The Clinical Illness Promotion Factor:  
A Third Ingredient  

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The interactions between a causative agent and a susceptible host involve a series of responses most of which are subclinical or asymptomatic but a few of which are manifested by clinical illness. The factor(s) which tip the balance are poorly understood in both acute and chronic diseases. It is designated here as the clinical illness promoting factor (CIPF), a third ingredient. Among infected persons some leads have been found as to why clinical illness develops: in tuberculosis genetic susceptibility plays a key role, as shown in twin studies; in EBV infections age at the time of infection, genetic, and psychosocial factors determine both the expression and the severity of illness; in poliomyelitis age, exercise in the incubation period, and genetic background are related to the development of paralysis. In the relationship between viruses and cancer, viruses and chronic diseases, or inanimate pathogens like tobacco and lung cancer, we know very little as to the factors that result in clinical disease among the many who are presumably susceptible and fully exposed. Epidemiologic study is urged to identify this CIPF or "third ingredient."

Our concepts of causation have, for the most part, focused on the establishment of the causal role of a given factor in the production of a disease or clinical syndrome by epidemiological and/or experimental means [1]. This proof often included reproduction of the disease in a susceptible laboratory animal or susceptible human host (as per the Henle/Koch postulates), the demonstration that the disease occurs more commonly in the presence of the suspected factor than in its absence (increased relative risk), or that removal of the factor decreases the incidence of the disease (attributable risk). These approaches to causative proof have concentrated mainly on two ingredients: the suspected factor and the human host.

I suggest it is time we focus on clinical illness promotion factors as "a third ingredient." In his short story of that title O. Henry relates the tale of a poor girl who has a piece of beef and a young man who has a potato. Together they join with these two ingredients to make a stew [2]. It is clear, however, that a third ingredient is necessary to make a good stew. In this instance the essential third ingredient is an onion. The rest of the story concerns the search for someone with this ingredient. In epidemiological studies we should also be searching for a third ingredient. The admixture of a "causative agent" fully clothed with all the potential antigens, on-
cogenic properties, or other putative pathogenetic factors necessary to produce disease with a fully susceptible host of the proper age, sex, socioeconomic, and nutritional status is often insufficient to result in clinical disease. A third ingredient, or even additional ones, may be needed. This is true of causative factors in both acute and chronic diseases. While the multi-factorial origin of disease has been recognized by many authors, I wish to focus on the factor(s) that result in clinical disease among those exposed to all the risk factors. This I will call “the third ingredient,” or the clinical illness promotion factor (CIPF).

**ACUTE INFECTIOUS DISEASES**

A major riddle in infectious diseases is why some individuals develop clinical illness as a result of infection while others do not. This variation in host response is true of most viral infections, although a few, such as rabies and measles infections, almost always result in clinical illness. Some of the clinical illness promotion factors influencing the host response are listed in Table 1. Age at the time of infection is one important determinant of the host response, especially to agents such as poliomyelitis virus, hepatitis A virus, and Epstein-Barr (EB) virus. With these agents, greater age of the host at the time of infection correlates with a greater possibility of clinical illness. Variations in the virulence of strains of virus, in the size of the inoculum, in the portal of entry, and in the status of the host have also been incriminated in producing clinical illness among those infected. Marked host variations exist, however, even when all these factors are held constant. For example, hepatitis B virus contaminated one lot of yellow fever vaccine given to over 5000

