

# Psychoneuroimmunology

Robert Ader<sup>1</sup>

Center for Psychoneuroimmunology Research, Department of Psychiatry, University of Rochester School of Medicine and Dentistry, Rochester, New York

## Abstract

Psychoneuroimmunology is the study of the relationships among behavioral, neural and endocrine, and immune processes. Bidirectional pathways connect the brain and the immune system and provide the foundation for neural, endocrine, and behavioral effects on immunity. Examples of such effects are conditioned and stress-induced changes in immune function and in susceptibility to immunologically mediated diseases. These data indicate that researchers should no longer study the immune system as if it functioned independently of other systems in the body. Changes in immune function are hypothesized to mediate the effects of psychological factors on the development of some diseases, and research strategies for studying the clinical significance of behaviorally induced changes in immune function are suggested.

## Keywords

conditioning; immunity; stress

Once upon a time, the immune system was considered an autonomous agency of defense. Research conducted over the past 25 years, however, has provided incontrovertible evidence that the immune system is influenced by the brain and that behavior, the nervous system, and the endocrine system are

influenced by the immune system. Psychoneuroimmunology, a new hybrid subspecialty at the intersection of psychology, immunology, and the neurosciences, studies these interactions (Ader, 1981b).

The immune system's defense of the organism against foreign, "nonself" material (antigens) is carried out by white blood cells, primarily T and B lymphocytes, that respond in various ways to the presence of antigens and retain a "memory" of encounters with them. Different immune processes can be distinguished by the particular cells that mount the body's defense. Antibody-mediated immunity refers to the production of antibodies by B cells derived from bone marrow; cell-mediated immunity refers to the actions of a variety of T cells derived from the thymus gland. Typically, immune defenses involve interactions among T and B cells and other specialized white blood cells (e.g., macrophages) and substances (cytokines) secreted by activated T cells. Not all immunity is based on the body's recognition of a previously encountered antigen, however. Natural killer (NK) cells, implicated in protection against the spread of cancer cells and the recognition of and defense against viruses, are a type of lymphocyte capable of reacting against some antigens without having had prior experience with them. A readily accessible overview of immune system functions is provided at the following Web site: [rex.nci.nih.gov/PATIENTS/INFO\\_TEACHER/bookshelf/NIH\\_immune](http://rex.nci.nih.gov/PATIENTS/INFO_TEACHER/bookshelf/NIH_immune).

## BACKGROUND

Interactions between the brain and the immune system were first observed in the laboratory in the 1920s, when scientists found that immune reactions could be conditioned (Ader, 1981a). In the 1950s, there was a short-lived interest in the immunological effects of lesions and electrical stimulation of the brain. At the same time, research was initiated to study the effects of stressful life experiences on susceptibility to experimentally induced infectious diseases. Interest in this research was rejuvenated when, beginning in the 1970s, several independent lines of research provided verifiable evidence of interactions between the brain and the immune system.

We now know that the brain communicates with the immune system via the nervous system and neuroendocrine secretions from the pituitary. Lymphoid organs are innervated with nerve fibers that release a variety of chemical substances that influence immune responses. Lymphocytes bear receptors for a variety of hormones and are thereby responsive to these neural and endocrine signals. The best known of these signals are reflected in the anti-inflammatory and generally immunosuppressive effects of adrenocortical steroids (hormones released by the adrenal gland).

Lymphocytes activated by antigens are also capable of producing hormones and other chemical substances that the brain can detect. Thus, activation of the immune system is accompanied by changes in the nervous system and endocrine activity. Cytokines released by activated immune cells provide still another pathway through which the immune system communicates with the central nervous system (CNS). Although the precise site (or sites) at which cytokines act within the brain has not been identified, cytokines

cause changes in the activity of the brain, in the endocrine system, and in behavior.

At the neural and endocrine levels, then, there is abundant evidence of interactions between the brain and the immune system. At the behavioral level, the most notable evidence of interactions between the CNS and immune system is the effects of conditioning and stressful life experiences on immune function. Another important line of research (not elaborated here) concerns the effects of immune processes on emotional states and other behaviors such as activity, sleep, and appetite.

