NIA Workshop on Inflammation, Inflammatory Mediators, and Aging

Bethesda, Maryland
September 1–2, 2004

WORKSHOP SUMMARY

National Institute on Aging
National Institutes of Health
Department of Health and Human Services

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Table of Contents

NATIONAL INSTITUTE ON AGING WORKSHOP ON INFLAMMATION, INFLAMMATORY MEDIATORS, AND AGING

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EXECUTIVE SUMMARY

Inflammatory processes, particularly those mediating chronic inflammation, have been implicated as predictors or initiators of, or contributors to, chronic diseases and conditions of aging including cardiovascular disease, osteoarthritis, osteoporosis, Alzheimer's disease, insulin resistance and diabetes, muscle wasting, and frailty. However, the underlying biology connecting mediators of inflammation with these various disease processes is unclear. Altered cytokine profiles due to aging of the innate immune system and/or of non-immune cell types, and/or to age-related in body composition and other factors, have been proposed to contribute to age-related changes in the structure and function of tissues, pathophysiologic changes, and the development of chronic diseases of aging. Inflammatory cytokines and other mediators of inflammation can also be strong near-term predictors of mortality associated with age-related chronic diseases.

The National Institute on Aging (NIA) held a multidisciplinary Workshop on Inflammation, Inflammatory Mediators, and Aging on September 1-2, 2004 (1) to explore the current state of knowledge on the role of inflammation on aging and in the development of many chronic diseases of aging, (2) identify gaps in understanding, and (3) pinpoint opportunities for future research. The Workshop was jointly organized by the Geriatrics and Clinical Gerontology Program, the Biology of Aging Program, the Neuroscience and Neuropsychology of Aging Program, and the Intramural Research Program. The workshop participants included basic research scientists, clinical researchers, epidemiologists, and investigators in geriatrics, immunology, coagulation, cardiovascular and periodontal diseases, adiposity, musculoskeletal (osteoporosis, osteoarthritis) disorders, Alzheimer's disease, psychiatry and stress responses. State-of-the-art presentations focused on the basic biology of inflammatory mechanisms, integrative physiology, mediators of inflammation as risk factors in morbidity and mortality, and implications of interventions that modulate cytokine action. Topics included a review of age-related changes in innate and adaptive immunity, the relationships between mediators of inflammation and various age-related chronic diseases, and functional overlaps between inflammation and metabolic regulation. Other presentations highlighted inflammatory processes relating to periodontal disease, stress and depression, the protective effect of exercise and/or caloric restriction, and latent infections. A final presentation provided an overview of the lessons learned from medical intervention studies. This document -- "NIA Workshop on Inflammation, Inflammatory Mediators and Aging: Workshop Summary" provides synopses of the various presentations and related discussion sessions.

Inflammation has been classically described as an acute process characterized by heat, pain, swelling, and redness. Yet inflammation involves a wide variety of processes or mechanisms

including those related to traditional inflammatory reactions, immunity, complement activation, and coagulation, as well as some processes previously described as metabolic or endocrine, such as those involved in glucose and cholesterol metabolism. These processes are indicated or detectable through examination of biomarkers, including cytokines and acute-phase proteins. Recent research suggests that the expression of these markers can indicate underlying physiological responses in a graded and continuous manner.

The effect of aging on immunity and inflammatory processes is not well understood. Age-related changes in the levels of many pro- and anti-inflammatory cytokines have been documented, but the cause or source of this altered cytokine production has not been clearly identified. The inflammation hypothesis of aging proposes that acute inflammation, which involves a number of feedback mechanisms regulating homeostasis, confers necessary short-term benefits at the expense of accumulating long-term damage. In this scenario, structural damage accumulates from repeated episodes of acute inflammation until a threshold is crossed and function (altered production, metabolism, etc. of acute phase reactants) is affected. Thus, the injury itself may be less of a problem than the body's response to that injury.

One proposed mechanism for aging-related changes in inflammatory responses involves changes in responses mediated by T helper cells – a shift from a TH1 cytokine response, which promotes cellular immunity, to a TH2 cytokine response, which promotes humoral immunity. Another possible hypothesis is based on a change in the frequency or function of immune system cell subtypes. Although several studies have reported age-related changes in the function of T cells and B cells, it is not known how monocytes/macrophages, dendritic cells, neutrophils, natural killer (NK) cells, and NKT cells are affected. Furthermore, it is not clear whether there are intrinsic factors leading to age-dependent changes in these cell types or whether the aged tissue environment produces factors that contribute to those changes.

In addition to the contributions from the immune system, recent studies in obesity have identified adipose tissue as a dynamic endocrine tissue that secretes a number of factors that contribute to systemic and vascular inflammation. Studies on the role of adiposity in insulin resistance and type II diabetes have revealed abnormal inflammatory cytokine production in adipocytes, supporting the hypothesis of an inflammatory basis for these metabolic diseases. Other factors that change with age, such as physical activity, have been associated with differing levels of inflammatory mediators.

Although strong correlations have been shown between inflammatory markers and independent risk factors for disease, the associations between these markers and direct measures of disease are moderate at best. Whether inflammatory mediators represent independent risk factors for disease or simply mark the underlying process is not known. What is known is that adverse age-related health outcomes usually occur later in life, but the biology leading to these events begins much earlier. The key, then, is to identify earlier phenomena, to determine the overall effects of modulating the activity of inflammatory mediators such as cytokines, and to use this knowledge to develop targeted interventions.

Future Research

To date, most studies have focused on inflammatory markers in the context of individual processes or diseases. For example, much of what is known about C-reactive protein has been derived from studies of cardiovascular disease. Future research must account for the interconnectedness of the systems in which inflammatory mediators are involved. For example, research identifying genetic polymorphisms associated with age-related inflammatory changes and chronic disease is necessary but needs to be informed by further knowledge of the interactions among these polymorphisms, behavior, and the environment. Workshop participants identified a number of specific research needs.

Research Areas

Future research should address the following areas:

- **The impact of age on inflammation and immunity**. Existing knowledge on the impact of age on the production of cytokines and other effector molecules by cells of the innate immune system is limited. New research also is needed on the causes and effects of modest (sub-acute) age-related increases in specific inflammatory mediators.
- **Mediators of inflammation as risk factors or risk markers for disease.** An important question is whether altered levels of mediators of inflammation cause disease, are risk factors for disease, or simply mark disease processes. It is not clear how these mediators are related to other risk factors, or which markers are most important. The measurement of multiple markers may prove useful.
- **Inflammation and primary aging changes.** A central questions is how to distinguish primary aging changes in levels or activity of inflammatory mediators from secondary changes due to clinical or subclinical pathophysiology
- **Sources and mechanisms of cytokine production.** The initial triggers for cytokine production have not been identified. The role of systemic levels of cytokines should be distinguished from the role of local or tissue level production by macrophages and other tissues.
- **Effects of cytokines on other tissues, such as brain, liver, and arteries.** Cytokines are known to have a modulating role in the function of several tissues and changes in cytokine levels and profiles may well affect how aging tissues function.
- **The impact of age and subclinical disease on established cytokine-outcome associations.** Age-related differences in cytokine-outcome associations have not been characterized. It is not clear whether age-related effects on these associations are direct or the result of coexisting conditions. Nor is it clear whether cytokine-outcome associations are directly affected by disease, mediated by disease, or both.
- **Predisposition to disease and inflammatory changes.** Genetic factors predisposing people to greater or lesser age-related changes in inflammatory processes and to chronic disease must be identified. Characterization of these factors, in terms of their interactions with environmental and behavioral factors, is also a priority.
- **Adiposity.** Further study is needed on the role of adiposity in inflammation and chronic disease. In particular, the effects of total adiposity and the distribution of adipose tissue on cytokine levels need to be better understood.

- **Effects of cytokines on musculoskeletal processes.** The effects of cytokines on muscle strength, power, mass, and remodeling, as well as on bone mineral density and remodeling, need greater attention.
- **Viral latency.** The correlations between lifetime viral exposures and latent viral loads and later health events are not known. Whether virus-related events are independent of systemic increases in inflammation or related to these increases should be examined.
- **Oxidative stress.** Oxidative stress has been suggested as a contributor to inflammation and age-related chronic disease processes like atherosclerosis. However, it is currently not feasible to measure oxidative damage, let alone the effects of accumulation of oxidative damage on inflammatory processes.
- **Drugs and interventions modulating cytokine production.** How cytokine levels could be modulated safely is not known. It also is not clear whether modulating cytokines translates into improved health outcomes, nor is it clear that the health effects of current interventions are explained by modulated inflammation. Studies on the effects of long-term use of drugs that modulate inflammation are needed to fill important gaps in knowledge. There is a possible risk of inflammatory hyperreactivity when these drugs are stopped.
- **Pleiotropic effects of beneficial drugs.** The underlying biology of the pleiotropic effects exhibited by beneficial drugs is poorly understood. Understanding the biology connecting inflammation with disease processes will guide a finer targeting of therapies.

Study Design

Further understanding of the connections among aging, inflammation, and chronic disease will require the use of imaging to identify better phenotypes, and longitudinal changes in phenotypes with aging, as well as improved genetic methods. It is also crucial to develop laboratory methods that are accurate, reproducible, suitable for use on easily attainable samples, and interpretable by a wide range of biologists working in the field. Studies should include parameters related to known risk factors such as age, sex, race and ethnicity, smoking, and obesity. Stronger and clearer associations are needed between mediators of inflammation, phenotypes of subclinical disease, and health outcomes. Researchers must also find ways to address the increasing phenotypic heterogeneity that occurs with age and the lengthy interval between the initiation of the disease process and the onset of symptoms. Sufficient analytical power depends on sufficiently large study populations reflective of increasingly diverse population characteristics. Longitudinal studies may be particularly challenging, but many of the present surrogate markers for underlying disease processes are weak and/or inconsistent.

Consensus also is needed on the parameters defining cytokinemia versus those defining inflammation. New methods are needed for measuring circulating cytokine levels, tissue cytokine levels, and stimulated production, as recent studies have suggested that measuring circulating cytokine levels may not provide an accurate picture of the relationship between disease processes and local cytokine levels. Proteomic assays, similar to those used to diagnose some cancers, are needed to assess age-related changes in protein expression. An updated compendium on animal models, such as that produced by the Federation of Aging Research, is needed to assist investigators in model and age selection. A Web-based database offering information about different models, organ systems, and expertise is needed to foster collaboration.

Resources

The NIA has a colony of aged rats and may establish a colony of aged, knockout mice. Several population-based studies, offering a variety of measurements and well-characterized phenotypes, have been conducted by NIA's Intramural and Extramural Research Program, and investigators focused on animal models may refer to these studies for similar phenotypes.

In addition, the NIA Geriatrics and Clinical Gerontology Program has released two RFAs.

- *Developing Interventions for Multiple Morbidities* (application receipt date: January 13, 2005), with the ultimate goal of improving health outcomes related to interactions among multiple, co-occurring diseases or conditions in elderly patients.
- *Inflammation, Inflammatory Changes and Aging* (application receipt date: September 14, 2005) seeks applications evaluating age-related changes on inflammatory processes and the effects of such changes in inflammatory processes on the progression of physiologic and pathologic aging changes.

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LIST OF ACRONYMS

ACD	anemia of chronic disease
AFAR	American Federation for Aging Research
ARIC	Atherosclerosis Risk in Communities
AU	anemia unexplained
BDNF	brain-derived neurotrophic factor
CF	cystic fibrosis
CHD	coronary heart disease
CHF	congestive heart failure
CMV	cytomegalovirus
CNS	central nervous system
CR	caloric restriction
CRP	C-reactive protein
CVD	cardiovascular disease
DC	dendritic cells
E. coli	*Escherichia coli*
EBV	Epstein-Barr virus
ELISA	enzyme linked immunosorbent assay
EPO	erythropoietin
ER	estrogen receptor
FITC	fluorescein isothiocyanate
GFAP	glial fibrillary acid protein
HERS	Heart and Estrogen/Progestin Replacement Study
HPA	hypothalamic-pituitary-adrenal
HT	hormone therapy
IBD	inflammatory bowel disease
IDA	iron deficiency anemia
IFN-γ	interferon gamma
IGF-1	insulin-like growth factor 1
IgG	immunoglobulin G
IκB	inhibitor of nuclear factor kappa B
IKK	inhibitor of kappa kinase
IL	interleukin
IMT	intima-medial wall thickness
IRS-1	insulin receptor substrate 1

JAK	Janus kinase
JNK	c-Jun N-terminal kinase
LDL	low-density lipoprotein (cholesterol)
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinase
MBHS	Michigan Bone Health Study
mCSF	macrophage colony stimulating factor
MESA	Multi-ethnic Study of Atherosclerosis
MRI	magnetic resonance imaging
NFκB	nuclear factor kappa B
NK	natural killer (cells)
NKT	NK T (cells)
NSAID	non-steroidal anti-inflammatory drug
OA	osteoarthritis
OAK	OA of the knee
OPG	osteoprotegrin
OPG-Fc-FITC	FITC-conjugated osteoprotegerin-Fc
PAI-1	plasminogen activator inhibitor type 1
PAMP	pathogen-associated molecular patterns
PBMC	peripheral blood mononuclear cells
PIC	pro-inflammatory cytokines
PRR	pattern-recognition receptors
RA	rheumatoid arthritis
RANKL	receptor activator of NFκB ligand
STAT	signal transducers and activators of transcription
SWAN	Study of Women's Health Across the Nation
TGF-β	transforming growth factor beta
TH	T helper (cell)
TIR	Toll/IL-1 receptor (domain)
TLR	Toll-like receptor (proteins)
TNF-α	tumor necrosis factor alpha
TZD	thiazoledinedione
VA-HIT	Veterans Affairs High Density Lipoprotein Cholesterol Intervention Trial
WBC	white blood cell
WHI	Women's Health Initiative

NATIONAL INSTITUTE ON AGING WORKSHOP ON INFLAMMATION, INFLAMMATORY MEDIATORS, AND AGING: WORKSHOP SUMMARY

September 1–2, 2004

INTRODUCTION

The National Institute on Aging (NIA) held a multidisciplinary Workshop on Inflammation, Inflammatory Mediators, and Aging on September 1-2, 2004 (1) to explore the current state of knowledge on the role of inflammation on aging and in the development of many chronic diseases of aging, (2) identify gaps in understanding, and (3) pinpoint opportunities for future research. The Workshop was jointly organized by the Biology of Aging Program, the Geriatrics and Clinical Gerontology Program, the Neuroscience and Neurophysiology of Aging Program, and the Intramural Research Program. State-of-the-art presentations focused on the basic biology of inflammatory mechanisms, integrative physiology, mediators of inflammation as risk factors in morbidity and mortality, and implications of interventions that modulate cytokine action. Topics included a review of age-related changes in innate and adaptive immunity, the relationships between mediators of inflammation and various age-related chronic diseases, and functional overlaps between inflammation and metabolic regulation. Other presentations highlighted inflammatory processes relating to periodontal disease, stress and depression, the protective effect of exercise and/or caloric restriction, and latent infections. A final presentation provided an overview of the lessons learned from medical intervention studies. A summary of the presentations and discussions from the September 1–2 workshop follows. The hard work and enthusiasm of the participants are gratefully acknowledged. This document -- "NIA Workshop on Inflammation, Inflammatory Mediators and Aging: Workshop Summary" provides synopses of the various presentations and related discussion sessions.