| TABLE 1 | Third Ingredients |
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| **Factors That Might Influence the Occurrence and Severity of Clinical Disease among Infected Persons** |
| Age at the time of infection |  |
| Alcoholism |  |
| Anatomic Defects |  |
| Antibiotic or anti-viral resistance |  |
| Chronic disease: Either pre- or co-existing |  |
| Dosage of organism |  |
| Double infections (Viral, bacterial, parasitic, fungal) |  |
| Drugs: Self- or physician-administered |  |
| Genetic make-up, especially effect on the immune system |  |
| Iatrogenic influences: Other therapies, surgery, etc. |  |
| Immune status of host at time of infection |  |
| Immune response of host to infection (beneficial or detrimental) |  |
| Immunodeficiency: Natural, drug-induced, disease-induced |  |
| Mechanism of disease production: Lysis, hypersensitivity, immune-complex |  |
| Mutation of organism during course of infection |  |
| Perception of illness by patient |  |
| Physical status at time of infection |  |
| Physical exercise during incubation period or at time of onset |  |
| Portal of entry of organism |  |
| Pregnancy |  |
| Psychosocial factors |  |
| Stress |  |
| Temperature of body at site of entry and viral multiplication |  |
| Trauma |  |
healthy male soldiers of about the same age at Camp Polk. Each received the same dose in the same arm on the same day [3]. Of those inoculated 1004 (20 percent) developed clinical jaundice, and the rest did not. The incubation period from injection to clinical illness varied from 60 to 154 days (mean 96.4 days). What “third ingredient” influenced the variability in host response and incubation period among these soldiers? Unfortunately, we don’t know the answer to this, since no studies were made to analyze this “natural experiment,” and, even if they had been, the laboratory tools were not available at that time to identify susceptibility and immunity or to recognize subclinical infections.

A deliberate search for factors influencing EBV infection and disease (clinical infectious mononucleosis—IM) was made among a single class of cadets at the West Point Military Academy studied over a four-year period [4,5]. Psychosocial factors were measured on admission, and leadership and academic records were recorded during school. Among 432 susceptible cadets, 194 (44.9 percent) became infected over the four years, and 238 (55.1 percent) remained still susceptible four years later [4]. Among the 194 EBV-infected cadets, 48 developed clinical IM (24.7 percent), and 146 (75.3 percent) did not. The reasons for this difference were sought in psychosocial behavior patterns and in academic achievement. A high commitment to a military career was associated with a 57.1 percent clinical attack rate among EBV-infected cadets, and low military commitment with only a 10.7 percent rate of clinical IM. High military commitment influenced infection and disease in opposite directions, since it was associated with a low infection rate among susceptibles and high clinical IM rates among the infected. If one had not separately identified the susceptibles, the infected, and those with disease, this distinction would have been obscured. Academic performance also influenced the clinical attack rate in the third and fourth year. Those susceptible cadets whose academic performance was poorer in the second semester than in the first semester during the year prior to EBV seroconversion had a 50 percent clinical attack rate, as compared to only a 5.6 percent clinical attack rate in those with relatively good academic performance in the second semester compared with the first semester. The level of motivation toward a military career was inversely related to academic performance. Therefore, high motivation and poor academic performance correlated with high rates of clinical IM (43.5 percent). The serious, well-motivated student who failed in his academic expectations was, thus, especially susceptible to clinical illness after infection had occurred. This same student, however, was less apt to be exposed and infected. These studies indicate that if susceptibility, infection, and disease categories can be objectively identified, the clinical illness promotion factor—here, psychosocial factors—may emerge as a “third ingredient” rather than being obscured or diluted out by the presence of immune and unexposed individuals. It should be emphasized that the biological mechanisms by which psychosocial factors influence infection and disease in West Point cadets are not known, and these findings may not necessarily apply to other settings.

Genetics also play an important role in the response of the host to infection. Earlier investigations of twins and families had suggested that genetics played a role for the occurrence of paralysis in poliomyelitis [6] and in the occurrence of rheumatic fever following Group A streptococcal infections [7]. However, quantitative evaluation of exposure and the presence of prior immunity within family units were not adequately considered in these studies. More elegant were the twin and family analyses of Kallmer and Riesner [8] in tuberculosis, in which the corrected rates for manifest tuberculosis on exposure to an index case in the family were
7.1 percent in marriage partners, 11.9 percent in half siblings, 25.5 percent in dizygotic co-twins, and 83.3 percent in monozygotic twins. More recently, the importance of genetics in the control of the immune response to EBV infection has been established by Purtillo and co-workers [9] in the X-linked lymphoproliferative syndrome. The ability to identify human leucocyte antigens (HLA) and increasing knowledge of the genetic loci through which they operate may contribute greatly to our future knowledge of "the third ingredient," provided we can compare persons who are known to be infected with controls who are matched in all risk factors and from which group the immune and unexposed can be excluded.