### BEHAVIORAL INFLUENCES ON IMMUNE FUNCTION

Pavlovian conditioning of alterations of immune function provides the most dramatic illustration of a functional relationship between the brain and the immune system. In a prototypical study using a paradigm referred to as taste-aversion conditioning, animals consumed a novel saccharin solution, the conditioned stimulus (CS), shortly before they were injected with an immunosuppressive drug, the unconditioned stimulus (UCS). When all animals were subsequently injected with antigen, conditioned animals that were reexposed to the CS alone showed an aversion to it and an attenuated antibody response compared with conditioned animals that were not reexposed to the CS and nonconditioned animals that were exposed to saccharin (Ader & Cohen, 1975).

Studies have since documented the acquisition and extinction of conditioned nonspecific responses such as NK cell activity and various antibody- and cell-mediated immune responses (Ader & Cohen, 2001). Conditioning is not limited

to changes associated with taste-aversion learning, and there is no consistent relationship between conditioned changes in behavior and conditioned changes in immune responses. Also, conditioned immunosuppressive responses cannot be ascribed to stress-induced or conditioned elevations of adrenal hormones. More recently, the conditioned enhancement, as opposed to suppression, of immune responses has been observed using antigens rather than pharmacologic agents as UCSs.

Data on conditioning in humans are limited. The anticipatory (conditioned) nausea that frequently precedes cancer chemotherapy is associated with anticipatory suppression of the capacity of lymphocytes to respond to foreign stimuli, and multiple sclerosis patients being treated with an immunosuppressive drug show a conditioned decrease in total white blood cell count in response to a sham treatment. Healthy subjects show enhanced NK cell activity when reexposed to a distinctive flavor previously paired with injections of adrenaline. In another study, it was shown that repeated injections of saline (which do not elicit an immune response) could attenuate the response to a subsequent injection of antigen. Conversely, however, repeated injections of antigen may not precipitate a reaction to a subsequent injection of saline.

Psychosocial factors, including stressful life experiences, are capable of influencing the onset or severity of a variety of immune disorders and infectious diseases. Such factors are also capable of influencing immune function. The death of a spouse, other "losses" (e.g., divorce), and other chronic stressors (e.g., caregiving for a chronically ill person)—and even less traumatic events such as school examinations—elicit distress and associated declines in immune function, including a depressed response to a viral antigen.

Clinical depression tends to be

associated with some immunologically mediated diseases, and this fact has focused attention on the immunological effects of depression. Depressed patients show a decline in several measures of immunity, elevated antibody levels to herpes viruses, and a diminished ability to mount a specific cell-mediated response to varicella zoster virus, which is responsible for shingles (Herbert & Cohen, 1993). In none of these instances, however, has it been demonstrated that changes in immune function specifically cause the health effects of depression or other affective responses to stress.

Evidence documenting stress-induced alterations in immunity comes mostly from animal research. Early life experiences such as disruption of an animal's interactions with its mother, the social environment, exposure to predators, odors emitted by stressed conspecifics, and physical restraint or other noxious conditions induce neuroendocrine changes and modulate both antibody- and cell-mediated immunity. In general, stress suppresses immune function, but the direction, magnitude, and duration of the effects depend on the antigen, the nature of the stressful experience, and the temporal relationship between the stressful experience and the encounter with antigen. The effects of stress also depend on a variety of host factors, such as species, age, and gender.

The neural and endocrine changes presumed to underlie the immunological effects of stressful life experiences have not been delineated. Any number of hormones or the patterning of hormonal responses could influence immunity. Elevated levels of adrenocortical steroids, the most common manifestation of the stress response, are generally immunosuppressive, and there are many stressor-induced changes in immune function that are mediated by adrenal hormones. However, many stress-induced changes in immunity are independent of adrenal activity.

The response to stressful life experiences involves complex interactions among behavior, the nervous system, the endocrine system, and immune response (Rabin, 1999). As a result, the literature on the immunological effects of stress has yielded some equivocal or seemingly inconsistent findings. It should not be surprising, though, that different stressors—commonly thought to elicit a common stress response—can have different effects on the same immune response. Also, one particular stressor can have different effects on different immune responses. Another source of variability may relate to the direct translation of procedures used in immunological research to behavioral studies. For example, a concentration of antigen that is optimal for the study of cellular processes or immunizations against disease may not be optimal for studies designed to investigate the psychobiological interactions that appear to influence immunoregulatory processes. Thus, for the latter purpose, we need studies in which antigen concentrations are at the lower levels to which individuals may be exposed in natural settings. Varying antigen dose would reduce the risk of masking the contribution of those biopsychosocial factors that influence health and illness in the real world.

