ALZHEIMER'S DISEASE

Progress Report on Alzheimer's Disease: Taking the Next Steps

National Institute on Aging National Institutes of Health

Introduction

Alzheimer's disease (AD) is an age-related and irreversible brain disorder that occurs gradually and results in memory loss, behavior and personality changes, and a decline in thinking abilities. These losses are related to the breakdown of the connections between nerve cells in the brain and the eventual death of many of these cells. The course of this disease varies from person to person, as does the rate of decline. On average, patients with AD live for eight to 10 years after they are diagnosed, though the disease can last for up to 20 years.

AD is part of a group of disorders, termed dementias, that are characterized by cognitive and behavioral problems. AD advances progressively, from mild forgetfulness to a severe loss of mental function. In most people with AD, symptoms first appear after age 60. The earliest symptoms characteristically include loss of recent memory, later compounded by faulty judgment, and changes in personality. Often, people in the initial stages of AD think less clearly and tend to be easily confused. Later in the disease, they may forget how to do simple tasks, such as how to dress themselves or eat with proper utensils. Eventually, people with AD lose the capacity to function on their own and become completely dependent on other people for their everyday care. Finally, the disease becomes so debilitating that patients are bedridden and likely to develop other illnesses and infections. Most commonly, people with AD die of pneumonia.

Although the risk of developing AD increases with age, AD and dementia symptoms are not a part of normal aging. AD and other dementing disorders are caused by diseases that affect the brain. In the absence of disease, the human brain often can function well into the tenth decade of life.

The Impact of Alzheimer's Disease

AD is the most common cause of dementia among people age 65 and older. It presents a major health problem for the United States because of its enormous impact on individuals, families, the health care system, and society as a whole. Scientists estimate that up to 4 million people currently suffer with the disease, and the prevalence (the number of people with the disease at any one time) doubles every five years beyond age 65. It is also estimated that approximately 360,000 new cases (incidence) will occur each year and that this number will increase as the population ages (Brookmeyer et al., 1998).

These numbers are significant now and will become even more so in the future. Since the turn of the century, life expectancies have increased dramatically. An estimated 35 million people—13 percent of the total population of the United States—are now aged 65 and older. According to the U.S. Bureau of the Census, this percentage will accelerate rapidly beginning in 2011, when the first baby boomers reach age 65. By 2050 the number of Americans aged 65 and older will have doubled, to 70 million people.

Approximately 4 million Americans are 85 years old or older, and in most industrialized countries, this age group is one of the fastest growing segments of the population. The Bureau of the Census estimates that this group will number nearly 19 million by the year 2050; some experts who study population trends suggest that the number could be even greater. This trend is not only apparent in the U.S. but also worldwide. As more and more people live longer, the number of people affected by diseases of aging, including AD, will continue to grow. For example, one study shows that nearly half of all people age 85 and older have some form of dementia (Evans et al., 1989).

One of the most pressing current issues is determining possible differences in AD risk, incidence, and prevalence among various racial and ethnic groups. These differences are important to study for several reasons. One is that the percentage of non-Caucasians in the older U.S. population is growing rapidly (by the year 2050, the per-

centage of the population over the age of 65 that is non-Caucasian will have increased from 16 percent to 34 percent). Another is that the variations in prevalence may give us important future insights into the different roles that particular genetic and environmental factors play in the development of AD. Recent research has shown that African Americans and Hispanic Americans may have a higher overall risk of AD than do Caucasians (Tang et al., 1998), although other studies have found conflicting results (Fillenbaum et al., 1998). It is important to note that many factors may be responsible for these differing estimates, for these populations vary in many respects besides their racial or ethnic diversity. Differences in socioeconomic status, health care, education, events occurring before birth (prenatally) or right around birth (perinatally), and life history may all influence a person's eventual risk of AD. Even the ways in which diagnostic tests that measure language, memory, and cognitive function are constructed and applied may cause people to be diagnosed with AD if their level of education or cultural assimilation makes them score lower on the test than do people with a higher level of education who are more culturally assimilated. Clearly, further careful investigation is needed to examine the role that ethnic and racial differences may play in determining the risk of AD, and studies now ongoing should begin to provide some answers.

AD puts a heavy economic burden on society. A recent study estimated that the annual cost of caring for one AD patient is $18,408 for a patient with mild AD, $30,096 for a patient with moderate AD, and $36,132 for a patient with severe AD (Leon et al., 1998). The annual national direct and indirect costs of caring for AD patients are estimated to be as much as $100 billion (Ernst and Hay, 1994; Ernst et al., 1997; Huang et al., 1988).

Slightly more than half of AD patients receive care at home, while the remainder are cared for in a variety of health care institutions. Many spouses, relatives, and friends take care of people with AD. During their years of caregiving, these families and friends experience emotional, physical, and financial stresses. They watch their loved ones become more and more forgetful, frustrated,

and confused. Eventually, the person with AD may not even recognize his or her nearest and dearest relatives and friends. Caregivers—most of whom are women—must juggle child care, jobs, and other responsibilities with caring for relatives with AD who cannot function on their own. As the disease runs its course and the abilities of people with AD steadily decline, family members face difficult decisions about the long-term care of their loved ones. Frequently, they have no choice but to place their relative in a nursing home. The numbers of caregivers—and their needs—can be expected to grow significantly as the population ages and as the number of people with AD increases.

Alzheimer's Disease: An Urgent National Health and Research Priority

Given our aging population, the magnitude of AD as a national health problem is steadily increasing. This makes the disease an urgent research priority. Interventions that could delay the onset of AD would have an enormous positive public health impact because they would reduce the number of people with the disease. This in turn would reduce the personal and financial costs associated with caring for them. A recent analysis provides a vivid illustration of the impact of delaying AD by even a few years. In this paper, the authors report that an intervention that could delay the mean onset of Alzheimer's disease by approximately 5 years, would reduce the numbers of persons with AD by 50 percent by the year 2050 (Brookmeyer et al., 1998).

The AD Research Effort

AD research supported by the Federal Government is divided into three broad, overlapping areas: causes/risk factors, diagnosis, and treatment/caregiving. Research into the basic biology of the aging nervous system is critical to understanding what goes wrong in the brain of a person with AD. Understanding how nerve cells lose their ability to communicate with each other and the reasons why

some nerve cells die and others do not is at the heart of scientific efforts to discover what causes AD.

Many researchers also are looking for better ways to diagnose AD in the early stages and to identify the earliest brain changes that eventually result in AD. Investigators are striving to identify markers (indicators) of dementia, develop and improve ways to test patient function, determine causes and assess risk factors, and improve case-finding and sampling methods for population studies.

Other researchers are working hard to discover and develop drugs that may help to treat symptoms or slow the progress of the disease, and eventually delay the onset of and prevent AD. Many of these drugs are now being tested in clinical trials. Finally, scientists and many health care professionals are seeking better ways to help patients and caregivers cope with the decline in mental and physical abilities and the problem behaviors that accompany the disease and to support those who care for people with AD. The National Institute on Aging (NIA), part of the Federal Government's National Institutes of Health (NIH), has primary responsibility for research aimed at finding ways to prevent, treat, and cure AD. The Institute's AD research program is integral to one of its main goals, which is to enhance the quality of life of older people by expanding knowledge about the aging brain and nervous system. The 2000 Progress Report on Alzheimer's Disease summarizes AD research conducted or supported by NIA and other components of NIH, including:

- National Institute of Neurological Disorders and Stroke

- National Institute of Mental Health

- National Institute of Nursing Research

- National Institute on Alcohol Abuse and Alcoholism

- National Institute of Environmental Health Sciences

- National Institute of Child Health and Human Development

- Human Genome Research Institute

- National Center for Complementary and Alternative Medicine

Other more modest AD research efforts not summarized in this report are supported by the National Institute on Deafness and Other Communication Disorders, National Cancer Institute, National Institute of Diabetes and Digestive and Kidney Diseases, National Institute of Arthritis and Musculoskeletal and Skin Diseases, and the Fogarty International Center.

The manifestations of Alzheimer's disease have been recognized since ancient times. Greek and Roman writers described symptoms similar to those that we know as AD. In the 16th century, Shakespeare wrote about very old age as a time of "second childishness and mere oblivion," suggesting that the symptoms of AD, or something quite like it, were known and recognized then. Despite this long familiarity, relatively little was known until recently about the processes in the brain that lead to Alzheimer's disease. Most physicians assumed that AD dementia was merely an inevitable consequence of aging. Scientists have made enormous progress in the last 25 years. Today, we know much more about Alzheimer's disease—what it is, who gets it, how it develops, and what course it follows. We have also made significant progress in the critical area of early diagnosis and have some promising leads on possible treatments. All of this research has deepened our understanding of this devastating disease. It also has expanded our knowledge about other late-life neurodegenerative diseases, brain function in healthy older people, and ways in which to minimize normal age-related cognitive decline.

The 2000 Progress *Report on Alzheimer's Disease* describes this important research effort. It begins with a description of our current knowledge about AD. This provides the backdrop for the next two sections, which present

highlights of recent research conducted by NIA and by other NIH Institutes. The report closes with a section called "Outlook for the Future," which summarizes progress in the ongoing NIH Alzheimer's Disease Prevention Initiative as well as the new President's Initiative on Alzheimer's Disease. These initiatives are designed to accelerate laboratory and clinical research and collaboration across the Federal Government and the private sector and to turn research results into real advances for patients, families, and caregivers.

Alzheimer's Disease: More Pieces of the Puzzle Fall Into Place

In normal aging, nerve cells in the brain are not lost in large numbers. In contrast, AD causes many nerve cells to stop functioning, lose connections with other nerve cells, and die.

At first, AD destroys neurons in parts of the brain that control memory, including the hippocampus (a structure deep in the brain that helps to encode short-term memories) and related structures. As nerve cells in the hippocampus stop working properly, short-term memory fails, and often, a person's ability to do easy and familiar tasks begins to decline. AD later attacks the cerebral cortex, particularly the areas responsible for language and reasoning. At this point, AD begins to take away language skills and changes a person's ability to make judgments. Personality changes also may occur. Emotional outbursts and disturbing behaviors, such as wandering and agitation, begin to occur and become more and more frequent as the disease continues its course. Eventually, many other areas of the brain are involved, all these brain regions atrophy (shrink and lose function), and the person with AD becomes bedridden, incontinent, totally helpless, and unresponsive to the outside world.

What Are the Main Characteristics of AD?

Two abnormal structures in the brain are the hallmarks of AD: amyloid plaques and neurofibrillary tangles.

Though scientists have known about plaques and tangles for many years, more recent research has revealed much about their composition, how they form, and their possible roles in the development of AD.

Amyloid Plaques. In AD, plaques develop first in areas of the brain used for memory and other cognitive functions. They consist of largely insoluble (cannot be dissolved) deposits of beta-amyloid—a protein fragment snipped from a larger protein called amyloid precursor protein (APP)—intermingled with portions of neurons and with non-nerve cells such as microglia (cells that surround and digest damaged cells or foreign substances that cause inflammation) and astrocytes (glial cells that serve to support and nourish neurons). Plaques are found in the spaces between the brain's nerve cells. Although researchers still do not know whether amyloid plaques themselves cause AD or whether they are a by-product of the AD process, there is evidence that amyloid deposition may be a central process in the disease. Certainly, changes in the structure of the APP protein can cause AD, as shown in one inherited form of AD, which is caused by mutations in the gene that contains instructions for making the APP protein. Recent work has revealed much about the nature of beta-amyloid and the ways in which it may be toxic to neurons, the processes by which plaques form and are deposited in the brain, and ways in which the numbers of plaques can be reduced.

Neurofibrillary Tangles. The second hallmark of AD consists of abnormal collections of twisted threads found inside nerve cells. The chief component of these tangles is one form of a protein called *tau*. In the central nervous system, *tau* proteins are best known for their ability to bind and help stabilize microtubules, which are one constituent of the cell's internal support structure, or skeleton.

In healthy neurons, microtubules form structures like train tracks, which guide nutrients and molecules from the bodies of the cells down to the ends of the axon. *Tau* normally holds together the "railroad ties" or connector pieces of the microtubule tracks. However, in AD *tau* is changed chemically, and this altered *tau* twists into paired

helical filaments—two threads of *tau* wound around each other. These filaments aggregate to form neurofibrillary tangles. When this happens, the *tau* no longer holds the railroad tracks together and the microtubules fall apart. This collapse of the transport system first may result in malfunctions in communication between nerve cells and later may lead to neuronal death that contributes to the development of dementia. Recent research has shed much light on this abnormal aggregation of *tau* protein and on the role that certain genetic mutations play in changing *tau*'s structure and contributing to neurodegeneration.

What Causes AD?

One important part of solving the AD puzzle is knowing what causes it: What makes the disease process begin in the first place and what contributes to its development? Why are some neurons more vulnerable and likely to die than are others? Why does the prevalence of AD increase with age?

Some diseases, like tuberculosis, have clear-cut causes. Others, such as diabetes or arthritis, result from many interrelated factors, including genetic, environmental, and other factors. AD fits into this latter group of diseases. Scientists do not yet fully understand what causes AD, but it is clear that AD develops as a result of a complex cascade of events that take place over many years inside the brain. The disease may be triggered by any number of small changes in this cascade, probably as a result of the interaction of different genetic and non-genetic factors in different individuals. Scientists have come a long way toward elucidating the genetic and non-genetic factors that contribute to the development of AD.

Genetic Factors in AD Development. Two types of Alzheimer's disease exist: familial AD (FAD), which follows a certain inheritance pattern, and sporadic AD, where no obvious inheritance pattern is seen. Because of differences in the age at onset, AD is further described as early-onset (occurring in people younger than 65) or late-onset (occurring in those 65 and older). Early-onset AD is rare (about 5 to 10 percent of cases) and generally affects people aged

30 to 60. Some forms of early-onset AD are inherited and run in families. Early-onset AD also often progresses faster than the more common, late-onset form.

All FAD known so far has an early onset, and as many as 50 percent of FAD cases are now known to be caused by defects in three genes located on three different chromosomes. Some families have mutations in the APP gene located on chromosome 21, which causes an abnormal APP protein to be produced; others have mutations in a gene called presenilin 1 located on chromosome 14, which causes an abnormal presenilin 1 protein to be produced; and still others have mutations in a very similar gene called presenilin 2 located on chromosome 1, which causes an abnormal presenilin 2 protein to be produced. Even if one of these mutations is present in only one of the two copies of a gene inherited from the parents, the person will inevitably develop that form of early-onset AD (this is called autosomal dominant inheritance). However, the total known number of these cases is small (between 100 and 200 worldwide), and there is as yet no evidence that any of these mutations play a major role in the more common, sporadic or non-familial form of late-onset AD. Scientists are now working to reveal the normal function of APP and presenilins and to determine how mutations of these genes cause the onset of FAD.

Although there is no evidence that autosomal dominant inheritance of mutated genes causes late-onset AD, genetics does appear to play a role in the development of this more common form of AD. In the early 1990s, researchers at the NIA-supported Alzheimer's Disease Center at Duke University in Durham, North Carolina, found an increased risk for late-onset AD with inheritance of one or two copies of the apolipoprotein E epsilon4 (APOE e4) allele on chromosome 19 (Strittmatter et al., 1993). Different alleles of particular genes produce variations in inherited characteristics, such as eye color or blood type. In this case, the variations are in the APOE gene that directs the manufacture of the APOE protein. This protein helps carry blood cholesterol throughout the body, among other functions. It is found in glial cells and neurons of healthy brains, but it is also associated in excess amounts

with the plaques found in the brains of people with AD. Researchers are particularly interested in three common alleles of the APOE gene: e2, e3, and e4. The finding that increased risk is linked with inheritance of the APOE e4 allele has helped explain some of the variations in age of onset of AD based on whether people have inherited zero, one, or two copies of the APOE e4 allele from their parents. The more APOE e4 alleles inherited, the lower the age of onset. The relatively rare APOE e2 allele may protect some people against the disease; it seems to be associated with a lower risk for AD and a later age of onset if AD does develop. APOE e3 is the most common version found in the general population and may play a neutral role in AD.