VIRUSES AND CANCER

How can a "third ingredient" be identified in the virus-cancer relationship? The three leading candidates for producing a human cancer, Epstein-Barr virus (EBV), herpes simplex type 2 (HSV-2), and hepatitis B virus (HBV) are all ubiquitous agents. Infection with them is very common in the settings where the cancers are most common. The presence or absence of antibody may thus be difficult to interpret in relation to causation, since both cases and controls have antibody. In EBV-related malignancies the antibody titer has been significantly higher in cases than in healthy controls. This has been shown for over 80 percent of cases of both African Burkitt lymphoma and nasopharyngeal cancer and for 30-40 percent of Hodgkin's cases. Initially, it was not known whether these results were due to viral multiplication in the tumor itself, to the immunosuppressive therapy given for it, or to an etiological role for the virus. There are several epidemiological approaches to this dilemma. One is to demonstrate that EBV infection and high EBV antibody titers (but not titers of other viral antibodies) preceded the disease, and, thus, might be involved in its pathogenesis. This type of prospective serological study has been done of EBV antibody titers in African Burkitt lymphoma (ABL) [10] and, in a preliminary way, in Hodgkin's disease [11] and nasopharyngeal carcinoma (NPC) [12-14]. In the Burkitt lymphoma study 42,000 children were bled, among whom 31 cases of tumor developed over a five-year period. Pre-tumor sera were available for 14 of these. The EBV VCA-IgG antibody titer was equal or higher than in controls in 10 of the 14 ABL cases in serum samples obtained seven to 54 months before the tumor was diagnosed [10]. Other herpes and viral antibody titers were not elevated. There was a 30-fold increased risk of later Burkitt tumor development in healthy persons with a twofold or greater EBV antibody titer over controls. In another study, two cases of Hodgkin's disease developed among 26,000 normal persons who had been bled in Washington County, Maryland, and whose sera had been stored away [11]. EBV antibodies were uniquely and significantly elevated over controls in sera from these two persons obtained 12 and 21 months prior to diagnosis as compared with four age/sex matched controls for each. In other studies elevated EBV IgA antibody titers have been shown in three individuals two and one-half to five years before diagnosis of NPC [12], in one of seven Alaskans who subsequently developed NPC [13], and in two Chinese who developed NPC 10 months later [14]. The presence of elevated antibody titers prior to illness certainly does not establish that the virus necessarily caused the tumor, but it does suggest that it may have played a role directly or indirectly in its pathogenesis.

A second epidemiologic approach is to study those persons already possessing high EBV antibody levels to determine if a "third ingredient" can be identified that results in the malignancy. In ABL an added factor is clearly needed to account for the geographic, seasonal, temporal aspects of the tumor. Most evidence suggests
that holoendemic malaria plays this role. EBV has been termed the “initiator” and malaria “the promoter” in this tumor. However, holoendemic malaria is, like EBV, an almost universal infection in early life in this setting. Its occurrence alone would be unlikely to account for ABL in persons so widely infected with both agents, unless strong quantitative differences in the intensity of parasitemia could be shown. Some other ingredient must be inducing the tumor in those who are doubly infected. The search for it is the epidemiological challenge now. In areas where NPC flourishes, EBV infection is also almost universal [15]. Here genetics have been shown to play a role because the highest incidence of the tumor occurs in Chinese living in, or derived from, southern China. In addition, there is almost a fivefold increased risk of NPC among Chinese themselves in the presence of certain HLA configurations (HL-A2, SIN2) as compared with Chinese without these HLA characteristics. In one study the combination of high-risk Cantonese Chinese and the presence of the HLA characteristics resulted in a 30-40-fold higher incidence in this group than in the population of India [15]. Thus, high EBV-IgA antibody levels and genetic background certainly set the stage for NPC, but what third ingredient results in the tumor?