If we are not always able to predict the direction, magnitude, or duration of the effects of stressful life experiences, it is clear nevertheless that stressful life experiences can influence immune functions; they can increase or decrease susceptibility to immunologically mediated diseases, permit an otherwise inconsequential exposure to some viruses to develop into clinical disease, or contribute to the reactivation of viral infections to which the individual was exposed in the past. Unfortunately, there are relatively few studies that have measured the relationship between susceptibility to a particular dis-

ease and those immune responses that are relevant to that disease.

#### BIOLOGICAL IMPACT OF BEHAVIORALLY INDUCED ALTERATIONS OF IMMUNE FUNCTION

The effects of conditioning and of stressful experiences on immune function have been referred to as "small." The changes in immune function have remained within normal limits, and it is argued, therefore, that the effects of behavior on immune function have no clinical significance. Although there may be reason to question the selective application of the criterion of effect size, a concern for the biological impact of behaviorally induced changes in immune function is quite legitimate. The association between stressful life experiences and susceptibility to disease and the association between stressful life events and changes in immune function do not establish a causal chain linking stress, immune function, and disease. Thus, a central question that remains to be addressed concerns the biological (clinical) significance of behaviorally induced changes in immunity.

There is little, if any, human research in which an altered resistance to disease has been shown to be a direct result of changes in immune function induced by stressful life experiences. Animal studies of experimentally induced or spontaneously occurring diseases, however, are being developed to address this issue. Stressful stimulation delays the production of virus-specific antibodies in mice infected with influenza and suppresses NK cell activity and the development of some T lymphocytes in animals inoculated with herpes simplex virus (HSV). Although physical restraint is ineffective in reactivating HSV infections, disruption of the social hierarchy within a colony of

mice increases aggressive behavior, activates the HPA axis,<sup>2</sup> and results in reactivation of HSV in a significant proportion of infected animals. When the spread of a lung tumor is related to NK cell function, several different stressors can decrease NK cell activity and increase lung disease.

Inflammatory processes, an essential component in the healing of wounds, can be modulated by the sympathetic nervous system and HPA axis. It is not surprising, then, that experimentally produced wounds heal more slowly in caretakers of Alzheimer's patients than in control subjects and in students tested before an examination rather than during summer vacation. Mice restrained for several days before and after they are wounded show a diminished inflammatory response, an elevated level of adrenocortical steroids, and a dramatic delay in healing.

Additional work with animals will enable studies of the mechanisms through which stressful life experiences affect health and determine whether disease susceptibility can, as hypothesized, be influenced by behaviorally induced alterations in immune function.

The biological impact of conditioning was examined using mice that spontaneously develop a disease similar to systemic lupus erythematosus in which there is an overreactivity of the immune system. In this case, a suppression of immunological reactivity would be in the biological interests of these animals. CS presentations without active drug were provided on 50% of the pharmacotherapy trials on which animals were scheduled to receive immunosuppressive drug. By capitalizing on conditioned immunosuppressive responses, it was possible to delay the onset of lupus using a cumulative amount of drug that was not, by itself, sufficient to alter progression of the autoimmune disease. Similarly, resistance to ex-

perimentally induced arthritis was achieved by exposing animals to a CS previously paired with immunosuppressive treatments. Among mice previously conditioned by pairing a CS with an immunosuppressive drug, reexposure to the CS following transplantation of foreign tissues delayed the immunologically induced rejection of the tissues. There is one clinical case study of a child with lupus who was successfully treated using a conditioning protocol to reduce the total amount of immunosuppressive drug usually prescribed. Although the effects of conditioning have been described as small, conditioned immunological effects can have a profound biological impact on the development of disorders resulting from an overreactive immune system, some cancers, and the survival of tissue transplants.