Until recently inflammation has been described as a single, acute process involving the immune system and characterized by heat, pain, swelling, and redness. Yet recent data have shown that inflammation can also comprise a chronic process, albeit operating at lower than acute levels, as demonstrated by the enhanced expression of several mediators such as interleukins 1 and 6 (IL-1, IL-6) and tumor necrosis factor alpha (TNF-α) with aging. Inflammatory processes, particularly those mediating chronic inflammation, have been implicated as predictors or initiators of, or contributors to, chronic diseases and conditions of aging. Although most studies demonstrating associations with inflammation have focused primarily on cardiovascular disease (CVD), recent investigations have shown links between inflammation and osteoarthritis, osteoporosis, Alzheimer's disease, muscle wasting and frailty, cancer, insulin resistance and diabetes, and rheumatoid arthritis (RA) – diseases or conditions which are primarily associated with aging. Recent studies also have shown inflammatory changes associated with aging. Studies on the role of adiposity in insulin resistance and type II diabetes have revealed abnormal inflammatory cytokine production in adipocytes suggesting that obesity may exert strong effects on inflammatory responses and the risk for developing these chronic diseases.

Although the literature provides evidence connecting inflammation or inflammatory mediators and aging, with chronic disease(s), most of these studies are correlative. Because the direct effects of aging on inflammatory responses and disease physiology are poorly understood, it is not surprising that a direct causal role of inflammation in the diseases of aging has yet to be demonstrated. It has been hypothesized that acute inflammation, which involves a number of feedback mechanisms regulating homeostasis, confers necessary short-term benefits at the expense of long-term damage and that aging represents the long-term accumulation of damage from repeated episodes of acute inflammation. Aging also may involve a gradual shift from a pathway favoring cellular immunity to one favoring humoral immunity, as evidenced by reduced T-cell function. Or the inflammatory response may become dysregulated with age, and altered cytokine production therefore may mark non-inflammatory processes.

The classic paradigm of inflammation has given way to a view in which traditional inflammatory processes, such as those involved in immunity, complement activation, and coagulation, are interconnected with processes previously described as metabolic or endocrine. Because of these interconnections, it has been difficult to establish whether inflammatory mediators directly cause disease or whether they simply mark the underlying pathology. Importantly, the effects of age on the immune system are not well understood, which further hampers identification of the source of cytokine production associated with aging or chronic disease processes. Further understanding of the role of inflammation and inflammatory mediators on aging and disease will facilitate the identification of early events in disease pathophysiology and, ultimately, the development of more effective targeted therapies.

INFLAMMATION AND AGING: A BIOLOGIST'S PERSPECTIVE

Russell Tracy, Ph.D., University of Vermont

Inflammation was first linked to aging through its association with vascular disease, but this association is not a new story. Almost 150 years ago, Rudolf Virchow wrote, in *Cellular Pathology as Based upon Physiological and Pathological History*, that "inflammation of the inner arterial coat [is] the starting point of the so-called atheromatous degeneration" (reviewed in Nieto 1998). In 1976, in the *New England Journal of Medicine*, Ross and Glomset stated that atherosclerosis was a response to injury. Now researchers are beginning to understand what was observed 150 years ago: that inflammation is a large part of disease, and that the response to injury is as much of an issue, if not more so, than the injury itself.

Inflammation has been classically characterized by the cardinal signs of heat, pain, swelling, and redness. But inflammation also operates at a much lower level than described by the classic acute definition, and it involves a wide variety of connected processes. Inflammation can be summarized by plasma biomarkers (acute-phase proteins) such as C-reactive protein (CRP), that represent underlying physiology in a graded and continuous, although not always linear, manner. For example, CRP concentrations above 10 mg/L usually represent acute inflammation, but even in "normal" people, the CRP distribution carries pathophysiological meaning. Each of these plasma biomarkers may or may not participate in the causal pathway in the particular inflammatory process of interest. For example, an anticoagulant biomarker such as protein C may have more of an association with coagulation than with inflammation, but that does not necessarily mean that this anticoagulant is not involved in inflammation at all, or in the CVD

process in general. Overall, this marks a shift from the old view of inflammation (either present or absent) to the new view (always "present" but to varying degrees).

Using CRP as representative of inflammation plasma biomarkers, what we know about this protein comes primarily from studies of CVD outcomes, particularly myocardial infarction. However, several population studies also have demonstrated associations between high levels of CRP and CVD mortality, stroke, and coronary heart disease (CHD). The correlation between CRP concentration and the risk for CVD appears to begin early in life (Ford 2003, Jarvisalo et al. 2002), and may be strongly affected by exercise and weight loss (Heilbronn et al. 2001). CRP is related to a number of risk factors for heart disease, including ethnicity, gender, age, hypertension, glucose tolerance, obesity or adiposity, insulin sensitivity, cigarette smoking, and coagulation activity. The associations between CRP and metabolic components such as glucose tolerance or insulin sensitivity are particularly strong.

However, the association between plasma CRP concentration and direct measures of atherosclerosis, such as intima-medial wall thickness (IMT) and coronary calcification, are relatively weak. Thus, inflammatory markers may be correlated strongly with risk factors for disease, but only moderately with the direct measures of the disease these markers are thought to represent. Furthermore, for the risk factor cigarette smoking, correlates have been demonstrated in various populations, but they are not additive in otherwise healthy individuals. It is possible that inflammatory mediators are governed in one way when people are healthy, but when a threshold is reached a regulatory switch occurs, resulting in a sharp increase in inflammatory mediator levels. The underlying biology of the connections between inflammatory responses and chronic disease need to be elucidated. However, the various and complex interconnected relationships between inflammatory markers and measures of chronic disease make it difficult to develop epidemiological models.

An important feature of our "new" view of inflammation is that there are many faces to inflammation and its connections to CVD. Inflammation involves systemic markers (often liver proteins) such as CRP and fibrinogen, paracrine/endocrine local markers such as IL-6 and TNFα, and cell-cell interaction markers such as E-selectin and intercellular adhesion molecule-1, all of which participate in traditional components of inflammation, such as innate and adaptive immunity, coagulation, and complement activation, as well as processes previously described as metabolic or endocrine, such as glucose or cholesterol metabolism.

Another feature is that, along with long-term prediction of CVD events such as myocardial infarction, inflammation biomarkers strongly predict near-term mortality associated with CVD in the elderly, as observed in the Cardiovascular Health Study. However, these biomarkers are not specific to CVD. They are also strongly predictive of near-term mortality associated with age-related chronic diseases such as diabetes, some cancers, pulmonary disease, renal disease, cognitive decline, and frailty. Importantly, a marker such as CRP and a subclinical disease such as atherosclerosis can be used as a "model system" to explore mechanisms and interventions. Vascular cell biology is a complex, longitudinal process, and CRP is, in part, a measure of the innate immune activity that clears oxidized low-density lipoprotein (LDL) cholesterol from the system. CRP and oxidative stress markers co-localize to atherosclerosis plaques, suggesting that

the body's response to oxidized LDL, possibly along with the oxidized LDL itself, poses a long-term problem.

One way to describe human biology is as a collection of metabolic "capacities," such as abilities to use glucose, fight infection, or respond to oxidative stress. The capacities associated with inflammation provide the interface between the individual and the environment, and interactions between these capacities and environmental exposures produce results that provide short-term benefit, but long-term damage. Feedback of this "damage" appears to generate an exponential "rate of decay" as people age. Thus, humans may age exponentially (rapid onset of frailty and death is an example), and this may most likely occur because of feedback loops among organ systems and among homeostatic mechanisms such as inflammation. If true, any paradigms that treat the study of aging and disease in a linear fashion therefore should be discarded.

One dimension of this interface between capacities and environmental exposures involves adaptive immunity. Tracy reported that weekly doses of IL-6 increased the burden of atherosclerosis in mice, suggesting that IL-6 may amplify T cell differentiation into T helper 1 (TH1) cells, which promote cellular immunity, and TH2 cells, which promote humoral immunity (Huber et al. 1999). Tracy further has shown that TH1 cells promote murine atherosclerosis but that TH2 cells do not, indicating a causal role for IL-6, most likely in accelerating adaptive immunity in the form of TH1 cells. It is not clear whether there is in humans an "immune deviation potential" inherent to TH1 cells that can predict susceptibility to atherosclerosis and other forms of CVD. An ancillary study in the Multi-Ethnic Study of Atherosclerosis (MESA) is under way to answer these questions.

All of this can be summarized by an inflammation hypothesis of aging, in which inflammation, as part of our daily interaction with our environment, causes long-term damage. The greater the response to environmental stress, or the more robust that person's responses are, the more long-term damage is done. Human beings therefore trade short-term benefit for long-term damage. This hypothesis is supported somewhat by the evolutionary biology concept of antagonistic pleiotropy, in which genetic variance, which is beneficial under one set of circumstances, becomes detrimental under another. Age represents a novel environment, and little is known about gene by age interactions. More genetic characterization of aging is needed.

For future research, the nature of the questions being asked will become more important as the complexity of the biology underlying the connections among aging, inflammation, and chronic disease is elucidated. As an example, public health questions, which involve risk-modeling, will use "age" as a covariate to summarize the biology, since "age" is an easily obtained variable containing significant information about risk. On the other hand, population biology questions should not use "age" as a covariate, since this would hide this biology. Future approaches should focus on imaging techniques, such as magnetic resonance imaging (MRI), functional MRI, positron emission tomography, and others, to define better phenotypes; the identification of biomarker-based longitudinal phenotypes, which reveal trajectories rather than cross-sectional snapshots; better genetic approaches involving larger populations, more specific populations, and familial studies; and the study of the organization between molecular and organismal biology.

Discussion

Discussion Leader: Lewis Kuller, M.D., Dr.P.H., University of Pittsburgh

How we study the interrelatedness of inflammation, aging, and chronic disease will depend on the use of proven laboratory methods that are reproducible and accurate and measurements of parameters in easily attainable samples (such as blood), that can be interpreted by "naïve biologists," and are related to known risk factors such as age, race and ethnicity, smoking, obesity, education, and sex. It is unclear whether the inflammatory markers correlated with chronic diseases reflect disease processes or simply measure systemic or local responses to the disease. It is also unclear whether inflammatory markers play a direct role in the pathogenesis of these diseases. If inflammation is involved directly in disease pathogenesis, we must determine whether the inflammatory process is primary or secondary.

Comparisons are needed to identify associations between markers and outcomes that are consistent across different populations and by age, race and ethnicity, and gender within populations. Associations consistent over time should be examined for short-term versus long-term effects and for consistency within the "incubation period" of disease. Specific cytokines or receptors related to diseases of interest, as well as the agents that stimulate increased production of these cytokines and receptors, should be identified. The ability of unique host susceptibility to predict disease should be determined, and associations between genetic polymorphisms and changes in inflammatory marker levels should be established. Therapeutic agents should be tested to determine whether they alter the levels of inflammatory markers as well as modify disease risk. Inflammatory markers such as CRP should be further studied to determine whether they have unique characteristics that affect specific diseases. Finally, animal models consistent with human epidemiology and clinical studies should be developed or improved.

It is unclear how much of the differences in current data and related observations depend on the specific tests, assays, and methods of evaluation used. For example, how different are the evaluations conducted in other places or populations? Inflammation has been studied in various populations around the world, and genetic variations and differences have been observed. However, most of these studies have been done in separate laboratories; for true comparisons, the data must be collected and analyzed more systematically. Two dimensions drive health outcomes: the existence of the perturbation, and the ability to mount a response. It may be that differences are noted across populations because of differences in perturbation, genetic architectures, or environments.

Further studies are needed to determine whether the increased comorbidities that occur with age result from a system overwhelmed by inflammation or the inability of anti-inflammatory responses to compensate. Pregnancy may have anti-inflammatory responses that are useful to model, because these responses have had to evolve for successful sexual reproduction. The danger of losing blood through damage or rupture to the placenta is so great that markers related to the coagulation pathway are extremely elevated. The high concentrations of markers plummet after birth. However, the advantages of the pregnancy model may be outweighed by its complexity.

The source of the inflammatory markers associated with aging or disease remains to be determined. The liver is a major source of cytokine production, but macrophages and adipocytes

also produce these markers. Future studies of the connections among inflammation, chronic disease, and aging should explore where the relevant cytokines are produced.

BASIC SCIENCE

Session Leaders: Elizabeth J. Kovacs, Ph.D., Loyola University Chicago, and Rebecca Fuldner, Ph.D., National Institute on Aging

The presentations and discussion in this section of the agenda provided participants with a basic foundation for further discussion on the roles of cytokines, innate and adaptive immunity, and obesity/adiposity in aging.

Overview of the Biology of Cytokines and Aging

Joost Oppenheim, M.D., National Cancer Institute

Cytokines are low-molecular-weight polypeptides used for cellular communication. They play roles similar to hormones, acting both locally and at a distance, but unlike hormones, they are not readily detectable in the serum. Kuhns and colleagues (1995) administered endotoxin to normal volunteers and then assayed their serum levels for cytokines. Some cytokine levels rose, but only transiently, and others remained stable, suggesting that most cytokine function takes place in the tissue, not the serum. Cytokines perform a variety of functions, including cell growth, differentiation, and death; the induction of non-responsiveness to other cytokines and cells; and the induction of the secretion of other cytokines. Cytokines act through autocrine, paracrine, and endocrine pathways, but their paracrine function is most notable. Several cytokines have been found in synovial fluid taken from patients with rheumatoid arthritis (RA), but these cytokines are not represented in the circulation, again pointing to cytokine function in the tissue rather than the serum.

Unlike other intracellular messengers such as hormones, cytokines are produced by cells as part of normal cellular activity and/or in response to environmental stimulus. Upon production they bind to receptors on the cell surface, inducing signal transduction pathways that initiate protein synthesis of immune-response markers. Cytokine receptors fall into seven superfamilies: type I cytokine receptors, which interact with such molecules as IL-2; type II cytokine receptors, which interact with such molecules as interferon gamma (IFN-γ); TNF receptors, activated by tumor necrosis factors; Toll/IL-1 receptors, activated by Toll-like receptor proteins 1 through 9 (TLR 1-9) or IL-1; receptor tyrosine kinases, activated by colony-stimulating factor 1; transforming growth factor beta (TGF-β) receptors; and chemokine receptors, activated by chemokines, β-defensins, and other mimetics.

Cytokine-mediated signaling is complex. Cytokines can induce themselves or regulate other cytokines and receptors. Combinations of cytokine function can become additive, inhibitory, synergistic, or cooperative. Cytokine effects may be influenced by other extracellular messengers, specific inhibitors such as soluble receptors and autoantibodies, and nonspecific inhibitors such as fungal products, hormones, and other cytokines. In addition, many cytokines perform redundant functions. For example, TNFα, IL-1, and IL-6 act in separate pathways, but they all participate in T- and B-lymphoctye activation and hepatocytic acute-phase response. Inhibiting or blocking one of these cytokine results in decreases in overall inflammation. Despite the fact that all the cytokines have their own specific functions and also show considerable

redundancies in activities, antibodies against single cytokines can have dramatic effects because cytokine in vivo effects are interdependent. For example, antibodies against TNF, which participates in acute immunoglobulin G (IgG) immune complexes in rats, induce pulmonary inflammation. This underscores the participation of cytokines in a network and their cascading effect.

