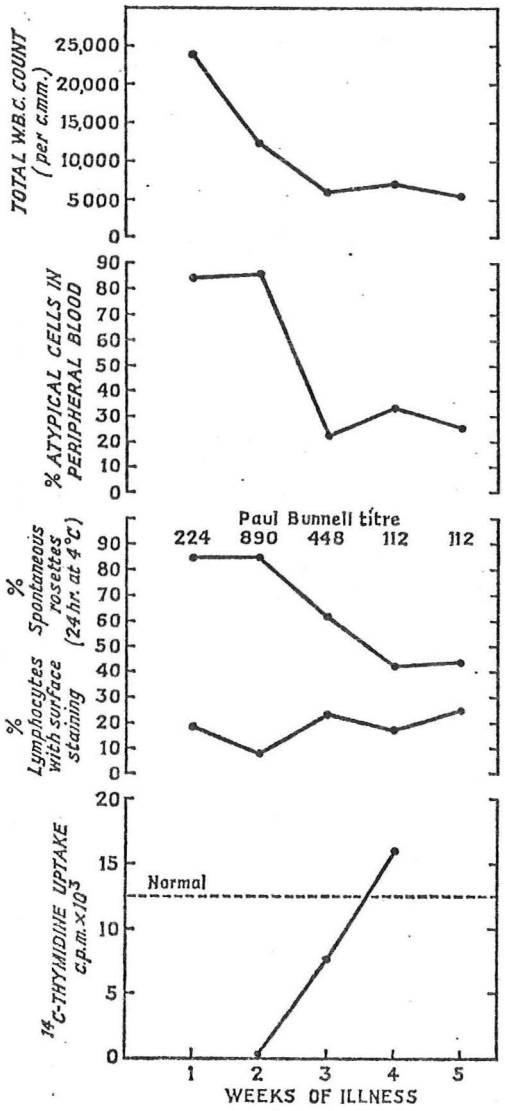

FIG. 7

Frequency of spontaneous rosette-forming cells, lymphocytes with surface staining, atypical mononuclear cells, and the changes in PHA responsiveness in a patient during the 5 weeks after onset of IM. The normal level shown is the mean for 16 controls (12,523 cpm).

The patient with IM can manifest anergy to tuberculin. The postulated explanation for this phenomenon may reside in competition for T cell activity.

When established in culture, lymphocytes from patients with IM produce interferon, macrophage inhibitory factor, and other lymphokines. They also synthesize immunoglobulin. Lymphocytes from patients with IM are easily converted into LCL. These LCL show the presence of the EBV genome. Approximately 50 to 90% of the lymphocytes in LCL can be determined to have immunoglobulin on their surfaces. Complement receptors are also found on the surfaces of a proportion of these lymphocytes. By analogy with the mouse animal model system, it can be presumed that the cells in established LCL have B lymphocyte population characteristics (*28*).

PATHOLOGY AND PATHOGENESIS

The fundamental pathological alteration in IM is lymphoreticular hyperplasia. This accounts for the tonsillar enlargement, the lymphadenopathy, the splenomegaly, the hepatic enlargement and the infiltration by mononuclear cells in other organ systems of the body. Atypical lymphocytes compose at least some part of these infiltrating cells. While lymph node architecture is generally preserved, a malignant lymphoma can at times be simulated. This has pertinent considerations since typical Reed-Sternberg cells have been found during the course of IM. Splenic subcapsular hematomas and rupture can occur and have been correlated with splenic enlargement and infiltration of the capsule and walls of the trabecular veins with mononuclear cells. Infiltrations of lymphocytes can be seen in the portal tracts of the liver, the perivascular areas of the brain, the meninges, the myocardium and in other organs like the kidney. The pathogenesis of the clinical manifestations of IM depends upon this lymphoreticular hyperplasia, the presumed presence of virus in lymphocytes and the immunological reactions that are occurring in various organ systems. On occasion these events may combine to produce a phenomenon like the cerebral edema that can complicate encephalitis due to IM and which may have a destructive potential.

CLINICAL MANIFESTATIONS

The typical course of IM in relationship to heterophile and EBV antibody titers is illustrated in Figure 8 (*91*).

