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UNDERSTANDING OF VIRAL RESPIRATORY ILLNESSES PROVIDED BY EXPERIMENTS IN VOLUNTEERS[1]

GEORGE GEE JACKSON

Department of Medicine, University of Illinois College of Medicine, Chicago, Illinois

DISCOVERY OF NEW RESPIRATORY VIRUSES

The discovery of agents assigned a causative role in respiratory diseases comes from the study of natural illness. Modern microbiological techniques have revealed a large number of viruses and nonbacterial organisms associated with the common respiratory diseases, and have greatly advanced our understanding of these illnesses (18). By the use of these techniques to study specimens from sick and well humans, so many agents have been discovered so rapidly that taxonomic crises have arisen over the relation among the viruses, and delight and debate have been stimulated among lexicographers who have assigned the viruses scientific names. ECHO virus, parainfluenza virus, reovirus, rhinovirus, and picornavirus are all newly coined terms. The profusion of viruses has required that some of them be labeled only by serial numbers or by an alphabetical jargon, and others are known as "orphan viruses" (viruses without a disease); removal from this category is sometimes difficult.

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NEED FOR VOLUNTEER SUBJECTS IN THE STUDY OF VIRAL RESPIRATORY INFECTION

It was in such a period of microbiological growth that Koch formulated his classic postulates (49) to distinguish the saprophyte, the contaminant, and the fellow traveller from pathogenic bacteria which caused disease. Besides isolation and identification of the microbe, the postulates included production of the disease with the isolated organism. In the case of the common viral respiratory infections, the fulfillment of this aspect of proof of etiology requires use of volunteer subjects; no other experimental model for the production of the common cold and related respiratory illnesses is presently known. Some have suggested that human tissue cultures grown in the laboratory could represent the human host in the demonstration of the etiological relationship of a virus isolate to a disease. The tenuous basis for such a conclusion is apparent from the increasing number of latent viruses in tissues, recognition of virus interference, cellular interferon, and the finding of filterable pleuropneumonia-like organisms (PPLO) in cell lines.

Society has wisely not made it easy for the experimental production of disease in humans (6). Man as a species for experimental study is probably the most cantankerous, unreliable, neurotic,

and variable that could be chosen. There is always the risk from unknown variables. Nearly all academic investigators are both ethical and careful in the design of experiments with human volunteers, but the excesses of the daring, unscrupulous, or sadistic have been witnessed. The welfare of the individual must always be placed above the needs of the experiment, and various social and legal measures are in effect to protect the prerogatives of the volunteer subject. In my opinion, it is important to the advancement of our understanding of disease, particularly viral respiratory diseases, that a social attitude which permits experiments with volunteers be encouraged. Observation of common viral respiratory infections over centuries and the trial and error of medication led only to myth and misunderstanding. Within the past decade, the combination of virological identification of disease-associated agents, the epidemiological description of specific diseases, and controlled studies in volunteers have begun to give definition to the puzzle of common respiratory infections and better reason to hope for effective means of prevention and cure.

RECOGNITION OF UNKNOWN AGENTS AND ETIOLOGICAL RELATION OF VIRUS AND DISEASE BY TRANSMISSION OF CLINICAL SYNDROMES

Examination of the role played by volunteers in the study of respiratory diseases shows a rapid evolution in the nature of their contributions as sophistication in virology has increased. At the beginning of recent studies, volunteers were used to test whether or not a filter-passing infectious agent could be shown to cause the common cold and other respiratory disease syndromes (2, 11, 12, 13, 20, 24, 34, 43). Such a conclusion is now a matter of fact. It is worthy of note, however, that all of the newly described viruses together cause only about one-half of the respiratory illnesses; the others are caused by infectious agents not yet identified and which can be shown only by the transfer of infection and illness to volunteers. Thus, the usefulness of this role of the volunteer has not been exhausted.