The inheritance of one or two APOE e4 alleles does not predict AD with certainty. That is, unlike early-onset FAD, which is caused by specific genetic mutations, a person can have one or two APOE e4 alleles and still not get the disease, and a person who develops AD may not have any APOE e4 alleles. APOE e4 increases the risk of developing AD; it does not cause the disease. The ways in which APOE e4 increases the likelihood of developing AD are not known with certainty, but one possible mechanism is that it facilitates beta-amyloid buildup in plaques and this contributes to lowering the age of onset of AD. Other theories involve interactions with cholesterol levels and effects on nerve cell death that are independent of its effects on plaque buildup.

Studies over the last several years strongly suggest that there are additional risk factor genes for late-onset AD, and candidates continue to be identified in this exciting new area of research. Building on the improving understanding of AD genetics, scientists will continue to look for clues as to which protein structures hasten the initiation of the disease process, what mechanisms cause AD, and what the sequence of events is. Once they understand these, they can then look for new ways to diagnose, treat, or even prevent AD.

Aging and AD Development. Getting older, in and of itself, is the major risk factor for AD. During the course of normal aging, the brain undergoes a number of changes:

- some neurons in some brain regions die, although most neurons important to learning do not die;

- some neurons and their processes shrink and function less well, especially neurons in areas important to learning, memory, planning, and other complex mental activities;

- tangles develop in neurons and plaques develop in surrounding areas in particular brain regions;

- the mitochondria in cells become more susceptible to damage (mitochondria are tiny organelles within the cell that break down glucose to release energy, which is then used by the cell to carry out its functions);

- inflammation increases; and

- oxidative stress increases.

In healthy older people, the impact of these changes may be modest, resulting in various degrees of age-related memory decline. In people who develop AD, on the other hand, some of these changes are much more extreme and have devastating consequences. Many scientists are studying the processes involved in normal aging of the brain in hopes of learning more about them and the differences between normal brain aging and AD.

For example, scientists are intensively studying the increase in oxidative stress that occurs in the aging brain when mitochondria in cells become more susceptible to damage. During normal metabolism, the body produces a kind of molecule called a free radical. Free radicals may help cells in certain ways, such as in fighting infection. However, free radicals are highly reactive, and the production of too many is called oxidative stress. Oxidative stress, which can injure cells, resulting in nerve cell damage and death, is now believed to be a major contributor to the aging process.

Because AD almost always develops in older people, who often have other conditions, such as heart disease or

high blood pressure, scientists are also interested in exploring whether these conditions play any role in the development of AD. For example, cerebrovascular disease is the second most common cause of dementia and there is some evidence that brain infarctions (strokes) and AD may possibly be linked. A brain infarction is an area of injury in brain tissue that usually occurs when the blood supply to that area is interrupted, depriving neurons of essential oxygen and glucose and causing vital circuits to die. Although major strokes have obvious consequences, small ones may go undetected clinically. Another lifestyle factor that may be important is blood cholesterol levels. Scientists are showing that high blood cholesterol levels may increase the rate of plaque deposition in special breeds of genetically engineered, or transgenic, mice.

Finally, it is becoming clear that there are parallels between AD and other progressive neurodegenerative disorders that cause dementia, including prion diseases, Parkinson's disease (PD), and Huntington's disease. All involve deposits of abnormal proteins in the brain, and new research is showing that these diseases have a number of important overlapping characteristics.

What Do We Know About Diagnosing AD?

Currently, clinicians use a range of tools to diagnose "possible AD" (dementia could also be due to another condition such as stroke) or "probable AD" (no other cause of dementia can be found) in a patient who is having difficulties with memory or other mental functions. These tools include a patient history, physical exam, and tests that measure memory, language skills, and other abilities related to brain functioning. Much is known about the clinical and behavioral characteristics of the disease and this also helps in diagnosing AD. Sometimes, brain scans are used to rule out the presence of strokes or tumors. The diagnostic process is crucial not only to accurately identify AD but to rule out other conditions that might be causing cognitive problems or dementia, such as stroke, PD, or inappropriate doses of medications. However, at

the present time, AD can be diagnosed conclusively only by examining the brain after death in an autopsy to determine whether the levels of plaques and tangles in certain brain regions are characteristic of AD.

The earlier an accurate diagnosis of AD is made clinically, the greater the gain in managing symptoms. An early, accurate diagnosis of AD is especially important to patients and their families because it helps them plan for the future and pursue care options while the patient can still take part in making decisions.

Researchers have made major progress in developing accurate diagnostic tests and techniques. In specialized research facilities, trained clinicians can now diagnose AD with up to 90 percent accuracy. Scientists are now working in several areas that may improve the ability of clinicians to make accurate diagnoses of AD even earlier and that are providing important insights into the earliest changes that occur in the brain of an AD patient even before a clinical diagnosis is made. These insights will help scientists determine the natural history of AD and understand the ways in which the changes in memory and other cognitive functions that occur in AD differ from those of normal aging and of other dementias. They will also help researchers pinpoint early changes that could be targets for drug therapy.

One area of very active research is mild cognitive impairment (MCI). Individuals who have a memory problem but who do not meet the generally accepted clinical criteria for AD are considered to have MCI with memory loss. They are becoming an increasingly important group for AD researchers to study because it is now known that about 40 percent of them will develop AD within 3 years. Some, however, whose memory loss is due to other causes, never develop AD. Understanding the different characteristics and clinical courses of MCI and AD will be essential in helping clinicians diagnose AD early and accurately.

One of the most important developments in neuroscience research during the past 10 years has been the refinement of techniques that allow scientists to look at changes in structure and function in the living brain. One

of these techniques is magnetic resonance imaging (MRI), which can be used to measure the size of various structures in the brain. Many studies have shown that AD causes some brain structures, particularly the hippocampus, to shrink early on in the disease, and scientists are exploring exactly how early this shrinkage can be detected. Several teams of NIA-funded scientists have established the usefulness of MRI as a research tool to help determine which people with memory problems are in the earliest stages of AD; to identify people who later will be diagnosed with AD; and to distinguish between people with MCI and those with no memory or learning problems, and between people without AD and those with very mild AD.

Another set of imaging techniques allows scientists to visualize the activity and interactions of particular brain regions as they are used during cognitive operations such as memorizing, recalling, speaking, reading, learning, and other sorts of information processing. This window on the living brain can help scientists measure early changes in brain function or structure to identify those individuals who are at risk of Alzheimer's disease even before they develop the symptoms of the disease. These imaging techniques include positron emission tomography (PET) scans and single photon emission computed tomography (SPECT) scans. Although these various imaging techniques are still used primarily as research tools, they hold great promise, along with other diagnostic measures, for earlier identification of persons at risk of developing AD.

How Can Alzheimer's Disease be Treated?

For those who are already suffering from the effects of AD, the most immediate need is for treatments to control their symptoms, including cognitive loss as well as problem behaviors such as verbal and physical aggression, agitation, wandering, depression, sleep disturbances, and delusions. Treatments are needed that work on many patients, remain effective for a long time, ease a broad range of symptoms, improve patients' cognitive function

and ability to carry out activities of daily living, and have no serious side effects. Eventually, scientists also hope to develop drugs that attack fundamental AD processes, preventing them from progressing to the state where they damage cognitive function and quality of life.

The Food and Drug Administration (FDA) has approved three medications for AD. All act by inhibiting acetylcholinesterase, an enzyme that normally breaks down acetylcholine, a key neurotransmitter involved in cognitive functioning. This neurotransmitter is produced by one set of neurons whose function is gradually lost in AD. The first of these medications, approved in 1993, was tacrine (Cognex). The second, approved in 1996, was donepezil hydrochloride (Aricept). Aricept is the drug most commonly used now to treat mild to moderate symptoms of AD. However, like Cognex, Aricept does not stop or reverse the progression of AD, and it appears to help only some AD patients for a period of time ranging from months to about 2 years, so its usefulness is limited. In April 2000, the FDA approved rivastigmine (Exelon) for the treatment of mild to moderate AD symptoms. In clinical trials involving more than 3,900 patients worldwide, the drug improved patients' ability to carry out activities of daily living, such as eating and dressing. Patients also had fewer or less severe behavioral symptoms, such as delusions and agitation, and showed improvement in cognitive functions such as thinking, memory, and speaking. However, like the other two drugs, Exelon will not stop or reverse AD.

Many other investigators are working to improve the quality of life for both patients and caregivers through research to develop better behavioral management techniques and caregiver skills.

One of the primary characteristics of the NIH AD research effort over the last 25 years has been support for a wide range of studies by a large and multidisciplinary cadre of researchers. Some of these investigations have developed to the point of suggesting new ways of treating AD. All have contributed to building the solid base of knowledge that exists today. The continuing expansion of this base is pointing scientists in new and productive research

directions. It is also helping investigators ask better questions about the issues that still remain unclear. The remaining sections of the *2000 Progress Report on Alzheimer's Disease* describe some of these exciting areas of research and the results that have emerged in the last year.

2000 AD Research Advances: Taking the Next Steps

During the last year, researchers supported by NIA and other NIH Institutes made advances in a number of areas important to Alzheimer's disease, including:

- understanding the etiology of AD—the biological events that cause the changes in brain cells and tissues that lead to AD;

- improving early diagnosis;

- developing drug treatments;

- improving support for caregivers; and

- building the research infrastructure.

Understanding the Etiology of AD

In the last year, scientists continued to improve their understanding of how different forms of AD develop, how mutations in the AD early-onset genes initiate the cascade of biological events that eventually lead to the death of a person with AD, in what order events in the cascade take place, what brain regions are affected earliest and why, and how genetic and environmental factors may interplay to determine the overall likelihood of AD developing. Answers to questions about the fundamental nature of the disease and the way in which it evolves will help investigators create improved methods for diagnosing AD before a patient has any behavioral symptoms, develop effective treatments, or perhaps someday, even prevent this devastating disease.

Amyloid. Scientists have known for many years that amyloid plaques in the brain, formed by the aggregation of individual fragments derived from a larger protein, APP, are a prominent and diagnostic feature of AD. It has been hypothesized that an approach to preventing AD is to block the production of amyloid in the brain, though there is as yet no formal proof that this would prevent the development of the clinical symptoms of AD. An important focus of scientific research, therefore, is finding out how to block the formation of beta-amyloid. Another is finding ways to inhibit amyloid's deposition into insoluble plaques once it is formed. NIA-supported investigators in a number of laboratories made significant progress in these areas, including headway on a possible anti-amyloid vaccine. In addition, in one of the most important stories of the year, several teams of industry-, foundation-, and NIH-supported researchers identified two of the long-elusive enzymes that clip APP and create the beta-amyloid fragments (see the sidebar on p. 17 for more on this discovery).

Pursuing an Anti-amyloid Vaccine. The *1999 Progress Report on Alzheimer's Disease* described recent breakthrough studies that took the first steps toward a possible AD vaccine. Using transgenic mice that carry mutant human forms of APP and show extensive amyloid plaque formation with advancing age, researchers at Elan Pharmaceuticals showed that repeated administration of an amyloid vaccine to generate an immune response can almost eliminate formation of amyloid plaques in these mice (Schenk et al., 1999). Research this year by these same scientists has shown encouraging results in the further development and testing of the amyloid vaccine approach. For example, they found that the vaccine is not toxic in a variety of animals studied, including non-human primates. Preliminary safety studies in humans have shown that one vaccination is well tolerated, and safety testing of multiple injections has begun. If additional testing of the vaccine approach reveals that it is safe in humans, the company plans to start efficacy trials in 2001 (Helmuth, 2000). Two major hurdles remain: Will the vaccine effectively clear amyloid in humans? Will clearing amyloid improve the

clinical symptoms of AD? Promising results presented by NIH researchers at the World Congress on Alzheimer's Disease in July 2000 replicated Elan's findings in other APP transgenic mice. One finding indicated that the vaccination prevented memory loss in another transgenic mouse model of AD. Additional research is clearly needed to determine whether these findings in mice will hold true for humans.

A recent study by NIA-funded researchers at Harvard Medical School provided a second approach to using the immune response to remove amyloid plaques. These researchers used the same amyloid as that used in the vaccine but administered it nasally rather than through injections (Weiner et al., 2000). They found that the nasal administration induced an immune response in the transgenic mice that develop extensive amyloid plaques at later ages. When young transgenic mice were given the human beta-amyloid by this route, they had a much lower amyloid burden at middle age than did animals that did not receive the vaccine. Although the nasal administration was not as effective as the original vaccination method, these results open the door to an alternative approach that may be better tolerated long-term than the injected amyloid vaccine.

Removing Amyloid. Other researchers are exploring ways in which brain microglia may destroy amyloid naturally. Certain kinds of scavenging microglia can engulf beta-amyloid, which suggests that they have the potential for clearing amyloid from the AD brain even without being activated by an immune response against amyloid. Scientists at the University of California, Irvine, showed that microglial uptake of beta-amyloid can be reduced by some forms of complement, a component of the inflammation cascade that occurs in the AD brain (Webster et al., 2000). Fibrillar (more damaging) complement-containing plaques may develop as AD progresses. These results suggest that mechanisms that inhibit the inflammatory process may increase the capacity of certain types of microglia to engulf amyloid and may be of therapeutic value (see p. 25 for more on studies focusing on inflammation).

Preventing Beta-amyloid From Forming. One way to prevent amyloid plaque formation is to stop beta-amyloid production. There are many ways of doing this. In one example, scientists at Rockefeller University in New York City built on previous test tube studies indicating that estrogen might reduce the risk of developing AD by lowering beta-amyloid secretion. They showed that treating cultured neurons with estrogen reduced the secretion of beta-amyloid peptides (see p.33 for more on clinical trials involving estrogen). In other studies, testosterone also decreased secretion of beta-amyloid (Gouras et al., 2000). It is not known whether these sex hormones have this effect in the body or whether the effect would be large enough to reduce amyloid accumulation and make a difference in the rate at which AD develops. Scientists are pursuing studies to try and answer these questions as well as exploring a number of other approaches to preventing formation of beta-amyloid.

Breaking Down Beta-amyloid. Another way to prevent amyloid plaque formation is to break beta-amyloid into pieces once it is released from cells and before it has a chance to aggregate into insoluble plaques. Researchers from Harvard Medical School found that an enzyme called "insulin degrading enzyme" can do this in tissue culture (Vekrellis et al., 2000). The enzyme regulated extracellular amyloid levels, suggesting that it might do the same in the brain. Finding ways to increase the activity of this enzyme could conceivably be a therapy for AD.

Another approach that scientists have taken to prevent plaque formation is to develop short peptides called "beta peptide sheet breakers," which inhibit beta-amyloid from forming plaques. A research team at New York University showed that beta peptide sheet breakers also reduce plaque formation when injected into the amyloid plaque-containing brains of rats. The peptide breakers reduced plaque size, neuronal shrinkage, and microglial activity around plaques and broke up amyloid deposits even after they were formed (Sigurdsson et al., 2000).

All of these findings raise a fundamental question: If plaque deposits could be removed from the brain of a person with AD, would that stop the progression of the

disease or even allow the brain to regain some of its lost function? Current and future studies should shed light on these critically important questions.