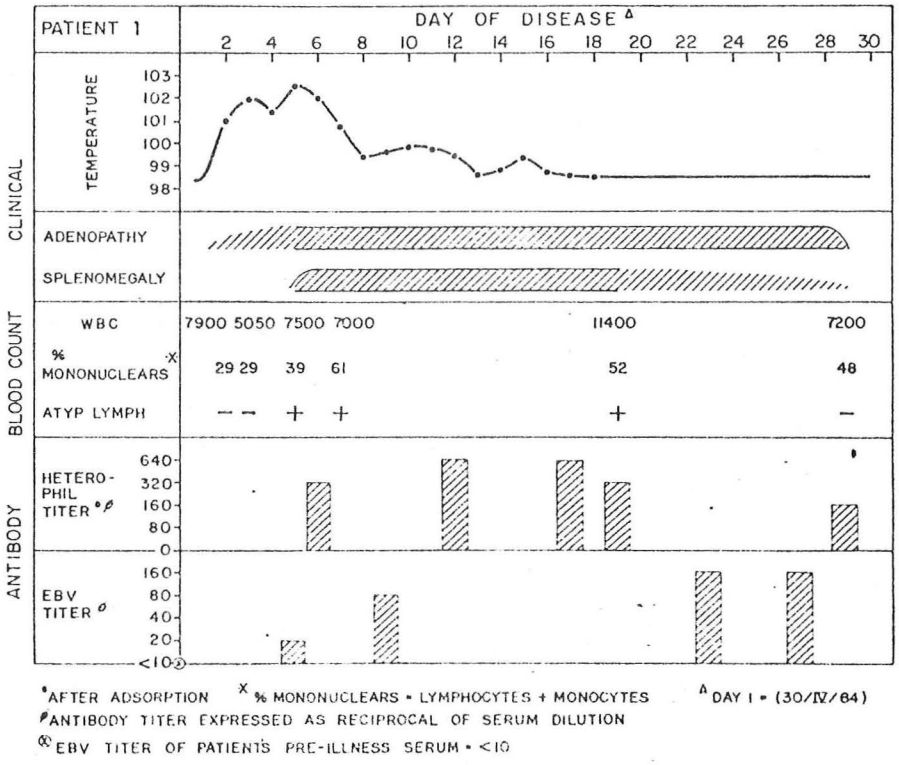

FIG. 8

Time relationships between clinical features, hematologic changes, and antibody levels (EBV and heterophile) in a typical case of infectious mononucleosis in an 18-year-old boy

All of the classical parameters of the disease become positive relatively early, and the typical course indicates resolution generally within a four-week period of time (*Fig. 9) (66)*.

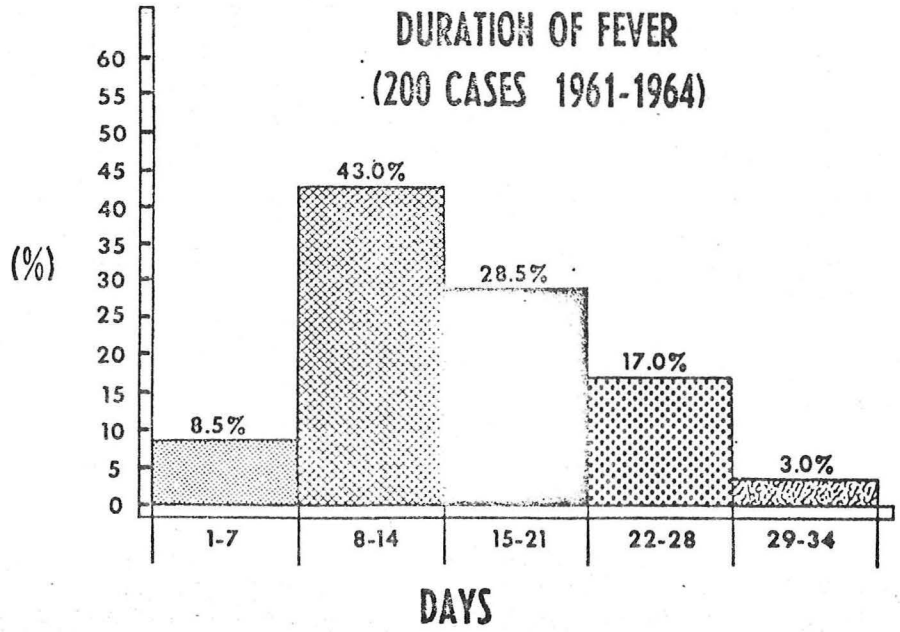

FIG. 9

The clinical and laboratory features of HA+ IM, as they have been elucidated in six series of more than 100 patients, each has been analyzed (*Table 8*). The onset is usually gradual, with the patient, in the typical pharyngeal form of the disease, experiencing sore throat and feverishness. Malaise, headache and fatigue are noted. A lesser number of patients may present with fatigue and malaise and a fever of undetermined origin but without symptoms referable to pharyngeal involvement. These have been classified as the so-called typhoidal, or essential, form of IM. Patients can also present with icterus as the major complaint. Then, regardless of whether a sore throat is present or not, the patients are classified as icteric cases of IM.