With the transmissibility of respiratory viruses to volunteers well established, considerably more attention has been given to the conditions of infection with specific viruses and the clinical syndrome in volunteers (3, 4, 7, 8, 10, 14, 15, 23, 24, 28, 30, 32, 35, 36, 37, 38, 40, 41, 42, 44, 45, 46, 47, 48, 50). Although the symptoms produced from infection with respiratory viruses are quite similar, it has been possible to show differences in the infectious dose, incubation period, and the relative preponderence of nose, throat, chest, and constitutional symptoms in volunteers (23, 27). Also, the relative severity of respiratory symptoms from rhinoviruses, myxoviruses, reoviruses, adenoviruses, and enteroviruses has been of interest. Occasionally, unexpected manifestations of infection, such as hemorrhagic bullous myringitis from *Mycoplasma pneumoniae*, have identified a previously unrecognized cause for a known clinical syndrome (10). Infection without symptoms under conditions in which other volunteers become ill has also given emphasis to the need for consideration of factors that distinguish infection and illness.

SOME HOST FACTORS IN VIRAL RESPIRATORY ILLNESSES

Psychological

In studies concerned with the psychological contribution of the host to respiratory illnesses, some persons were found who were stoical symptom-deniers and who never had colds, and others had hyperactive rhinosecretion from mild stimulation (23). By serological reaction, they were equally susceptible to infection and had evidence suggesting a similar rate of infection with prevalent viruses. As volunteer subjects, the same dose of infectious virus caused the same number of symptomatic infections in all but the psychologically most deviant from normal. Thus, although having a cold may be "all in your head," it affects most of us similarly when an infectious virus is the cause, and only some of us when other stimuli are the cause.

Constitutional

Among the large proportion of persons whose reactions are psychologically normal and among whom a uniform virus challenge is equally infectious, there is variation in the severity of the symptoms from infection and there are marked differences in the number of respiratory illnesses per year (23). These differences are in part related to the constitutional endowment of the subject. As a measure of this, persons with a personal history of allergy manifested by seasonal hay fever or by hives from specific foods were contrasted with others who had only a family history

of such allergy and with a third group of persons who had neither personal nor family history of allergy. When a uniform virus challenge was given, the subjects with an allergic diathesis had an excess of about one-third more symptoms than did nonallergic subjects. Volunteers who reported allergy only among family members showed a symptomatic response midway between those with and without allergy. Other constitutional factors may be even more important than allergy in determining the symptomatic response of a person to a respiratory virus infection. Such a thesis is supported by recognition of families that as a unit are relatively susceptible or immune to respiratory infections under similar living conditions and with apparently comparable disease exposure (5).

Physiological

Variation in the susceptibility of individuals on the basis of physiological status could be shown in relation to the menstrual cycle. The middle period between menses was the time of greatest susceptibility to respiratory viruses and, conversely, of equal or even more importance was the increased resistance to viral respiratory infection during the period of menstruation (14, 23). Logically, one would assume this is a result of the cyclic hormonal effect on the epithelium of the upper respiratory tract, similar to that known to occur in the vagina and uterus, but this has not been proven. Overall, there was little or no difference in the susceptibility of males and females to a standard virus challenge. To a lesser extent, another physiological factor, sleepless fatigue, was shown in males to increase symptomatic infection, but not to the degree traditionally assigned it by common belief. These two physiological states must be merely examples of unnumbered physiological events that determine the host response upon contact with a virus at different times and under variable conditions.

EFFECT OF CHILLING ON SUSCEPTIBILITY TO THE COMMON COLD

Although mothers in general remain unconvinced and colleagues dutifully are scientifically skeptical about the applicabilty of the experimental proof, cold weather, chilling, wet feet, and drafts neither cause the common cold nor increase the susceptibility of volunteers challenged with virus (15, 23). Volunteers were chilled as early as 24 hr before challenge with a suspension of a rhinovirus or from immediately after to as long as 24 hr after virus challenge. The chilling was accomplished experimentally by total body exposure to a temperature of 60 F for 2 to 4 hr or by respiration of frigid air and immobility with full dress at 10 F. In this experience, chilling alone with instillation of a noninfectious solution did not initiate symptoms of respiratory infection, except for transient rhinorrhea. Instillation of a uniform virus suspension resulted in illness among both the chilled and nonchilled volunteer subjects with the same frequency.