Presenilins. Scientific interest in the presenilin proteins heightened dramatically when scientists discovered that mutations in the genes that code for presenilin I and presenilin 2 account for approximately 40 percent of cases of familial Alzheimer's disease. The recent discovery that presenilin 1, in fact, may be one of the enzymes that clip APP into beta-amyloid generated much excitement in the scientific world and provides a perfect example of how a genetic finding can lead to other, non-genetic insights into particular cellular pathways that may be important in the early preclinical stages of AD development. Investigators have found that presenilins have a number of other possible functions besides clipping APP. Understanding more about these functions will shed further light on the nature and development of AD, possibly leading to new targets for prevention or treatment strategies.

One of the other ways presenilins may be important is their involvement in cell death pathways. Studies conducted by a number of research teams, including intramural investigators at the NIA, indicate that in a transgenic mouse model, neurons expressing presenilin 1 mutations causing AD in humans are more vulnerable to stress-related cell death. Neurons in these mice can be rescued by treating them with inhibitors of programmed cell death (apoptosis) that work through channels in the cell that transport calcium (Mattson et al., 2000). Normal changes in cellular calcium levels are involved in regulating the activity of many cellular pathways and proper regulation of these levels is essential because abnormal increases in calcium can lead to cell death.

Yet another function suggested for the presenilins is that they are involved in cell-cell communication through maintaining synapses (the tiny gaps between neurons across which neurotransmitters travel). A study conducted by scientists at Mt. Sinai Medical Center in New York showed that presenilin 1 is located at the synapse and that it may be necessary for proper connections between

neurons (Georgakopoulos et al., 1999). Thus, presenilin 1 may be important in the way neurons connect with one another and how they maintain their contacts. Presenilin mutations that cause AD could possibly affect presenilin function at the synaptic connections between brain cells.

These studies have brought us a lot closer to understanding presenilins, but there is a great deal still to be learned. How can these molecules have so many functions? Which function(s) is the one that, when disrupted in persons carrying a presenilin mutation, initiates AD pathology? Knowing the answers to these questions will help investigators better understand the early stages of AD and then find effective therapeutic approaches for its prevention and treatment.

Programmed Cell Death (Apoptosis). AD is characterized by abnormal cell death of vulnerable neurons in regions of the brain that are essential to learning, memory, attention, and judgment. Understanding the mechanisms of cell death in response to different signals will give scientists clues about how to prevent it in AD. An important area of research into the etiology of AD, therefore, is the process of programmed cell death, called apoptosis. Apoptosis is a kind of cell suicide that is important for weeding out unnecessary cells in normal development, maintaining tissues in a continuously healthy state, and targeting cancerous cells in the adult. However, a high level of apoptosis in the adult brain results in irreversible loss of brain function because most neurons are irreplaceable. A family of enzymes called caspases is important in the apoptosis process, and in the past year, several teams of NIA-supported scientists made important advances in the understanding of the possible role that caspases play in AD.

In one series of studies, researchers at Columbia University College of Physicians and Surgeons sought to discover whether the activation of caspases could explain any of the features of AD's progression (Troy et al., 2000). They found that beta-amyloid induced one particular caspase not only to initiate the cell death pathway—to start apoptosis—but also to act as an effector of cell death, in essence becoming the "cell terminator." This study suggests

that gene or drug therapies that target this specific caspase might prevent the cell death that is associated with beta-amyloid and might be a therapeutic approach for AD.

There are several other potential targets for therapies that prevent apoptosis. One of them involves telomerase, an enzyme that maintains chromosome structure. Intramural scientists at the NIA have shown that under certain circumstances, telomerase can block specific apoptotic pathways and in tissue culture can decrease vulnerability to cell death induced by beta-amyloid (Zhu et al., 2000). It remains to be seen whether this also works in the brain. Another possible avenue for preventing cell death may be to mimic the changes in biology that accompany reduced food intake in animal models. NIA intramural researchers have shown that both normal mice and transgenic mice carrying the presenilin 1 mutation were less vulnerable to toxin-induced neuronal cell death when they ate 30–40 percent less food over time. This may occur because oxidative stress is reduced by consuming less food (Zhu et al., 1999).

APOE. Understanding the genetic factors associated with AD is important because through this work scientists will be able to better understand how the disease starts and progresses. Researchers are working to discover not only what gene mutations cause the disease and how they initiate the disease process, but also what risk factor genes might work together to make individuals more susceptible to late-onset disease.

As noted earlier, mutations in three genes—APP, presenilin 1, and presenilin 2—have been identified as causing early-onset disease, and the APOE e4 allele of the APOE gene has been identified as a major risk factor for the more common late-onset disease. For example, in a recent follow-up study of the APOE risk factor alleles in late-onset AD, a University of Washington at Seattle research team showed that having the e4 allele can make a difference of as much as 17 years in the age of onset of the disease (Warwick Daw et al., 2000).

This past year, exciting new data on why APOE e4 is a genetic risk factor for late-onset AD were provided by

scientists at the Washington University School of Medicine in St. Louis (Holtzman et al., 2000). These studies, using transgenic mice, focused on the cellular mechanism involved in APOE e4 function. Previous studies in transgenic mice with a mutated human APP gene suggested that an interaction between APOE and beta-amyloid is somehow linked to beta-amyloid deposition and amyloid plaque formation. In this new study, transgenic mice with the mutated APP were used to create mice in which both of their mouse APOE genes had been removed and replaced either with the human APOE e3 or APOE e4 allele. Mice with the APP mutation and no mouse APOE genes had fewer amyloid deposits and no neuritic plaques. When either of the human APOE genes was present, the pattern of beta-amyloid deposition changed. There was amyloid deposition in the hippocampus but few neuritic plaques until later ages. In mice with the human APOE e4 risk factor gene, there was more amyloid deposited as well as a large increase in the amount of fibrillar beta-amyloid compared to mice with the human APOE e3 gene. Thus, APOE e4 may be critical for the formation of plaques and consequent nerve cell damage and death. These findings could help in developing drugs that could alter the levels of the APOE protein in the brain and consequently prevent the formation of fibrils, inhibit beta-amyloid deposition, and promote the removal of amyloid. This could ultimately slow or prevent the development of AD.

Studies in similar transgenic mouse models by researchers at the University of California at San Francisco showed that different APOE genes had different effects on memory in mice (Raber et al., 2000). A spatial memory test was used to see how well the animals remembered cues from the environment in order to find a hidden platform placed in a water maze. (An example of "spatial memory" in people is remembering where things are around the home, like the bathroom, kitchen, or telephone. AD patients eventually lose this type of memory completely and this is a likely reason why they easily become disoriented.) APOE e3–bearing mice remembered better than the APOE e4 ones. This may help explain why hu-

man APOE e4 carriers are at greater risk of developing AD than are APOE e3 carriers.

By combining several powerful tools—molecular biology, genetics, and mathematical modeling—the University of Washington at Seattle researchers identified four new AD-related regions in the human genome where one out of the several hundred genes in each of these regions may be a risk factor gene for the disease (Warwick Daw et al., 2000). Their calculations showed that these as yet unidentified genes seem to make a contribution to the risk of developing late-onset AD that is at least as important as the contribution of APOE e4. In fact, one of these genes is calculated to make a contribution that is several times greater than the impact of the APOE e4 gene. There is much work still to be done here, for not only do the identities of the four new genes associated with AD need to be established, but scientists must then determine why certain alleles of these genes contribute to the likelihood of a person developing AD.

Additional Genetic Links to AD. Other investigators, working at Harvard and the Massachusetts General Hospital, have continued previous work that identified chromosome 12 as having a region that predisposes toward developing late-onset AD. The precise identity of the gene in the predisposing region is not yet known, but the alpha-2 macroglobulin gene has been identified as one that may confer risk (Tanzi et al., 1999) (see also p.46 for more on this area of research). Other possible nearby sites on chromosome 12 also could contribute to the risk of developing the disease. Research on one of these, identified by a research team at Duke University, indicates that the new site acts independently of APOE to increase the risk of late-onset familial AD and that it may be associated with Lewy body disease, a neurodegenerative disease that has features of both AD and Parkinson's disease (Scott et al., 2000).

Other genetic associations with AD, suggesting new risk factors, have been found in population-based studies. One possibility that has emerged is that specific polymorphisms in genes for pro-inflammatory agents increase

the risk of developing AD. For example, researchers at McClellan MemorialVeterans Affairs Medical Center, Little Rock, Arkansas, and Glasgow University, Glasgow, Scotland, found that one type of polymorphism in the interleukin 1 (IL-1) gene doubles the risk for AD in two separate populations (Nicoll et al., 2000). Investigators caution, however, that many of the genetic associations that have been identified may be spurious, caused by differences in genetic backgrounds and in environment between the control and AD groups rather than being associated with risk of AD.

The fact that families share the same genetic background reduces the possibility that associations of genes with the disease may be related to the genetic differences that exist between unrelated persons. Because of this, scientists have also analyzed data from families, though the statistical methods for detecting risk factor genes in genetically complex diseases such as AD are still evolving. Only as additional populations and families are studied with refined methodologies and as the various research groups share their data will it become clear exactly what genes are risk factor genes for AD.

In a related research area on genetic links to AD and aging, scientists have long been intrigued by the fact that some people retain healthy and vigorous cognitive function into very old age, while others become cognitively impaired Currently, some controversy exists as to whether the risk of developing AD increases even in very old age (in persons over 90 years of age). Data from another study done this year by investigators at the Mt. Sinai School of Medicine have fueled this debate by suggesting that people 90–102 years of age were actually *less likely* to develop late-onset AD than were younger individuals (60–89 years old) (Silverman et al., 1999). While the possibility of non-genetic protective factors cannot be ruled out, this study suggests the existence of genetic factors that protect specific long-lived individuals from becoming cognitively impaired. The hunt for genes that may protect against late-onset AD might specifically target nonagenarians and centarians who have experienced healthy aging.

Protein Aggregation in Other Neurodegenerative Diseases. A common thread in neurodegenerative disorders is the abnormal aggregation of proteins in the brain. Examples of these proteins include amyloid 'in AD; synuclein in Parkinson's disease; prions in prion diseases; huntington in Huntington's disease; BRI in familial British dementia; and *tau* in tauopathies, such as fronto-temporal dementia with Parkinson's disease (FTDP-17), progressive supranuclear palsy, and Pick's disease. Most researchers believe that the protein aggregates formed are toxic and give rise to the multiple brain changes that characterize the different neurodegenerative diseases. If one relationship between these diseases really is abnormal protein aggregation, then discovering ways to prevent aggregation, or the processes set in motion by the aggregation, may halt the disease process. In the last year, researchers made significant advances in understanding abnormal protein aggregation in a number of these diseases.

Tau. One major diagnostic feature of AD is the formation of neurofibrillary tangles in susceptible nerve cells in the brains of persons with AD. Tangles are composed of *tau*-containing paired helical filaments. Since the discovery in 1998 that mutations in the *tau* gene cause FTDP-17, scientists have rapidly initiated experiments to try to understand how changes in the structure of *tau* or how altered levels of specific forms of *tau* could result in the abnormal production of paired helical filaments and death of neurons in this disease. Finding out how changes in *tau* structure cause paired helical filaments and neuron death in FTDP-17 will help scientists to understand the similar process in AD brains.

Two types of transgenic mice have been used to examine how *tau* is involved in this process. One type of mouse, created by scientists at the University of Pennsylvania School of Medicine, overproduced one of the six forms of human *tau* (Ishihara et al., 1999). The mice showed aggregation of *tau* resulting in loss of microtubules in the neurons as well as degeneration of axons. The mice had pathology similar to that seen in FTDP-17 and the amyotrophic lateral sclerosis/Parkinsonism-dementia complex of Guam. These findings suggest that these

neurodegenerative diseases can result from altered ex-pression of normal forms of *tau*.

A second type of transgenic mouse was created with one form of the human *tau* gene containing the most common human mutation causing FTDP-17 (Lewis et al., 2000). Investigators at the Mayo Clinic, Jacksonville, Florida, found that this mouse had problems with walking and other movements and had behavioral deficits. The investigators found a direct relationship between the level of expression of the mutated gene, the number of neurofibrillary tangles, and the age of the mouse. This mouse model confirms the hypothesis that neuron loss can and does result from a mutation of the *tau* gene.

Synuclein. A protein called alpha-synuclein is known to accumulate abnormally in plaques in AD, in Lewy bodies in Lewy body disease and Parkinson's disease, and in a number of other diseases that are collectively called synucleinopathies. A rare form of inherited PD is caused by a mutation in the gene that directs the production of alpha-synuclein, and the mutated alpha-synuclein forms insoluble deposits. This strengthens the hypothesis that abnormal protein deposition is one common thread that links dementing diseases. When abnormal proteins, such as alpha-synuclein, accumulate on the pre-synaptic side of synapses, the chemical information between cells might not be transmitted properly and the circuit might be interrupted.

This year, three model systems have been used to study alpha-synuclein function. Using immature brain cells in a test tube, scientists at the University of Pennsylvania examined the way in which alpha-synuclein is expressed in development and its functions (Murphy et al., 1999). Alpha-synuclein was seen only after interneuronal connections were formed and then only in the pre-synaptic part of the synapse. Alpha-synuclein may, therefore, help regulate function at the synapse, but it is not yet known whether altering the amount of alpha-synuclein or mutating it will change how much neurotransmitter gets from one neuron to the next.

Researchers at the University of California at San Diego worked with a second model for alpha-synuclein.

Transgenic mice overexpressing the protein had problems with motor function similar to those found in Parkinson's disease (Masliah et al., 2000). These findings suggested that increased amounts or accumulation of alpha-synuclein within neurons may play an important role in the development of pathology seen in Parkinson's disease and related disorders such as Lewy body disease.

A third transgenic mouse that overexpresses abnormal forms of one kind of APP was created at the University of California at Los Angeles and researchers looked at altered alpha-synuclein expression in this mouse model of AD. Unlike control mice, these mice had large numbers of nerve processes containing alpha-synuclein, very similar in location to those found in Lewy body disease. This again suggests that there is likely to be a connection between AD, Lewy body disease, and Parkinson's disease (Yang et al., 2000).

Prion Diseases. The family of prion diseases, including Creutzfeldt-Jakob Disease in humans, bovine spongiform encephalopathy ("mad cow disease"), and sheep scrapie, are caused by abnormal folding of a prion protein, which then can infect another animal with the disease. Although AD is not infectious, many parallels exist between the prion diseases and AD, including the fact that both prions and beta-amyloid form amyloid structures in the brain. In a series of experiments, similar to those that inhibited beta-amyloid formation (see p. 16), researchers at New York University Medical Center showed that, as in AD, an amyloid peptide breaker custom-designed for the prion amyloid fibril reversed prion protein aggregation in the test tube, in living cells, and in a mouse infected with sheep scrapie (Soto et al., 2000). These results give investigators possible avenues for future treatments of these invariably fatal dementing diseases.

Familial British Dementia and Associated Disorders. Recent studies have shown that a number of dementias besides AD are associated with genetic defects that result in other kinds of amyloid deposits in the brain. For example, in 1999, scientists at the New York University School of Medicine and the National Hospital for Neurology and Neurosurgery and the Institute of Neurology, both in Lon-

don, identified a defect in the gene called BRI, which is located on chromosome 13 (Vidal et al., 1999). This gene is associated with the development of familial British dementia (FBD), a disease characterized by progressive dementia, paralysis, and loss of balance. It usually occurs around age 4050. Similar to AD, FBD patients have amyloid deposition associated with blood vessels and neurofibrillary tangles. The mutation in the BRI gene causes longer than usual forms of the protein to be made. Part of the new protein is clipped off and deposited in the brain as amyloid plaques, which leads to neuronal dysfunction and dementia. Several teams of scientists are intensively studying the BRI gene. One team, located at the University of Chicago and Rockefeller University, showed that an enzyme called furin and specific processing pathways are involved in the formation of fibrils in FBD (Kim et al., 1999).