Inflammation involves a cascade in which tissue injury stimulates cells to make pro-inflammatory cytokines, which in turn directly or indirectly stimulate hepatocytes to produce acute-phase proteins and inflammatory markers. At the cellular level, the generation of inflammatory products activates adjacent stroma and endothelium, eliciting chemokines and initiating the accumulation of inflammatory cells. If inflammation is severe enough, cytokines are produced in the plasma, thereby activating the release of cortisol, which participates in a feedback mechanism. T-cell- and macrophage-derived cytokines are involved in both innate and adaptive immune responses. In the TH1 response pathway, dendritic cells (DC) stimulate lymphocytes to produce cytokines, such as IL-2, TNF-β, and IFN-γ, that are involved in cellular immunity. This response is involved in inflammatory diseases. The TH2 response pathway favors the production of cytokines, such as IL-4, 5, and 10, that are involved in humoral immunity and antibody production. This response is involved in allergic diseases. Bidirectional suppression between these two pathways is particularly notable. IFN-γ suppresses the TH2 response, and IL-4 and 10 suppress TH1.

Chemokines are involved in chemotaxis, a process by which activated leukocytes in the circulation become adhesive and infiltrate tissues. Like cytokines, chemokines interact with various receptors, act on a number of cell types, and display overlap and functional redundancy. Chemokines can attract immature DC, and as the DC mature, they lose these chemokines and produce new ones, thereby communicating with the lymph node and enabling the delivery of antigen to lymphocytes. However, chemokines are not the only proteins that interact with DC. Non-cognate ligands, such as β-defensins, also interact with these cells. Defensins and other mimetics serve as endogenous, multifunctional immune alarmins, which can activate immunity but are different from the normally exogenous and microbiotic danger signals. Autoantigens also mimic chemokines by interacting with G-protein-coupled receptors on DC, but they do not induce immature DC to mature.

The effect of aging on cytokine production and activities are controversial. The concentrations of some cytokines, such as IL-6, increase with age, but the concentrations of other cytokines, such as IL-2 and 10, decrease, and still others, such as IL-7, remain the same. This suggests a shift to TH2 responses at the expense of TH1 responses, although the pattern is not clear in general. T-regulatory cells, which modify immune responses and participate in bidirectional crosstalk with immature DC, also decrease with age (Tsaknaridis et al. 2003). The production of IL-6 interferes with T-regulatory cell function, as does blocking the production of IL-2 or cluster of differentiation 25 (CD25). IL-2, among other cytokines, stimulates T-regulatory cell function. Studies of the role of cytokines in regulatory interactions between DC and T-regulatory cells may enhance our knowledge of age-related effects on cytokines and immunity.

Overview of Innate Immune Cells and Cytokine Production: Changes that Occur with Age

Elizabeth J. Kovacs, Ph.D., Loyola University Chicago

Innate immunity differs from adaptive immunity in that it is a constitutive, nonspecific, rapid-response system that has evolved over billions of years. The innate immune system consists of monocytes, macrophages, DC, neutrophils, eosinophils, basophils, mast cells, natural killer (NK), and NK T (NKT) cells. Neutrophils, which make up 60 percent of circulating leukocytes, arrive at the injury site first. These cells adhere to activated endothelium and migrate across the vessel wall to the affected tissue, where they engulf bacteria, induce respiratory burst, release mediators, and perpetuate the inflammatory response cascade. Monocytes, macrophages, and DC, antigen-presenting cells that are longer lived and less prevalent in the circulation, arrive at the site hours later and perform many of the functions of neutrophils, in addition to presenting antigens, which aids in the development of adaptive immune responses. These cells are a potent source of inflammatory cytokines such as IL-1β, TNF-α, IL-6, IL-8, and IL-12.

The innate immune system mounts inflammatory responses. How it distinguishes the self from the infectious non-self and how it responds so quickly is just beginning to be understood. The process is thought to involve pathogen-associated molecular patterns (PAMPs) and pattern-recognition receptors (PRRs). PRRs, which are present at the cell surface, within the cell, or in secreted form, make foreign cells more susceptible to phagocytosis, activate complements and the coagulation cascade, activate pro-inflammatory signaling, and induce apoptosis. Toll-like receptors (TLRs) comprise a family of type I transmembrane receptors that contain extracellular, leucine-rich receptors and intracellular Toll/IL-1 receptor (TIR) domains. The TIR domain is involved in recruiting adaptor molecules that activate downstream events. TLR4 signaling, for example, begins with the activation of the TLR4 by microbial lipopolysaccharide (LPS), which triggers mitogen-activated protein kinase (MAPK) signaling and ultimately leads to the degradation of inhibitor of nuclear factor kappa B (IκB), leaving nuclear factor kappa B (NFκB) free to travel to the nucleus and induce transcription of inflammatory and immune-response genes. This signaling cascade leads to a variety of processes including tissue repair, antibacterial defense, adaptive immunity, and apoptosis.

There are several possible mechanisms for age-dependent changes in innate immunity. The number or distribution of cells may change. On the other hand, the function or activation state of the cells may change. Some studies have shown that the number of NK and NKT cells increase with age, whereas the number of neutrophils or antigen-presenting cells do not. In contrast, more evidence of altered function has been reported for neutrophils, antigen-presenting cells, and NK and NKT cells, particularly in phagocytosis, chemotaxis, and cytokine and chemokine production.

Future research efforts must distinguish between circulating levels of cytokines and macrophage production of those same mediators. Aged subjects exhibit increased concentrations of pro-inflammatory cytokines, but macrophages stimulated *in vitro* with LPS and other TLR ligands have lower levels of TNF-α and IL-6, independent of ligand concentration, duration of exposure, source of macrophages, or mouse strain. Some evidence of defects in TLR, MAPK, and Janus Kinase/signal transducer and activator for transcription (JAK/STAT) has been reported

(Boehmer et al. 2004; Yoon et al. 2004), but it is not clear whether these age-dependent defects are universal. This is being investigated further in a handful of laboratories.

Secondly, future studies should determine whether age-dependent defects in innate immune response are intrinsic to the cells or the result of environmental exposures. If these changes are extrinsic, how long the cells need to reprogram following a shift to a younger environment must be determined. If these changes are intrinsic, their cause should be determined. Dr. Kovacs' laboratory is conducting adoptive-transfer experiments, in which innate immune cells are transferred from aged environments to younger ones and vice versa. They also are conducting experiments in which the age environment is altered by estrogen replacement, glucocorticoid inhibition, and the inhibition of aberrant cytokine production.

Finally, decisions should be made on which animal models are best for the questions being asked. Experimental models vary by mouse strain, age, gender, and cell type. Moreover, most animal models are housed in genetically pure, germ-free environments. Whether or not this is appropriate needs to be considered. Future studies in which animals are placed in different environmental scenarios will be useful.

Discussion of Cytokines and Innate Immunity

Because cytokines are produced in response to danger signals and not constitutively, one view proposes that aging may provide a type of stimulus and that most observed biological effects stem from an ongoing inflammatory response. Humans do not live in a germ-free environment; thus, low levels of stimulation could occur constitutively. Moreover, some longitudinal studies have found increased IL-6 production with age, suggesting that something stimulates a spike in IL-6 production. Epidemiologists have observed associations between health outcomes and gradients of higher-level cytokines, but these gradients exist at a level lower than what is considered inflammatory. Increased cytokine production may stem from loss of regulation or a leakage in the system, leading to biological effects that are not part of the inflammatory response. Studies of aging using animal models raised in germ-free environments may distinguish between these views.

Finally, the above discussions underscore the problem of measuring inflammatory responses in the blood versus the tissue. Most epidemiological studies use serum, and other studies use tissue, leading to conflicting data. While it is not realistic to sample a variety of tissues from aged humans, there needs to be a better way to correlate data from animal models and human studies.

Aging and the Adaptive Immune System

Donna M. Murasko, Ph.D., Drexel University

Adaptive immune response declines with age. T-cells shift from a naïve phenotype to a memory phenotype, and this alteration represents the most consistent and dramatic change in immune response. Alterations in cytokine production may mediate these changes. However, although data from murine studies generally support an age-associated shift from a TH1 response to a TH2 response, a similar shift is not readily apparent in humans. A review of more than 60 studies evaluating type 1 and type 2 cytokines suggests inconsistencies in age-associated changes in cytokine production in humans. These inconsistencies hinder useful cross-study comparisons.

Several factors may account for these variations. The heterogeneity of phenotypes in aged people is one factor. Some studies focus on people with multiple chronic diseases, and others focus on frail or nursing-home populations. Conclusions from these studies may not apply to healthy aged people, and vice versa. Even subjects comparable in health status demonstrate considerable heterogeneity in aging-associated phenotypes. The differences in assays used to quantify cytokines form another factor. Bioassays may not necessarily produce the same results as enzyme linked immunosorbent assays (ELISA), and mRNA-based assays may not reflect what happens at the protein level. For example, bioassays have shown comparable concentrations of IFN-γ in aged people and young people, but mRNA data suggest that aged people produce more IFN-γ than younger ones. The stimulus used to induce cytokine production is yet another factor. Many studies in both humans and mice have used mitogenic stimulation of mononuclear cells or isolated subpopulations of T cells. Although this stimulus generates data suggestive of the T-cells' potential to produce the cytokine, it may not correlate with the T-cell's ability to respond to a natural stimulus *in vivo*.

It is not clear whether the source of cytokine production affects the disease process. With the exception of IL-2, the cytokines associated with T-cell responses are produced by multiple subsets of cells. IFN-γ, for example, is produced by NK cells as well as by CD4 and CD8 T cells. Recent data show that the overall production of IFN-γ in response to viral challenge decreases in aged mice and that the source of IFN-γ production shifts from CD4 cells in young mice to CD8 cells in aged mice. Likewise, more CD8 cells stain positively for IL-4 in aged people than in young when peripheral blood mononuclear cells (PBMC) are stimulated with phorbol myristate acetate and ionomycin.

Whether mechanisms operate in aged people to compensate for the decline in immune response is not clear. Nor is it clear whether the "effector stage" of immune response differs from the onset of disease; thus it is not known whether interventions are better targeted to prevent disease initiation or the end stage. Potential age-related changes in susceptibility to regulatory processes such as apoptosis also should be studied.

Discussion

Autoimmunity is another area of research on aging, inflammation, and inflammatory markers that remains to be investigated. It is clear that autoantibodies and paraproteins increase with each decade of life, although the conventional clinical wisdom is that these proteins do not affect diseases such as RA or lupus. Many autoimmune diseases begin in middle age, and some, like RA, characterize old age. A decline in regulatory cell function may contribute to overall immune decline. Most agree that the concentration of IL-2, which is necessary for stimulating CD25 T cells, decreases and that the concentration of IL-6 increases, interfering with the stimulation of T cells. Whether T-regulatory cells use other cytokines to control immune response is not clear.

Compensatory mechanisms should be explored further. A concomitant upregulation of anticoagulation pathways has been observed with the upregulation of coagulation pathways. Epidemiological and clinical trials have been initiated with the assumption that people who experience heart attacks exhibit low anticoagulation levels. However, the opposite has been observed. It is possible that as IL-6 levels increase, whatever regulates this cytokine increases as well to maintain homeostasis.

Adipocytes, Inflammatory Responses, and Metabolic Regulation

Gökhan S. Hotamisligil, M.D., Ph.D., Harvard University

The survival of multicellular organisms strongly depends on their ability to fight infection and store energy for times of low nutrient availability. Hence, metabolic and immune systems are among the most basic requirements across the animal kingdom, and many metabolic and innate immune pathways have essentially remained unchanged throughout evolution. Metabolic and immune pathways also have evolved to be closely linked and interdependent. Many signaling proteins, transcription factors, and even energy molecules can function in both metabolic and immune roles, perhaps most prominently in adipocytes and macrophages. Metabolic stress such as obesity is linked to insulin resistance, CVD, diabetes, and cancer, among others; these diseases also are linked to aging. Because of the interconnectedness of the metabolic and immune pathways and the chronic diseases linked to both aging and obesity, adipocytes, alone or in concert with immune cells such as macrophages are key players in these processes.

During the past decade it has become clear that alterations in inflammatory response are critical in controlling metabolic homeostasis, especially in the context of obesity. Recent studies have demonstrated that in obese people, abnormal inflammatory cytokine production results in abnormal insulin action. Many proteins used by adipocytes for communication are integral to innate immune response. Studies in genetic models also have shown that inflammatory response is critical in insulin resistance and diabetes. This was first demonstrated in the discovery that TNF-α influences metabolic homeostasis. Since then, many inflammatory mediators have been identified as products of adipocytes.

Inflammatory signals including cytokines induce a serine phosphorylation event on insulin receptor substrate 1 (IRS-1), the principal substrate of the insulin receptor. This modification of IRS-1 is a critical step in negatively regulating the insulin action induced by multiple cytokines or free fatty acids. The c-Jun N-terminal kinase (JNK) has been recently identified as the major serine/threonine kinase responsible for insulin insensitivity in obesity. In several models of obesity, JNK activity is increased at all sites critical to peripheral insulin sensitivity. Mice deficient in JNK1 exhibit a dramatic resistance against the development of insulin insensitivity and type 2 diabetes. This suggests that JNK is involved in mediating the tight interaction between inflammation and insulin response. In addition to JNK, inhibitor of kappa kinase (IKK), which activates NFκB, also is critical in obesity-induced insulin insensitivity through this mechanism. These observations further demonstrate the inflammatory etiology and the role of adipocytes in common chronic metabolic diseases.

Discussion

Macrophages have been shown to infiltrate adipose tissue, especially in obese people. Macrophages are the major source of IL-6 production, but T cells and adipocytes also produce this cytokine. As people age, muscle tissue is replaced by fat tissue, increasing the number of fat cells producing IL-6. It is not clear, however, which source plays a primary role in increased IL-6, particularly because muscle cells themselves produce IL-6. It also is not clear what role macrophages play in adipose tissue. There is about half as much IL-6 in fat, but the mass of adipose tissue (approximately 20 percent of body weight even for a lean animal) is enormous compared to any other tissue type. Epidemiological studies may be useful in addressing this question, especially if body composition data are available. One study has shown relationships

among IL-6, visceral fat, and fat accumulated in the muscle, but whether this phenomenon is related to age is not clear, because the relationships also hold for physically inactive children and therefore may be related to disuse.

Other questions for further study include the signals leading to chronic inflammation, differences in cytokine production based on the source of adipose tissue; the mechanism underlying the replacement of muscle and liver tissue with adipose tissue; and age-related mechanisms, other than energy underutilization, that stimulate fat production.

MEDIATORS OF INFLAMMATION AS RISK FACTORS FOR MORBIDITY AND MORTALITY

Session Leaders: Jeremy Walston, M.D., Johns Hopkins University, and Sherry Sherman, Ph.D., National Institute on Aging

In a conceptual model, disease or aging triggers inflammatory changes, leading to physiological and molecular changes such as those described during the Basic Science Session. These changes ultimately give rise to poor health outcomes such as frailty, disability, and death. The presentations in this session focus on human and epidemiological studies suggesting roles for inflammatory mediators in these poor outcomes.

Inflammation Reflects Molecular Processes in Fat and Multiple Organ Systems

Tamara B. Harris, M.D., National Institute on Aging

Until recently, interest in the connections between inflammation and disease was primarily cardiocentric. The research paradigm is now changing. In old age, the inflammatory cytokine IL-6 predicts cardiovascular disease (CVD)-related and non-CVD–related mortality equally, suggesting that inflammation is important in areas other than CVD. Whether an increase in pro-inflammatory cytokines is an intrinsic aging process and relates to this is still controversial.