Physical examination generally reveals fever and generalized lymphadenopathy. Of diagnostic import is the presence of posterior cervical adenopathy. The nodes are firm, nontender, not matted together, and there is no overlying redness to the skin. Pharyngeal inflammation and tonsillar hypertrophy are common manifestations. There may be an exudate present in the oropharynx and on the tonsils. In about 50% of the patients, palatal petechiae occur. These are discernible usually by careful search with a bright light and are at the margin of the hard and soft palates. In about 30% of the patients, eyelid edema occurs. This edema has been postulated to have the same pathogenetic mechanism as the edema which can be present in the uvula and soft palate, and may reflect lymphatic obstruction. Splenomegaly is present in from 30 to 71% of patients. Hepatomegaly is less common, and is generally never marked. Jaundice is seen in 5 to 11% of patients. A skin rash, generally a maculopapular eruption, has been considered to be infrequent in the course of IM. Its frequency in different series may reflect the prior administration of drugs such as ampicillin.

In the clinical laboratory, a relative and absolute lymphocytosis are seen. At some time or other in the course of IM, most patients have greater than 4,500 lymphocytes per mm^3 and greater than 50% lymphocytes. The majority of these cells are at some time during the course of IM atypical. Since the five series are defined in terms of their heterophile antibody positivity, all of the cases had the presence of specific heterophile agglutinin.

Hepatic functional disturbances are extremely common. Forty-one to 70% of patients with HA+ IM have disturbances related to hepatic function abnormalities as manifested by increased SGOT and/or SGPT. The results of the liver function determinations in one series of patients are shown in Table 9 (*73*).

TABLE 8

CLINICAL AND LABORATORY FEATURES OF INFECTIOUS MONONUCLEOSIS

	Feature	*Per Cent of Patients*					
Symptoms	Sore throat	75	89		85		100
	Feverishness				78		
	Malaise				71		
	Headache				52		
	Myalgias		7		46		
Signs	Fever	33*	98	100	95	71	100
	Lymphadenopathy	93	100	85	91	92	100
	Pharyngeal inflammation		85		89	87	
	Exudative tonsillitis	40	31	30	33		
	Splenomegaly	40	75	49	71	30	45
	Hepatomegaly			29	48		15
	Palatal enanthem	(14/27) 52	50		42		
	Eyelid edema		~ 33		36		
	Jaundice		1	5	4	6	
	Rash	1	3.5	8	2	41¶	6
Clinical Laboratory	> 4500 lymphocytes/mm^3	100†	100				
	> 50% lymphocytes	100†	100				100
	Atypical lymphocytes		100	100	100	~ 97	100
	Heterophile antibody	100	100	97	100	100	100
	↑ SGOT and/or SGPT		(58/70) 41	(20/45) 14	70§		
	β-hemolytic streptococci		11	30	(7/19) 37	12	29
Complications	Splenic rupture	0	0.2	1	0		0
	Central nervous system	0	1.6	0	3	0.9	
	EKG abnormalities		6	(4/39) 10			
	Hemolytic anemia	0	3	0	36‡		
	No. of patients studied	176	500	105	102	575	776
	Characteristics of study group	College	Young adult	Pediatric	6-28 years	Atlanta,Ga. population	Rochester, Minn. population
	Reference	36	66	4	28	50	51

* > 101°F

† Combined lymphocyte and monocyte count

§ 1 or more tests abnormal (SGOT, LDH, alkaline phosphatase)