This year, the New York University School of Medicine research team identified a close variant of FBD in a small Danish population (Vidal et al., 2000). Severe accumulations of amyloid around blood vessels, cataracts, deafness, loss of balance, and dementia characterize this variant. The genetic defect is a single base mutation in the same gene as FBD. Its presence, too, results in the formation of a longer than usual protein that is clipped to form amyloid. Future studies of animal models that have this specific mutation may be helpful in understanding why the Danish form of the variant has abnormal amyloid deposition that is closely associated with specific blood vessels in the brain. By studying the different amyloid types, the product of distinctly different genes and processing pathways, scientists hope to gain a better understanding of the effects of amyloid in the AD brain.

Possible Therapeutic Approaches for Abnormal Protein Deposition. One approach to therapeutic targets for preventing protein aggregation might be to use members of a large family of proteins that are collectively called chaperones. Members of this family help proteins fold properly and discourage improper folding and abnormal aggregation. By assisting in proper protein folding, chaperones help cells survive in the face of stress insults

that might otherwise kill them. One member of the chaperone family, which so far has only been found in lower organisms, dramatically affects abnormal folding of a prion-like protein in yeast (Lindquist et al., 2000). Researchers at the University of Chicago supported by NIH's National Institute for General Medical Sciences (NIGMS) introduced a chaperone family member called Hsp 104, along with the abnormal protein segment that gives rise to Huntington's disease, into *C. elegans, a* kind of worm that is commonly used in biology research. When expressed alone, the abnormal protein segment aggregated and was toxic to the worm. When it was expressed along with Hsp 104, however, both the aggregation and the toxicity were reduced (Satyal et al., 2000). These observations show that chaperones can affect the abnormal folding of a protein fragment that causes Huntington's disease. Perhaps ways of increasing the production of certain chaperone family members could be another way of limiting deposition of the toxic protein aggregates that are found in many neurodegenerative diseases.

Aging and AD Development. A certain number of changes in the way the brain functions can be expected during normal aging, and investigators are looking at how these processes differ from what occurs during AD. A recent study by intramural scientists at the NIA, for example, showed that in normal healthy aging, there was a loss of cell markers for neuron remodeling (or plasticity). On the other hand, in AD brains there were much more dramatic changes in these markers (Hatanpää et al., 1999). Determining how the brain changes in normal aging and what relevance this has to development of AD is an important area of research.

Another area of active investigation is how to limit the production of free radicals, those highly reactive molecules whose overproduction can injure cells. Brain metabolism requires large amounts of oxygen, which can be converted into damaging free radical molecules. It is now believed that oxidative stress, which occurs when too many free radicals are produced, is a major contributor to the aging process.

Brain aging is an added risk factor for the accumulation of free radicals. With age, the compensatory mechanisms that have evolved to cope with and eliminate free radicals become less efficient. Neurons are particularly vulnerable to attack by free radicals because they have a high metabolism and are low in natural antioxidants. DNA and RNA are prime targets of free radical attack, as are brain lipids and proteins. Part of the heterogeneity of brain aging in different individuals may, in fact, be due to the generalized effect of free radicals on the brain as people age. Several ongoing clinical trials have been designed to determine whether treatment with antioxidants can slow age-related cognitive decline or development of AD (see p. 35 for more details on these clinical trials).

Early Life Events and Other Factors. Another way to look at the cause and development of a disease is to examine it from a population-based perspective. Does the disease occur more or less often in certain racial or ethnic groups? Does it occur more or less often in groups who live in particular environments, follow certain lifestyle patterns, or have particular experiences during their lives?

A number of investigators have conducted these types of epidemiologic studies to learn more about whether and to what extent early life events and other factors have an impact on the development of AD. For example, studies have examined the relationship between level of education and childhood rural residence as possible risk factors for AD in older African Americans (Hall et al., 2000); the association of AD with mother's age at the patient's birth, birth order, number of siblings, and area of residence before age 18 (Moceri et al., 2000); the potential interactions among total serum cholesterol, APOE genotype, and risk of AD in older African Americans (Evans et al., 2000); and head injury as a risk factor for AD (Guo et al., 2000; Plassman et al., 2000). Other studies have examined cognitive function and risk of dementia in a group of several thousand Japanese-American men who have been followed for a period of 36 years (Launer et al., 2000; Petrovitch et al., 2000) and have analyzed data pooled from several large-scale existing studies to determine the as-

sociation of AD risk with family history of dementia, female gender, low levels of education, smoking, and head trauma (Launer et al., 1999).

Several intriguing possibilities have emerged from these studies. For example, investigators in the cholesterol study found that high blood cholesterol was a risk factor for AD in those with no APOE e4 alleles, but not in those with one or two e4 alleles. Blood cholesterol, therefore, may be a potentially modifiable risk factor for AD in some people (Evans et al., 2000). Results from one of the other studies indicated that rural residence in childhood, together with fewer than 6 years of school, was associated with increased AD risk (Hall et al., 2000).

Though epidemiologic findings such as these can be suggestive and interesting, they can be conflicting or incomplete as well, partly because investigators looking at the same issues may use different study methods, but also because of the complexity of the issues and the large number of variables involved. For example, the low educational attainment that emerged as a risk factor in the study mentioned above may actually be a surrogate or marker for other deleterious socioeconomic or environmental influences in childhood. Nevertheless, epidemiologic research is a valuable complement to basic research on AD, and ongoing and future studies show promise for shedding further light on the relationship among AD risk, early life events, and other factors.

Improving Early Diagnosis

The clinical diagnosis of AD has improved significantly in recent years. However, important gaps in knowledge remain, and work continues on the search for reliable, valid, and easily attained ways to identify AD very early in the course of the disease. Early diagnosis is important for a number of reasons. First, to understand the cause(s) of the diseases and how best to intervene at early stages, scientists need to know what is happening in the brain during that time. Tests are also needed that can reliably separate people with Alzheimer's disease from those with cognitive problems that stem from other causes. Finally,

when more effective treatments become available, it will be important to identify people at the very earliest stages of AD so that treatment can be started before brain changes result in cognitive deficits.

The 1999 Progress Report on Alzheimer's Disease described advances in our understanding of the early changes in AD that researchers made as a result of examining the brains of people who had died while still in the very early, preclinical stage of AD. In the last year, researchers continued to make significant progress in several areas related to early diagnosis, including improving neuroimaging techniques, improving the predictive ability of neuropsychological tests, focusing on correlations between clinical signs of possible AD and pathological changes in the brain, and understanding the biological markers of early disease.

Neuroimaging. Researchers at Brigham and Women's Hospital in Boston used magnetic resonance imaging (MRI) measurements to determine whether healthy older persons and persons in the presymptomatic phase of AD could be identified before they developed clinically diagnosed AD (Killiany et al., 2000). MRI scans can image a living person's brain and are used to measure the size of different structures in it. In this study, which was jointly supported by NIA and the National Center for Research Resources, healthy people and those with mild memory difficulty received an MRI scan at the beginning of the study ("at baseline"). Over the next 3 years, the researchers determined which of the participants later met clinical criteria for AD. The investigators looked at differences in brain tissue volume in a number of areas, focusing on the regions involved in memory and executive functions, such as organizing, planning, and switching back and forth among tasks and ideas. The researchers found that they could identify people who would develop AD over time based on measurements of these brain regions. The MRIs were 100 percent accurate in discriminating between the participants who were healthy and those from a third group who already had mild AD. They were 93 percent accurate in discriminating between participants who were

healthy and those who initially had memory impairments and ultimately developed AD. In the case of the people "converting" to AD, one of the regions involved with memory had about 37 percent less volume than that of the individuals who remained healthy, probably reflecting a loss of brain cells. Other comparisons showed a relatively high accuracy rate as well, although it was more difficult to distinguish the people who continued to have memory problems but did not progress to AD from those who eventually converted to AD.

In a second, similar study, conducted at New York University Medical Center, healthy older people and those with mild cognitive impairment received an MRI and a clinical and cognitive evaluation at baseline and follow-up (3.2 years) (Convit et al., 2000). Again, results indicated several specific sites in the brain that may be affected in preclinical AD, and that atrophy in these areas may indicate future AD in these individuals.

Another neuroimaging study, carried out by investigators at the Mayo Clinic Alzheimer's Disease Center/ Alzheimer's Disease Patient Registry in Rochester, Minnesota, focused on demonstrating relationships between certain brain structures, especially the hippocampus, and cognitive function in AD patients and healthy people (Petersen et al., 2000). The scientists found that the volume of the hippocampus (as measured by MRI) predicted performance on most acquisition and recall measures across the spectrum of normal aging and AD.

An additional MRI study, not funded by NIH, contributed important insights into the potential use of imaging to assess disease progression and response to drugs. In this study, conducted by researchers at the National Hospital for Neurology and Neurosurgery in London, England, the rate of brain atrophy was assessed from two MRI scans separated by 12 months in AD patients and healthy individuals (Fox et al., 2000). The second scan was compared with each individual's first scan, and the volume of cerebral tissue loss was calculated based on the difference between the two scans. Results indicated that the mean rate of brain atrophy was 2.4 percent per year for the AD patients and 0.4 percent per year for the controls. This

method can be used to quantify brain atrophy over time and might be a tool to monitor progression of AD in clinical trials over a shorter time course than possible using neuropsychological tests.

Two other studies focused on trying to develop methods for imaging plaques in the living brain. One, conducted by scientists at Duke University Medical Center and involving brain tissue from individuals who had died, used magnetic resonance microscopy to try to distinguish plaque-specific signal from noise (Benveniste et al., 1999). The other, conducted by University of Pennsylvania School of Medicine researchers, used mouse tissue and transgenic mice to explore the potential usefulness of a special type of probe (a radioligand probe) to image plaques (Skovronsky et al., 2000). Both of these studies are early developmental studies that may eventually lead to imaging studies in humans and ways to monitor plaque levels in the brain in response to vaccine or other treatment.

Brain activity may decline before brain atrophy becomes noticeable and both may predict later cognitive decline and dementia. A number of research teams supported by NIA and NIMH have measured brain activity as a predictor of cognitive change (see p. 46 for more on studies supported by NIMH; several of these are co-funded by NIA).

Neuropsychological Testing. In the past year, NIA-supported investigators also have looked at ways to improve standardized tests of memory, language, and other neuropsychological components in hopes of being able to better predict future development of AD.

In one study, scientists at Harvard and the Massachusetts General Hospital examined whether it was possible to identify aspects of the Clinical Dementia Rating (CDR) scale to predict which people with "questionable" AD have a high likelihood of converting to a diagnosis of AD over time (Daly et al., 2000). The CDR is a semi-structured clinical interview that stages AD from 0 (normal) to 0.5 (questionable), 1.0 (mild), 2.0 (moderate), and 3.0 (severe), based on an assessment of six categories of function (memory, orientation, judgment and problem solving,

community affairs, home and hobbies, and personal care). Results of this study indicated that the likelihood of progressing to AD was strongly related to the sum of the individual scores in each category. For example, more than 50 percent of individuals with a total CDR score of 2.0 or higher at baseline developed AD during the 3–year follow-up, whereas only about 10 percent of individuals with a score of 1.0 or lower developed AD during this interval.

A second study also examined whether initial performance on a variety of neuropsychological tests in healthy older people could accurately predict subsequent decline to dementia over a period of nearly 4 years (Kluger et al., 1999). This research team from the New York University School of Medicine found that a small set of neuropsychological measures, especially a paragraph delayed recall test, significantly differentiated those who later developed AD from those who did not. This assessment may be particularly useful in predicting the future cognitive status of older people with mild cognitive impairment.

Clinical-Pathological Correlations. Another important focus for scientists working on the early diagnosis of AD is improving the understanding of the relationship between early pathological damage to the brain and outward clinical signs. For example, the Oregon Brain Aging Study follows healthy individuals without cardiovascular or other diseases of aging who are 85 years or older. Investigators made measurements of senile plaques and neurofibrillary tangles in certain regions of the brain after participants had died and compared these measurements to the individuals' previous clinical status, cognitive measures, and rate of cognitive change (Green et al., 2000). Results indicated that there was an increased burden of these neuropathological markers even in those individuals who had cognitive decline but were not functionally impaired and did not meet diagnostic criteria for dementia. The strong relationship between the cumulative pathological changes and the rate of decline suggests that these markers have clinical consequences and are not just benign indications of aging.

Another study, conducted by scientists at the Rockefeller University and Mt. Sinai School of Medicine, New York, assessed levels of beta-amyloid variants and *tau* in the cortex of subjects with no, questionable, mild, moderate, and severe dementia (Naslund et al., 2000). Results indicated that total levels of two types of amyloid peptides were elevated early in dementia and that these levels were strongly correlated with cognitive decline. Additionally, in the frontal cortex, beta-amyloid was elevated before the occurrence of significant *tau* pathology. These findings support an important role for beta-amyloid in the initial pathological events in AD dementia.

Researchers at the Washington University School of Medicine, St. Louis, Missouri, looked at tangle formation in various stages of aging and AD (Uboga et al., 2000). Results indicated that fibrillar tangles, corresponding to what are generally understood to be classical tangles, increased exponentially with age and severity of AD, whereas diffuse tangles seemed to represent an earlier form of tangles. The density of diffuse tangles peaked around preclinical AD and then decreased in more severe stages of AD.

Markers. Scientists are also trying to establish whether there are biological markers for AD. If so, clinicians could eventually use them to determine whether a person is entering an early, preclinical stage of AD. Thus, treatments could be started earlier, perhaps when they might be more effective.

In one such study, researchers at the University of Washington School of Medicine determined whether changes in a person's sense of smell (using the Cross-Cultural Smell Identification Test) were able to predict cognitive decline over a 2–year period in older Japanese-Americans enrolled in a community-based longitudinal study of memory and aging (Graves et al., 1999). Results indicated that unexplained loss in the sense of smell in the presence of one or more APOE e4 alleles was associated with a high risk of cognitive decline.

In another longitudinal study of older individuals, Columbia University investigators found that plasma levels

of beta-amyloid were higher at the beginning of their study in those who subsequently developed AD than in those who did not (Mayeux et al., 1999). Thus, plasma levels of beta-amyloid may be detected several years before the onset of symptoms.

Developing Drug Treatments

Improvement in the understanding of AD is making it possible for scientists to design new therapeutic strategies to intervene at multiple stages of the disease process. Insights into the neurochemistry and neurobiology of the disease and epidemiologic studies pinpointing risk factors have resulted in a marked expansion of the types and numbers of drugs that are being developed and are now being tested or may be tested in the future. Today, it is estimated that NIA, other NIH Institutes, and a number of pharmaceutical companies are or will be testing 50 to 60 compounds in human trials. They focus on three major aspects of AD: treatments for short-term maintenance of cognitive function in patients with AD; treatments to slow the progress of the disease, delay its onset, or prevent it; and treatments for AD-associated behavioral problems.