Most diseases associated with old age involve the deposition of ectopic material that triggers inflammation. This endogenous response to ectopic material may reflect the evolution of beneficial immune defenses against bacteria and viruses. However, when directed against stable, non-infectious material within the body, this inflammatory effect may be deleterious. For instance, advanced glycosylation end-products are formed within the dermis in long-standing diabetes and are thought to contribute to inflammation. This same process, at a lower chronic level, may also occur in people with reduced insulin sensitivity. Other examples of antagonistic pleiotropy can be identified for inflammation. Singer and colleagues (2004) discuss multiorgan failure as an adaptive response to overwhelming systemic inflammation. Cigarette smoking has been shown to promote higher levels of IL-6, even in the absence of chronic obstructive pulmonary disease. A report from the Radiation Effects Research Foundation also has shown that early-life radiation doses correlate with increased IL-6 levels later in life.

Another issue pertinent to inflammation and aging is the "domino effect," in which inflammation in one system affects other cells, tissues, and organs. Acute-phase phenomena associated with IL-6 production promote hematopoietic, metabolic, and hepatic changes, as well as changes in non-protein plasma constituents. Adipose tissue secretes proteins that have both paracrine and endocrine effects on other organs. For middle-age individuals, CRP and IL-6 concentrations are

higher across increasing levels of obesity, leading to impaired glucose intolerance and hyperinsulinemia. Inflammatory effects related to obesity have been demonstrated even in children, which will allow the dissection of inflammatory effects from age-related ones.

Weight and height tend to increase into middle age, and the amount of body fat increases, even if weight is held constant. With advancing age, central fat increases, particularly visceral fat, and lean mass decreases. Even people who lose weight exhibit higher concentrations of fat. Kirkland and colleagues (2002) suggest that in old age, adipose cells experience faulty differentiation, resulting in mesenchymal adipose cells with higher potentials to secrete cytokines and increase inflammation. With increased levels of obesity, fat infiltrates into the liver; aging research should thus account for the liver as a major regulator in old age. The lymphatic system is another consideration. Data from Pond (2002, 1998) demonstrate that in activated lymph nodes, fat secretes a tremendous amount of cytokine, triggering paracrine effects.

Integrative interactions between inflammation in one system and effects in other organ systems are another issue in aging. Here, cystic fibrosis (CF) in children presents an interesting model of this phenomenon. It has been established that CF patients given non-steroidal anti-inflammatory drugs (NSAIDs) exhibit better outcomes, not because the drugs affect the disease, but because inflammation associated with the disease is better controlled. These data suggest that the inflammation itself, apart from the underlying mechanisms of the disease, may contribute to health outcomes related to the disease. Likewise, it also has been shown that CF patients who are better controlled on NSAIDs have increased muscle mass and bone-marrow content, reflecting lower metabolic rate, better appetite and ability to endure exercise.

Finally, the balance between pro- and anti-inflammatory mechanisms is important. Multiple health conditions involve pathways that activate pro-inflammatory cytokines. A number of drug interventions act by decreasing pro-inflammatory cytokine production. However, it is much easier to measure pro-inflammatory mechanisms than anti-inflammatory ones, so the key pieces are still poorly understood.

Future investigations into inflammation, inflammatory mediators, and aging should determine whether tissue-specific biomarkers are secreted into the circulation, how inflammation in one physiologic system affects other systems, and whether increased inflammation or anti-inflammatory failure is dominant in old age or in complex, multisystem disease.

Discussion

The anti-inflammatory content of fat, particularly with regard to TGF-β, is a matter of some debate. Some data suggest that adipose tissue produces as much TGF-β as other tissues, but it is not known whether the fat cell itself or the surrounding stroma produces it. With current methods, fat tissue samples also include endothelium and macrophages, making it difficult to identify adipocyte-specific effects. Adipocyte interactions are another topic of interest. As is discussed by Sundeep Khosla, M.D. (see below), adipocytes and osteoblasts stem from the same precursor in the bone marrow, suggesting a reciprocal relationship that may be applied to aging. Finally, the potential effects of weight cycling on inflammation are not clear. Likewise, inflammatory changes associated with interventions in which people lose a significant amount of weight in a short period of time are not known.

Inflammation, Homeostasis, and Frailty

Luigi Ferrucci, M.D., National Institute on Aging

Frailty is a global deterioration of health status that affects anatomical integrity and function of multiple physiological systems in older persons. Frailty predicts multiple negative health outcomes, and is strongly associated with physical and cognitive function decline. Main features of this condition also include reduced functional reserve, reduced ability to respond to stress and disease and a high prevalence of frailty at the extreme of the age spectrum. Inflammation shares with frailty a number of characteristics. Elevated CRP, IL-6, or TNF-α levels are associated with increased mortality in the elderly, and IL-6 also has been associated with high risk of mobility disability. IL-1 and IL-6 concentrations increase with aging, but it is not known whether this mild pro-inflammatory state results from the burden of pre-clinical and clinical co-morbidity or is a characteristic of the aging process per se.

The concept of homeostasis assumes that biological organisms are constantly in a condition of dynamic equilibrium between entropic forces and mechanisms seeking to regain stability. If the balance between homeostatic mechanisms and entropic forces is adequate, as in many young adult and healthy older persons, adequate physical and cognitive function are maintained or easily and completely regained after acute diseases. In acute diseases affecting one physiological system, entropic forces overwhelm the homeostatic mechanisms to the point of clinical detection and, if the clinical condition progresses, disability can ensue. In the presence of frailty, however, the entropic forces continuously attack multiple systems and wear down the homeostatic mechanisms. Thus, understanding how the physiological systems that maintain the homeostatic equilibrium change with age is essential to understanding frailty. Homeostatic mechanisms, such as hormonal messaging and inflammation, are responsible for housekeeping and energy balance. Entropic forces include oxidative stress, poor nutrition, and lack of physical activity. Interestingly, parallel declines have been demonstrated in these multiple physiological systems as these regulatory systems become somewhat dysfunctional in older persons. However, what drives the decline in the homeostatic mechanisms is still unknown. Several researchers have suggested that oxidative stress and inflammation play an important causal role in the development of age-associated frailty.

The domains of frailty include poor nutritional status, reduced muscle strength, diminished lower-extremity performance, low physical activity, and a sense of exhaustion. A strong linear relationship between poor nutrition and age-associated frailty has been demonstrated, suggesting possible protective effects in diet. Physiologic concentrations of n-3 fatty acid are positively correlated with a high concentration of components in the anti-inflammatory pathway. Decreased muscle strength and performance, and poor lower extremity performance have been associated with elevated IL-6 plasma concentrations. Physical activity has been shown to have a protective effect on inflammatory responses. Mild depressive symptoms have also been found to be associated with amplified and prolonged inflammatory responses (Glaser et al. 2003). Thus each component of frailty has some association with inflammation or mediators of inflammation

Where and how this tendency towards a pro-inflammatory state in older persons originates is not known. One well-known hypothesis is that insult or injury leads to oxidative stress, which in turn activates the NFκB pathway, ultimately stimulating cytokine and interleukin production. In this

scenario, oxidative stress functions as a signaling mechanism. Age-related declines in immune efficiency could thus result from oxidative damage to the mitochondria, which sets up a vicious cycle between the secretion of TNF-α and IL-1 and mitochondrial defects. According to this hypothesis, excessive production of reactive oxygen species in the mitochondria may be the underlying cause of the increased pro-inflammatory response observed with old age.

Oxidative stress acting through the inflammation pathway can modulate the production and biological activity of several hormones and, according to recent literature, may also cause an ortho-parasympathetic imbalance. For example, data from the "Invecchiare in Chianti" (InCHIANTI) study, an epidemiological study based in Tuscany, Italy, demonstrate a correlation between insulin-like growth factor 1 (IGF-1) and muscle strength and power in older people. There is also clear evidence that high circulating levels of IL-6 are associated with insulin resistance. These data suggest that oxidative stress may represent an important connection between the frailty syndrome and age-related disease, and that both processes may be derived from the same core mechanism.

Discussion

The idea of oxidative stress as an underlying determinant of disease is a controversial one. The problem is that oxidative stress is a convenient mechanism to suggest for promoting disease processes, but it cannot be measured. It could be that oxidative stress does serve as a signaling mechanism for frailty, but the inability to measure oxidative stress makes this hypothesis difficult to prove.

The absence of vascular disease has been established as a major determinant of longevity. The production and delivery of energy is a key function of the cardiovascular system. Many of the observed effects in frailty may result from tissue malnutrition associated with vascular disease. Changes in the vasculature contribute considerable burden to aging processes, and virtually every risk factor for CVD has been associated with increased oxidative stress. Further understanding of the pathophysiology of vascular disease and frailty in the context of inflammation and oxidative stress may further our understanding of oxidative stress itself.

The notion of oxidative stress serving as an important signaling mechanism for frailty stimulates further questions about age-associated changes in inflammatory pathways. Frailty involves a number of stressors that activate NFkB, but these stressors exist for young people as well. The differences between the cells in younger people and those in older people remain unclear. The number of mitochondrial mutations appears to increase with aging, and inflammatory markers coexist with those of oxidative stress, suggesting oxidative stress as a key difference between younger people and older people. Again, this cannot be proven because of the inability to measure oxidative stress.

Inflammatory Cytokines and the Anemia of Frailty

William B. Ershler, M.D., Institute for Advanced Studies in Aging and National Institute on Aging

The prevalence of anemia increases with age and occurs in 10 to 20 percent of older populations and in almost 50 percent of nursing home patients. A third of cases are related to nutritional deficiencies (iron, B12, folate). Other underlying etiologies might include anemia of chronic

diseases (ACD), which is associated with chronic inflammatory conditions, myelodysplastic syndromes, chronic renal insufficiency, or miscellaneous causes. It is not clear whether there is an "anemia of aging." In previous studies of patients with anemia, there is a component that defies classification. The prevalence of this "anemia unexplained" (AU) increases with age. Data from several population studies show that AU occurs in at least a third of older study participants.

Recent evidence suggests that AU may reflect hematopoietic changes that coincide with aging. Erythropoietin (EPO) is a glycoprotein secreted from the renal system. Serum concentrations are usually less than 30 mIU/dL unless someone is anemic, when levels typically rise to more than 100 mIU/dL. Blunted EPO response to anemia is observed in patients with cancer, HIV, RA, IBD, prematurity, and aging. EPO gene expression and receptor binding are inhibited by IL-1, IL-6, and TNF-α. More than 90 percent of 49 AU patients participating in the National Geriatrics Research Consortium Anemia Survey exhibit low EPO concentrations. However, those with iron deficiency anemia (IDA) had high EPO levels; the IDA patients had a greater IL-6 level than nonanemic patients, but significantly less than AU patients.

An analysis of EPO and IL-6 levels obtained from archived samples of each patient visit over a 10-year period from the Baltimore Longitudinal Study of Aging shows that in general, hemoglobin levels fall with age, while serum EPO increases with age, with the rise more exaggerated in those who are to develop anemia. The EPO rise is less prominent in individuals who have diabetes or hypertension, perhaps reflecting disease-associated impaired renal secretion. The failure of EPO secretion may contribute to the age-associated AU.

IL-6 stimulates osteoclasts, inhibits EPO gene expression and ligand binding, and is a major inducer of hepcidin, a small peptide produced by hepatocytes that is a key factor in iron regulation and homeostasis, as well as innate immunity. Increased hepcidin production interferes with normal iron regulation. Under experimental conditions, sustained exposure to hepcidin results in anemia profiles typical of ACD. Thus, age-associated AU may involve renal changes associated with blunted EPO production, combined with IL-6–induced, hepicidin-mediated iron dysutilization.

Even in the absence of chronic disease or inflammation, increased levels of proinflammatory cytokines are found in older individuals. Age-associated cytokine imbalance may contribute to the overall frail phenotype, including low bone density, reduced lean body mass, anemia, cognitive impairment, and increased susceptibility to disease. This cytokine dysregulation may contribute to the development of anemia through direct inhibition of EPO, by interference with normal iron metabolism, or by marrow suppression and increased apoptosis.

A number of scenarios may explain AU. For healthy 65-year-olds, bone marrow reserve and renal endocrine (EPO) function generally are adequate, IL-6 and hepcidin are somewhat elevated, EPO levels are higher, and there is evidence of iron dysutilization, but anemia does not occur because EPO can stimulate the bone marrow reserve. In older individuals who are not frail, bone marrow remains good, but renal endocrine (EPO) function, EPO levels, and iron utilization are lower, and IL-6 and hepcidin are slightly elevated, and these changes are associated with a mild case of anemia. In late old age with frailty, moderate to severe anemia is observed, with

accompanying declines in renal endocrine (EPO) function, iron utilization, and EPO levels, as well as much higher IL-6 and hepcidin levels.

The factors associated with AU may account for the substantial amount of anemia observed in the frail elderly. However, it is not clear whether correction of anemia results in meaningful clinical improvement in other aspects of frailty.

Discussion

Although hepcidin is detrimental in aging and in anyone subjected to chronic inflammation, it is beneficial in acute-phase responses. This is another example of evolutionary factors working against older humans. Reproductive hormones exert important effects on hemoglobin, as well as negative effects on IL-6 expression. However, the IL-6 gene does not appear to contain response elements for androgen or estrogen. The mechanism underlying these hormonal effects on IL-6 is under study.

Periodontal Disease, Inflammation, and Cardiovascular Outcomes

Steven Offenbacher, D.D.S., Ph.D., University of North Carolina-Chapel Hill

Oral diseases and conditions are associated with other health problems such as diabetes, heart disease, respiratory infections, and osteoporosis. The mouth is both a source and a portal of infection. Bacteria live on the surface of the teeth in a complex biofilm as a multifaceted, self-replenishing ecosystem. The organisms in this biofilm have evolved with humans and therefore are designed to persist in a hostile environment capable of evading normal neutrophil host clearance mechanisms. Gingivitis is a local inflammatory process associated with an overgrowth of bacteria on the surface of the teeth, which involves an infiltration of white blood cells (WBC), particularly neutrophils. This is a common problem that leaves the underlying periodontal ligament and bone intact. However, if allowed to progress, gingivitis leads to periodontitis, in which these connective tissues are destroyed. Nationally, the prevalence of periodontal disease increases with age. About 35 percent of persons aged 30–90 years who participated in the Third National Health and Nutrition Examination Survey had periodontitis (Albandar et al. 1999). Diabetes, smoking, and heredity are major risk factors for periodontal disease.

The existence of a complex bacterial biofilm on the teeth and periodontal inflammation results in a recurrent barrage of bacteria in the bloodstream, which activates the liver and stimulates production of CRP and IL-6. A wide body of work has centered on associations between periodontal disease and increased risk for atherosclerosis. Cross-sectional and longitudinal studies have shown moderate associations, but case-control studies have demonstrated stronger correlations between periodontal disease and stroke than between periodontal disease and CHD. Data from the Atherosclerosis Risk in Communities (ARIC) study, a prospective study of 4,000 people aged 45 to 64 years that includes a dental component, show that people with severe periodontal disease exhibit a greater proportion of thickened carotid arterial walls, a sign of atherosclerosis and an increased risk for heart attack and stroke.