‡ Reticulocytosis and evidence of mild hemolysis

¶ Incidence of concomitant drug administration not stated

TABLE 9

RESULTS OF LIVER FUNCTION TESTS IN PATIENTS WITH INFECTIOUS MONONUCLEOSIS

Test	*Normal Value*	*Criteria for Abnormally High Test Results*	*Range Reported in This Study*	*No. Patients Having Test*	*No. Patients With Abnormal Test Results*	*% Patients With Abnormal Test Results*
BSP	< 5% retention after 45 min.	> 6.5% retention after 45 min.	0-42%	19	9	47
SGPT	< 40 units	> 60 units	10-386 units	20	13	65
Serum alkaline phosphatase	4.0 BU	6.0 BU	2.4-17.7 BU	18	7	39
Serum bilirubin	1.0 mg/100 cc	> 1.2 mg/100 cc	0.4-1.34	15	1	6
Cephalin-cholesterol flocculation	< 2+	2+ or >	0-4+	18	17	94
Thymol turbidity	< 4.5 units	> 4.5 units	4.3-13.2	18	13	72

As can be seen, the SGPT never rose above 386 units in this particular series and the serum bilirubin was elevated only to a minor degree. The functional hepatic disturbances can be correlated with the major pathological finding of round cell infiltration in the portal triads. β-hemolytic streptococci have been found in 11-37% of patients studied. This prevalence has been considered excessive by some authors but not by others. An occasional patient with IM may have microscopic hematuria and it is not known how this relates to the presence or absence of β-hemolytic streptococci on throat culture.

The usual course of IM is a stereotyped one, with the total duration of illness usually being a two- to four-week period of time. Relapses of IM are considered to be infrequent and genuine recurrences are not thought to occur. In Hoagland's view, chronic disease due to infectious mononucleosis, "chronic infectious mononucleosis", is about as tenable as "chronic measles".

Complications are infrequent in IM. Splenic rupture occurs in 0 to 1% of cases and may be antedated by left upper quadrant pain, as might occur in a subcapsular hematoma. Edema of pharyngeal and laryngeal structures may complicate IM and lead to upper airway obstruction. Abnormalties relating to the nervous system occur in 0 to 3% of patients with IM. The nervous system abnormalities may be divided into the following clinical syndromes: (1) aseptic meningitis, (2) encephalitis, (3) transverse myelitis, (4) polyneuritis (Landry-Guillain-Barre syndrome), and (5) mononeuritis. On occasion, patients with encephalitis may present predominantly clinically as an acute cerebellar syndrome. Neurological complications have been found to be more common in men and, on occasion, may so dominate the clinical picture as to be the presenting complaint. Encephalitis is characterized by headache, stiff neck, delirium and confusion. Irrational behavior, convulsions and papilledema may be seen. The cerebrospinal fluid findings indicate normal glucose values, a predominance of lymphocytes and an occasional case in which the protein concentration may be 100 mg.% or greater (*Table 10) (107)*. The cerebrospinal fluid may contain heterophile antibody as well as antibody to EBV. Peripheral polyneuritis can present with manifestations initially being related to cranial nerve involvement. Albuminocytologic dissociation is present. The case-fatality ratio with central nervous system involvement is low and full recovery can usually be anticipated.

Cardiac complications due to IM mainly revolve around electrocardiographic disturbances which can be seen in from 7 to 10% of patients. Clinically significant pericarditis due to IM is rare. Nonspecific ST-T wave changes are the usual abnormalities but PR and QT interval prolongation can be observed as well as various arrhythmias. The rate of occurrence of rhythm disturbances is low (2/500) but one patient had an AV nodal rhythm persisting for more than two years after its initial appearance during IM (*66*). This same patient, 10 years after IM, had a RBBB but had no limitation of physical activity.

Although reticulocytosis and evidence of mild hemolysis is probably common in IM, clinically significant hemolytic anemia is infrequent. Other rare complications include bullous myringitis, arthritis and arthralgias, thrombocytopenic purpura and agranulocytosis. If pneumonia occurs in IM, a search for other etiologies should be made.

TABLE 10

SUMMARY OF LABORATORY FINDINGS

Case	Peripheral Blood					Cerebrospinal Fluid*			
	Leucocytes			Heterophile Antibody Titers		Cells, no./mm³		Protein mg/100 ml	Sugar mg/100 ml
	No./mm³	Lymphocytes %	Leucocytoid Cells, % of mononuclear cells	Presumptive†	After Adsorption (guinea pig kidney)	Lymphocytes	Polymorphonuclear leucocytes		
1	13,200	81	50	1:1024	1:224	36	0	300	66
2	8,600	16	26	1:2048	-	33	1	120	100
3	6,900	60	52	1:4096	1:1792	54	3	100	72
4	16,200	52	52	1:64	-	13	16	75	-
5	6,400	32	-	1:1024	1:448	37	2	80	-
6	8,500	55	42	1:448	1:224§	29	10	65	-
7	9,800	38	48	1:1792	1:1792§	6	1	170	70
8	6,800	59	-	1:1024	1:896	-	-	-	-
9	9,900	55	89	1:512	1:112	-	-	-	-
10	7,700	80	59	1:2048	1:896	14	0	35	72
11	5,700	40	54	1:1024	-	7	1	56	62
12	8,100	51	36	1:2048	-	4	0	50	90