Short-term Maintenance of Cognitive Function. One of the most prominent features of AD is that levels of a neurotransmitter called acetylcholine fall sharply in patients. This is important because acetylcholine is crucial in the formation of memories and is used commonly by neurons in the hippocampus and cerebral cortex—regions devastated by AD. This discovery about acetylcholine, which occurred in the mid-1970s, led to many studies on the cells that use acetylcholine and the enzymes and other proteins that take part in its manufacture or activity—a network known as the cholinergic system. This system was the first major system to be targeted for drug intervention. A number of drugs that temporarily maintain the cholinergic system have been developed or are now being tested to treat the cognitive decline experienced by patients with AD. For example, all three of the drugs that have been approved by the FDA—tacrine (Cognex),

donepezil hydrochloride (Aricept), and rivastigmine (Exelon)—act by slowing down the metabolic breakdown of acetylcholine. These agents do not, however, alter the underlying course of the disease.

Slowing, Delaying, or Preventing the Disease. Scientists are working at a stepped-up pace to examine a number of compounds that might delay the onset of AD, slow its progress, or prevent it altogether. These compounds include estrogen, anti-inflammatory agents, antioxidants, and nerve growth factors.

Estrogen. Estrogen, a hormone that is produced by the ovaries during a woman's reproductive years, affects brain regions relevant to memory, such as the hippocampus. A large body of data gathered over the past 25 years in animal studies supports the notion that estrogen has some positive effects on memory function. In epidemiologic studies, estrogen use has been associated with a decreased risk of AD and with enhanced cognitive function. It also has both antioxidant and anti-inflammatory effects and enhances the growth of processes from particular neurons important for memory function. These data have created intense scientific interest in the relationship between estrogen, memory, and cognitive function in humans. In recent years, NIA has supported one AD clinical trial on estrogen in the hope that it might be able to provide evidence on whether estrogen actually affects the progression of AD. This trial, which was conducted through the Alzheimer's Disease Cooperative Study (ADCS) (see p. 43 for a description of the ADCS), was a pilot study of estrogen replacement therapy (ERT), using a commonly prescribed form of estrogen, in postmenopausal women who had had a hysterectomy and who had mild to moderate AD. Results of this study indicated that ERT did not slow progression of AD or improve cognitive or functional outcomes (Mulnard et al., 2000). Even if given for a full year, estrogen was not helpful for these women. It should be noted that the findings apply only to a very specific population of older patients who had had AD for some time.

A closely associated study by researchers at the University of Southern California that was not funded by NIA

provided similar results (Henderson et al., 2000). In this study, postmenopausal women with mild to moderate AD were treated for 16 weeks with 1.25 mg/day of estrogen. At both 4 and 16 weeks there were no significant differences between treatment and placebo groups on measures of cognition or caregiver-rated functional status. Estrogen did not slow the functional decline associated with AD, and did not improve mood or other symptoms of AD.

Another clinical trial, not funded by NIA, examined the effects of estrogen on cognition, mood, and blood flow to the brain in women with mild to moderate AD (Wang et al., 2000). Blood flow is important because nutrients such as glucose and oxygen reach the brain through the blood stream. The better the blood flow, the more likely a person is to have good cognitive function. This study tested the effect of 1.25 mg of estrogen given without any progesterone to women in Taipei, Taiwan, who had a diagnosis of mild to moderate AD. Women were given the hormone for 3 months. Again, there was no beneficial effect, either on blood flow or cognitive decline in these women. When taken with data from clinical trials done in the U.S., the data suggest that the negative estrogen findings might be generalizable to older women with AD who are of different races.

Though these three studies have found that estrogen does not have a beneficial effect on women who already have AD, they do not answer the question of whether normally aging women who take estrogen after menopause will be protected from developing AD or age-related cognitive decline. Separate studies are underway to examine this question. For example, an ongoing study, the Women's Health Initiative Memory Study, is a component added to the NIH's Women's Health Initiative. This component, which is being supported by Wyeth-Ayerst Laboratories, will determine whether hormone replacement therapy decreases the incidence of cognitive decline and dementia in cognitively normal women aged 65 and older (Shumaker et al., 1998). Another element of this study, called the Women's Health Initiative Study of Cognitive Aging, is investigating whether hormone replacement

therapy protects against age-associated memory and cognitive decline.

A similar study on possible preventive measures during normal aging is now in the patient recruitment phase. This multi-site, NIA-supported clinical trial will determine whether the use of estrogen in cognitively normal older women with a family history of AD (and therefore a twofold to threefold increased risk of developing the disease) may prevent the development of AD. Scientists at Columbia University are coordinating this study.

Other clinical studies involving estrogen include a study by intramural NIA researchers who examined the effects of estrogen and progesterone on memory and other cognitive functions in normally aging women as part of the Baltimore Longitudinal Study of Aging. Women taking hormones did better on tests involving verbal learning and memory than women who had never taken them (Maki et al., 2000). NIA intramural researchers also have conducted studies on the effects of estrogen replacement therapy in which PET measurements of cerebral blood flow in brain regions important to learning and memory showed significant improvement after one year of estrogen treatment. This study further supports the beneficial effects of the hormone in normally aging women (Maki et al., 2001).

Anti-Inflammatory Agents. One of the hallmarks of AD is inflammation in the brain, but whether it is a cause or an effect of the disease is not yet known. As the new vaccine work indicates (see p. 15), in some circumstances, inflammation—stepping up the activity of brain microglial cells—might actually help curb amyloid accumulation. However, epidemiologic evidence strongly suggests that anti-inflammatory agents, such as prednisone (a steroid) and NSAIDs, including ibuprofen and indomethacin, are associated with a decreased risk of AD. Recent studies in transgenic mice suggest that an NSAID can limit plaque production in the mouse brain (Lim et al., 2000).

The NIA has supported a study to compare the effects of prednisone versus a placebo (inactive pill) on patients with diagnosed AD to see whether progression of

the disease can be slowed. Results of this study, which was conducted through the ADCS, indicated that there was no difference in cognitive decline between the prednisone and placebo treatment groups (Aisen et al., 2000). Thus, a low-dose regimen of prednisone does not seem to be useful in treating AD.

While results of this study were negative, they point the way to additional research. For example, one trial is testing NSAIDs, as opposed to steroids like prednisone, in clinical trials. An ADCS study with AD patients, which began at the end of 1999, will compare treatment with a traditional NSAID (naproxen), which blocks the activities of both COX-1 and COX-2 enzymes (see inflammation section, p. 25), to treatment with a COX-2 inhibitor (rofecoxib), which is a more specific type of NSAID with pain relieving qualities but without some of NSAIDs' side effects on the gastrointestinal system. A second trial will determine whether NSAIDs might act earlier in the disease process to block the development of AD, as suggested by the epidemiologic studies. The NIA has just funded such a trial to determine if naproxen or another COX-2 inhibitor (celecoxib), can slow or prevent development of AD in cognitively normal elderly persons with a family history of AD.

Antioxidants. Over-production of free radicals can result in oxidative damage to cells (see p. 10 for a more detailed explanation of free radicals). Because free radicals may play a key role in both normal aging and AD, researchers are studying agents that inhibit and protect against oxidative damage. Several experimental studies have shown that these agents, called free radical scavengers or antioxidants, can inhibit the toxic effects of beta-amyloid in tissue culture. Investigators are now exploring whether the scavengers may indeed delay or prevent the disease. Many free radical scavengers are known. These include vitamins E and C, ginkgo biloba, melatonin, flavonoids (chemicals found in many plants, including tomatoes), and carotenoids (chemicals found in plants such as carrots). One free radical scavenger, vitamin E, is now used therapeutically as it staved off progression to certain important endpoints in AD patients by 6 months in an NIA-funded clinical trial. The beneficial effects of estrogen on

AD and on the aging process in healthy women may be partly due to its antioxidant activity.

An NIA-supported clinical trial, the Memory Impairment Study, is testing the ability of two agents—vitamin E and donepezil—to intervene earlier, before signs of memory impairment have developed into clinically diagnosed AD. Investigators want to see whether these agents can delay or prevent the onset of AD. The study is being carried out over a 3–year period in more than 700 people with MCI. People with MCI have memory problems but do not meet the accepted clinical criteria for AD.

Another compound being tested is ginkgo biloba, an extract derived from the leaves of the ginkgo tree. It appears to have antioxidant properties as well as anti-inflammatory and anticoagulant properties. The NIH's National Center for Complementary and Alternative Medicine, along with several other Institutes, is supporting a new clinical trial of this compound to determine whether it can delay or prevent dementia in older individuals (see p. 55 for a description of this study).

Nerve Growth Factor (NGF) and Other Neurotrophic Factors. NGF is the best known of a class of compounds known as neurotrophic factors and has been well studied in animal models for its ability to maintain viability in specific neuronal classes. In one study with aged rhesus monkeys (the best animal model of human aging) researchers at the Salk Institute, La Jolla, California, found that cholinergic neurons in a particular region of the brain exhibited age-related shrinking and loss of the ability to make acetylcholine (Smith et al., 1999). The investigators were able to reverse most of the shrinkage and loss of cholinergic properties in the monkeys by using specialized cells called fibroblasts that were genetically modified to secrete NGF. The fibroblasts were grafted directly into the affected regions in the brain. Based on the results of this study, researchers have now initiated privately-funded human phase I gene therapy clinical trials to test the safety of this grafting procedure.

Treating Behavioral Symptoms. Behavioral symptoms—agitation, aggression, wandering, and sleep disorders—are common in AD patients and can be serious. Physicians

now have several treatments for these symptoms, such as antidepressants, antipsychotic drugs, and sedatives, but researchers continue to search for better treatments, including non-drug approaches for AD patients.

One ADCS clinical trial is focusing on alleviating sleep disturbances, a common problem for AD patients. Nighttime wandering and agitation can result in injury for patients and disrupted sleep for caregivers. In this study, groups of patients were given either a slow-release preparation of melatonin (a naturally occurring hormone that can induce sleepiness), an immediate release preparation of melatonin, or a placebo. This trial has finished and data are being analyzed.

Another ADCS study focused on agitation, a problem affecting 70 to 90 percent of AD patients and one that can make caring for a patient at home very difficult. Drugs are commonly used to control signs of agitation, but they can have distressing side effects. This study involved people with AD who were living in the community (not in a nursing home or other care facility). Participants were randomly assigned to four groups, receiving either non-drug behavior management techniques, medication (haloperidol, an antipsychotic, or trazodone, an antidepressant), or a placebo (Teri et al., 2000). Over a 4–month period, study investigators followed the participants to see which intervention was most effective in reducing irritability, restlessness, pacing, wandering, and physical and verbal abuse of caregivers. The investigators found that about one-third of the study participants in each of the two medication groups and in the behavior modification group showed improvement by the end of the study. However, about one-third of the group that received the placebo also improved over the course of the study, meaning that a portion of the study participants got better regardless of the treatment they received. These results underscore the need for additional research into new treatments that might more effectively relieve this difficult problem for people with AD and their caregivers.

Future Considerations for AD Clinical Research. Scientists engaged in designing and developing clinical trials to test

potential treatments for AD face a number of challenges. One is the need to recruit large numbers of participants—those with diagnosed AD, those earlier in the course of the disease (before clinical diagnosis), and healthy older people—so that the effects of the treatment and its safety and effectiveness at different stages of the disease can be measured with confidence. Close collaboration with existing research and treatment facilities, such as the Alzheimer's Disease Centers and Alzheimer's Disease Cooperative Study sites, helps to ensure a sufficient pool of potential study participants. Recruitment strategies being developed for trials such as the Memory Impairment Study will also help to ensure strong participation.

A second challenge is the need to incorporate into study designs the special characteristics of people with AD. In many respects—cognitively, behaviorally, psychologically, and medically—this patient population is different from patients who participate in clinical trials for other diseases, and a number of research programs are now looking at issues relating to the ethical aspects of research on dementia patients (Earnst et al., 2000; Marson et al., 1999). Because of their dementia, many AD patients at later stages of the disease may not even be fully aware that they are participating in clinical research. Their disease means that family members and other caregivers; need to be included as full partners in the research effort, and extra care must be taken to accommodate AD patients and protect their interests and rights. Institutional Review Boards, which judge the risks and benefits to patients and approve research involving human participants, are an important player in this process for they ensure that adequate protections for the rights of the research participants have been incorporated into the research plan, and they ensure that Federal and State regulations for the protection of human research subjects are being followed.

The discovery of genetic mutations that increase the risk of developing AD has raised an important new ethical issue for clinical research in AD. Although this discovery has brought new promise of predictive testing for the disease, the information yielded by genetic testing for AD and the implications it has for the person tested and for

other family members raises important questions about the use of such tests. Access to genetic information could affect a patient's insurability if disclosed and could affect employment status and legal rights. The National Bioethics Advisory Commission (NBAC) is currently reexamining the Federal regulations to see whether AD patients participating in clinical research need any further protections and to ensure that protection of sensitive information is part of every research plan. (See p. 54 for more information on research in this area conducted by the National Human Genome Research Institute).

Improving Support for Caregivers

Perhaps one of the greatest costs of Alzheimer's disease is the physical and emotional toll on family, caregivers, and friends. As Alzheimer's disease makes inroads into a person's memory and mental skills, it also begins to alter his or her emotions and behaviors. Patients can experience extreme agitation and feelings of anger, frustration, and depression. They can begin to exhibit bizarre behaviors such as pacing, wandering, screaming, and physical or verbal aggression. These changes in a loved one's personality, the need to provide constant, loving attention for years on end, and the physical demands of bathing, dressing, and other caregiving duties are major reasons for caregiver exhaustion and depression and for placing AD patients in nursing homes.

A recent study analyzing data from more than 1,500 caregivers who participated in the 1996 National Caregiver Survey provides details on the physical and other costs of caregiving (Ory et al., 1999). These data show that dementia caregivers spend significantly more time on caregiving tasks than do people caring for those with other types of illnesses. In addition, they report that this type of caregiving has a greater impact in terms of employment complications, caregiver strain, mental and physical health problems, time for leisure and other family members, and family conflict than do other types of caregiving.

Other research shows that the information and prob-

lem-solving needs of caregivers evolve over time as the disease progresses and caregiving issues shift. These findings point to a need for programs and support services tailored to the unique and evolving challenges faced by AD caregivers. This suggestion is supported by a recent study conducted by researchers at Thomas Jefferson University, in Philadelphia. These investigators examined particular characteristics of caregivers that might predict whether they would start and stick with an intervention designed to help them with caregiving (Gitlin et al., 1999). Knowing these characteristics may help in the future to create better ways of assisting family members and other caregivers to care for persons with AD. The researchers found that being older and female predicted participation in the intervention. Those who found it easier to adhere to the intervention were older, were less depressed, and had a less dependent person to care for. Caregivers who reported depressive symptoms were unable to adhere to strategies that involved behavioral change or manipulations to the physical environment, and the authors suggest that these caregivers should be treated for their depression before they are asked to learn environmental modification techniques.

Research focusing on the safety of the home environment as an important component of caring for persons with dementia has recently been reported by this same team from Thomas Jefferson University (Gitlin et al., 2000). Persons with dementia living alone or with a family caregiver must deal with six basic safety concerns: injury from falls, injury from ingesting dangerous substances, leaving the home and getting lost, injury to self or others from sharp objects, fire or burns, and inability to respond rapidly to crisis situations, A wide range of environmental strategies can be introduced to maximize home safety. As everyday competencies decline with memory loss, persons with dementia may have increasing difficulty navigating physical spaces and processing and interpreting environmental cues and stimuli. As a result, care-givers may need periodically to reevaluate the physical safety of the home and introduce new strategies for keeping the home safe.

REACH

In 1995, the NIH established a major five–year initiative to carry out social and behavioral research on interventions designed to help caregivers of patients with AD and related disorders. Resources for Enhancing Alzheimer's Caregiver Health (REACH) is co-sponsored by NIA and the National Institute of Nursing Research (NINR).