ARIC data demonstrate that periodontal disease (assessed using serum antibodies as a marker for periodontal infection) is associated with increased serum IL-6 levels and particularly with diabetic status. Serum IgG concentrations above the median for oral organisms are positively associated with thick carotid artery walls. The relationship between atherosclerosis and systemic

exposure to oral organisms becomes more significant with age and appears to be independent of traditional risk factors. Similar relationships have been demonstrated for serum antibody concentrations and CHD. These data point to the level of serum antibodies to oral organisms as an index of systemic exposure of these organisms.

Preliminary data demonstrate a positive effect of periodontal therapy on inflammatory responses and heart disease. D'Aiuto and colleagues (2004) have shown that periodontal therapy leads to a reduction in serum inflammatory markers such as IL-6 and CRP and decreases the risk for CVD. Future studies should address whether periodontal disease is an unrecognized source of inflammatory stress leading to increased morbidity and mortality in aging. Opportunities to add measures of periodontal disease (based on clinical measurements or laboratory assessment of serum antibody levels) to aging studies, , should be explored. Finally, associations between underlying genetic deficiencies in inflammatory responses and increased risk for periodontal disease should be explored, particularly with regard to their relationships with age, infectious exposure and disability..

Discussion

The relationship between periodontal disease and aging is not clear. There is clearly a shift between TH1 and TH2 responses resulting from periodontal inflammation—hepatic cytokines and placental cytokines shift from TH2 to TH1 in association with *Porphyromonas gingivalis* infection—but this has not been further explored in the context of CVD. Furthermore, autoimmunity has not been addressed in the context of hygiene. It is possible that genetic disposition, rather than an exaggerated inflammatory response to infectious challenge, is the predominant trigger, but this genetic difference has not yet been identified.

Chronic Stress, the HPA Axis, and Inflammation

Janice Kiecolt-Glaser, Ph.D., Ohio State University

Caregiving for family members with dementia is a good model for chronic stress. Compared with controls, caregivers exhibit several changes in health. Caregivers' response to influenza vaccine is lower than non-caregivers' responses, and although both groups mount similar responses to pneumococcal vaccine, caregivers are less able to maintain this protective response (Kiecolt-Glaser et al. 1996; Vedhara et al. 1999). Moreover, in wound-healing experiments, caregivers take an average of 24 percent longer to heal than non-caregivers. In these experiments, even more commonplace stressors affect wound healing. Students take longer to heal during final exams than immediately following summer vacation. Adults who show poorer responses to vaccination and other antigenic challenges experience higher rates of clinical illness (Burns and Gordon 1990; Gravenstein et al. 1994; Hobson et al. 1972). Stress enhances poor health habits such as sleep deprivation, less exercise, poorer dietary choices, and more alcohol use and smoking. These behaviors in turn affect inflammatory responses. People with higher body mass exhibit higher levels of CRP, and sleep deprivation alters IL-6 production, contributing to immune system dysregulation. On the other hand, physical activity may help attenuate chronic inflammatory processes.

Stress and depression can directly alter inflammatory cytokine production as well. In a community sample of older adults, reversible depressive symptoms lead to increased IL-6 production. Likewise, chronic stressors like caregiving are associated with higher IL-6. These

associations are enhanced with increasing age. In a longitudinal community study, the age-associated increase in IL-6 production in caregivers is about four times as large as that of non-caregivers. No systematic differences in health behaviors can account for this difference. Even more striking, these inflammatory alterations do not "rebound" when caregiving ends. Immediately following a spouse's death, bereaved former caregivers show a higher incidence of depression and anxiety. Follow-up data show no difference in depressive symptoms between bereaved and non-bereaved groups. However, former caregivers' average rate of change in IL-6 production do not differ from that of current caregivers, even several years after the death of the impaired spouse (Kiecolt-Glaser et al. 2003).

The biological cause underlying these phenomena is not well understood. Johnson and colleagues (2002) suggest that chronic stress may sensitize or prime people such that they are more vulnerable to exposure later in life. In this scenario, caregivers' stress history places them at greater risk for infection, and their inflammatory response is exaggerated or prolonged. This is supported by the correlation of higher levels of depressive symptoms with higher levels of IL-6. Studies in women who have just given birth show that those with prior history of major depression have greater increases in IL-6 after delivery (Maes et al. 2001). One hypothesis is that the chronic stress suffered by caregivers induces a premature aging of the immune response. This is particularly striking in light of the higher levels of fasting insulin, higher risk for hypertension and CHD, and higher risk for all-cause mortality observed among caregivers.

IL-6 influences the endocrine system and may therefore affect the hypothalamic-pituitary-adrenal (HPA) axis. Potent stimulators, such as corticotropin-releasing hormone, stimulate heightened HPA activity and increase cortisol levels, which initiate, perpetuate, and aggravate depression and depression-like behaviors. Thus stress can enhance the risk of depression through interactions between inflammatory and endocrine processes.

Discussion

It is not clear how stress influences inflammatory changes. For example, the source of increased IL-6 is not known, and how stress would promote hepatic activity is not understood. In addition, several studies have examined connections between the immune system and the central nervous system (CNS). In these studies, placing a rat in a novel environment results in increased IL-6, particularly in the liver, most likely as a result of activating the CNS. It may be useful to study a group of caregivers who are not stressed, if such a group exists, to determine whether other aspects of caregiving are involved in the observed associations. Persistence is another issue. For example, in a study of current and former smokers, using a CRP enzyme-linked immunosorbent assay as a surrogate, former smokers have elevated levels of CRP, similar to current smokers, regardless of how long ago they stopped smoking. The idea of persistence should be explored further, particularly interactions between persistence and age.

INTEGRATIVE PHYSIOLOGY

Session Leaders: Caleb E. Finch, Ph.D., University of Southern California and Jill Carrington, Ph.D., National Institute on Aging
The following presentations focused on connections between inflammatory processes and other physiologic systems.

Caloric Restriction, Inflammation, and Aging

Richard Weindruch, Ph.D., University of Wisconsin
Caloric restriction (CR), a 30 to 50 percent reduction in caloric intake without malnutrition, is the only dietary intervention shown in mammals to extend maximum lifespan and retard or prevent a broad spectrum of age-associated pathophysiological changes across species. This field of study began in 1935, and since then interest has risen rapidly. The mechanisms underlying energy metabolism, the prevention or delay of aging by CR, the influences of CR on aging and diseases in primates, and the development of CR mimetics are under study.

Early-onset CR studies provide the best available dietary model for studying the biology of decelerated aging in mammals. Adult-onset CR studies, on the other hand, are most relevant for potential human application. CR can be effective even if initiated as late as middle age in animal models, and autopsies on extremely long-lived CR mice seldom reveal age-related pathologies. Candidate mechanisms underlying the CR phenotype include decreased free-radical production and damage, shifts in energy metabolism, decreased adipose tissue and altered functional characteristics, decreased systemic inflammation, decreased body temperature, changes in gene expression, and hormonal changes. It should be noted that these mechanisms are not mutually exclusive.

Gene-expression profiling studies have been done to define genomic shifts related to the CR phenotype. This work has involved the brain (Lee et al. 2000), the gastrocnemius (Lee et al. 1999), the heart (Lee et al. 2002), and adipose tissue of mice (Higami et al. 2004). Profiling of epididymal fat shows that a large number of genes involved in energy metabolism are upregulated by CR, whereas genes involved in inflammation are downregulated. Histological examination of adipose tissue shows that adipocytes are smaller in CR mice.

Studies of CR also have been done in primates. Two trials, sponsored by the NIA and the University of Wisconsin, are under way. Indications are that rhesus monkeys subjected to CR are less prone to develop diabetes and exhibit favorable changes in plasma lipids and less oxidative damage in muscle. Adiposity is reduced, and CRP levels are also lower in these monkeys. However, the effects of CR on longevity among nonhuman primates are unclear. For humans, there is no direct evidence of CR effectiveness, but there is some indirect evidence such as a higher incidence of centenarians among Okinawans, who reportedly eat fewer calories. It also has been observed that high-calorie intake increases the risk for cancers of the prostate, breast, gastrointestinal tract, and brain as well as for Alzheimer's and Parkinson's disease. Recent data have demonstrated an improved risk-factor profile for CVD in people practicing CR.

Discussion

There are a number of questions for future research, including the psychological and emotional effects of CR, the effect of intermittent CR regimens, and the effect of CR on reproduction.

Connections between CR and insulin also should be explored further. Mutations in the insulin–IGF-1 pathway have been associated with longevity, and CR enhances insulin sensitivity. However, because of the myriad effects observed in the CR animal, it is virtually impossible to determine what percentage of the overall effect stems from insulin sensitivity. Finally, in the NIA-University of Wisconsin studies, some of the early deaths observed in control monkeys resulted from endometriosis. Angiogenesis has not been examined widely in the context of CR, although some of the downregulated genes in adipose tissue are involved in this process.

Inflammatory Processes in Brain Aging

Caleb E. Finch, Ph.D., University of Southern California

The origins of inflammatory processes during "usual" aging that appear to arise in the absence of specific pathological lesions in the brain is an open question. Our understanding of inflammatory changes in the human brain during usual aging is confounded by the emergence of Alzheimer's disease at later ages, because senile plaques are loaded with inflammatory markers. However, in the aging rodent, which does not accumulate amyloid and plaque material, a nearly identical gene-expression profile is observed with age, although at a lower level of induction (Prolla and Mattson, 2001). This suggests a continuum of inflammation and neurological diseases during aging (Finch, 2005). In some respects, Alzheimer's disease represents intensifications of changes more generally present in the brain upon aging. There may not be an increase in inflammatory cells, but a change in activation. At the protein level, the brains of healthy controls in an Alzheimer's disease study show no evidence of cognitive decline or senile plaques, although all exhibited complement-protein deposits associated with diffuse types of amyloid. *In vitro* culture studies have shown that the inflammatory induction profile persists for 3 weeks in primary culture. Elevations that are greatest with age reside in regions of the brain susceptible to Alzheimer's disease.

Several studies have manipulated these inflammatory aging changes by caloric restriction (CR) which slows the activation of white matter microglia and which attenuates amyloid deposits in transgenic AD mice (Patel et al., 2005). Conversely, diets high in fat and cholesterol have been associated with Alzheimer's disease and promote Alzheimer's-like changes in transgenic mice. CR attenuates deposits of amyloid, suggesting that CR performs in an anti-inflammatory manner. This hypothesis is supported by data showing that ibuprofen exerts some of the same effect as CR. Further study should address the convergent pathways involved in CR and anti-inflammation.

Anti-inflammatory drugs and statins have provided unexpected general protection against the chronic diseases of aging. Aspirin and statins have been used to prevent or delay the onset of CVD, and NSAIDs have shown a protective effect against colorectal and esophageal cancers (Finch, 2005). Whether anti-inflammatory compounds affect the progression of Alzheimer's disease is not clear, although there is some suggestive evidence. In a 25-year follow-up to the Honolulu Asia Aging Study, early elevations of CRP predict higher risk for later dementia (Schmidt et al. 2002). In invertebrate models, aging flies exhibit a 5- to 100-fold increase in antimicrobial gene expression, suggesting that inflammatory changes are generalized in aging (Landis et al. 2004).

Even in the absence of neurological disease, the brain exhibits age-related changes, such as the progressive loss of dopamine receptors. Animal equivalents of humans aged 40 to 60 years display a subset of inflammatory changes, even in healthy, active animals. For one marker, glial fibrillary acid protein (GFAP), mRNA increases progressively in the brain. Although GFAP is not classified as an inflammatory gene, astrocytes can mount a large inflammatory response. *In vitro* studies in which astrocytes are obtained from both young and old brains and grown to confluence in culture demonstrate that astrocytes from older brains are less able to support neurite growth. These deficits can be reversed by using short interfering RNA to downregulate GFAP. In addition, GFAP contains response elements involved in NFkB activation (Rozovsky et al., 2005). These results suggest that aging involves an accumulation of oxidized lipids and proteins in brain and other tissue, which can be attenuated by CR, and that GFAP may drive age-related changes in the brain.

Another open question is the extent to which reduced chronic inflammation accounts for the progressive increase in lifespan during the past 200 years (Finch and Crimmins, 2004). As countries industrialized in the 19th century, life expectancy progressively increased as a result of public health and hygiene movements. Increased life expectancy could therefore be explained by a reduction in population exposure to infectious agents. An examination of age-specific mortality patterns for cohorts born from 1751 to 1931 reveals a downward shift in mortality. In particular, cohorts with high childhood mortality had shorter lifespans, suggesting infant mortality as one of the strongest predictors of cohort lifespan. These results also suggest that survivors of population-wide infections carry inflammatory loads (such as elevated CRP and IL-6 levels) even if the infections are latent or cured, leading to increased early mortality and vascular disease. Future studies examining processes in the brain will have to address both systemic interactions and external environments.

Discussion

Aspirin or ibuprofen has not been tested in experiments examining neurite outgrowth. If the hypothesis about childhood infections is true, then one would expect that countries in which these diseases are still prevalent would carry elevated cytokine profiles. However, under historical conditions, survivors may not necessarily carry the infection, even though people in their cohorts have died. It is important to demonstrate that over time, the infectious potential of the environment in which the cohort matures is a factor. Lower birth weight and maternal infections also should be considered. Finally, it is not known whether brain studies have involved interfering with inflammation to determine the effects on aging.

Inflammatory Cells, Cytokines, and Cognition in Aging

Steven F. Maier, Ph.D., University of Colorado

Inflammatory processes in the periphery and brain are not separate; inflammatory processes in the periphery initiate a process that communicates to the brain, thereby inducing the production of pro-inflammatory cytokines (PICs) by glial cells. This peripherally driven induction of PICs in the brain is exaggerated by aging. These observations help in the understanding of a number of features of the cognitive declines that sometimes occur with aging, and grew from knowledge about physiological adjustments to sickness, such as fever and increased acute-phase protein levels, and behavioral adjustments, such as reduced food and water intake and reduced physical

activity. Many of these adjustments affect or are affected by the brain, suggesting that pathways exist by which activated immune cells communicate with the brain.

Inflammatory processes have been shown to initiate communication with the brain. Neutralizing pro-inflammatory cytokine production blocks sickness and neural activation. Likewise, administering pro-inflammatory cytokines peripherally produces sickness and neural activation. Cytokines communicate with the brain by blood-borne routes and neural routes, inducing the brain to produce its own cytokines. Glial cells are largely responsible for cytokine production, and every synapse in the brain is encapsulated by glial cells. In short, peripheral cytokines, primarily IL-1β, stimulate the production of cytokines in the brain. Thus, inflammation in the periphery induces inflammation in the brain. How this relates to aging has been unclear, but some insight can be gained by research on murine prion disease that has found that after administation of disease-producing proteins into the brain, glia, although activated for months, only make cytokines for days (Perry et al. 2003). If peripheral immune challenge occurs during this period, the brain glial cells overexpress cytokines. Likewise, aging glial cells in the human brain can become primed, leading to an exaggerated response with subsequent challenges. Hippocampal IL-1β protein levels after *Escherichia coli* (*E. coli*) injection have been shown to remain elevated in older rats for much longer than in younger ones.

In connecting these findings to cognition, Maier noted that the induction of IL-1 in the hippocampus selectively interferes with declarative memory, which includes memory for facts, ideas, and events); blocking IL-1 receptors blocks those effects. Considering that aging primes glial cells to overexpress pro-inflammatory cytokines, that peripheral inflammation leads to glial activation, and that hippocampal IL-1 interferes with the formation of long-term memory, one can hypothesize that elderly people are vulnerable to memory disruptions produced by peripheral inflammatory events such as infection and injury.

