Mycoplasmal Studies in Infectious Mononucleosis* (33324)

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Infectious mononucleosis is thought to be caused by a microorganism. Efforts to isolate the causative agent have included culture of pharyngeal secretions, blood, and stool for bacteria and viruses and attempts at transmission of the disease to volunteers. Recent work (1, 2) suggests that the etiologic agent may be identical or related to a herpes-like virus found in cultures of Burkitt's lymphoma cells. As another approach to etiology, pharyngeal secretions, saliva, and leukocytes from patients with early infectious mononucleosis were cultured for mycoplasmas. Media capable of supporting the growth of *M. pneumoniae* and T-strain mycoplasmas and techniques found to provide the most accurate picture of the pharyngeal mycoplasmal flora (3) were used. Metabolic-inhibition neutralization antibody tests of sera from patients and controls employed patent-derived strains of *M. pharyngis* (orale I), *M. salivarium, M. hominis*, and a T-strain mycoplasma.

Materials and Methods. Clinical. Members of the study group were students at the University of Virginia. Mycoplasmal flora was characterized in patients and controls seen by one student health physician (A.J.A.) during the winter and spring, 1966–1967. The diagnosis of infectious mononucleosis rested on a compatible clinical and hematologic picture and a heterophil agglutination titer of >1:14 after guinea pig erythrocyte absorpotion. Controls were students with nonrespiratory complaints selected at random. Students with a past history of mononucleosis or illness resembling mononucleosis that was heterophil negative were excluded.

Mycoplasma culture. Pharyngeal flora of patients and controls was characterized using media and procedures previously described (3). Anterior oral pool secretions (predominently saliva) expectorated into carrying broth and leukocytes obtained from 3–5 ml of whole blood (4) were cultured for mycoplasmas in the same fashion as pharyngeal secretions. Pharyngeal and anterior oral pool results were combined for analysis.

Serology. Paired sera from patients and single sera from controls were screened for antibodies to mycoplasmas. Additional *M. pharyngis* antibody studies were performed with paired and single sera from patients seen in the spring of 1966 and with 12 pairs randomly selected from an earlier student study (5).

Metabolic inhibition (MI) antibody titers were measured with the method of Purcell *et al.* (6) employing 3× cloned strains of *M. salivarium, M. pharyngis, M. hominis*, and a T-strain, all isolated from patients with mononucleosis. Media and methods were as described except that the pH of the starting broth was 6.5, the 50% endpoint was read at pH 7.0–7.1, and $10^{1.5}$–$10^{3.0}$ color change units of organism were used in each well. Unheated guinea pig serum was added in tests with *M. salivarium, M. hominis*, and the T-strain at a final concentration of 4.5%. Some human sera enhanced the growth of *M. pharyngis*, making interpretation difficult. Inactivated human serum from an individual without demonstrable MI antibody to *M. pharyngis* was included in the broth at 4.5% final concentration to minimize this effect.

Results. M. pharyngis and/or *M. salivarium* were isolated from the majority of both patients and controls, and no significant diff-

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TABLE I. Carriage of Mycoplasmas by Mononucleosis Patients and Controls.

		Organism			
		M. pharyngis		*M. salivarium*	
	No. tested	No.	%	No.	%
Patients	16	8	50	12	75
Controls	12	9	75	8	67

erences in the isolation rates of either organism was observed between the two groups (Table I). Both organisms were obtained from 5 to 16 mononucleosis patients and from 6 of 12 controls; no mycoplasmas were isolated from 1 patient and 1 control. The secretions of one patient yielded four mycoplasma species: *M. pharyngis, M. salivarium, M. hominis,* and a T-strain. No mycoplasma strains were isolated from preparations of peripheral blood leuocytes from 11 patients.

One fourfold antibody rise, to *M. pharyngis,* was detected in 21 serum pairs from patients. Titers in a single serum sample (the acute phase) from each patient were compared to the titers in a single serum from each control (Table II). Eight (38%) of 21 patients had a titer of $>1:4$ to the strain of *M. pharyngis* whereas 1 (6%) of 16 controls had a similar antibody level. In contrast, the proportions of sera with a titer of $>1:4$ to *M. salivarium* from patients and controls were similar (24% and 19% respectively). Significant antibody to the *M. hominis* strain was detected in one control serum; no metabolic inhibition of the T-strain mycoplasma was produced by any serum.