* CSF heterophile antibody titer was 1:1792 in case 2 (1:896 after absorption); not determined in other cases

† Presumptive titers, except in cases 6 and 7, are based on serum dilutions of 1:8, 1:16, 1:32, etc. Titers for cases 6 and 7 are based on serum dilutions of 1:7, 1:14, 1:28, etc.

§ Titers after adsorption on beef erythrocytes: Case 6, 1:7, case 7, 1:112.

It is generally held that sequelae do not eventuate after IM. Recently, however, investigators have been interested in the relationship between IM and Hodgkin's disease (2). This interest has been generated by case reports detailing the occurrence of Hodgkin's disease after IM, the potential of EBV as an oncogenic agent, reports of increased EBV antibody titers in Hodgkin's disease and the epidemiology of Hodgkin's disease in adolescence and early adult life which suggests that an infectious etiology may be involved. Kaplan at Stanford University Medical Center in California has reported over an eight-year period 29 patients who have given a "reasonably well documented history" of infectious mononucleosis. Except for one older man, the patients were between 14 and 33 years of age and the period between the diagnosis of IM and the diagnosis of Hodgkin's disease ranged from two months to 14 years, with a median interval of four years. Among veterans who had IM in 1944, there were five who died of lymphoma by 1965, as compared with 2.3 to be expected on the basis of national morbidity data. Among 4,516 cases of IM in Connecticut, there were five who were subsequently reported to have Hodgkin's disease, a rate of 13 per 100,000 person years of observation. This is higher than the incidence of Hodgkin's in any age group of the general population. However, in a study of 776 patients in Rochester, Minnesota, followed over a two-decade interval, none developed lymphoma or other neoplasm. Further widescale investigative studies are presently in progress to decide the issue.

When IM occurs in the course of leukemia, a betterment of the underlying disease process has been reported. However, other reports do not confirm that such betterment invariably follows IM.

CLINICAL LABORATORY

A leucocytosis is generally present with maximum counts being reached between the 12th and 18th days of illness. Unless bacterial infection supervenes, the majority of cells making up this leucocytic response are lymphocytes. Absolute lymphocytosis, > 4,500 cells/mm^3, and relative lymphocytosis, > 50% of the cells, is the rule. The absolute granulocyte count tends to decrease during the illness. In infrequent cases the absolute granulocyte count can dip below 500/mm^3 (*Fig. 10) (16)*. The absolute eosinophile count may be normal or may be slightly increased. The monocyte count is probably slightly increased. The platelet count tends to be decreased in IM and in rare cases, thrombocytopenic purpura may occur *(Fig. 11) (19)*.

