

Epidemiologic Investigations with Respiratory Disease Virus RI-67

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The complexity of the problem of the acute respiratory diseases is well illustrated by this report upon a new and widespread entity herein called the RI-67 group of infections.

‡ During January, 1954, we reported¹ the recovery of a new virus, designated RI-67, from the throat washings of a patient with primary atypical pneumonia in an epidemic of acute respiratory illness which occurred among the soldiers at Fort Leonard Wood, Mo., during the winter of 1952-1953. The designation RI-67 was derived from Respiratory Illness, case number 67, in the epidemic. The new virus was cytopathogenic for HeLa (human epidermoid carcinoma) cells² in tissue culture and the agent could be propagated readily in these and other cultivated human epithelial cells. Patients who were infected with the virus developed significant increases in neutralizing and complement-fixing antibodies against the agent in the progress of their maladies.

The illnesses in the patients who were hospitalized with RI-67 infection in the epidemic at Fort Leonard Wood^{1, 3} belonged in two clinical entities. These were "undifferentiated acute respiratory disease (ARD)"^{4, 5} and "primary atypical pneumonia"^{6, 7} of the kind that is unassociated with the development of cold agglutinins. Patients from other epidemics with common cold or with

"primary atypical pneumonia" in which there was an increase in cold or streptococcus MG agglutinins failed to show a rise in complement-fixing or neutralizing antibody for the RI-67 agent.

A total of five HeLa cytopathogenic viruses were recovered from patients with ARD or with primary atypical pneumonia in the epidemic at Fort Leonard Wood and additional strains were isolated from sick persons in more recent outbreaks of respiratory illness in military installations throughout the United States. Serological comparisons of the recovered strains indicated that these agents comprise a family of viruses which we have referred to as the "RI-67 group." The viruses of the group are heterogeneous antigenically when examined by the serum neutralization method but share a complement-fixing (CF) antigen that is common to the entire family. The CF antigen is a soluble substance which can be separated readily from the virus particles

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This paper was presented before a Joint Session of the Epidemiology and Laboratory Sections of the American Public Health Association at the Eighty-second Annual Meeting in Buffalo, N. Y., October 12, 1954.

by differential centrifugation or by filtration.⁸ The RI-67 family is closely related to the group of adenoid and tonsil degenerative agents described by Rowe, Huebner, et al.,⁹ and shares the group-specific soluble antigen with them. To serve the need for simplicity, the data presented in the first part of this paper have been limited to the findings obtained with a single virus strain, viz. RI-67. Specific information relative to the group relationships of the RI-67 family has been reserved for a later portion of this report.

At the time of our first observations of the epidemic at Fort Leonard Wood,^{1, 3} it was suspected that influenza was present in the outbreak. This suspicion was readily substantiated by virus isolation and serological tests which showed that influenza A prime was involved. It was found, however, that influenza was responsible for only a minority of the cases and that this disease was prevalent during but a short period of the episode while the noninfluenzal illnesses, on the other hand, occurred over several months and comprised the majority of the sicknesses. When the RI-67 agent was discovered and the appropriate serological tests were developed, all sera collected from the patients in the epidemic were re-examined for increase in amount of antibody against this virus. The tests showed that nearly all of the patients who did not have influenza were, indeed, suffering an infection with the RI-67 or a related virus. In a few of the individuals, there was evidence of simultaneous infection with both the RI-67 and influenza agents.

One of the problems that attracted our attention very early in the study of the RI-67 agent was that of establishing more definitively the relationship between RI-67 infection and human disease. To explore this question, a group of adult patients with advanced neo-

plastic disease were inoculated via the respiratory route or parenterally with the tissue culture passage virus. The aim was twofold with a view toward finding out whether the agent would cause respiratory illness in the recipients and whether there would be a manifest oncolytic effect on and regression of the tumors as a result of viral infection. The results¹⁰ obtained were quite definitely negative. None of the persons developed significant illness and there was no conspicuous destruction of the tumors. The majority of the volunteers did show, however, an RI-67 neutralizing and CF antibody response.