Memory has been explored in rats using a fear-conditioning paradigm. In one set of experiments, the Maier laboratory is challenging mice with *E. coli*, subjecting them to fear conditioning, then testing memory after 1 or 24 hours to determine whether bacterial challenge induces memory loss. Neither aging nor *E. coli* affect short-term memory, but older animals challenged with *E.coli* exhibit decreased long-term memory. Contextual fear conditioning induces brain-derived neurotropic factor (BDNF) mRNA in the hippocampus (Hall et al. 2000). *E. coli* challenge reduces the BDNF mRNA increase produced by conditioning. Likewise, hippocampal IL-1β interferes with the BDNF mRNA induced by contextual fear conditioning.

Future research should address whether age-related cognitive vulnerability to peripheral infection extends to other cognitive processes other than the formation of long-term, declarative memories, whether the observed effects can become permanent after repeated challenges, whether inhibited glial activation or interference with pro-inflammatory cytokine production eliminates age-related vulnerability, and whether blocking the signal from the periphery to the brain prevents age-related cognitive decline.

Discussion

Studies involving brain ischemia would be useful, as one would expect the same set of predictions discussed above to apply. Research in PBMC has documented that estrogen inhibits

the production of IL-1; thus repeating the above experiments in female animals may be useful. However, the attendant costs are prohibitive. Long-term production of IL-1 in early infections has been shown, but whether IL-1 production is delayed in older animals, and whether the above results might be explained by a sensitized peripheral response, is not clear. Prolonged expression of inflammatory cytokines may be observed in some systems, depending on dose. It is not clear whether that is the case here. *E. coli* clearance does not differ between younger rats and older ones. All animals receive the same dose, but differences in weight may affect the concentration of bacteria. It is not clear whether structural changes occur in response to age and peripheral challenge, but this issue is being explored.

It is not known whether aged mice deficient in the IL-1 receptor exhibit better memory. However, the role of IL-1 in memory is complicated. Basal IL-1 is required for memory formation, but eliminating IL-1 prevents memory formation. Signal to noise is another issue; expressing IL-1 in too many places at too high a level may make the effects difficult to discern.

Inflammatory Cytokines and the Cardiovascular System

Jeffrey Bender, M.D., Yale University

Most coronary patients have elevated CRP levels, and at high concentrations of CRP, the probability of event-free survival decreases (Ridker et al. 2002). This is also true for LDL cholesterol, and even at relatively low levels of LDL, elevated CRP increases the risk for cardiac events. The standard of care involves the free use of statins, but Ridker's data raise the question of whether statins should be used even in patients with low LDL.

It is not clear whether CRP is a risk factor for CVD or a marker of disease. If it is indeed a risk factor, then the question is whether inflammatory markers directly cause atherosclerosis or whether classical risk factors lead to atherosclerosis, inducing elevated levels of inflammatory markers. At present, there is little evidence that CRP directly causes atherosclerosis. However, Paul and colleagues (2004) inserted a transgene for CRP into an atherosclerotic strain of mice and found that at all levels, the burden of atherosclerosis was higher in mice carrying the transgene, suggesting a causative role for CRP.

Normal endothelial function in the blood vessel is antithrombotic and minimally permissive of leukocyte adhesion, and it relaxes the muscle cells and promotes the release of various vasodilating substances. When endothelium becomes dysfunctional, such as in aging or in response to periodontal disease, it becomes permissive of leukocyte adhesion. The leukocytes infiltrate, monocytes differentiate into macrophages and produce free radicals, constricting substances are produced, and muscle cell proliferation is promoted. In addition, nitric oxide, which acts as a potent vasorelaxant, is produced less in dysfunctional endothelium. The stages of leukocyte adhesion involve leukocyte rolling, which is mediated by selectins and other ligands; mutual activation of endothelium and leukocytes, leading to additive adhesion and infiltration into the interendothelial space; and the production of chemokines to recruit more leukocytes.

Leukocyte adhesion is a key event, but what triggers pro-inflammatory cytokine production is not known. A panel of cytokines is involved in atherogenesis and allograft rejection. The transcripts encoding these cytokines contain regions in the 3′-untranslated region that are rich in adenine and uridine. These elements are potent targets of degrading enzymes, such as

ribonucleases, thereby making transcription very labile. The protein product for these cytokines is minimal unless something stabilizes the messenger RNA. If transcription is triggered in human T cells, RNA is induced, but it fades after a few hours. Thus mRNA stabilization markedly enhances ultimate gene expression and pro-inflammatory protein production. In stable plaques, pro-inflammatory proteins induce the production and activity of macrophage matrix metalloproteinases, resulting in the conversion of the plaque to an unstable one.

Lower survival related to congestive heart failure (CHF) is observed at the highest quartiles of TNF and IL-6. Thus, inhibiting the activity of these cytokines should reduce the risk for CHF. Yet two clinical trials have tested TNF-α inhibitors, etanercept and infliximab, in the prevention of CHF, and both trials have been terminated for lack of clinical benefit. One reason for these failures may be that TNF-α production is a peripheral phenomenon. Another reason is that physiologic levels of TNF-α may be involved in tissue remodeling and repair. Yet another possibility is that infliximab is directly cytotoxic to cells with membrane-bound TNF-α. Finally, patient selection may not have been optimal for these trials. Those with highest TNF-α levels may be the best candidates.

In studies by Ridker et al. (1999) of CRP levels over a 5-year period of statin treatment, CRP levels rise with placebo, but fall with the use of statins, suggesting a use for statins in reducing inflammation. Likewise, increased ejection fraction in heart failure patients on statins led to the use of statins in CHF patients. Statins inhibit the 3-hydroxy-3-methylglutaryl coenzyme A reductase mevalonate pathway, which contains components important in targeting lipids toward cell membranes as well as components important for inflammation.

Future research should address whether CRP is a risk factor in atherosclerosis; what effect aging has on pro-inflammatory cytokine production; whether the endothelium is more sensitive to pro-inflammatory mediators in aging; whether cytokines are pathogenic in heart failure and, if so, why inhibitor trials have failed; and whether the anti-inflammatory effects and benefits of statins in CVD justify their use for aging patients.

Discussion

The failure of TNF-inhibitor trials is somewhat surprising in light of the effectiveness of these therapies in RA and inflammatory bowel disease (IBD). Circulating TNF concentrations in cardiac patients are fairly close to what are observed in IBD patients, so it is not clear why these trials have failed. CRP associations with CVD outcomes have been somewhat inconsistent. In some cohort studies, CRP has been strongly predictive, whereas in others, it has been a poor predictor. However, IL-6 is strongly and consistently predictive. It is not clear what the source of these mediators is in aging patients.

Muscle Effects Related to the Modulation of Cytokine Action by Aging, Diet, and Physical Activity

Helle Bruunsgaard, M.D., Ph.D., University of Copenhagen, Denmark

The role of contracting muscles in mediating metabolic effects on health has been discussed for some time. Of particular interest is the identification of the muscle-contracting factor that mediates exercise-induced changes. The inflammatory mediators TNFα and IL-6 are tightly linked, so much so that separating their effects is often difficult. However, a 100-fold increase in

IL-6 concentration is observed in response to physical activity, as well as in the inflammatory cascade, but no change is observed in TNF-α. It has been shown that muscle IL-6 mRNA increases in working muscles in relation to physical exercise without muscle damage, and that muscles themselves express IL-6. Whether IL-6 is released from the working muscle to the blood is not known.

In one previous study of young, healthy men, catheters were placed in the femoral artery and the femoral vein to measure IL-6 release. When the test subjects performed physical activity, IL-6 was released from the exercising leg and could fully account for the increase in plasma IL-6. This suggests that IL-6 serves as an energy sensor that mobilizes the substrate for oxidation in working muscles. In a second study, young, healthy men performed a one-leg exercise to deplete glycogen in that leg and then performed a two-leg exercise following a carbohydrate-restricted diet. Muscle biopsies from both legs revealed that the transcription rate for IL-6 was increased in the presence of normal glycogen levels. However, in the glycogen-depleted leg, the transcription rate was even higher, suggesting that IL-6 production is related to glycogen content.

An examination of fat tissue showed that IL-6 induces lipolysis to mobilize fatty acids. Young, healthy men infused with high doses or low doses of IL-6 exhibited increased concentrations of fatty acids. In addition, the rates of appearance and disappearance of fatty acids, as well as whole body-fat oxidation, increased, suggesting that IL-6 mediates an increase in arterial fatty acid concentration during exercise (Van Hall et al. 2003). In the liver, IL-6 infusions alone do not affect glucose homeostasis (Febbraio et al. 2004)), but IL-6 related to muscle contraction does.

Because physical activity offers protection and improves symptoms in diseases associated with low-grade inflammation, one might conclude that physical activity exerts an anti-inflammatory effect. Starkie and colleagues (2003) exposed subjects to a low dose of endotoxin and found small elevations of TNF-α concentrations. However, when subjects exercised before endotoxin exposure, TNF-α production was blocked, supporting the hypothesis that exercise is indeed an anti-inflammatory process. IL-6 infusion also blocked the elevation in TNF-α induced by endotoxin, suggesting that IL-6 mediates the anti-inflammatory effects of exercise.

Unpublished data suggest that the amount of IL-6 released from working muscle is no different between young and old people, indicating that the IL-6 mechanism is preserved in healthy adults. TNF-α expression in muscles increases in frail elderly and in people with type 2 diabetes, but after 12 weeks of resistance training, local TNF-α is decreased at both the mRNA and protein levels (Greiwe et al. 2001). In a similar intervention in nonagenarians, however, the systemic anti-inflammatory effects of physical activity are not readily apparent (Bruunsgaard et al. 2004). This study may be underpowered, or it might be more effective for these adults to perform lower-intensity endurance exercise for a longer duration. Treadmill exercise for 3 months reduces CRP concentrations in patients with intermittent claudication (Tisi et al. 1997), and 9 months of endurance training reduces CRP concentration in subjects preparing for a marathon (Mattusch et al. 2000). On the basis of these data, exercise interventions may still be valuable for the elderly.

It is paradoxical that IL-6 appears to mediate the beneficial effects of exercise, but plasma TNF-α and IL-6 are associated with low muscle mass and strength, physical inactivity, insulin

resistance, and mortality. However, there are some differences between exercise-induced IL-6 and that observed during chronic, low-grade infection. Plasma IL-6 therefore must be reconsidered in elderly populations. Whether IL-6 is more beneficial or more detrimental remains to be seen, but it appears that balance among organs is important.

Discussion

People who undergo exercise training show different exercise-induced IL-6 levels than those who do not. This may be due in part to a higher glycogen content in relation to regular training. There is some disagreement about the role of IL-6 in exercise-mediated health benefits. Opioids and endorphins, as well as IL-1 (which is only produced in exercise models that involve muscle damage), also are produced during exercise. Most likely, there is a multiplicity of effects produced by exercise. Animal models as well as an assessment of the kinds of exercise models used (and whether or not there is muscle damage) are needed to further study the underlying factors responsible for exercise-mediated health benefits. Otherwise, the claim that IL-6 is beneficial may be an overinterpretation of the data.

Inflammatory Cells, Cytokines, Estrogen Effects, and Bone

Sundeep Khosla, M.D., Mayo Clinic

The large amount of bone loss following the cessation of ovarian function has been well established, as has the preventive effect of estrogen replacement. The cessation of ovarian function is associated with an increase in bone resorption. However, bone formation and resorption are coupled; as one increases, so does the other. The relative increases in formation markers are smaller in magnitude, compared to markers of resorption.

Although there is less data about sex hormones in men, increasing evidence now suggests that estrogen is protective against bone loss both in women and in men. A study of castrated Czech men revealed comparable losses of bone in men following gonadectomy as has been found in women following oophorectomy. In another study (Falahati-Nini et al. 2000), in which elderly men made deficient in sex hormones received estrogen alone, testosterone alone, both, or neither in the presence of an aromatase blocker, bone resorption markers increased when men were made hypogonadal. Estrogen alone almost completely prevented the rise in bone resorption, while testosterone was unable to do so in the presence of the aromatase blocker. Estrogen, then, is the dominant sex steroid regulating bone resorption, even in men.

Based on a number of studies done primarily in rodents, it has been shown that the stromal cell or osteoblast precursor in the bone marrow orchestrates the differentiation and activation of osteoclasts. Stromal cells produce a number of factors, including receptor activator of NFκB ligand (RANKL), which is necessary and sufficient for differentiation and activation of osteoclasts in the presence of macrophage colony stimulating factor (mCSF). Stromal cells also produce inhibitory factors; the balance, for example, between RANKL and osteoprotegrin (OPG) determines how many osteoclasts are formed. TGF-β dampens the stimulatory effects of a number of cytokines on osteoclasts. Monocytes produce IL-1 and TNF-α, which stimulate osteoclast precursors to differentiate and become activated osteoclasts. Likewise, T cells and B cells produce RANKL. Thus, a number of immune cytokines and cells regulate bone turnover, and there is increasing interest in the cross-talk between bone and the immune system.

Most of what is known about the reproductive steroid effects on bone is based on animal studies. Human studies are sparse and contradictory. One study (Scheidt-Nave et al. 2001) examined the relationship between serum IL-6 levels and bone loss in postmenopausal women and found no significant association in late menopausal women (10 years or more postmenopause). Serum IL-6, however, was the single most important predictor of femoral bone loss in early postmenopausal women (less than 10 years). Abrahamsen, Bonnevie-Nielsen, and colleagues (2000) measured serum cytokine levels and related them to the rates of bone loss over 5 years in perimenopausal women randomized to hormone therapy (HT) or placebo. In this study, high serum IL-6 in women receiving placebo was associated with slower rates of bone loss (the exact opposite of that found in the study of Scheidt-Nave and colleagues). Ralston (1994) found that untreated postmenopausal women or women with osteoporosis exhibited higher concentrations of IL-1, TNF, and IL-6 mRNA levels in bone biopsies than young, healthy women and postmenopausal women on HT. This was consistent with rodent models, but there was a great deal of variability in the measurements. Abrahamsen, Shalhoub, and colleagues (2000), on the other hand, studied one group of untreated postmenopausal women who lost bone slowly and another group who lost bone quickly, as well as a group of postmenopausal women on HT. No differences in IL-1β or TNF-αmRNA levels in bone biopsy samples were observed among these groups. One reason for these contradictions may be that serum cytokine levels are affected by a number of factors and may not reflect changes in the bone microenvironment. Secondly, most studies used bone biopsies or whole-marrow cultures; these studies therefore are limited by the mixture of cell types, many of which may be irrelevant to the regulation of bone metabolism.

To get around these difficulties, Eghbali-Fatourechi and colleagues (2003) developed a method for assessing cytokine and growth factor production in the bone microenvironment *in vivo* in humans and have focused their efforts on RANKL. This method takes advantage of two-color flow cytometry using fluorescein isothiocyanate (FITC)-conjugated osteoprotegerin-Fc (OPG-Fc-FITC) as a probe to identify bone marrow mononuclear cells expressing RANKL on the surface. The amount of RANKL expressed per cell increases in estrogen-deficient, postmenopausal women and correlates directly with serum resorption markers and inversely with serum estrogen. Serum RANKL, however, shows no pattern, underscoring the caveat that phenomena measured in the serum do not necessarily reflect what happens in the microenvironment.