The issue of clinical significance has occasioned a lot of misplaced breast-beating and apologies in the name of scientific conservatism. Except, perhaps, for extreme and rare circumstances, the notion that a conditioned stimulus or psychosocial conditions could, by themselves, perturb the immune system to an extent that exceeds normal boundaries and leads to overt disease is somewhat simplistic from either an immunological or a behavioral perspective. Given the complexity of the cellular interactions within the immune system and the interactions between the immune and nervous systems, a behaviorally induced deviation from baseline that did not exceed the normal boundaries would seem to be the only response that could reasonably be expected. As far as susceptibility to a particular disease is concerned, however, it would not be unreasonable to theorize that changes capable of altering immune responses relevant to disease could have clinical consequences when interacting with environmental pathogens or when superimposed upon existing pa-

thology or an immune system compromised by host factors such as age or external influences such as immunosuppressive drugs of abuse. The potential importance of psychoneuroimmunological interactions, then, requires that we adopt research strategies that capitalize on individual differences; high-risk populations (e.g., the very young or old, people whose immune systems are compromised, those with genetic predispositions to particular diseases, those with existing disease); systematic variation of the magnitude of the antigen; and the measurement of responses that are demonstrably relevant to particular diseases.

## CONCLUSIONS

Psychoneuroimmunology is an interdisciplinary field that has developed and now prospers by ignoring the arbitrary and illusory boundaries of the biomedical sciences. As a result of the integrative research conducted in recent years, a paradigm shift is occurring; researchers can no longer study immunoregulatory processes as the independent activity of an autonomous immune system. These processes take place within a neuroendocrine environment that is sensitive to the individual's perception of and adaptive responses to events occurring in the external world.

Research predicated on the hypothesis that there is a single, integrated defense system could change the way we define and study certain diseases. Theoretically, it is likely that behavioral, neural, and endocrine interventions are relevant in the treatment of some immune system-related diseases (e.g., arthritis) and that immune system activity may contribute to the understanding and treatment of behavioral, neural, and endocrine disorders (e.g., depression or even schizophrenia).

We cannot yet detail the mechanisms mediating the effects of conditioning or stressful life experiences on immune responses, and further studies are needed. However, we do know that neural and endocrine changes are associated with changes in behavior and that there is a network of connections between the brain and the immune system. The existence of these bidirectional pathways reinforces the hypothesis that changes in the immune system constitute an important mechanism through which psychosocial factors could influence health and disease.

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**Acknowledgments**—Preparation of this article was supported by a Research Scientist Award (K05 MH06318) from the National Institute of Mental Health.

## Notes

1. Address correspondence to Robert Ader, Department of Psychiatry, University of Rochester Medical Center, Rochester, NY 14642.
2. This term comes from the structures involved in the secretion of so-called stress hormones. During a stress response, the brain's hypothalamus (H) releases a chemical that affects the pituitary gland (P). The pituitary then se-

cretes a hormone that causes the adrenal glands (A) to release corticosteroids (cortisol in humans, corticosterone in rodents) into the bloodstream.

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# Exploration of Environmental and Genetic Risk Factors for Alzheimer's Disease: The Value of Cross-Cultural Studies

Hugh C. Hendrie<sup>1</sup>

Department of Psychiatry, Indiana University School of Medicine, Indianapolis, Indiana

## Abstract

Advances in molecular genetics have revolutionized epidemiological research. It is now possible to combine the techniques of population genetics with research on risk factors to construct genetic-environmental interactive models that explain geographic-ethnic variations in disease rates. Cross-cultural studies involving populations from developing and developed countries offer a unique opportunity for constructing these models by providing a wide diversity of environmental exposures. Results from a comparative Indianapolis-Ibadan study suggest that Alzheimer's disease incidence rates are lower in Yoruba than in African Americans and that these lower rates may be due to a combination of genetic and environmental, primarily dietary, influences.

## Keywords

Alzheimer's disease; cross-cultural; genetics

Advances in molecular genetics have revolutionized epidemiological research. Vague, ambiguous concepts such as race and ethnicity need no longer be considered as risk factors. Instead, epidemiologists are now able to combine the techniques of population genetics with more traditional research on risk factors to formulate etiological hypotheses involving specific genes and specific environmental factors that can explain geographic or ethnic variations in disease rates.