While our own attempts to reproduce the respiratory illness in human volunteers using the cultivated virus were unsuccessful, Dingle and his co-workers¹¹ have recently presented serological evidence, obtained in retrospect, which clearly showed a relationship between RI-67 virus and the illness ARD which occurred in patients in epidemics as long ago as 1942 and in human volunteers inoculated with the throat washings from a patient with ARD in 1945. As in our investigations,^{1, 3} no relationship to the common cold or to primary atypical pneumonia associated with cold agglutinins was found. These studies by Dingle's group not only substantiated the role of RI-67 virus in human disease but, in addition, showed that RI-67 infection was not a new malady; instead it was a disease which was prevalent at least 10 years ago.

Evidence for widespread contemporary occurrence of infection with the RI-67 and related viral strains was found in studies of epidemics which occurred in U. S. Army camps during the past two years. In Table 1 are presented the results of serological (CF) tests for RI-67 infection among soldiers who were hospitalized with febrile respiratory illness at seven different U. S. Army camps during the winter, spring,

Table 1—Occurrence of RI-67 Infection in Outbreaks of Respiratory Illness Among Military Personnel, 1952–1954

Installation	Time Period							
	1952		1953				1954	
	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer
Fort Dix, New Jersey					4/6 *	46/53 (87%)	34/72 (47%)	8/53 (15%)
Fort Ord, California			2/6	8/26 (31%)		22/49 (45%)	4/10 (40%)	4/5
Fort Leonard Wood, Mo.	20/33 (61%)	106/138 (77%)				27/34 (80%)		
Fort Riley, Kansas						7/12 (58%)		
Camp Roberts, California				4/14 (29%)				
Camp Carson, Colorado					0/14 (0%)	0/14 (0%)	0/5	
Fort Lewis, Washington				0/7				

* Numerator = Number serum pairs positive; Denominator = Total number pairs tested. Winter = January-March; Spring = April-June; Summer = July-September; Fall = October-December.

summer or fall of 1952, 1953, or 1954.* It is seen that during this period, RI-67 group virus infections were highly prevalent at Fort Dix, Fort Ord, Fort Leonard Wood, Fort Riley, and Camp Roberts while, by contrast, the disease appeared to be absent from Camp Carson and Fort Lewis. These findings are quite in keeping with what one might expect based on the activities at the seven posts. It has long been known that the incidence of acute respiratory disease (ARD) and primary atypical pneumonia, unlike influenza which attacks the newly inducted and old soldiers alike, is consistently higher among recruits than among "seasoned" cadremen or operating personnel at a post.^{4, 12, 13} Thus, during the time

period of these observations,† Forts Dix, Ord, Leonard Wood, Riley, and Camp Roberts were populated principally by inductees who were in training, and a high incidence of infection was evident. Camp Carson and Fort Lewis, on the contrary, which had little or no training activity, were occupied by "seasoned" soldiers and there was no appreciable occurrence of the disease.

It is of importance to note further in the table that infections with RI-67 group viruses were present throughout the year including the warm months as well as the cold season. This was in striking contrast to influenza which usually occurs in epidemic form during late autumn, winter or early spring and only rarely in the summer. Finally, the data in Table 1 revealed that the percentage of RI-67 group infections among the hospitalized respiratory disease cases was highest at Fort Dix, N. J., during

* We are indebted to T. O. Berge, Lt.Col., MSC; Dr. E. H. Lennette; J. J. McCue, Capt., MC; H. E. Shuey, Lt.Col., MC; and L. G. Thomas, Col., MC, for the serum samples from patients at Fort Ord, Calif.; to R. Stragnell, Capt., MC, and J. P. Sargent, 1st Lt., MC, for the materials from Fort Leonard Wood; and to R. L. Cavanaugh, Lt.Col., MC, for the specimens from Camp Carson, Colo.

† Information furnished by G. R. Carpenter, Col., MC, Preventive Medicine Division, Office of the Surgeon General, U. S. Army, Washington, D. C.