The experience of Andrewes and his colleagues (3, 4), who studied the effect of wet socks, drafts, and an uncomfortable cold humid climate upon susceptibility to the common cold, was similar in that they were unable to affect the frequency or severity of colds by these measures. The early epidemiological studies of Paul and Freese (39) on the occurrence of respiratory illnesses among inhabitants of the arctic island of Spitzbergen can be given the same interpretation. These studies clearly show that frigid weather did not sustain a high frequency of respiratory diseases during isolation of the island by the winter freeze.

From these various studies on host and environmental factors in volunteers, it has been recognized that, in addition to the large number of viruses that cause respiratory infections, the illnesses resulting from infection and the development of similar symptoms, sometimes without infection, can be influenced by psychological, constitutional, and physiological factors in the subject. The epidemiological effect of the environmental climate on respiratory disease was not related in volunteers to temperature *per se* or to chilling of the host. The epidemiologists and the physiologists must find better keys than these to unlock the mystery of the high incidence of winter respiratory illnesses.

SPECIFIC IMMUNITY TO THE COMMON COLD

One of the important discoveries from studies in volunteers was the demonstration of specific immunity against respiratory viruses (21, 22). Among all of the previously considered host and environmental factors, only the virus and the specific immunity of the individual are regularly of major importance. The absence of symptomatic illnesses in volunteers who were rechallenged with the same strain of virus showed the existence of relatively complete immunity for periods as long as 44 months. Rechallenge of a sufficient number

of subjects after more than 1 to 2 years to determine the duration of immunity to the same inoculum has not been done.

Data based on the neutralizing effect of convalescent sera on the infectivity of a common cold virus in other volunteers reveal some unusual characteristics of the immune reaction that have not been entirely explained (26). The appearance and increase of protective antibody in the serum may require several weeks. On the other hand, a serological response in neutralizing antibody for tissue cultures sometimes occurs in 1 or 2 weeks (45). Natural infections with rhinoviruses have shown the same phenomenon of eliciting early and late antibody responses, but they commonly have caused only very late rises in serum antibody or none at all. Volunteers rechallenged with the same secretion also were observed to be more immune at 3 months than at 3 weeks. The differences do not appear to be only that of primary or secondary infection, although this may be in part the explanation.

The protective capacity of serum against an unidentified common cold virus was observed to start to decrease beyond 1 year after infection (26). Other experience has shown variable persistence of neutralizing antibody in tissue cultures (45). Observation of the presence of prechallenge serum antibody in volunteers has established the fact that reinfection of the respiratory tract occurs in persons with circulating antibody, especially in the case of respiratory syncytial virus (9, 30, 31, 37). Minor antigenic differences in the viruses might account for some of the reinfections, but it seems definite that infection with many respiratory viruses will not produce life-long immunity as is sometimes expected and apparently true for certain virus infections. Although the titer of naturally acquired serum antibody has shown poor correlation with the susceptibility of a person to reinfection, the protective influence of specific antibody usually can be shown, by modification, if not prevention, of the illness. A mechanism for the asymptomatic control of nasal reinfections was suggested by the immunoelectrophoretic estimation of γ-globulin in nasal secretions from volunteers before and after virus infection (1). Exudation of γ-globulin after infection preceded the onset of symptoms, and could account for the limitation and modification of reinfection owing to the secretion of specific serum antibody.

USE OF IMMUNITY ON RECHALLENGE TO ESTABLISH VIRAL RELATION TO DISEASE

Identity of Viruses

The immunity of the volunteer to rechallenge with the same virus has been a useful tool for the identification of similar and distinct viruses for which specific antisera are not available and difficult to produce. We were able to show by cross-immunity in volunteers the nearly complete antigenic identity of 2060 and JH viruses before high-titer sera were made and the relationship was established (24). Similarly, two unknown agents collected from different sources during an epidemic of respiratory disease for which no typing sera were available were readily shown to be antigenically the same by cross-challenging volunteers (33).