Cooper and Kaufman (1998) recently proposed a disease model involving contributions from genes and the environment to explain disease rates in populations. They emphasized that genetics and the environment interact in such a way that the presence of the two factors does not always imply that their influence amounts to the sum of the two effects (see Fig. 1). The propensity to develop an illness does not depend solely on the presence or absence of a specific gene mutation or variant. Rather, it depends on the biological products of that gene

(gene expression), which can be influenced by environmental as well as other factors.

Cross-cultural studies of Alzheimer's disease (AD), particularly those involving subjects in developing countries, offer a unique opportunity for applying this proposed disease model to AD because such studies provide a much wider diversity of environmental exposures than do studies of populations solely in industrialized countries, where important risk factors may be missed because of their very pervasiveness. The environmental diversity cross-cultural studies bring into play makes it possible not only to identify new environmental factors, but also to explore the effect of this environmental diversity on gene expression. Since 1992, research teams from the Indiana University School of Medicine and the University of Ibadan, Nigeria, have been collaborating on a longitudinal comparative study of the prevalence and incidence rates of AD and other dementing illnesses and their associated risk factors. Our study's participants, who are all age 65 years and older, include African Americans residing in Indianapolis and Yoruba living in the Idikan wards of Ibadan, Nigeria. In this review, I discuss how Cooper and Kaufman's model might be applied to cross-cultural research in AD using data primarily from this Indianapolis-Ibadan project. The major methodological problems that exist with respect to cross-cultural research are not discussed.

$$\begin{aligned} \text{Observed Phenotypic Variation} = & \text{Genes} + \text{Environment} + \\ & \text{Genes} * \text{Environment} + \\ & \text{Genes} * \text{Genes} + \\ & \text{Environment} * \text{Environment} \end{aligned}$$

**Fig. 1.** Modeling the contribution of genes and the environment. Asterisks indicate interaction between factors (from Cooper & Kaufman, 1998, p. 815).

### COMPARISON OF RATES OF AD BETWEEN POPULATIONS

Many studies have been conducted on the prevalence of AD throughout the world, but few have been conducted in nonindustrial countries and fewer still have used the same methodologies used in studies of industrial countries (Hendrie, 1998). We have reported significantly lower rates of dementia (8.24% vs. 2.29%) and of AD (6.24% vs. 1.41%) in Yoruba than in African Americans. Our prevalence rates for African Americans are approximately the same as those reported in the large cross-Canada national prevalence study. Our prevalence rates for Yoruba are at the lower end of previously reported rates.

Prevalence rates, however, depend on factors in addition to incidence rates of illness. Differences in life expectancy or in survival of demented and nondemented subjects between sites could also affect prevalence rates. Incidence rates, which are the numbers of new cases occurring over a fixed period of time, are a better indication of true rates of illness than prevalence rates. In our 5-year incidence study, the age-standardized incidence rates for both dementia and AD were significantly lower for Yoruba than for African Americans (for dementia: Yoruba, 1.35%, and African Ameri-

cans, 3.24%; for AD: Yoruba, 1.15%, and African Americans, 2.52%; Hendrie et al., in press). It should be noted that although prevalence and incidence rates were consistently lower among the Yoruba, the association with age was identical between sites; that is, prevalence rates in both sites roughly doubled every 5 additional years of age.

### COMPARISON OF FREQUENCY OF PUTATIVE RISK FACTORS BETWEEN POPULATIONS

Great cultural and socioeconomic differences exist between the impoverished, predominantly Muslim residents of the Idikan wards of Ibadan and the elderly African Americans living in Indianapolis. The elderly Yoruba have relatively limited access to health care and live, for the most part, in large extended families. In contrast, most of the African Americans in our study reported good access to health care and either live in single-family dwellings with members of their immediate family or are widowed and live alone. Many studies report a high level of education as a protective factor against the development of AD. In Ibadan, more than 80% of the subjects had received no education. In Indianapolis, the mean num-

ber of years of education for our cohort was 9.6. This finding is counterintuitive in view of the low rates of AD in the Yoruba. It might suggest that education level is not directly related to AD risk but instead serves as a marker for other influences in childhood. In the African Americans, for example, it was the combination of low education and childhood residence in the rural South that increased the risk of AD (Hall, Gao, Unverzagt, & Hendrie, 2000).