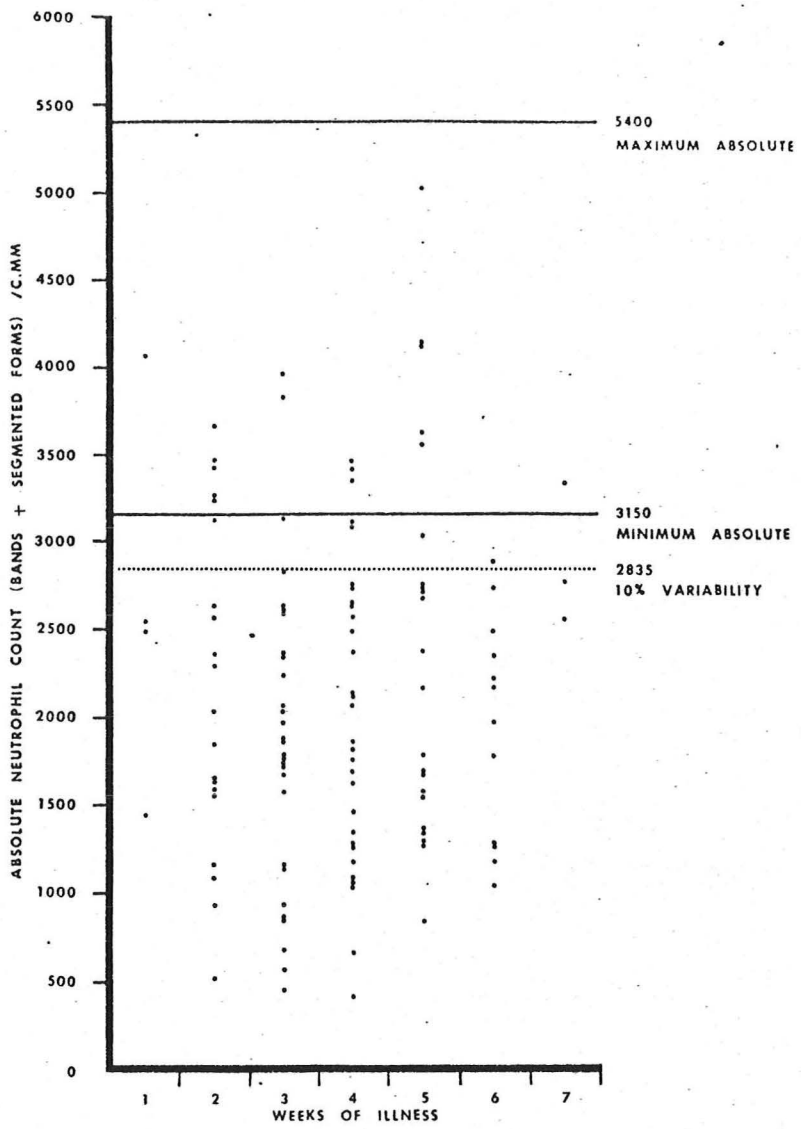

FIG. 10

Absolute neutrophil counts per week of illness in infectious mononucleosis

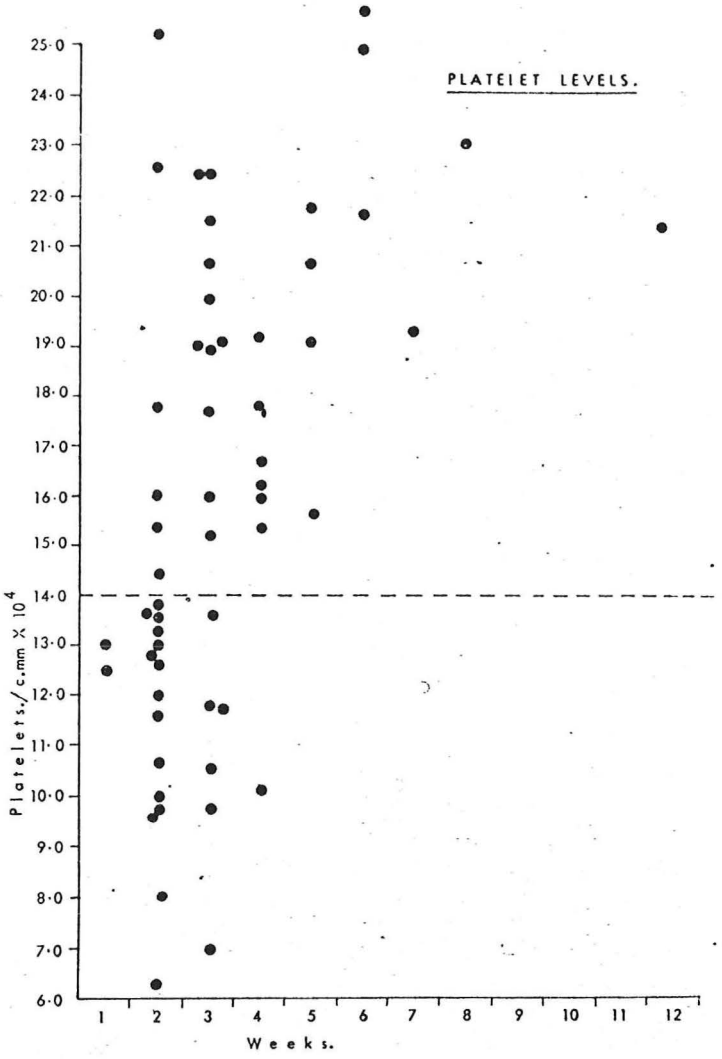

FIG. 11

Platelet levels in infectious mononucleosis

At some phase of IM, the majority of lymphocytes are considered atypical. Of practical significance is the demonstration that lymphocyte morphology is altered by duration of anticoagulant contact. A fingerstick preparation should be used or else films of anticoagulated blood should be made soon after collection. The most widely used morphologic classification of atypical lymphocytes has been patterned after Downey (*33*). This investigator was particularly concerned with the differentiation of atypical lymphocytes from leukemic cells. He stressed the pleomorphic character of the morphology of the atypical lymphocyte and its categorization as a mature, differentiated cell. It should be stressed that the morphologic types described by Downey are not distinct and that gradations between all three types can be observed in a single film. A patient during the course of IM may have differing proportions of each cell type. Before considering Downey's classification, a few general comments are in order. Atypical lymphocytes are pleomorphic in appearance in contrast to the monotonous regularity of the morphology of leukemic lymphocytes. In keeping with their designation as mature, differentiated cells, atypical lymphocytes (1) tend to have a low nuclear/cytoplasmic ratio, (2) the nuclei are often indented or lobulated, (3) the cytoplasm may be abundant and can be seen at times to

flow around the surface of adjacent erythrocytes, (4) the cytoplasmic color is often deeply basophilic but may be sky-blue in color, and (5) cytoplasmic vacuoles and azurophilic granules may be present.

The Downey Type 1 cell (monocytoid lymphocyte) is characterized by an eccentrically placed nucleus whose long axis may be at a 90° angle to the long axis of the cell. The nucleus is indented or lobulated, nucleoli are not generally present and the chromatin-parachromatin margin is blurred. The cytoplasm is deeply basophilic and may contain vacuoles and azurophilic granules. The Downey Type 2 cell probably corresponds best with the plasmacytoid lymphocyte. The nucleus tends to be centrally placed with the nuclear chromatin approximating that of the plasma cell. The cytoplasm is abundant and tends to have a sky-blue color. Fewer vacuoles and inclusions are seen. The Downey Type 3 cell has a tendency to be lymphoblastic in appearance. The nuclear/cytoplasmic ratio may be increased and nucleoli can be seen. These cells can be differentiated from lymphoblasts by the indentation of the nuclei, the quantity and the vacuolization of the cytoplasm.