Additional pairs and single sera from patients and controls were tested for *M. pharyngis* antibody alone. No rise to *M. pharyngis* was found in 10 serum pairs from patients; 1 of 5 controls showed a four-fold rise (this individual had a similar rise to *M. salivarium*). Single serum *M. pharyngis* titers (acute phase from pairs) of all patients and control sera were combined and are shown in Fig. 1. Seventeen (40%) of 42 patients had

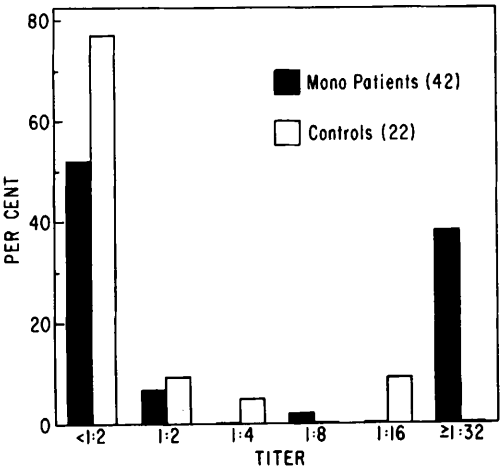

FIG. 1. *M. pharyngis* antibody titer in acute phase of single serum specimens from patients with mononucleosis and controls.

antibody levels of $\geq 1:4$ to *M. pharyngis* and virtually all had high levels. In contrast, 3 (14%) of 22 controls had titers of $\geq 1:4$ ($p < 0.05$), and 1:16 was the highest tite found.

Discussion. No appreciable difference in the mycoplasmal flora of the pharynx and anterior oral pool of mononucleosis patients and suitable controls was found, and this portion of the investigation was consequently terminated. Leukocyte preparations from patients produced no growth in mycoplasma media, although the presence of heparin might have inhibited growth had there been mycoplasmas present.

A significantly greater proportion of patients than controls had antibody titers of

TABLE II. MI Antibody Titers of $\geq 1:4$ in a Single Serum from Mononucleosis Patients and Controls.

		Organism						
		M. pharyngis		*M. salivarium*		*M. hominis*		T-strain
	No. tested	No.	%	No.	%	No.	%	(no.)
Patients	21	8	38	5	24	0	—	0
Controls	16	1	6	3	19	1	6	0

$\geq$1:4 to *M. pharyngis*, but the importance of this finding is not clear. Continued elevation of titers in convalescence and insignificant alteration of MI titers by absorption with bovine red-cell antigens served to differentiate antibody to *M. pharyngis* from the heterophil antibody. The finding that titers to *M. pharyngis* in serum pairs were stable and that only 40% of patients had antibody titers of $\geq$1:4 suggests that the observed difference is unrelated to the pathogenesis of infectious mononucleosis. Conversely, the sensitivity of the MI test for *M. pharyngis* antibody and the importance of antigenic differences among strains, as found with *M. hominis* (8), are not known. It might be hypothesized that antibody to *M. pharyngis* represents yet another early abnormal immunoglobulin response in some patients with infectious mononucleosis. Further work is needed for clarification.

Summary. Mycoplasma flora of the pharynx and anterior oral pool of 16 patients with early infectious mononucleosis did not differ significantly from that of 12 controls. Patient-derived strains of *M. pharyngis, M. salivarium, M. hominis,* and T-strain mycoplasma were employed in metabolic inhibition antibody tests of paired and single sera from patients and controls. The only difference observed was the presence of MI antibody titers of $\geq$1:4 to *M. pharyngis* in 40% of 42 patients' sera compared to 14% to 22 sera from controls. Antibody responses to mycoplasmas were not observed during the course of illness.

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A Direct Radioisotopic Microassay for Cholinesterase (33325)

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Radioisotopic methods for the determination of cholinesterase[1] (1) activity in microquantities of biological tissue and fluids are undoubtedly advantageous over manometric (2), polarographic (3) or microchemical methods (4). Winteringham and Disney (5, 6) were first to use acetate ^{14}C labeled acetylcholine as a substrate for the enzyme. They incubated blood with acetylcholine-^{14}C and measured the amount of the remaining unhydrolyzed substrate after careful removal of the volatile acetate under vacuum. Reed *et al.* (7) also used the labeled substrate for incubation with enzyme but removed the unhydrolyzed portion with an ion-exchange resin batch treatment method.

A simple and sensitive isotopic method will be described for repeated routine microdetermination of cholinesterase activity in a large number of samples from biological material utilizing acetylcholine as a substrate.

Method and Procedure. Rat brain was used for tissue material. A 10% rat brain

[1] Cholinesterase is used in this manuscript to designate enzymatic hydrolysis of acetylcholine by eserine-sensitive esterases.