There are many lifestyle differences between the two populations in our study. For example, dietary intake varies widely: The elderly Yoruba in the Idikan wards consume a low-calorie, low-fat diet consisting mainly of grains, roots, and tubers, supplemented with a small amount of fish. Ascorbic acid levels have been reported to be relatively high among the Yoruba, probably because of the high consumption of peppers. The African-American diet, however, is high in fat and sodium and low in fiber. These lifestyle differences are reflected in significant differences in biological and medical variables (see Table 1) that are often associated with risk of circulatory problems such as heart attack and stroke.

There is mounting evidence that not only is vascular disease associated with stroke-related dementias, but it may contribute to the development and progression of AD as well. The relatively lower incidence of vascular disease and vascular risk factors in the Yoruba than in the African Americans may account for the differences in rates of AD and dementia between the two sites. As our study is longitudinal in design, and enables us to conduct predictive analyses on the effects of these risk factors, we hope to test this hypothesis. It is also possible that the interaction of these vascular risk factors with other genetic risk factors may account for the differences between the two populations.

**Table 1.** Summary of biological and medical variables that are significantly different between Yoruba and African-American subjects

Variable	Yoruba (n)	African Americans (n)
History of hypertension	19% (2,470)	61% (2,204)
History of diabetes	2.5% (2,482)	24% (2,206)
History of stroke	1.3% (2,480)	11% (1,960)
Ever smoked	24% (2,472)	63% (2,205)
Mean cholesterol (mgs/dl)	166 (71)	221 (117)
Mean body mass index	21.4 (1,104)	28.9 (1,115)
Mean systolic blood pressure	135 (1,213)	146 (945)

## GENETIC RISK FACTORS

Early-onset inherited forms of AD have been associated with gene mutations on three chromosomes, 21, 14, and 1. These findings are extremely important for understanding the pathophysiological mechanisms of AD but account for only a very small proportion of all Alzheimer's cases (about 2%).

Apolipoprotein  $\epsilon$  (ApoE), a fat-bound protein circulating in the blood, is known to play an integral role in cholesterol transport. This protein exists in several slightly different structures with similar function; these three *isoforms* are encoded by separate forms (alleles) of a gene on Chromosome 19. The three alleles are termed  $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$ . Inheritance of these alleles is similar to that of blood groups (i.e., everyone inherits two alleles, one from each parent). The  $\epsilon 4$  allele has now been identified as an important risk factor for the most common forms of late-onset AD; specifically, individuals possessing two copies of this allele have a greater risk for AD (up to eightfold or more) than do individuals with no copies, and individuals with a single copy have an intermediate risk (2 to 4 times that of individuals with no copies).

The association between ApoE  $\epsilon 4$  and AD is one of the most consistent findings in AD research, being confirmed in many studies throughout the world. One of the

intriguing features of the studies conducted so far is the marked variation in frequency of the ApoE  $\epsilon 4$  allele found in different population and ethnic groups. Reported frequencies of the  $\epsilon 4$  allele have ranged from 5% or less in the Amish to more than 40% in some Aboriginal populations. It is not clear yet what effect this variation in population frequency has on the rates of AD in these populations.

In our Indianapolis-Ibadan study, the frequencies of the ApoE alleles were almost identical in the two populations (Indianapolis:  $\epsilon 2$ –10%,  $\epsilon 3$ –68%,  $\epsilon 4$ –22%; Ibadan:  $\epsilon 2$ –8%,  $\epsilon 3$ –71%,  $\epsilon 4$ –21%). In contrast to previous studies of Caucasian and other population groups, our study showed only a weak association between  $\epsilon 4$  and AD in African Americans; this association attained significance only for those individuals who possessed two copies of the  $\epsilon 4$  allele. In the Yoruba, so far we have found no association between  $\epsilon 4$  and AD for individuals with either single or double copies of  $\epsilon 4$ .