Participating researchers are from universities and medical centers around the country:

- University of Alabama and University of Alabama at Birmingham;

- Veterans Affairs Medical Center and University of Tennessee at Memphis;

- Center on Adult Development and Aging at the University of Miami, Florida;

- Veterans Affairs Palo Alto Health Care System and Stanford University, California;

- Center for Collaborative Research at Thomas Jefferson University in Philadelphia, Pennsylvania;

- Hebrew Rehabilitation Center for the Aged and Boston University; and

- University Center for Social and Urban Research at the University of Pittsburgh, Pennsylvania.

REACH projects focus on characterizing and testing the most promising home- and community-based interventions for helping caregivers, particularly in minority families. The interventions include support groups, behavioral skills training programs, family-based interventions, environmental modifications, and computer-based information and communication services. Information about the project is available on the REACH website (www.edc.gsph.pitt.edu/reach/).

A recent study supported by REACH concerns clinical trials recruitment, an issue vital to the continuing success of AD research. Researchers often experience difficulty in recruiting adequate numbers of participants, and this can add to the cost of the study, the time necessary to complete it, and the usefulness of the results. Recruiting older participants and their caregivers, particularly those in minority communities, presents a special set of challenges for AD researchers. Unfortunately, recruitment outcomes are not routinely reported in the literature and the documentation of recruitment costs is rare. This REACH study compared the cost-effectiveness of methods used by the project's Boston site at the Hebrew Rehabilitation Center for the Aged to recruit participants for a study of AD caregivers (Tarlow et al., 2000). The results of this study indicate that a well-planned, multi-pronged, and flexible recruitment plan is the most productive for recruiting AD caregivers. Effective planning includes budgeting for enough personnel, materials, and time to conduct the recruitment process; using realistic estimates of the potential pools; and establishing contingency plans for under-enrollment.

Building a Research Infrastructure

An important component of the success of NIH's AD research effort is its vibrant network of research institutions and investigators who work together, as well as independently, to continue the process of discovery in AD. Building this research infrastructure is an ongoing effort and it ranges from developing a variety of innovative mechanisms for funding research, to sponsoring research conferences on cutting-edge issues, to enhancing the effectiveness with which research is conducted and data gathered, to ensuring that AD research has a broad and comprehensive focus.

Pursuing Innovative Mechanisms for Funding AD Research. Because of the cost, time, and effort involved, relatively few medications or treatment strategies are tested in full-scale clinical trials. However, it is important to provide as

many opportunities as possible to explore the potential of multiple compounds and strategies. NIA has therefore developed a number of mechanisms for funding research aimed at each stage of drug development, from efforts to identify useful drugs, through testing in animals and pilot clinical trials, to full-scale clinical trials. At each step, the NIA is fostering industry participation.

Small Business Innovation Research Grants (SBIRs). SBIRs are grant mechanisms designed to establish the merit and feasibility of ideas that may eventually lead to commercial products or services, and to support in-depth development of those whose feasibility have been established. A number of these grants have been funded in the area of AD research and they provide an important way for small businesses to participate in the research process. They also serve as a bridge between laboratory work and commercial development.

Drug Development Contract. NIA maintains a contract mechanism for funding investigators or small companies who have a potentially interesting candidate AD treatment drug but who lack the means to begin the formal drug testing process. This contract, Investigational New Drug Toxicology for Drugs to Treat Alzheimer's Disease and Other Diseases of Aging, funds a contractor to conduct animal studies to evaluate drugs for toxicity. If the toxicology screening is successful, the data generated are used to file a request to the Food and Drug Administration for approval to carry out initial tests for safety and efficacy in humans. This contract mechanism has already yielded several potentially promising compounds, and applications to test several more drugs are in process.

Pilot Trial and Trial Planning Grants. These grant mechanisms give investigators funding to plan future large multi-site clinical trials and conduct smaller-scale clinical trials aimed at treating AD's cognitive and behavioral symptoms as well as slowing the progression of and ultimately preventing AD. The trials allow investigators to develop recruitment strategies and diagnostic procedures, test drug responses, and generate data necessary to apply for funding of a full-scale clinical trial.

"Add-on"Components to Ongoing Clinical Trials. One effi-
cient way to conduct AD clinical trials is to add a cognitive
or dementia component to an ongoing trial. For example,
an NIA-funded cognitive component has been added to
the Women's Health Study, which is a clinical trial funded
by the National Heart, Lung, and Blood Institute (NHLBI)
in which healthy older women are taking aspirin or the
antioxidant vitamin E to measure possible effects on car-
diovascular disease. A cognitive component has also been
added to a second NHLBI trial, the Women's Antioxidant
Cardiovascular Study, in which older women at high risk
of cardiovascular disease are taking either antioxidants
or a combination of folate and vitamins B6 and B12. In
both of these trials, the add-on studies are testing the ef-
fect of these compounds on age-related cognitive decline.
In an add-on to a third NHLBI-funded study, the
Physician's Health Study 11, researchers will examine
whether the antioxidant supplement or multivitamin the
participants are taking to measure effects on cardiovas-
cular function have an influence on their age-related cog-
nitive changes.

Enhancing the Efficiency and Effectiveness With Which
Research is Conducted

Alzheimer's Disease Centers (ADCs). The ADCs Program
promotes research, training and education, technology
transfer, and multicenter and cooperative studies in AD
and other dementias and normal brain aging. Each of
these centers enrolls and performs longitudinal studies
on AD patients and healthy older people. Autopsy is an
important part of this research effort. Many NIH-sup-
ported research projects depend on the ADC Program
to provide access to patients and biological fluid and
tissue samples. In addition to individual research grants,
several other major initiatives depend on the ADCs,
including the Alzheimer's Disease Cooperative Study,
CHORD (Caregiving, Health Services, and Outcomes Re-
search in Dementia) and the National Alzheimer's Co-
ordinating Center. Many of the Centers have satellite
clinics that target minority, rural, or other under-served

populations in order to expand the numbers of persons from diverse population groups who participate in research protocols and clinical drug trials associated with the parent Center.

National Alzheimer's Coordinating Center. The National Alzheimer's Coordinating Center (NACC), established in 1999, is designed to facilitate research on Alzheimer's disease by providing a facility for the analysis of combined data collected systematically from all of the existing ADCs as well as other sources. Until NIA established the NACC, each ADC collected and stored its own information separately. Now that there is a central data coordinating center, data from all ADCs are being combined, analyzed, and made available to researchers in the ADCs and soon to other qualified scientists in the wider research community. The NACC assembles, maintains, analyzes, and disseminates data; catalogs brain tissue and other biological samples stored at the ADCs; serves as a study design and statistical consulting resource for ADCs; and coordinates semi-annual meetings of the ADCs to discuss progress and planning for shared initiatives. Research activities that use NACC resources are supported by the NACC itself and by the NIH and other Federal and non-Federal sources. Ongoing NACC initiatives include:

- A collaborative study by 16 ADCs that is exploring the possibility of defining neuropsychological characteristics that might predict which of the healthy participants in a study will get AD. Such predictions could dramatically shorten the time needed to conduct prevention trials, decrease the number of study participants needed, and decrease trial costs.

- A study by five ADCs that will enroll up to 500 sibling pairs, one member of whom has AD and the other does not. This will permit the investigators to identify gene differences between the affected and the unaffected siblings as part of a larger effort to find risk factor genes for late-onset AD.

- A study by five ADCs that is collecting data on the clinical expression of Alzheimer's disease in Hispanics of Mexican and Caribbean origin. Hispanics are the fastest growing minority group in the United States and estimates are that they will be the largest minority group by the middle of this century. Data suggest that the onset of AD may be earlier in this population than in others. Better and more complete information on cognitive and behavioral function as well as on demographic, acculturation, and medical variables that might affect the age at which symptoms begin are needed to determine if disease onset is actually earlier in this population and, if so, what factors may contribute to the earlier onset.

Alzheimer's Disease Cooperative Study (ADCS). NIA first funded the ADCS in 1991 to build an organizational structure so that many Centers could cooperate in investigating promising drugs for AD and develop and improve tests for evaluating AD patients in clinical trials. The studies funded by the ADCS are carried out largely through the ADC clinical cores. During the first 5–year grant period, the ADCS began four drug studies and two studies of cognitive impairment assessment tests for Alzheimer's disease clinical trials (one each in English and Spanish). In 1996, NIA funded the ADCS for another 5 years. The new studies include the Memory Impairment Study; the study of two different kinds of anti-inflammatory compounds; the study of melatonin and sleep disorders in AD; and research on divalproex sodium (Depakote), an antiseizure drug, as a possible therapy for agitation and dementia in nursing home residents.

Broadening the Focus of AD Research. NIA also supports some AD research at ten Exploratory Centers on the Demography of Aging. The goal of the Centers is to stimulate innovative and policy-relevant research on the health and economic circumstances of individuals as they age, and on the growing population of older persons in the

United States and worldwide. The Centers have helped to identify and describe some of the most important aging-related trends in the population in the areas of health, longevity, disability, retirement, economic circumstances, and family support. Several of the Centers have projects on the demography or biodemography of AD.

For example, two centers—one at Duke University and one at the University of Michigan—are exploring the potential for integrating survey and biological data to create a substantial new resource for relating individual physiology and genetics on the one hand, with cognition, functional ability, and the progression of illness on the other. At the Duke Center, NIA is supporting a supplement to the National Long-Term Care Survey (NLTCS) to collect biological data (blood and/or saliva) from about 7,500 of the NLTCS participants. These data will help to identify the genotypes that are associated with chronic illness and dementia in a population sample. They will also help to compare the onset and progression of AD among those with different APOE genotypes.

Researchers at the Demography Center at the University of Pennsylvania are exploring the relationships between APOE genotype, AD, and heart disease as they differ across population subgroups through integrating a diversity of data sources. Researchers at the USC-UCLA Demography Center are also exploring the interactive influences of the APOE genotype with social, demographic, biological, and health behavior factors, using new data from the MacArthur Study of Successful Aging. They are comparing the increased risk of cognitive and physical decline from the APOE e4 genotype across populations with different characteristics, including gender, education, exercise , and social environment. In a closely related project, the UCLA researchers are estimating the incidence of cognitive change and cognitive impairment in the United States population as a whole. This analysis will serve as a baseline for understanding future population trends, and the effects on cognition of various interventions, such as drugs for AD, or behavioral change interventions.

Support for AD Research by Other NIH Institutes

National Institute of Neurological Disorders and Stroke (NINDS)

Scientists supported by NINDS conduct studies aimed at increasing our knowledge of the brain processes responsible for a variety of neurodegenerative disorders, including AD. It is well known that cells in the brain die in chronic neurodegenerative diseases. Because the mature brain cannot normally replace lost nerve cells, an important goal of treatment and prevention is to minimize nerve cell death. To do this, we must understand the molecular mechanisms involved in cell death, also called apoptosis.

NINDS-supported scientists are seeking a more complete understanding of the molecular mechanisms that damage cells and trigger apoptosis as well as the metabolic steps that carry out the processes. Such knowledge will provide the foundation upon which to base new therapeutic strategies. (See p. 18 for more on apoptosis research.)

In one study, NINDS-supported investigators at Harvard Medical School reported that the interaction of APP with beta-amyloid can result in neurotoxicity (Lorenzo et al., 2000). Beta-amyloid is found in two forms: fibrillar, which is neurotoxic, and soluble, which is not. The investigators sought to determine the mechanism by which fibrillar beta-amyloid becomes neurotoxic. They found that when soluble beta-amyloid is converted into a fibrillar form, it is much more likely to bind to certain protein receptors on the surface of neurons, including one for APP. Neurons without APP were less vulnerable to beta-amyloid's neurotoxicity than neurons with APP, indicating that the interaction of beta-amyloid with APP (and certain other surface proteins) may mediate beta-amyloid's toxicity. The researchers theorized that this binding may either abnormally inhibit or activate cell systems, leading to toxicity. Beta-amyloid did not interact as readily with a secreted form of APP that is known to protect the brain against a variety of toxic events, as it did with another form called holo-APP. These studies indicate fibril forma-

tion significantly increases the likelihood that beta-amyloid will bind with APP. The study showed that APP may be implicated in neurodegeneration and that beta-amyloid toxicity is at least partially linked to its interaction with APP. Determining the mechanism(s) by which beta-amyloid becomes toxic may one day allow researchers to block its lethal effects and prevent the death of nerve cells.

In a second study, NINDS-supported investigators at the University of Cincinnati reported that neurotoxicity associated with the ApoE peptide may be tied to its fragmentation (Tolar et al., 1999). They placed full-length ApoE, a fragmented form of ApoE, and a synthetic ApoE-related peptide into cultures of chick and rat neurons; all three forms were toxic to the neurons, and the full length ApoE became fragmented. When the scientists added a mixture of protease inhibitors to block the action of enzymes that cut ApoE into fragments, both the neurotoxicity and fragmentation of full-length ApoE were blocked, but the protease inhibitors had little or no effect on the other two forms of ApoE. This suggests that the neurotoxicity seen with the full-length ApoE is related to fragmentation.

The investigators also found that exposure to the ApoE peptide caused a significant influx of calcium into the neurons, causing the cell bodies to swell and their neurites to fragment. Eliminating calcium found outside the cells reduced, but did not eliminate, this effect. Several calcium channel blockers, each of which works using different cell surface receptors, were then tested for their effect on the cells' calcium response. Only MK-801, which works by way of specific neurotransmitter receptors, was able to significantly decrease the cellular influx of calcium and prevent cell death in rat neurons exposed to the ApoE peptide. However, MK-801 did not protect chick neurons against the toxic effects even though it did affect the influx of calcium, indicating that something other than the calcium load itself may be involved in the toxic effects of ApoE. These experiments substantiate the hypothesis that ApoE is involved in neuronal degeneration in AD. Characterizing the neurotoxic effects of ApoE and determining the cellular receptors and systems involved may some day lead to a way to prevent AD.

In a third NINDS-supported study, investigators at McGill University in Canada focused on a group of enzymes, called caspases, that are known to be involved in apoptosis and APP processing (LeBlanc et al., 1999). Their preliminary findings indicate a possible role for caspase-6 in APP metabolism and beta-amyloid production in AD. Using cultures of human neuronal cells, the researchers showed that serum deprivation, which is known to induce apoptosis and consequent changes in APP metabolism, causes caspase-6 to become activated and that, some hours after this activation, the production of beta-amyloid is increased and cells die. Inhibition of caspase-6 prevents an increase of beta-amyloid in serum-deprived neurons. They also determined the process whereby caspase-6 increases production of beta-amyloid. Caspase-6 generates beta-amyloid indirectly by cutting APP into beta-amyloid-forming fragments. This is believed to be the first study to link caspase-6 to neuronal cell death. While additional studies are needed to fully understand the molecular mechanisms by which neurons die, the McGill research suggests that caspase inhibitors may one day be adapted to interrupt or prevent a cascade of processes that lead to neuronal cell death. The McGill team hypothesizes that caspases may cause APP/beta-amyloid processing changes that sicken, but do not kill, neurons. Over time, these damaged neurons would increase the production of beta-amyloid in the brains of people with AD.

National Institute of Mental Health (NIMH)

NIMH supports research on the causes of AD, its clinical course, and treatment and services for patients with AD. In the last year, researchers supported by NIMH have made advances in a number of areas, including the use of advanced imaging techniques, improvements in understanding mental illnesses in both caregivers and patients, and the basic molecular and genetic underpinnings of the disease.