In Hodgkin’s disease prior tonsillectomy, socioeconomic and educational levels, birth order, prior infectious mononucleosis, and elevated EBV antibody titers have been incriminated as important risk factors [16]. However, these risk factors have not been compared in persons with or without high EBV antibody titers to determine if one of them might represent the third ingredient.

Thus, the epidemiological approach to “the third ingredient” in EBV-related malignancies is to compare persons with the malignancy to those persons who have all the pertinent risk factors of the case, including high EBV antibody levels, but who do not have the tumor. Other viral candidates that will require similar investigations are HSV-2 in relation to cervical and vulvar cancer, HBV in relation to hepatocellular cancer, CMV in relation to Kaposi’s sarcoma and prostatic cancer, and retroviruses in relation to leukemia.

VIRUSES AND CHRONIC DISEASE

The role of viruses in the pathogenesis of certain chronic diseases is being increasingly recognized. These involve chronic diseases of the central nervous system (Kuru, Creutzfeld-Jakob disease, subacute sclerosing panencephalitis, progressive multi-focal leukoencephalopathy, multiple sclerosis and allied neurological diseases), of the connective tissues and arteries (systemic lupus erythematosus, sarcoidosis, peri-arteritis nodosa, rheumatoid arthritis), of the kidney (immune complex nephritis), and of the pancreas (Coxsackie and juvenile diabetes). These exciting advances must be evaluated epidemiologically with methods that recognize susceptibility, infection, and other risk factors in selecting controls. It must be stressed that (1) no one of these putative causes is likely to cause all cases of the disease, (2) other factors (“a third ingredient”) are needed in addition to a susceptible host and the putative agent, (3) both the causative agent and the co-factors may be different in different settings without diminishing their important role in causation in a particular setting, and (4) a given agent may operate either directly or indirectly in causation and at different points in the pathogenesis.

DISCUSSION

Advances in molecular virology have yielded very sophisticated techniques to identify the virus, its genome, or its footprints in tissues and to identify particular
genome segments that control particular antigenic activities. Second, advances in producing antibody components of high sensitivity and high specificity, particularly the use of monoclonal antibodies, have created a new set of tools to examine the humoral immune response. Third, developments in the study of cell-mediated immunity and its genetic control have permitted a better understanding of the immunoregulation of viral infections. We have learned how this system can both cause and prevent clinical disease. These new virological, immunological, and genetic advances are yielding new insights into disease causation and pathogenesis, and they provide new techniques to the epidemiologist. Causation is increasingly recognized as a multi-factorial and complex phenomenon with different sets of risk factors operating in different settings. Many of the causes of disease are so ubiquitous that almost everyone has been exposed to them. This is also true of (direct or indirect) exposures to certain agents, such as tobacco smoke, which are associated with chronic diseases. What then makes disease develop in some who have been exposed, but not in others? It is the search for a clinical illness promotion factor, “a third ingredient,” that I urge epidemiologists to pursue. It may be external or internal to the host, it may vary from disease to disease, and it may vary within a single disease in various epidemiologic settings. To discover it, one must study a disease intensively within a single ecological setting and compare persons with the disease with exposed and “infected” controls who have all the same risk markers as the cases. In this effort the epidemiologist should join hands with the virologist, the clinician, the statistician, the immunologist, the biochemist, and the social scientist. If we can identify and modify the clinical illness promotion factor(s), then our efforts at control and prevention can be directed only at those few persons who develop the disease rather than at the total group who are exposed as is our current practice.

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