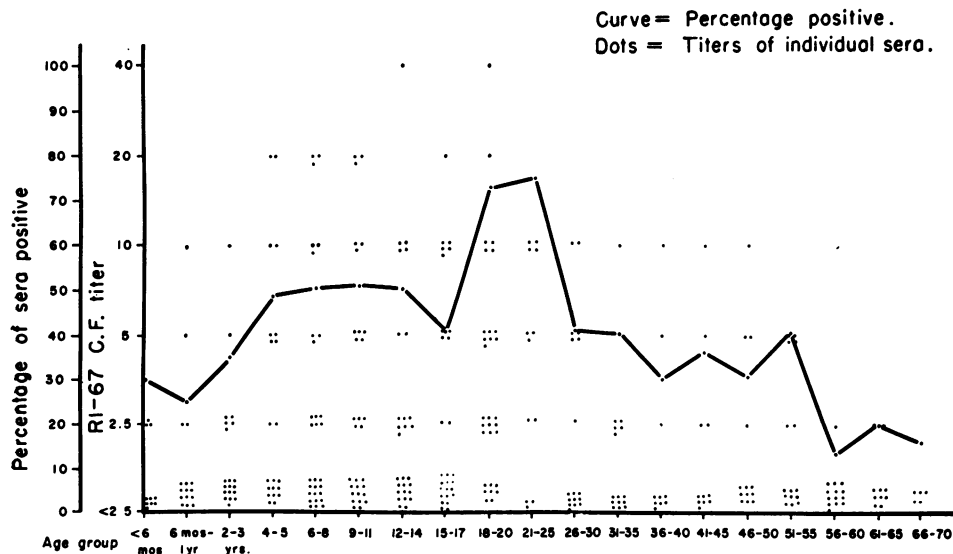


Figure 1—Age-frequency Distribution of RI-67 Complement-fixing Antibody in Sera of Normal Population

the winter (87 per cent positive) with a gradual tapering off in the spring (47 per cent) to a low summer prevalence (15 per cent). At Fort Ord, Calif., by contrast, the percentage of cases caused by RI-67 group virus infection remained at a fairly constant level (31 to 45 per cent) throughout the year. Whether this difference in seasonal occurrence at the two forts is significant and related, perhaps, to their contrasting climates or whether it is merely a reflection of the sampling of the cases for study remains to be determined.

Although epidemics of respiratory disease caused by the RI-67 family of viruses is most commonly observed in military training camps, experience with these and related agents is not limited to recruit populations alone. This is shown in Figure 1 which presents the results of a study to determine the age-frequency distribution in the normal population of CF antibody against the group-specific soluble antigen which is shared by the RI-67 family of viruses and the latent adenoid and tonsil de-

generative agents of Rowe, Huebner, et al.⁹ The sera that were employed in the investigation were collected principally from military persons or their dependents who were sick with a nonrespiratory illness during the summer months of 1951 to 1953 and were considered normal insofar as respiratory disease was concerned. The dots represent the individual titer determinations and the line shows the percentage of sera found to contain RI-67 group antibody, i.e., with a CF titer of from 1 to 2.5 or greater.

It is evident in the figure that there was a gradual increase in the percentage of sera which were positive, starting from 21 per cent in persons one year of age or less and ranging upward to 74 per cent in the 18- to 25-year group followed by a decline to 23 per cent among 61- to 70-year-old persons. These data are interpreted to indicate that many individuals of all age groups in the population have had prior experience with viruses of the RI-67 family or with other related agents. It appears, further, that

the greatest prevalence of infection is during mid-life with less occurrence in the years preceding or following. The great peak of positive test results, which were obtained with sera from 18- to 25-year-old persons, probably reflected the origin of the sera chosen for study, that is, specimens from military sources. Thus, the 18- to 25-year-age group consisted mainly of soldiers who were not more than a few months to a few years beyond their basic training experience when RI-67 group infection and resultant acquisition of antibody is so frequent.

The findings reported thus far made it clear that respiratory disease due to RI-67 group virus infections occurred commonly among recruits and that there was widespread experience with these viruses in the population. It appeared important, then, to determine what percentage of a typical sample of recruits became infected with the RI-67 agents during training and to find out what proportion of the men did or did not exhibit clinical disease along with their infection. To obtain information along these lines, an investigation was undertaken to follow a company of new recruits, both clinically and serologically, from the beginning to the end of their 17 weeks of basic and advanced military training. For this study, all the persons in a newly formed company of men at Fort Dix, N. J., were bled during the second, fourth, eighth, and seventeenth weeks after introduction into the company. Additionally, all the men who were hospitalized with respiratory disease during this period were bled at the time of entrance into the hospital and again during convalescence two or three weeks later. All the sera were tested for RI-67 CF antibody and a portion was also examined for neutralizing capacity. The hospital and dispensary record of each recruit was inspected to determine the occurrence of respiratory illness and to relate such sickness, where possible,

to a significant (fourfold or greater) rise in RI-67 antibody during the period following. The study took place in the time of the cold months of the year and extended from February through May, 1954.