For example, in a study conducted by a team from Massachusetts General Hospital and Harvard Medical

School, researchers showed that the genes for the protease known as beta-secretase map to chromosome 11 (BACE1) and the Down's region of chromosome 21 (BACE2) (Saunders et al., 1999). The beta-secretases cleave the amyloid precursor protein to produce beta-amyloid in the brains of patients with AD and Down's syndrome, and they represent powerful new targets for drugs to treat these disorders. The mapping of their genes has now allowed NIMH-supported scientists to continue to search for Alzheimer gene mutations in these genes using the NIMH Alzheimer family sample, a national resource of clinical data and biomaterials (tissue and DNA samples) collected from individuals with AD, schizophrenia, or bipolar I disorder, This database was established to aid researchers in understanding the genetic bases of these disorders and is known as the NIMH Human Genetics Initiative. This study is important because the BACE genes are candidates for genes whose mutations could cause AD. Inhibiting the proteases that are made by these genes could represent a novel treatment for Alzheimer's disease (See the sidebar on p. 17 for more about these proteases).

Tau and neuronal thread protein (AD7C-NTP) are two proteins that are found at elevated levels in the cerebrospinal fluid of patients with AD. Assays for AD7C-NTP and *tau* are marketed commercially by two separate firms to aid in the diagnosis of AD. However, the relative utility of these two tests in diagnosing AD has never been compared. NIMH-supported scientists at Stanford University compared the ability of AD7C-NTP and *tau* tests to correctly differentiate patients with AD from healthy individuals and from patients with other neurologic disorders such as Parkinson's disease (Kahle et al., 2000). When used together, the tests resulted in an overall diagnostic accuracy for AD of 81 percent. However, this combined diagnostic accuracy was only slightly better than that achieved with either test alone. These results demonstrate that AD7C-NTP and *tau* are both potentially useful markers for AD.

In 1998, in a highly publicized report, a team at Harvard University asserted that a polymorphism in the gene for alpha-2 macroglobulin (A2M) was a major risk factor for

AD. This finding generated a great deal of interest because the A2M protein had been linked in other reports to the basic biological process thought to give rise to AD pathology in the brain. Two studies have carried these findings to the next stage. The first, conducted by scientists at Massachusetts General Hospital and Harvard Medical School, confirmed the previously published association of the A2M gene with late-onset AD (Blacker et al., 1999). The original association and the confirmation were observed using the NIMH Alzheimer sample families. A2M can enhance the clearance of amyloid from the brains of AD patients, and the association of this gene with AD can provide clues about this process as well as ideas for new therapies for treating the disease.

In the second study, investigators at Indiana University working with other researchers from Munich University, Stanford University, and the Eli Lilly Company, tested the association between the A2M mutation and Alzheimer's disease in a sample of almost 600 patients with AD and healthy older people (Dodel et al., 2000). While the A2M mutation was indeed a risk factor in the patient sample, individuals with the A2M mutation had about 1.5 times the risk of AD as did those without the mutation, suggesting that the risk of developing AD is not as great as previously reported. In comparison, APOE e4 increases the risk for AD by about 5 times. These results show that A2M mutations result in a small but significant increase in the risk for AD.

NIMH-supported researchers at UCLA have combined genetic testing with PET scanning to determine brain function in people at risk for AD who still have normal memory function. The researchers found that adults carrying the AD risk factor allele APOE e4 show significant decline in brain function over a 2–year period—without symptoms of the disease (Small et al., 2000). The genetic testing was performed on 54 adults, aged 50–84, with very mild and common age-related memory complaints. Half of the participants carried the APOE e4 allele. Initial PET scans revealed that the group carrying this allele had significantly lower function in the regions of the brain important to memory and learning, compared with the group

without the APOE e4 allele. The researchers then conducted a 2–year follow-up on 20 study participants. The group carrying the APOE e4 allele demonstrated a 5 percent function decline in these same regions of the brain compared with their initial PET scans. Consistent with normal aging, the group without the APOE e4 gene demonstrated decline only in the frontal cortex, where executive functions reside. Results of memory testing, when researchers ask questions to measure forgetfulness and changes in memory, appeared normal for both groups at the beginning of the study and at the 2–year follow-up. The investigators found on follow-up examination that a single copy of the APOE e4 gene was associated with lowered brain function in people with normal memory performance. The UCLA study is the first to report long-term PET results for individuals with the genetic risk for AD who have no disease symptoms. The research team has just announced a new UCLA Memory Clinic for testing treatments to prevent age-related memory loss. Studies will continue research into exploring the use of PET scanning and genetic testing for identifying persons who may be at high risk of developing AD.

Scientists at Good Samaritan Regional Medical Center and Mayo Clinic, Scottsdale, Arizona, the University of Arizona, and Arizona State University used PET to characterize AD-related reductions in brain activity before the onset of symptoms in persons who carry the APOE e4 gene (Reiman et al., 2000). The study focused on determining the power of PET to rapidly test treatments to prevent this disorder. After only 2 years, cognitively healthy, late middle-aged APOE e4 carriers had significant reductions in regional brain activity, and these reductions were significantly greater than those in people who did not have this gene. The scientists estimated that fewer than 30 cognitively healthy APOE e4 carriers would be needed per active and placebo treatment group to test a candidate prevention therapy after only one year and that fewer than 100 cognitively normal APOE e4 carriers would be needed per treatment group to test a candidate prevention therapy after only 2 years. This study leads the way to potential testing of treatments to prevent AD without having to

study thousands of individuals or wait many years to determine whether or when treated individuals develop symptoms.

Behavioral disturbances are very common among patients with Alzheimer's disease and contribute substantially to the morbidity of the illness. These symptoms include delusions (the false beliefs that patients may have), hallucinations, and agitated or aggressive behavior. Scientists supported by NIMH are conducting many studies on aspects of depression and other mental illnesses in AD patients and caregivers. For example, approximately one-half of the AD patients who were participating in a study conducted by researchers from the University of California, San Diego, and University of Iowa developed psychotic symptoms of delusions and hallucinations over a period of 3 years (Paulsen et al., 2000). Such symptoms cause considerable distress for the patients and their caregivers, and frequently result in the decision to place the patients into a nursing home or other care facility. Patients with signs of parkinsonism and more rapid decline in cognitive function were at the highest risk of developing psychotic symptoms. From practical and theoretical viewpoints, understanding the pathophysiology and risk factors of psychotic symptoms is important because of their common association with severe behavioral problems, such as agitation and aggression, that lead to expensive institutionalization. These findings suggest a very high incidence of such symptoms, and possible identification of a subtype of AD characterized by parkinsonism and rapid cognitive decline.

In a study among 307 middle-aged and elderly psychiatric outpatients (a third of whom had AD dementia with psychosis or severe agitation that was being treated with relatively low doses of medications), scientists at the University of California, San Diego (UCSD), and the VA San Diego Healthcare System (Jeste et al., 1999a) found that 22 to 37 percent developed tardive dyskinesia. Tardive dyskinesia is a serious movement disorder that tends to persist and may even be irreversible in some patients. This incidence is much higher than that reported for younger adults with schizophrenia (4 to 5 percent per year).

The high risk of tardive dyskinesia in AD patients treated with rather low doses of typical medications has clinical implications for the use of these drugs, which are among the most commonly prescribed agents for this problem in this population.

In a second study, the UCSD/VA research team found that the risk of tardive dyskinesia was significantly (about sixfold) lower with the atypical antipsychotic drug, risperidone, compared to the typical drug, haloperidol, in 122 middle-aged and elderly outpatients (21 percent of whom had AD dementia, with psychosis or severe agitation treated for 9 months) (Jeste et al., 1999b). The marked difference in the risk of tardive dyskinesia with typical versus atypical antipsychotics suggests that the latter may be preferred in the treatment of elderly AD patients with psychosis or severe agitation who are at a high risk of tardive dyskinesia.

Neuropsychiatric symptoms are known to play an important role in patient distress, caregiver burden, nursing home placement decisions, and health care costs of patients with AD. Unfortunately, the factors that contribute to the expression of these symptoms are not well understood. Why some patients develop certain symptoms and others do not is not clear. A group of investigators at the UCLA School of Medicine used 18F fluorodeoxyglucose (FDG) PET to examine the metabolic activity in critical brain regions in patients with AD (Sultzer, 1999; Sultzer, 1996). This noninvasive technique can reveal specific areas of brain dysfunction in living patients. Recent results from this UCLA laboratory demonstrate that delusions are associated with dysfunction in specific brain regions: the anterior cingulate and areas of the prefrontal cortex. These parts of the brain are important in the ability to compare internal ideas with previous experience and external reality. Additional results indicate that dysfunction in other specific areas of the brain are associated with depression and agitated behavior in AD. These findings demonstrate that behavioral symptoms of AD are a fundamental expression of specific changes in brain region function, rather than random consequences of global brain disease. Current activities by this research group include examining how dysfunction in specific regions of the brain may pre-

dict whether patients with AD respond to treatment for cognitive deficits or behavioral symptoms. They are also examining whether regional brain function improves in association with successful treatment.

Recent advances in basic science research have provided clues to mechanisms by which genetic vulnerability, aging, and other factors lead to the neuropathologic changes of AD. At the other end of the AD research spectrum, clinical trials have shown that pharmacologic interventions can help treat the symptoms of the disorder. Unfortunately, treatment may not always be helpful, and psychotropic medications used to treat behavioral disturbances in the elderly often have adverse effects. These investigators are continuing research on ways to clarify why specific symptoms occur, which can facilitate development of more targeted and effective treatments. By examining critical brain mechanisms involved in the expression of clinical symptoms, these investigators are conducting "intermediary" work that provides a bridge between basic science and clinical research. This work has the potential for identifying neurobiologic markers that predict response to treatment. Such work allows researchers an opportunity to translate and apply our new understanding of basic mechanisms in AD to the clinical care of patients and development of more effective treatments.

Finally, as part of a project entitled the Sources and Mediators of Alzheimer's Caregiver Stress, NIMH-supported researchers at the University of Maryland are examining the impact of caregiving on the lives of adult children who provide assistance to a parent impaired by AD. These scientists are exploring the effects of caregiving on health by observing its disruptive and stressful consequences for roles, relationships, and activities outside of actual caregiving, such as social and leisure life, relationships with children or spouses, or conflicts between the simultaneous fulfillment of job and caregiving responsibilities. Because of the progressive character of AD, it often happens that increasing caregiving involvement eventually comes to exert these kinds of consequences. The resultant negative health effects, in turn, may lead to an increased use of the medical care system.

National Institute of Nursing Research (NINR)

NINR supports research on biobehavioral aspects of AD and related dementias. The primary focus of current studies is on behavioral, physical, and functional problems such as wandering, agitation and aggression, and maintaining activities of daily living.

Family members are usually the primary caregivers for people with dementia. An NINR-supported study conducted by researchers at the University of Texas, Houston, involved a controlled trial of an intervention to ease the burdens of family caregivers who are living with relatives with dementia (Ostwald et al., 1999). Although many family members willingly assume the caregiving role, they often discover that it imposes particular burdens. For example, caregiving has been linked to substance abuse, isolation, family stress, and depression. This study randomly assigned pairs of caregivers and relatives with dementia to either a treatment or a control group. Caregivers in the treatment group participated in seven weekly 2–hour sessions that taught them about dementia, developed their practical skills, increased their confidence and coping abilities, and improved communication with their affected family members. Meanwhile, the relatives with dementia participated in daycare activities that gave clinicians opportunities to assess their disruptive behaviors. At 5 months, researchers collected data from this group of caregivers and relatives as well as from the control group, all of whose members were awaiting treatment, Even though approximately 3 months had elapsed since the first group had completed treatment, these caregivers reported fewer burdens and negative reactions to disruptive behaviors than did caregivers in the untreated control group. This difference was particularly striking in light of the fact that the two groups of relatives with dementia showed no difference in numbers of disruptive behaviors and had suffered similar declines in cognitive functioning over the course of the study. This study demonstrates that a short-term intervention can reduce caregivers' burdens and negative reactions to disruptive behaviors displayed by family members with dementia. These findings suggest

that reducing the burdens of caregiving can delay the institutionalization of patients with dementia. Ongoing studies will investigate this possibility.

In a second NINR-supported study, researchers from the University of Michigan examined a training program to assist nurse aides in detecting agitation and aggression in patients with dementia. Episodes of agitation become increasingly frequent as dementia progresses (Whall et al., 1999). Agitation that goes unnoticed and untreated can lead to acute episodes of aggression during which patients may injure themselves or others. Aggression in dementia is a major problem, causing individuals to be placed in nursing homes, nursing staff members to burn out, and nursing homes to use physical and pharmaceutical restraints. Estimates suggest that as many as one-half of demented patients exhibit aggression. Nurses' aides provide the majority of care in nursing homes, and consequently, they are usually in the best position to detect early signs of agitation in demented patients. However, nurses' aides are sometimes unable to provide accurate observations of patients' behavior. In this study, nurses' aides were taught in two sessions that totaled just 50 minutes how to recognize and rate degrees of agitation. Because patients' shower baths often elicit agitation, the study focused on this routine event. Nurses' aides and nurse experts rated patients' agitation during their shower baths. After the training, the nurses' aides agreed with the nurse experts more than 90 percent of the time about the signs of agitation that they were seeing. This kind of assessment and early detection can be crucial in preventing agitation from escalating to physical aggression. With relatively little training, nurses' aides can recognize the first signs of agitation in nursing home patients with dementia.

National Institute on Alcohol Abuse and Alcoholism (NIAAA)

Dementia in older adults can be caused by a number of factors, including AD, vascular disease, or alcohol. In fact, alcohol-related dementia is frequently misdiagnosed as AD and is a significant cause of dementia in the United States. Because alcohol-related dementia is thought to be

at least partly reversible with abstinence, its prognosis is very different from that for AD. Establishing criteria for differential diagnoses of these two dementias thus has major practical and public health implications. NIAAA-supported researchers are concerned with establishing criteria for differential diagnoses.

NIAAA-funded investigators at the University of Pittsburgh School of Medicine compared elderly alcoholics with dementia with AD patients in an effort to distinguish the two conditions from the standpoint of types of cognitive impairment and long-term outcome (Saxton et al., 1999). The investigators found evidence that these are two quite distinct conditions. For example, disorientation in space and time is an early problem encountered by all patients with AD, whereas it was observed in a milder form in only 3 out of 10 patients with alcohol-related dementia. In contrast with AD, where memory impairment typically is profound, memory impairment in alcoholics with dementia appears to be mild and slowly progressive and verbal intelligence and language skills are preserved. Common deficits in alcoholics with dementia include impaired abstraction, poor short-term memory, and visuospatial difficulties, whereas patients with AD do not manifest the same degree of visuospatial impairment but do manifest profound memory loss that involves loss of both recognition and recall as well as word-finding deficits. The investigators caution that these findings are based on a study of only 10 alcoholics with dementia and 10 AD patients. A much larger study is needed to establish and validate diagnostic criteria to distinguish the two types of dementia. The ultimate outcome of this project will be to provide diagnostic criteria for distinguishing AD from alcohol-related dementia, This will have both practical and medical significance for the development of treatment plans and interventions that target the two different groups.

National Institute of Environmental Health Sciences (NIEHS)

Scientists supported by NIEHS are examining the ways in which metals and other compounds found in the envi-

ronment may affect brain tissues and possibly contribute to the development of AD.