Of major diagnostic importance in IM is the presence of heterophile antibody that has specific absorption characteristics. This antibody has an affinity for sheep, horse, beef or ox erythrocytes and differs from the Forssman antibody that appears during serum sickness in that it is not absorbed by extracts of tissue that contain the Forssman antigen, viz., guinea pig or horse kidney. The heterophile antibody of IM, however, is absorbed by beef erythrocytes and these features enable a differential absorption test to be done (Paul-Bunnell-Davidsohn test). Davidsohn's criterion for a positive differential absorption test indicating the presence of heterophile antibody specific for IM is the persistence of agglutinins against sheep erythrocytes not more than 3 dilutions or 8-fold from the original titer after absorption with guinea pig kidney extract. Absorption of the serum with beef erythrocyte stromata should reduce the titer by more than 4 dilutions or 16-fold.

At the present time in most hospitals in the United States, a screening procedure (Monospot test) is used as the presumptive test for IM specific heterophile antibody. The test is simple and easily performed and consists of reacting serum with guinea pig extract on one side of a slide and with beef erythrocyte stromata on the other. Formalinized horse erythrocytes are then added to both sections of the slide. If the agglutination occurring after absorption with guinea pig kidney is greater than that occurring after absorption with beef erythrocyte stromata, the test is considered positive. The Monospot test is sensitive and gives $\leq$ 1% false negative reactions. False positive reactions may occur, however, and, in the usual circumstances, the technician will then perform the more specific classical Paul-Bunnell-Davidsohn test.

The heterophile antibody specific for IM is generally always present in the third or fourth week of illness. If the clinical and hematologic features suggest IM and the Monospot test is initially negative, it should be repeated at this time. A positive Paul-Bunnell-Davidsohn test *per se*, however, is not diagnostic of IM as specific heterophile antibody may be present from a previous episode of that disease or else may be related to a resurgence of antibody during an unrelated infection. It is also evident that inapparent EBV infection may be associated with positive Monospot and Paul-Bunnell-Davidsohn tests.