It was found in the study that 169 or 81 per cent of the 209 men in the company showed a fourfold or greater rise in CF antibody titer for the RI-67 strain during the 17-week training period, while only 40 or 19 per cent failed to do so. This finding is interpreted to indicate that at least 81 per cent of the men suffered an infection with RI-67 or a related virus during the time period indicated. Thirty-nine per cent of these titer increases occurred by the fourth week of residence in the company and 38 per cent took place between the fourth and eighth weeks; only 4 per cent occurred during the final eight- to 17-week period. It is of importance to add that the RI-67 CF antibody titer increases in the serum samples from 16 of the men tested were accompanied also, in 14 instances, by a rise in neutralizing antibody against the RI-67 strain.

The attempt to relate, precisely, the development of a rise in CF antibody titer and the occurrence of respiratory illness among the men in the company was not without difficulty. In the cases which were hospitalized, the increase in RI-67 antibody could be correlated exactly with the appearance of the illness since blood specimens were taken at the time of hospital admission and again during convalescence. The connection between titer elevation and the occurrence of respiratory disease which was treated at the dispensary without hospitalization, however, was far less precise since only the routine two, four, eight, and 17-week blood specimens were collected from these men. Thus, it was possible only to make a rough estimate of the mutual relationship between antibody increase and respiratory illness handled at the dispensary.

The results of the study showed that 29 per cent of the men who developed a significant rise in antibody titer were hospitalized with a respiratory disease which corresponded temporally to the serological data. Roughly 28 per cent of the soldiers showing such elevation in titer were treated at the dispensary for a respiratory complaint which could be related to the positive serological findings. On the other hand, 43 per cent of the individuals who showed an increase in CF titer failed either to report to the dispensary or to enter the hospital. It is inferred that the majority of these persons suffered either a mild or an inapparent infection. The significance of the relationship between the occurrence of respiratory disease and a rise in antibody titer was supported by the data obtained when the sera from all the hospitalized cases in the company were examined. Thus, 54 men entered the hospital with respiratory illness during the first eight weeks of training and of these, 46 or 85 per cent showed an increase in CF titer which was related in time to the development of the disease.

It was observed, further, that roughly half the soldiers in the company had detectable RI-67 CF antibody (titer 1:2.5 or greater) in their two-week serum specimens and the other half did not. The occurrence of subsequent CF antibody titer increase and of respiratory illness, however, was not appreciably different in the two groups. Thus, it appeared that the virus was circulating widely in this population of recruits, some of whom had antibody initially while the others did not, and the incidence of infection did not appear to be influenced materially by the presence or absence of CF antibody in the initial serum specimen.

A similar investigation was carried out during the past summer to determine the RI-67 infection rate among recruits who underwent their training during the warm months of the year. Prelim-

inary tests performed with sera from a group of 30 men in the study revealed that RI-67 infection was very infrequent during the summer as compared with the winter. Thus, only 10 per cent of the men showed a significant titer rise during the first nine weeks in company. The findings in both the winter and summer experiments are being analyzed more completely and will be presented in detail in a later report.¹⁴

The seemingly ubiquitous distribution of agents of the RI-67 group has posed a considerable problem to determine which virus strains play a role in human disease and which, on the other hand, lead a more saprophytic existence. We have attempted to gain some insight into the problem by testing acute and convalescent sera from patients with respiratory illness for antibody against a selected group of viral strains. To illustrate the kind of results obtained, the typical findings in a small representative sample of the titrations are shown in Table 2. In the tests, the paired sera from four patients with primary atypical pneumonia or ARD were assayed for neutralizing antibody against strains RI-67, RI-218, and RI-C1 which were recovered from patients with respiratory disease and against Rowe's and Huebner's latent adenoid degenerative agent AD-6. It is seen that the neutralizing antibody titer of the sera from patient 1 rose from 0 to 128 when tested with the three respiratory disease agents but showed no rise for the adenoid virus AD-6. The paired sera did, however, present a titer increase when tested with the soluble antigen of each of the four agents by the complement-fixation method. In patient 2, a rise in neutralizing antibody titer occurred against strains RI-218 and RI-C1 and not against RI-67 or AD-6. Patients 3 and 4 showed an augmentation of the neutralizing capacity for RI-67 and RI-C1 but not for RI-218 or AD-6 strains. It is also evident that patient 2 had neutralizing