Inflammatory processes are considered to be a critical factor at various stages in the development of AD, and the microglial cells involved may play both positive and negative roles in the process (see p. 25 for more detail on inflammation). The research of one NIEHS intramural laboratory is directed toward understanding the role that microglia play in neurodegeneration and the mechanisms involved in microglia regulation. Such understanding may lead to the development of a treatment to minimize progression of the disease following acute trauma to the brain. In one series of studies, the investigators found that early activation of microglial cells may not be associated with an injury response. However, the presence of microglia appears to be required in the cascade of events, and the lack of a prominent microglial response can lead to an exacerbation of the neuronal damage (Bruccoleri et al., 2000; Bruccoleri et al, 1999; Harry et al., 2000). The microglial response was altered in the APOE transgenic mouse model for AD. However, the response was dependent upon the age of the animals. The importance of these observations in AD research is that previous efforts toward a therapeutic intervention have been focused on minimizing the microglial response. This work demonstrates that always reducing the microglial response does not necessarily lead to a positive outcome and that the basal level of the pro-inflammatory cytokines needs to be taken into consideration before such intervention is attempted (see the sections on inflammation and vaccine development on pp. 25 and 15 for more about the microglial response).

Another area of investigation by NIEHS-supported researchers has been the role of substances called growth factors and inhibitors. Scientists at the University of Utah are studying the structural properties of a protein known as human neuronal growth inhibitory factor (GIF) (Faller et al., 1999). GIF inhibits survival and growth of cultured neurons and has been reported to be decreased in the postmortem brains of patients with AD. GIF is a member

of a class of metallothionein proteins that bind and regulate metal ions within cells. In comparing the structural features of GIF with other metalloproteins, the research team found evidence for increased conformational flexibility of both domains of the GIF protein, a property desirable for receptor-ligand interactions and metal exchange. The increased conformational flexibility of GIF may underlie its function as an inhibitory growth factor, as less flexible metallothionein proteins do not share this biological activity. Because AD is a neurodegenerative disease characterized by the buildup of plaques, there has been great interest in understanding how these plaques are formed. Knowing more about the GIF protein may help investigators to understand the details of plaque formation.

In recent publications, scientists at the University of California, Irvine, have described alterations in key neural proteins following treatment with aluminum compounds. In one study, levels of beta-amyloid and ubiquitin were increased in mouse neural cell cultures after aluminum administration (Campbell et al., 2000). These two proteins have been found in increased amounts in the pathological lesions of AD. The data suggest that aluminum might play a role in AD by promoting the formation of neuronal beta-amyloid and ubiquitin. Aluminum has long been suspected to play a role in several neurological diseases associated with aging including AD, but the link has never been unequivocally identified. Possible mechanisms include enhancement of iron-induced free radical formation by aluminum and changes in protein structure and function.

In another report from the University of California, Irvine, researchers describe depressions of glial fibrillary acidic protein (GFAP) after injections of aluminum lactate were given to laboratory rats (Guo-Ross et al., 1999). GFAP is a component of the protein structure or cytoskeleton of astrocytes that are found in the brain. Normally, astrocytes mount a vigorous inflammatory response to brain injuries, which includes increased levels of GFAP. The reported depression of GFAP levels may reflect impairment of astrocyte function and suggests that these cells may be the primary targets for aluminum neurotoxicity. The

results of this study may help to elucidate the role of aluminum in neurodegenerative diseases like AD.

National Institute of Child Health and Human Development (NICHD)

NICHD supports research related to AD primarily through its programs involving neurobiology and mental retardation and developmental disabilities. NICHD supported advances in basic neurobiology stem from the Institute's efforts to understand the processes underlying both normal and abnormal human development. These advances are helping researchers understand how brain functions, such as memory and thought processing, are established in early embryonic development. They hope this knowledge will ultimately lead to drugs that can address the gradual loss of memory in patients with AD. For example, scientists have traditionally believed that the number of brain cells was established at birth and unresponsive to signals outside the cell later in life, leaving little hope for efforts to replace old or damaged brain cells. However, the recent identification of neural "precursor" cells and persistent cell development in the mature brain raises the possibility that the number of neurons in certain regions of an individual's brain is actively maintained throughout life, rather than being diminished over time.

Scientists sponsored by NICHD have found that a protein in the body, basic fibroblast growth factor (bFGF), regulates nerve cell growth in the brain of newborn rats by crossing the blood-brain barrier to stimulate nerve cell division (Wagner et al., 1999). These researchers also discovered that the effects of bFGF were not restricted to the perinatal period, but also stimulated brain cell growth in older animals, indicating that cells continue to be responsive to bFGF later in life. In adult animals, peripheral bFGF increased cell division threefold in the forebrain and areas related to the sense of smell, indicating that bFGF regulates ongoing generation of nerve cells by a unique pathway, potentially providing new approaches for treating damaged brain cells during

development and into adulthood. Researchers also found that the peripheral injection of small doses of bFGF increased the proportion of early nerve cells and stimulated the growth of new nerve cells in the neonatal rat brain. The existence of a biological pathway transporting growth factors to the nervous system has potential implications for developing treatments for brain damage associated with neurodegenerative conditions, such as AD, in addition to congenital conditions and acquired brain disease.

National Human Genome Research Institute (NHGRI)

The NHGRI supports research aimed at carrying out the Human Genome Project, an international research program designed to construct detailed genetic and physical maps of the human genome and develop a resource of detailed information about the structure, organization, and function of human DNA. Another major component of NHGRI's mission is to analyze the ethical, legal, and social implications of the genetic information uncovered through research and to develop policy options for public consideration.

Now that it is known that an allele of a risk factor gene (APOE) is linked to late-onset AD, controversy has surrounded the issue of presymptomatic testing for this disorder. NHGRI-supported investigators at Johns Hopkins University are developing a survey to determine how the public, particularly those at increased risk, understand the current information regarding inheritance of AD risk and the role of other risk factors. In addition, they will study physician understanding and interest in genetic testing for AD. Expectations of the genetic testing process will be compared between the public and physicians. Participants will include adult offspring of previously studied AD patients and physicians who treat these patients. The study focuses on knowledge of AD genetic risk and attitudes toward presymptomatic genetic testing, where false negatives and false positives are a well-known feature. Focus groups of physicians and high-risk offspring will be conducted to explore the

educational needs of these populations. This research will help in the development of effective methods to integrate genetic testing for AD into health care.

In a second NHGRI-supported project, Georgia State University Memory Assessment researchers will examine the factors that influence an individual's choice to obtain susceptibility genotyping for AD and what the consequences of that information would be. Clinicians will assess the benefits and risks of providing this information to adult children of people with AD. Determination of APOE status will be used in a format that parallels likely clinical usage and will permit the development of guidelines for clinicians for genetic testing, risk assessment, and appropriate counseling scenarios. Because of its inherent uncertainties, APOE determination and counseling is an ideal model to develop new guidelines for whether and how best to use susceptibility gene markers in this and other diseases where such markers are or will be available in the future. This research will examine how genetic testing for AD is perceived by physicians and patients at high risk. It will also aid in the development of guidelines for the effective integration of genetic testing for AD.

A third NHGRI project, conducted by researchers at Massachusetts General Hospital and the University of Alabama, addresses the ethical, legal, and social implications of AD genetics from the critical perspective of a group at high risk for the disease: currently unaffected relatives of individuals with AD. The investigators have been working together since 1990 as part of the NIMH Human Genetics Initiative to identify families with AD. Nearly 350 such families, predominantly affected sibling pairs and over 300 of their unaffected siblings, will be included in the study, which will examine knowledge, attitudes, and behavior related to genetic testing in the unaffected individuals in these AD families and their primary care physicians. Researchers will develop educational materials for genetic testing for AD for individuals at high risk and examine attitudes toward such testing on the part of physicians and these high-risk individuals.

National Center for Complementary and Alternative Medicine (NCCAM)

NCCAM conducts and supports basic and clinical research and research training on complementary and alternative medicine (CAM) and develops other programs to further the investigation and application of CAM treatments that show promise. The Center is currently pursuing several projects related to AD. In one, conducted at Oregon State University, Corvallis, researchers will use a recently developed transgenic mouse model of AD to investigate whether oxidative stress plays a role in the deposition within the brain of beta-amyloid plaques and the brain's reaction to deposited beta-amyloid. Oxidative stress is known to be a feature of aging, the major risk factor for AD. Matched transgenic and wild-type mice will be studied at various ages that are relevant to plaque deposition. Treatment with antioxidants, vitamin E, and ginkgo biloba extract (GBE) will be compared to treatment with an oxidative stressor and with no treatment to assess the degree of plaque deposition. This project may elucidate possible causes of AD as well as suggest therapeutic interventions. (See p. 10 for more on oxidative stress and p. 35 for details on clinical trials in this area.)

A second project conducted by NCCAM-supported researchers at the Oregon Health Sciences University, Portland, is a randomized, placebo-controlled, double-blind study of the effect of standardized GBE on preventing or delaying cognitive decline in people age 85 years or older (the oldest old). The study focuses on this population because of their high risk for developing MCI, which can be a precursor to dementia. Approximately 200 elderly cognitively healthy people will be enrolled and followed to see whether researchers can detect conversion to MCI. The study will ascertain whether GBE has a disease-modifying effect on the brain and will assess the antioxidant effects of GBE and the magnitude of the biological effect of the treatment using volumetric quantitative MRI. Older individuals who exhibit general cognitive decline are at a higher risk of developing AD. Preventing this cognitive decline may slow conversion to AD.

Another study of the effects of ginkgo biloba is under way, jointly supported by NCCAM, NIA, NHLBI, and NINDS. This is a multi-center, randomized, double-blind, placebo-controlled trial to determine the effect of 240mg/day of ginkgo biloba in decreasing the incidence of dementia in general and AD specifically. The participants will be aged 75 years and older. Secondary outcomes will be changes in cognitive function, incidence of cardiovascular disease, and total mortality. Because there is no approved therapy for preventing AD, if ginkgo biloba extract is found effective, individuals at high risk of developing dementia will have an inexpensive and safe prevention option. However, if the extract is ineffective as a prevention agent, these data will provide important information to consumers and allow for informed decision-making concerning continued use of this botanical. The study is also one of the largest primary prevention trials of AD. As such, valuable information will be obtained on the progression of AD in a healthy population.

Outlook for the Future

In the last 25 years, scientists have produced an extraordinary body of research findings on AD. Many of these findings have defined the genetic and biologic changes that underlie AD and offer possible targets for treatment. Researchers have identified drugs and other agents that could potentially counteract the pathologic changes that occur in AD and are testing many in clinical trials. They have made gains in defining people at high risk of developing AD. As methodologies are refined, scientists and clinicians will be able to investigate and understand the very earliest pathological and clinical signs of AD—perhaps 10 to 20 years before an actual clinical diagnosis is made. A variety of approaches also have been applied to improve methods of providing quality care for AD patients, reduce caregiver burden, and decrease the need for institutionalization. In seeking to understand AD, investigators are also defining normal aging. Research is beginning to shed light on healthy cognition and how to minimize normal, age-related cognitive decline.

Federal support of AD research has been the foundation of many of these breakthroughs in our understanding of the disease. This funding also has helped establish an infrastructure that will continue to facilitate research advances. Novel grant award mechanisms, such as the Alzheimer's Disease Centers Program, the Leadership and Excellence in Alzheimer's Disease award, and the National Alzheimer's Coordinating Center, have attracted distinguished scientists to AD research, promoted interdisciplinary research collaborations, enhanced coordination of research data from multiple studies, developed patient examination facilities and biologic resources that are necessary for research on the disease, and enabled patient outreach efforts.

These advances have made it possible for the NIH to launch two new initiatives that build on current activities and give a new focus to future work. The first initiative, the NIH Alzheimer's Disease Prevention Initiative, is designed to expedite the progress toward finding effective medications and other approaches to delaying or preventing the onset of Alzheimer's disease. In collaboration with other Federal agencies and the private sector, this initiative is moving forward on several fronts simultaneously:

- fostering new approaches to basic biologic and epidemiologic research;

- increasing the focus on drug discovery and development;

- improving methods to identify early those people who are at increased risk of developing AD;

- facilitating movement of possible new treatments into the clinic for testing in clinical trials; and

- actively pursuing research into drug and non-drug strategies for treating behavioral disturbances in AD patients.

Candidate interventions for AD prevention have been identified. These include estrogen-like compounds, anti-

inflammatory agents, and antioxidants, as well as drugs that target cell death, the accumulation of abnormal insoluble molecules like plaques and tangles, and other harmful processes involved in AD. The evidence upon which these interventions are based was largely unknown only a few years ago, and the pace of discovery is accelerating. The AD Prevention Initiative will stimulate laboratory and clinical research in these areas.

Some of the clinical trials that are part of the AD Prevention Initiative are already underway, and many more are planned. For example, the first NIH clinical trial aimed at preventing or delaying the onset of clinically diagnosed AD in persons at risk—the Memory Impairment Study— was launched'in March 1999, and a major prevention trial of two anti-inflammatory drugs has been started. Other trials will be added to already ongoing trials that are investigating treatments or prevention strategies for other conditions. This 11 piggy-backing" approach will produce results much more swiftly and cost-effectively than will newly initiated, freestanding studies.

The second initiative, called the President's Initiative on Alzheimer's Disease, was announced by President Clinton on July 16, 2000. In this effort, the NIH will set aside $50 million over the next 5 years to support new research on AD. The NIH will be soliciting applications to support meritorious research, including both basic research as a part of pre-clinical studies and clinical interventions to treat or prevent AD by targeting the production of disease-associated processes, such as formation of amyloid plaques and neurofibrillary tangles. A major component of the President's Initiative will be efforts to address promising immunological strategies to prevent amyloid deposition.

Importantly for those who now have the disease, NIH also is intensifying its AD research and information efforts on issues related to supporting patients and the family members, friends, and providers who care for them. These efforts will include a special emphasis on the needs of a diverse patient population.

A defining aspect of these AD initiatives is collaboration among NIH Institutes and with other Federal agen-

cies, private pharmaceutical companies, and other entities in the private sector, such as foundations. The major funders of AD research—the National Institute on Aging, the National Institute of Neurological Disorders and Stroke, the National Institute of Mental Health, and the National Institute of Nursing Research—make up the NIH AD Working Group that will coordinate and direct these efforts. Other NIH institutes that fund AD research also will be closely involved.

For several years, NIA staff have worked with other Federal agencies, including the Health Care Financing Administration, the Department of Veterans Affairs, the Food and Drug Administration, and the Centers for Disease Control and Prevention on various areas related to AD. These areas include developing data sets for research purposes, collaborating in research, developing appropriate standards for testing drug efficacy, and pursuing outreach and education efforts. NIH will continue this collaboration, as well as efforts to develop relationships with State and local agencies, so that effective AD prevention and treatment strategies can be successfully carried out in the community.

The NIH also will continue to cooperate with pharmaceutical companies in basic research, drug development, and testing and, in particular, will continue to encourage small companies to apply for drug development grants. As part of this effort, NIH will continue to identify partners for collaboration and to encourage its grantees to build collaborative research relationships with the private sector.

Last, but by no means least, the NIH will continue to work closely with voluntary organizations such as the Alzheimer's Association. One example of this partnership is NIH/Alzheimer's Association co-sponsorship of conferences on different aspects of AD research. The Alzheimer's Association also collaborates in research, education, and outreach at the local and national levels with Alzheimer's Disease Centers, NIH-supported AD investigators, and the NIA's ADEAR Center. The Institute for the Study of Aging, Inc., (ISOA) a non-profit organization recently established primarily to facilitate development

and testing of effective drugs for AD, co-sponsored with NIA a workshop on maintaining cognitive vitality, and future joint initiatives are being discussed.

These multifaceted collaborative initiatives, which combine an accelerated search for causes, an assault on the effects of the disease, and vigorous efforts to prevent onset, will energize the fight against AD and bring us closer to the day when we will be able to prevent or even cure this terrible disease, which robs our older relatives and friends of their most precious faculty—their minds.

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