Future research is needed to identify the key target cells that mediate the pro-resorptive effects of estrogen deficiency on bone, as well as the most important cytokines in humans, which may then serve as therapeutic targets. Are these cytokines also involved in mediating the defect in bone formation observed in the context of estrogen deficiency? Does estrogen modulate the production and activity of these cytokines in other conditions associated with bone loss?

Discussion

The effect of estrogen on RANKL transcription is not clear. The RANKL promoter is difficult to study, because the most relevant response elements are far upstream. However, it is likely that estrogen indirectly regulates RANKL through suppression of other cytokines. The role RANKL plays in the immune system also is not clear. RANKL is involved in the maturation of dendritic cells, and there is substantial crosstalk between RANKL/OPG system in bone and the immune system, but clearly more studies are needed to further refine our understanding of this.

Reproductive Hormones, Inflammatory Cytokines, and Inflammatory Markers in the Onset of X-ray Defined Osteoarthritis of the Knee

MaryFran Sowers, Ph.D., University of Michigan

Rheumatoid arthritis (RA) affects 1 percent of the population and the prevalence for this disease may be declining. Osteoarthritis (OA), on the other hand, affects 30 to 50 percent of the population aged more than 50 years and is one of the leading causes of disability among people aged 15 years or older (MMWR 1994). OA is defined by the degeneration of articular cartilage, combined with changes in subchondral bone accompanied by intra-articular inflammation. It is characterized by the softening, fibrillation, ulceration, and loss of articular cartilage in the joint, as well as the formation of osteophytes and bony cysts. These processes lead to joint pain, tenderness, limitations in movement, crepitus (the cracking sound from bone rubbing against bone), and effusion (fluid in the joint space). The involvement of localized inflammation in OA is somewhat controversial, as inflammation is not part of the textbook definition.

The Southeast Michigan Osteoarthritis Populations program is conducting population-based studies to identify biomarkers associated with prevalent and incident OA of the knee (OAK). The program specifically considers how arthritis develops in mid-age women. The Michigan Bone Health Study (MBHS) is an observation study that has enrolled 602 Caucasian women aged 25 to 45 years in 1992 (baseline), with 2.5-year follow-ups from 1996 through 2002. The Study of Women's Health Across the Nation (SWAN), an observational study of pre- or perimenopausal women, has enrolled women aged 42 to 52 years at 1996 baseline, 60 percent of whom are African American, with 2.5-year follow-ups through 2002. Both the MBHS and the SWAN studies employ parallel assessment protocols in which X-rays are scored using Kellgren-Lawrence scales based on the presence of osteophytes, the narrowing of joint space, the severity of sclerosis, and the presence of deformity.

These studies reveal no discernible prevalence of arthritis prior to age 40. Between the ages 40-54, however, the picture changes entirely, and prevalence of x-ray-defined knee OA increases to around 15 percent. A striking difference in mean CRP concentration is also noted between study enrollees with and without OAK. Those with OAK exhibit hsCRP concentrations close to those observed in older people with CVD. CRP is also predictive of subsequent incident OAK, but it is not exclusively a predictor of this disease as CRP concentrations increase with greater body size and the presence of diabetes. OAK adds to the burden of increasing CRP levels with greater body size and diabetes. In addition, IL-6, which is associated with CRP concentration, also varies by OAK status. The same is observed with TNF-α. When these markers are examined in relation to MRI indicators of bone marrow edema, CRP concentration appears to change, depending on the degree of severity, penetration of the cartilage, and bone marrow edema.

Potential roles for reproductive hormones have been studied in relation to OAK. Estrogens act as antioxidants, anti-inflammatory agents, and agents that inhibit bone resorption, and they play a strong role in chondrocyte metabolism. The Southeast Michigan Osteoarthritis Populations program has studied testosterone, estradiol, estrone, and the estrone byproducts 2-hydroxyestrone and 16-hydroxyestrone. The concentration of 2-hydroxyestrone is higher in women with high CRP concentrations, and even higher in women with prevalent or incident OAK. On the other hand, 16-hydroxyestrone is high in women with higher CRP, and even higher

in women with prevalent or incident OAK, but only when circulatory estradiol concentrations are at the lower levels most likely observed in the late menopausal transition.

The role of age in these studies of inflammation and hormones is not well understood. The differences reported are among women at the midlife, and the impact among persons more than 60 years of age is yet to be determined. Also yet to be developed are measures of dose response for CRP and hormones that could be translated into clinical practice. Finally, as with other diseases, it is not clear whether the CRP association, even in relation to incident OAK, represents a causal agent or is a marker of an underlying process whose cumulative sequelae are awaiting visualization on x-ray.

Discussion

The mechanism underlying the estrogen metabolite associations with greater CRP and increased OAK prevalence is not clear. A review of the arachidonic pathway suggests that the hydroxyestrones are involved in the breakdown of cyclooxygenase 1 and 2 (COX1, 2), which might suggest a role for estrogen in arthritis. The pro-inflammatory effects of 16-hydroxyestrone is surprising, though one could assume that an association would arise because of its capacity to bind estrogen receptors (ER) or another, unrelated pathway. Finally, increased weight is a predominant factor in these associations. Lean women with OAK have lower concentrations of CRP, suggesting that their OAK may incorporate other mechanisms and that OAK among women with greater body size really represents an additional component of the health risk constellation associated with obesity.

General Discussion—Integrative Physiology

Discussion Leader: Caleb E. Finch, Ph.D., University of Southern California

The "estrogen conundrum"

Although a large amount of data demonstrates positive effects of estrogen on endothelium and muscle at the cellular, molecular, and whole-animal level, HT trials have yielded unexciting data in terms of protection against heart disease. With regard to aging, primate data demonstrate that if estrogens are used at the time of ovariectomy, they have an overwhelmingly positive effect. However, if estrogens are started 2 years after the ovariectomy, which is approximate to 6 years postemenopause, the positive effect is absent. These data indicate that timing is critical. This may be an issue in studies such as the Women's Health Initiative (WHI) where women were started on HT at age 65, which might be already too late. The conversion to acute coronary syndromes may be a different story. Here, estrogens affect interactions between adhesion molecules and the endothelium. Tissue type may be a factor in this conundrum. For example, some of the aging effects observed with vascular disease do not appear to have as much of an impact in bone. Some cytokines apart from IL-1, IL-6, and TNF-α may respond differently to estrogens in terms of the ER, the timing of estrogen, or the presence of estrogen catabolites.

Animal models

It is somewhat daunting to consider the myriad ways in which inflammation affects chronic disease. One crosscutting issue in sorting this out is the choice of animal model. Diet often has wide variation within normal limits in terms of phytoestrogens and fatty acids, but little attention has been paid to this issue. Likewise, genetics is a consideration in every inflammatory disease. Each animal model has unique features of its genotype, and perhaps diet and other aspects of

husbandry. Future study design must incorporate controls for diet components, particularly phytoestrogen and fat content, as well as behavioral interactions. The facility in which these animals are housed must also be considered in terms of how tightly it is controlled. For example, mice exposed to moderately halophilic bacteria outbreaks may experience premature aging and therefore serve as good models for chronic infection and inflammation.

Models of exhausted pro-inflammation

It is not clear whether models exist in which the pro-inflammatory cytokine response has been exhausted. AIDS patients with several complications might lose the pro-inflammatory response, but there may be other conditions in which humans do not mount a pro-inflammatory response. From a vascular standpoint, one study has drawn endothelial cells from different people and examined their response to pro-inflammatory mediators, in terms of transcription induction. Response is remarkably different among individuals, but it is not clear why. This points to a genetic predisposition in a person's response to inflammatory mediators.

Genetic studies

A broad range of TNF-α and IL-6 polymorphisms, all of which are related to inflammatory pathways, have been measured in terms of health outcomes, but no single association has been identified. However, strong interactions with behavior or environment have been observed. These factors should be considered in future genetic research, especially in research on aged populations. One problem is that intermediate phenotypes are only weakly or moderately associated with outcomes, and genotypes explain only a small percentage. Thus many of these studies have no statistical power. Another problem lies in the heterogeneity of phenotypes that increase with age, which makes the association of genetics with events even more difficult. Approaches to genetic study must be improved, perhaps by examining additive or synergistic effects among genes or looking through different pathways.

A great deal of study has focused on markers such as IL-6, but the range of study should be expanded to identify other biomarkers of inflammation and aging. IL-6 functions between pro-inflammation and anti-inflammation; thus its measurement characteristics are better. However, other markers with similar characteristics should be identified. Tissue-specific assays would be needed for these efforts. Genomic and proteomic screening analyses also would be useful. To perform powerful human genetic studies, extreme phenotypes are needed. For example, gene expression profiling in peripheral monocytes or PBMC from people without atherosclerosis could be compared with profiling done on cells from people with atherosclerosis. Likewise, proteomic analyses might facilitate longevity studies by examining the proteins produced in long-lived humans. Proteomic analyses of serum proteins produced in certain cancers may serve as a model for this effort. Gene expression profiling has been done in monkeys and mice, as a function of age and tissues, and shown striking differences in tissue, as opposed to cells. These studies also can serve as another model.

In conjunction with improved genetic methods, better cellular methods are needed as well. For example, what is feasible as far as biopsies in healthy people should be determined, and less invasive methods should be developed to allow for larger sampling.

IMPLICATIONS OF INTERVENTIONS THAT MODULATE CYTOKINE ACTION

Inflammation and Aging: Lessons Learned from Medical Intervention Studies

Russell Tracy, Ph.D., University of Vermont

As the presentations in this meeting have shown, linear thinking should be abolished when considering inflammation, inflammatory mediators, and aging. In many cases, humans appear to age exponentially, most likely because of feedback loops among organ systems and homeostatic mechanisms such as inflammation. Each component plays a vital role, and as components begin to degrade over time, they feed back to each other, affecting health. Inflammation markers can reflect underlying disease, causal pathways, or both. Interventions and medications can provide insight into the crosstalk between systems. The following interventions have been explored for CVD.

Warfarin

The Thrombosis Prevention Trial (Medical Research Council, 1998) and Warfarin after Myocardial Infarction Trial (Anand et al. 1998) identified beneficial effects of warfarin. In the Thrombosis Prevention Trial, warfarin reduced the risk for fatal myocardial infection by 39 percent, compared to aspirin, which reduced the risk for non-fatal myocardial infarction by 32 percent. In the Warfarin after Myocardial Infarction Trial, moderate- to high-intensity warfarin reduced the risk for stroke, myocardial infarction, and death. Low-intensity warfarin showed no benefit. Warfarin, however, is difficult to control and can profound effects, limiting usefulness in these settings.

Aspirin

Data from the Physicians Health Study show that aspirin confers a benefit in people with higher CRP levels (Ridker et al. 1997). Aspirin now has become a paradigm in terms of preventive medications. However, Feng and colleagues (2000) observed little change in CRP with the use of aspirin, at best a small decline in the short term. Likewise, Ikonomidis and colleagues (1999) did find that high-dose aspirin lowered CRP in patients with chronic stable angina. Thus aspirin confers a benefit over longer periods of time in people who are very ill; at that time significant drops in both IL-6 and CRP were observed, suggesting that aspirin is an anti-inflammatory drug.

Activated protein C

Activated protein C, an anticoagulant enzyme that inactivates factor VIIIa and factor Va, is extremely effective in late-stage sepsis as an anticoagulant (Bernard et al. 2001). While there are fewer data on the anti-inflammatory effects of this protein, there are some survival curve differences, as well as decreases in serum IL-6, that strongly support this position, identifying an important link between the coagulant and inflammation systems.

Lipid-lowering drugs

Secondary prevention data from the Cholesterol and Recurrent Events trial (Ridker et al. 1998) showed that statins are highly effective in people with a high level of inflammation. The effects of statins on CRP fall within a broad range, and for some men, statins provide no benefit. These findings have been strongly supported in a number of studies including AFCAPS-TexCAPS (Ridker et al., 2001).

Fibric acid derivatives

Data on these drugs are contradictory. In the context of hyperlipidemia, gemfibrozil has been shown to lower fibrinogen but not CRP (de Maat et al. 1997), increase fibrinogen and plasminogen activator inhibitor type 1 (PAI-1, Durrington et al. 1998), and reduce fibrinogen, IL-6, and CRP (Staels et al. 1998). As a peroxisome proliferator-activated receptor alpha ligand, fenofibrate inhibits IL-1–induced expression of IL-6 in human aortic smooth muscle cells (Staels et al. 1998) and reduces CRP-induced expression of monocyte chemoattractant protein 1 by human umbilical vein endothelial cells (Pasceri et al. 2001). In the Veterans Affairs High Density Lipoprotein Cholesterol Intervention Trial (VA-HIT) nested case-control study, inflammation in men with low LDL and CHD is associated with an increased risk for recurrent coronary events, and inflammation appears to be a non-lipid target of gemfibrozil in these patients (Cushman, in preparation). Clinical application of these findings requires further investigation.

Estrogen

Hormone Therapy (HT) data have been interesting from a coagulation standpoint. Most of this data come from studies on oral estrogen; many of the observed effects with oral estrogen are not mimicked by the patch, suggesting a first-pass liver effect. Estrogen has been shown to increase HDL, protein C, triglycerides, factor VIIc, and prothrombin and to decrease LDL, fibrinogen, PAI-1, and antithrombin. The Heart and Estrogen/Progestin Replacement Study (HERS) has found that estrogen increases the risk for early CHD mortality (Hulley et al. 1998). These data are supported by data from the WHI. Prior to HERS, the Postmenopause Estrogen/Progestin Interventions trial showed that hormone use doubled or tripled CRP levels in these women. Selective estrogen-receptor modulators do not mimic these effects, as demonstrated in three separate studies (Cushman et al. 1999; Cushman et al. 2001; Walsh et al. 2000). The current paradigm suggests that oral estrogen is an anti-inflammatory compound, but the cause of the small but real early CVD risk is unknown, and the rise in CRP is interesting in this regard.

Insulin and thiazolidinediones (TZDs)

While not a lot of data have emerged, TZDs appear to reduce IL-6, CRP, and MMP-9 concentrations, as well as the number of WBC (Haffner et al. 2002). Insulin therapy appears to lower CRP, but it is the most important driver of plasma PAI-1, so mixed effects seem likely.

Exercise and/or weight loss

Geffken and colleagues (2001) have found that in the general population, increased physical activity is associated with lower CRP, fibrinogen, factor VIIc, and WBC count. In a study on inflammation, thrombosis, and metabolic syndrome, Heilbronn (2001) showed that a 12-week caloric restriction (CR) regimen resulted in an average weight loss of 7.9 kg that led to some reduction of CRP. Calles-Escandon and colleagues (1996) showed that CR-induced weight loss decreases PAI-I and plasmin/antiplasmin complexes.

Blood pressure

Pahor and colleagues (2002) have noted that blood pressure medications exert no large effects on inflammatory markers but that they do exert a large apparently beneficial effect on PAI-1.

Inhibitors of bone resorption

A study of oral alendronate therapy, which reduces osteoporosis, reveals significant decreases in IL-1, IL-6, and TNF-α after 30 days. These decreases persist even after 90 days. This therapy also significantly reduces CRP after 90 days.

Anti-TNF therapy

Although infliximab, a chimeric monoclonal immunoglobin antibody to TNF-α, has changed the course of therapy for Crohn's disease (Rutgeerts et al. 2004), it has not demonstrated benefit in patients with symptomatic heart failure (Feldman and McTiernan 2004). Work in this direction should continue, taking into consideration routes of administration, different anti-TNF reagents, reagents addressing different cytokines, and improved or alternative selection of patients.