Other tests have been proposed for the detection of IM specific heterophile antibody. These have never gained wide acceptance in the United States. The ox hemolysin test is considered positive if serum at a 1:40 dilution, in the presence of an optimal dilution of complement, can hemolyze a predetermined number of erythrocytes. The test is sensitive and specific and has been mainly used in Europe. Enzyme tests have also been used and rest upon the principle that papain treatment of sheep red blood cells removes the IM receptor site but accentuates the non-IM receptor site. If IM specific heterophile antibody is present, papain treatment should lower the titer. A second test revolves around pretreatment of sera with papain treated sheep red blood cells. IM specific heterophile antibody should not be affected by this procedure whereas the non-IM antibody titer should be significantly lowered.

HA- IM

EBV is now thought to be the etiology of HA+ IM. The etiology of HA- IM can next be considered. It must be emphasized that the heterophile reaction may be negative in the early phase of illness and that repeat determinations are necessary during the third or fourth week after onset to determine definitely that a case is HA-. Numerically, the most important etiologies of HA- IM are CMV and EBV. Other less important causes are *Toxoplasma gondii*, adenoviruses, rubella virus, herpes simplex virus, mycoplasma, and other undetermined agents.

The etiology of HA- IM has been investigated most intensively in Finland (*75*). In the initial Finnish study, 275 patients were accepted with an illness compatible with IM by clinical and hematological criteria. Sixty of these persons were HA-. Patients with studies indicating toxoplasmosis were excluded from this group. 44/60 had sufficient specimens available for further serological study. 19/44 (43.2%) had evidence of recent CMV infection. Eight of the remaining 25 patients had EBV antibody titers (anti-VCA) $\geq$ 1:60, a level suggesting recent infection. Not more than 10% of a normal Finnish population has EBV antibody titers of this magnitude. 12/25 patients had intermediate EBV antibody titers 1:40-1:80. In the remaining 5 cases, EBV infection could be excluded. This study has now been extended to 494 patients. Of 73 persons $\geq$ 15 years of age with HA- IM, 33 (35%) had evidence of recent CMV infection.

In the Finnish study, persons with HA- IM due to CMV all were $\geq$ 15 years of age and presented with the essential or icteric form of IM. Pharyngitis was not present. Workers in the United States have recently reported a series of patients with CMV mononucleosis and did note the presence of pharyngitis and lymphadenopathy. In further studies, exudative tonsillitis has been observed.

Toxoplasmosis can present with a syndrome that is essentially indistinguishable from the essential form of IM. Its exact incidence in the United States can only be estimated, but it probably accounts for less than 5% of HA- IM cases (*103*). The contribution of adenoviruses, rubella virus, herpes simplex virus and mycoplasma to the etiology of HA- IM appears limited.

THERAPY

Usually symptomatic measures are all that are needed in IM. If splenomegaly is present, caution should be urged against undue physical exertion because of the possibility of splenic rupture. Double-blind studies assessing the effect of prednisolone vs aspirin have been performed in uncomplicated IM with the results indicating no significant difference between the two therapeutic modalities (*Table 11) (36).*

TABLE 11

SUMMARY DATA ON THERAPY

	Prednisolone (Group A)	*Aspirin (Group B)*
A. Prior to Therapy		
No. of patients	9	15
Age	20.1	19.1
Duration of illness in days at 0 point	9.6	10.3
Fever at start of test period	100.6	99.3
No. of patients with fever over 101 at start	3	2
Signs at start: % of patients showing:		
Sore throat	50	78
Exudate	25	28
Cervical nodes	87	78
Tender liver	0	0
Enlarged spleen	12	36
Heterophile positive during illness	77	60
B. Results (in days)		
Days in hospital	9.9	9.0
Total duration of disability*	19.5	19.3
Duration after starting therapy:		
Fever	3.0	0.5
Sore throat	2.2	4.9
Exudate	1.0	1.3
Cervical adenopathy	3.6	4.9
Relapse (number)	0	1

* Onset to hospital discharge

Although controlled data are lacking, most authorities, however, would use short term steroid therapy if IM should become significantly complicated. Indications include upper airway obstruction, central nervous system involvement, clinically significant hemolytic anemia, thrombocytopenia and agranulocytosis. The rationale for steroid therapy resides in their capacity to induce lymphocytolysis and to aid the resolution of inflammatory edema. These effects outweigh the theoretical disadvantages of steroids in blunting host defense mechanisms.

PREVENTION

Prospects of a vaccine appear limited at the present time. Inactivated vaccine would require the necessity of repeated dosages. A live vaccine does not appear indicated because of the potentiality of EBV as an oncogenic agent and the capacity of this virus to enter into a latent state with subsequent reactivation.

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