## POSSIBLE GENETIC-ENVIRONMENTAL INTERACTIONS

The lack of association between  $\epsilon 4$  and AD in the Yoruba may explain in part some of the difference in rates of AD between the Yoruba and the African Americans. It may

be that there are differences between the two populations in other genes that regulate the expression of AD. We are currently exploring this possibility.

The differences in cholesterol levels between sites and the role that ApoE plays in cholesterol processing make an ApoE-cholesterol interaction resulting in a differential AD risk an obvious possibility. Two prior studies have reported a significant interaction between ApoE and cholesterol in determining risk for AD (Jarvik et al., 1995; Notkola et al., 1998). Notkola et al. (1998) suggested that cholesterol, in fact, mediates some of the effects of ApoE  $\epsilon 4$  on AD. In a preliminary study, increasing levels of cholesterol increased the risk of AD in African Americans, but only in those subjects who did not possess an  $\epsilon 4$  allele (Evans et al., 2000). The relationship was not seen in the Yoruba, perhaps because they had much lower levels of cholesterol. It is too early to tell whether or not this mechanism accounts for the different rates between the two sites.

Cell functioning is dependent on the metabolism of oxygen. However, this metabolic process throughout life can create toxic by-products, which can eventually cause cell death. This potentially cell-destroying process is referred to by the term *oxidative stress*. As one of the most metabolically active organs, the brain is particularly vulnerable to oxidative stress. Low-calorie diets produce less evidence of oxidative stress than high-calorie diets; oxidative stress also appears to be reduced by diets containing high levels of antioxidants (e.g., vitamins C and E), which act as scavengers for the toxic oxidative end products. It might be anticipated, therefore, that the diet of the Yoruba would produce less evidence of cell damage due to oxidative stress than would the diet of African Americans. In fact, in a very small study involving cells from 8 Yoruba subjects (5 with probable

AD and 3 normal subjects), there was little evidence of oxidative stress (probably because of diet), which might explain at least in part the lower incidence rates of AD in Yoruba (Lahiri et al., 1999).

Both of these preliminary studies suggest that diet or dietary products may be a mediating influence on the association between ApoE  $\epsilon 4$  and AD. Again, it must be emphasized that these results are only very preliminary and will need confirmation in our longitudinal studies. Moreover, it is likely that ApoE  $\epsilon 4$  will not be the only genetic factor that confers risk for AD. Already there is evidence to suggest that a gene located in Chromosome 12 may be implicated in AD. Many other possible interactions that may alter the association of  $\epsilon 4$  with AD have yet to be explored.

Also, we have as yet studied only the Yoruba. It is entirely possible that other African groups would show different gene-environment associations with AD. There is at least as great a genetic diversity among Africans as among non-Africans. The variation in frequencies of  $\epsilon 4$  is at least as great within African populations as across populations around the world.

## CONCLUSION

The contrast between the advances in the understanding of the role of genetics in AD and the inability to identify environmental factors clearly, together with the lack of clear evidence of differences in incidence rates between countries, has led many investigators to speculate that AD is predominantly or exclusively genetic in origin. Cross-cultural transnational research, by providing greater environmental diversity, offers the

opportunity to identify hitherto unsuspected environmental influences and interactions between environmental and genetic factors that may alter gene expression. Cross-cultural studies such as ours are still in their infancy. So far, we have established two populations with different incidence rates of AD and have identified some potential environmental risk factors. Our next major step is to assess the association of these risk factors (using more accurate biological measurements of them) to AD in both populations and to explore how they may interact with genes in a longitudinal study. We hope to establish a disease model that will account for differences in AD incidence in both populations.

By the year 2005, about 70% of all elderly worldwide will be living in developing countries. If the prevalence rates of AD in developing countries in any way parallel the rates in developed countries, the burden of caring for these AD patients in these countries will be staggering. We hope that by including two populations, one from the developing world and one from the developed world, our model for AD will be more generalizable than models constructed primarily from populations in Western societies.

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**Acknowledgments**—The study discussed in this article was supported by Grant No. AGO-9956 from the National Institute on Aging and Alzheimer's Association Grant No. IRG-95-084.

## Note

1. Address correspondence to Hugh C. Hendrie, Department of Psychiatry, 541 Clinical Dr., Room 298, Indianapolis, IN 46202-5111; e-mail: hhendri@iupui.edu.

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