Table 2—Neutralizing and Complement-Fixing Antibody Response Against Selected Virus Strains in Patients with RI-67 Group Infection

No.	Patient Specimen	Neutralization Titer Against Strain				C.F. Titer Against Strain			
		RI-67	RI-218	RI-CI	AD-6	RI-67	RI-218	RI-CI	AD-6
1	Acute	0 *	0	0	0	0	0	0	10
	Conv.	128	128	128	0	80	80	80	80
2	Acute	8	0	2	2	5	0	5	5
	Conv.	8	32	8	2	40	40	80	40
3	Acute	0	8	0	8	10	10	10	20
	Conv.	32	8	32	8	40	80	80	80
4	Acute	0	0	0	0	5	5	10	10
	Conv.	32	0	32	0	80	80	160	160

* Numbers are reciprocals of serum titers. 0 equals titer less than 1:2 in neutralization and less than 1:5 in C.F. tests.

antibody against the RI-67 and AD-6 agents and patient 3 had antibody against RI-218 and AD-6 viruses in the acute specimens; these titers were not increased during convalescence. This antibody probably resulted from previous experience with these viruses. As with patient 1, the sera from the latter three cases all showed a titer rise against the soluble CF antigen of all four strains.

Summary and Conclusions

Infection with viruses of the RI-67 group appears to be responsible for a large portion of the cases of cold agglutinin negative, primary atypical pneumonia and undifferentiated acute respiratory disease (ARD) among U. S. Army populations during the contemporary period and constitutes a problem of no small proportion among newly recruited soldiers undergoing basic training. These illnesses, unlike influenza, are prevalent in some areas during the warm months as well as the cold season of the year and epidemics, in which both influenza and RI-67 infections are of etiological importance, may occur simultaneously in the same population.

Most recruits in a company of men

who underwent basic training at Fort Dix, N. J., during the winter of 1954 presented serological evidence of infection with RI-67 or related viruses during the first eight weeks in company. In about one-fourth of the men, the illness was severe and required hospitalization, while the disease in another one-fourth of the persons was sufficiently mild to permit treatment at the dispensary. The remaining half of the soldiers suffered either a very mild or inapparent infection.

Antibody surveys showed that prior experience with RI-67 group or other related viruses, such as the adenoid and tonsil degenerative agents of Rowe, Huebner, et al., is widespread in the population and that infection is most common during the middle years of life with less occurrence during the periods which precede or follow. While infection with these viruses certainly occurs among civilian as well as military persons, the magnitude of the problem in the former group remains to be determined.

The RI-67 family of viruses comprises a group of agents that are heterogeneous antigenically when compared by the serum neutralization method but share a common group-specific complement-fixing antigen, which is also elaborated

by the latent tonsil and adenoid degenerative agents. The RI-67 CF procedure provides a simple diagnostic test for infection with this group of viruses which is ready for widespread employment by the routine serology laboratory at such time that the antigen becomes more readily available. The neutralization test, which may also be used to detect increase in antibody, is more laborious and expensive to perform and requires the use of a "battery" of antigenically different strains of viruses of the group. For this reason, the neutralization test is less readily applicable for routine diagnostic purpose than the CF method, but it remains the only procedure presently available for identification and antigenic analysis of newly recovered viral strains.

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Experiment in Diabetes Control

The New York City Health Department has begun a pilot project in control of diabetes in the area of the city that has the highest death rate from diabetes. The District Health Center of the Brownsville Health District, Brooklyn, offers all residents 25 years of age or over and younger residents who are relatives of diabetics, or who are referred by private physicians, a free diabetes test. After blood and urine analyses for excess amounts of sugar have been made, the patient receives notification of results by mail. If the test so indicates the patient is advised to see a physician or go to a treatment clinic. No treatment is given at the Diabetes Control Center.

By way of informing the district residents of the service a leaflet "Are You One of the Hidden Million?" has been widely distributed. It explains diabetes briefly, with particular reference to the fact that it is easily controlled when treated early and that when controlled, its sufferers can live normal, satisfying lives.

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