Etiological Relation of Attenuated Strains

Immunity against rechallenge has added another potential postulate to those of Koch for proof of the etiological role of a virus in illness. The need for this approach is twofold. Passage of a virus in nonhuman tissue cultures for isolation and characterization can cause attenuation so that infection does not reproduce the disease in volunteers; nevertheless, it provides immunity, which is the basis for live-virus immunization (28, 48). Thus, immunity rather than disease is the result of infection and the means of proof of etiological relationship. The second need for the use of immunity in this respect is that unknown and unrecognized viruses coexist with known and recognized ones. The latter may be erroneously assigned an etiological role. Failure of a prior challenge with the recovered virus isolate to protect against infection and illness on rechallenge with the original respiratory secretion has permitted recognition of a second unknown disease-causing agent in a single respiratory secretion. The lack of immunity to illness revealed a false assumption with regard to the etiological relationship between a virus and disease.

Recognition of Unknown Dual Virus Infections

Nasal secretions from different individual common cold donors have been shown by means of challenge and rechallenge experiments to contain a rhinovirus and one of the following: ECHO-11 virus, reovirus type 1, parainfluenza virus type 1,

or respiratory syncytial virus. In each case, challenge with tissue-culture harvests of the reovirus and parainfluenza virus isolates failed to protect against a rechallenge with the nasal secretion from which these viruses could be isolated. Furthermore, it has been possible to demonstrate that the titer of serum-neutralizing antibody before challenge against each of two viruses in a single secretion predetermined the likelihood of infection with one or the other virus when two were present. Some dual infections occurred, but the data strongly suggested the in vivo operation of viral interference between two infectious viruses given simultaneously. Usually, only one of them caused the major amount of clinical illness, whereas infection with the other appeared to prevent illness.

From these studies in volunteers, it has been possible not only to fulfill Koch's postulate of production of illness by the isolated virus, but also to show the acquisition of immunity against symptomatic reinfection. Use of the latter index added further fulfillment of the spirit of the Koch postulates by showing proof of an etiological relation in the case of an asymptomatic virus infection and disproof of an etiological relation by failure to elicit immunity against an unrecognized virus.

Protective Effect of Live Vaccine and Chemoprophylaxis Against Influenza

Another important role of volunteer studies has been and currently must continue to be the evaluation of methods for the prevention or treatment of viral respiratory infections. The ability to have controlled uniformity in the dose and time of administration of the infectious inoculum, as well as the vaccine or chemotherapeutic agent, permits more rapid and definitive answers regarding the efficacy or nonefficacy of these procedures (16, 19, 25, 29).

Using attenuated live influenza virus vaccine, workers in this laboratory were recently able to show approximately 75% protection against reinfection with the homologous strain 8 months after immunization (17). Administration of an anti-influenza compound, amantadine, to volunteers by mouth before giving them a challenge with influenza virus showed definitively for the first time in man that it was possible to prevent such a respiratory infection with a small amount of drug given orally (29). Such experiments in volunteers under conditions of uniform challenge and controlled trial of the drug permit some basis for hope for the successful chemotherapy of respiratory virus diseases.

Summary and Conclusions

Experiments in volunteer subjects were first used to demonstrate the infectivity for man of unknown filter-passing agents. This function of the volunteer has decreased but is still necessary. The rapid increase in the number of identified viruses obtained from respiratory secretions has increased the need for rigorous proof of the etiological role of each virus and the clinical respiratory symptoms it causes. A social attitude that fosters ethical, controlled experiments in volunteers will accelerate a more complete understanding of these infections. The challenge of volunteers has shown some of the interrelations among virus, host, and environmental conditions in infection and illness. Among the host factors, psychological, constitutional, and physiological factors played a role. Chilling could not be shown to be an important environmental condition related to infection or illness from common cold viruses. Immunity of volunteers against illness on rechallenge with the same virus was shown, and the relation to time and serum antibody was observed. The specificity of the immunity suggested its suitability as another postulate for the proof of identity of different viruses, as evidence for an etiological role in disease from attenuated viruses, and as a means for the detection of unrecognized viruses coexisting with known isolates in respiratory secretions. The uniform and controlled measures permitted in experiments with volunteers showed the protective effect of an attenuated live virus vaccine or a chemoprophylactic drug against influenza.

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