The data surrounding these drugs suggest several considerations for future research. The antagonistic pleiotropy discussed during this workshop may be especially important in drug safety, when different members of a compound class are treated the same. Routes of administration and characteristics of a single reagent may be critical. It is also important to note that not all markers reflect drug action equally, especially when high doses of medications are used. Finally, pharmaceutical companies should note that if a drug works better than expected, it may be an anti-inflammatory drug.

Discussion

With respect to activated protein C, it is not clear how blood clots trigger inflammation. Clots may or may not trigger inflammation, and activated protein C could be involved in effects outside coagulation. However, the most likely function for this protein is in anticoagulation and in thrombin generation. The literature supports the role of thrombin outside blood clotting. However, this enzyme is promiscuous, as is plasmin, and their functions are not clear.

The long-term use of medications, such as statins and oral estrogen, should be explored further. Women who take oral contraceptives may become hypercoagulable and exhibit increased CRP concentrations. The CRP increase remains unexplained, while the hypercoagulation observed in these women may be explained by changes in coagulation factors. However, in terms of IL-6, some mouse data and *in vitro* data show that low levels of estrogens result in decreased IL-6. In pregnant women, where estrogen levels are much higher, these mediators are markedly overproduced. More may be learned from studies like SWAN. As far as statins are concerned, late in life and especially among frail people, the incidence of cancer increases, then at later ages, drops. Statins are involved with farnesylation pathways, and may provide a benefit related to tumor activation and proliferation. However, the effects of long-term use of statins are unknown. Finally, in general, there is a risk for rebound when inhibitors of processes are removed after long-term use, possibly resulting in hyperreactivity; this is of some concern and should be factored into clinical research planning.

RFA Announcement

The NIA Geriatrics and Clinical Gerontology Program has released an RFA, *Developing Interventions for Multiple Morbidities*, to stimulate the development of interventions in the context of multiple conditions or diseases (National Institutes of Health, 2004). The application receipt date is January 13, 2005.

FUTURE OPPORTUNITIES FOR RESEARCH

Innovative Strategies for Basic Research

Discussion Leader: Elizabeth J. Kovacs, Ph.D., Loyola University Chicago

Colonies of aged mice and rats, available through the NIA (contact: Nancy Nadon, Ph.D., Biology of Aging Program, NIA), supplies whole animals or tissues representing multiple ages. The colonies have switched from one facility to another; thus some phenomena observed in the original group are no longer reproducible. For example, in animals from the original group, macrophages spontaneously produce cytokines with higher levels of production by cells from older animals. However, following the move to a new facility, in our hands similar levels of cytokine are produced by macrophages from aged and young mide. This underscores the need to consider animal-model suppliers when designing future studies. At present, most investigators do not think about supplier-dependent differences in adiposity, diet, presence of infections, or environmental considerations. Differences between ex-breeders and virgin animals also should be considered. Additionally, sex differences in responses have yet to be addressed.

The development of aged knockout mice will further our understanding of the effects of aging. Dr. Kovacs' laboratory aging IL-6 knockout mice in the BALB/c background to study inflammatory responses after burn injury. Because this model is being developed through an NIA grant, cells and tissues that are not a part of Dr. Kovacs' studies are available to other investigators. The NIA also is considering the development of a colony with aged, knockout mice. The selection of the types of knockouts will be decided by a committee.

Interdisciplinary communication and collaboration is vital to future research. The development of a Web-based database can facilitate discussion about animal models and organ systems and encourage sharing and collaboration. The NIA Intramural Program has conducted population studies characterizing several phenotypes and performing several measurements. Investigators working with animal models that exhibit similar phenotypes could communicate with the Intramural Program and vice versa, again to facilitate collaboration. In addition, investigators with expertise in certain assays can share that expertise with others to further aging research.

Communication also can help investigators to determine whether the age they choose to study is appropriate for the research question. For example, inducing a disease associated with old age in a 2-month-old knockout mouse may yield different results, when compared to inducing the same disease in an 18-month-old mouse. This gap points to the need for a resource that permits comparisons of animal models with respect to age and, in some cases, sex, thus assisting investigators in their consideration of study design. The American Federation for Aging Research (AFAR) published such a compendium in 1990–1991, and investigators applying for AFAR grants were required to justify their selection of models based on information in the compendium. This resource should be updated and made available to all researchers working on the biology of aging.

A key question raised throughout this workshop is the source of the proinflammatory cytokines including IL-6. Adipose tissue, macrophages, and the liver all appear to produce IL-6 and other mediators, but distinguishing these as sources in healthy aged subjects of a particular disease system under study will require new methods. Collecting organs from old and young mice may not be enough. Nor is the collection of inflammatory cells. On the other hand, the tissue- or cell-

type–specific features predisposing an organism to disease also must be studied. The attempt to understand chronic disease in the context of inflammation is somewhat complicated by the fact that some people age without developing these diseases. Finally, signaling pathways, including oxidative pathways and free-radical generation which triggers NFκB, should be further explored.

Methodological Issues and New Studies for Investigating Risk Factors Related to Inflammation

Discussion Leader: Marco Pahor, M.D., Wake Forest University

The questions emerging from the meeting fall in five main areas: (1) measurement, (2) health-related outcomes; (3) age, gender, race effects; (4) body composition effects; and (5) interventions.

Measurement is one key consideration in the study of inflammatory markers and their roles in aging and chronic disease. It must be determined which cytokines are most important and whether the measurement of multiple markers is more informative than focusing on a single one. In addition, measurement must account for variation between and within individuals: sampling schedule (for example, time of day and single versus multiple sampling); test sensitivity and variation; the parameters defining cytokinemia versus those defining full inflammation; and differences among circulating cytokines, stimulated production of cytokines, and tissue levels.

The role of inflammatory markers in health-related outcomes is another key consideration. Cytokines do not exist in a vacuum, but they participate in a complex matrix of biological, comorbidity, behavioral, socioeconomic, and environmental factors. Whether the inflammatory markers discussed at this workshop participate in the causal pathway to disease or simply mark an underlying physiology remains to be determined. The time of measurement versus the occurrence of the outcome must be considered, as well as the specificity of the observed phenomena and the possibility of multi-organ failure leading to comorbidity. Whether inflammatory markers are directly related to other risk factors for disease must be examined. Further study is needed on the impact of subclinical disease on the observed associations, as well as the initial trigger for cytokine production.

Age, gender, and race or ethnicity effects form another key question. Normal values should be determined for population subgroups. Any assessment of variations in cytokine levels according to age, gender, and race or ethnicity should account for direct effects related to these factors, as well as the effects of co-existing conditions. This will further fine-tune age-, gender-, or race or ethnicity-related differences in the associations between cytokines and health outcomes.

The effects of body composition also should be considered in future research on inflammatory markers. The effects of adiposity and of total versus direct distribution of fat (subcutaneous, visceral, intramuscular) on cytokine levels are not clear. The effects of cytokines on sarcopenia, bone mineral density and remodeling, and other tissues such as brain, liver, and arteries are also unclear.

The challenge is to assess the causative role of cytokines in disease. Temporal and dose-response relationships must be explored, and the observed associations between cytokine levels and disease processes, their strength, their consistency, and their biological plausibility must be

assessed. Intervention studies and genetic studies, including the use of knockout animals, will further this research.

The design of potential interventions should consider the sources and mechanisms of cytokine production to be targeted, whether the cytokine can be modulated safely, and whether modulation of the cytokine translates into improved health outcomes. Multifactorial interventions may also be useful. There is a long list of interventions that should be tested in animal models, including anticoagulants, antihypertensives, antioxidants, aspirin and NSAIDs, hormones, glucose control, statins and fibrates, and weight loss and CR.

There is some disagreement on whether the size of effects is important. On the one hand, a small but statistically significant difference may not necessarily be biologically relevant. On the other hand, a small difference in cytokine level may not be biologically relevant at one point in time, but it may matter over time. For example, in the context of hepcidin, even low levels of IL-6 appear to have biological effects. Likewise, small changes in estradiol appear to accumulate over time, and the level of change might depend on the number of receptors present in the cell under study. This question is further complicated by differences among *in vitro* studies, animal models, and humans. The importance of differences depends on how long these differences last and on the sensitivity of the assay. High doses often are needed to induce an effect *in vitro*, but lower doses are needed *in vivo*. If a cytokine is increased in both scenarios, the change may be small.

Several intervention trials may be promising, but criteria must be established to choose the intervention to pursue. With respect to aging, it is especially critical to determine which outcome to study or whether to combine outcomes, because focusing on one single disease may not produce a complete answer. As several organ-focused Institutes have ongoing clinical trials, an opportunity for collaboration may exist if questions about age or inflammation can be added to a study already under way.

The timing of disease processes versus disease events may complicate further study. For example, MESA aims to study atherosclerosis and cardiovascular events. Atherosclerosis begins in middle-age, but CVD events occur at much older ages. This makes it difficult to gather information within the same study, and the current surrogate markers for earlier processes are weak at best. Identifying causal relationships means designing longitudinal studies across a broad age range, which can prove costly. In addition, chronic disease most likely stems from several processes, and future research must find a way to capture these processes with the event, accounting for the multidimensionality of health. Molecular imaging, which tags dynamic molecules and follows them during a process, will help in this regard.

Finally, randomized clinical trials have described the pleiotropic effects of drugs. Although beneficial effects are observed, the biology underlying those effects is not clear. Defining the underlying triggers will help in the development of more finely directed therapies.

The establishment of a forum for sharing thoughts and ideas after this workshop will be useful. The American Association for the Advancement of Science has a Web site, Science of Aging Knowledge Environment (www.sageke.org), which provides a forum for people to continue these types of conversations. However, there is a cost to gain access.

Integrative Physiology: New Perspectives on Tissue Interactions

Discussion Leader: Ronald Glaser, Ph.D., Ohio State University

Biological rhythms reflect the body's attempt to return to some baseline (homeostasis). These fluctuations not only reflect diurnal changes but seasonal changes as well. The regulation of homestasis of the immune system is influenced by neuropeptides and hormones. However, if challenged by a pathogen for example, the immune system is activated. Significant increases in replication of immune cells takes place and significant increases in cytokines accompany this process. It is known that cortisol increases simultaneously with the activation of the immune system, and it is thought that the production of this stress-suppressing hormone plays an important role in down-regulating the immune response to return to a baseline level of activation (Besedovsky, et al., 1986). It is likely that these fluctuations change as an individual gets older since there is a rich literature on the impact of aging on the immune response.

There is now increasing evidence that psychological stress can induce dysregulation of many aspects of the immune response and that chronic stress, particularly in older individuals, may actually "reset" the immune system such that important processes, such as inflammation, may be dysregulated resulting in higher levels and prolonged production of proinflammatory cytokines. Since levels of some proinflammatory cytokines, like IL-6, normally increase with aging, any up-regulation of the synthesis of this cytokine could have important health implications since many diseases found in older populations are associated with increased levels of IL-6. For example, Kiecolt-Glaser and colleagues (2003) have shown that the chronic stress of being a spousal caregiver of a patient with Alzheimer's Disease (AD) can reset the production of IL-6 to a significantly higher level than those found in the well matched control subjects not caregiving. Increased levels of serum IL-6 in older individuals has been associated with risks for rheumatoid arthritis, diabetes, cardiovascular disease and cancer. In a follow-up study, it was shown that caregiving may sensitize individuals in such a way that when exposed to an antigen, they over-express IL-6 and that these interactions could explain the data obtained in the first study (Glaser, et al., 2003).

Viral latency may present a new way to think about aging and inflammation. There have been several reports that show that stress reactivates latent herpesviruses such as Epstein-Barr virus (EBV) and cytomegalovirus (CMV). These studies are reviewed in Glaser, et al. (2005). A majority of people are infected with EBV and CMV by the time they become adults as demonstrated by the presence of antibodies to the viruses. The cellular immune response plays an important role in controlling the expression and replication of these latent herpesviruses.

If a person is immune-suppressed and the cellular immune response to the latent virus is impaired, the latent virus can be reactivated. The cellular immune response in older individuals is not as vigorous as the cellular immune response in young individuals and, therefore, older people are less able to control the steady-state expression of, for example, latent EBV. Healthy older individuals show evidence of an increase in "leakage" of latent EBV proteins leading to a memory immune response and higher levels of antibody to the virus (Glaser, et al., 1985). The same has been shown for CMV (McVoy and Adler, 1989). Chronic stress exacerbates these interactions as reflected in even higher antibody titers (Kiecolt-Glaser, et al., 1991). It is possible

that the increases in the production of viral proteins could play a role in chronic inflammation and the production of proinflammatory cytokines, like IL-6.

Recent studies suggest that some EBV-encoded proteins including the latent membrane protein-1 (LMP-1), the membrane antigen gp350, and the deoxyuridine triphosphate nucleotidohydrolase (dUTPase) can induce immune dysregulation of the immune response (Dukers, et al., 2000; D'Addario, et al., 2000; Padgett, et al., 2004). There are data that suggest that these interactions can up-regulate the production of proinflammatory cytokines, including IL-6 (Glaser, et al., 2005). The data from these series of studies suggest that EBV-encoded proteins, by themselves and independent of their function in virus replication, can induce immune dysregulation in virus-infected patients. Another way of looking at this relationship is that people may carry, within their own latent viruses, a source of viral antigens that can produce inflammation when the virus is reactivated. As already mentioned, this process may be enhanced in older individuals. It is possible that the connection between stress and inflammation may be part of the process linking inflammation with cardiovascular disease.

It has been suggested that approximately 15 percent of all human cancers are associated with inflammation. As already discussed, there are many common illnesses observed in older individuals that have been associated with increased serum levels of IL-6, including cancer.

In thinking about viral latency in this context and more globally, the T-cell response is known to decrease with aging. This is reflected in the studies that show higher antibody titers to latent EBV and CMV in older individuals. Whether the "leakage" of viral proteins associated with the reactivation process is directly linked in some way to increases in serum IL-6 levels is yet to be shown, but studies are underway to determine if this connection can be made. It may be possible to explore these relationships using genomic approaches and study the expression of viral proteins that may be modulated by the aging process. It is possible, through studies like this, that a new way of looking at herpesvirus latency may provide insight into understanding the pathophysiology of latent viruses and diseases, such as cardiovascular disease.

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APPENDIX 1

National Institute on Aging
Workshop on Inflammation, Inflammatory Mediators and Aging
Bethesda, Maryland
September 1–2, 2004

Agenda

WEDNESDAY, SEPTEMBER 1

8:30 a.m.	Welcome and Introductions	**Sherry Sherman, Ph.D.** *National Institute on Aging*
		Richard J. Hodes, M.D., *Director* *National Institute on Aging*
8:40	Inflammation and Aging: A Biologist's Perspective	**Russell Tracy, Ph.D.** *University of Vermont*
9:10	*Discussant*	**Lewis Kuller, M.D., Dr.P.H.** *University of Pittsburgh*
9:20	*General Discussion*	

Basic Science
Session Leaders: Elizabeth J. Kovacs, Ph.D., and Rebecca Fuldner, Ph.D.

9:30	Overview of the Biology of Cytokines and Aging	**Joost Oppenheim, M.D.** *National Cancer Institute*
10:10	*Discussion*	
10:15	Overview of Innate Immune Cells and Cytokine Production: Changes that Occur with Age	**Elizabeth J. Kovacs, Ph.D.** *Loyola University, Chicago*
10:35	*Discussion*	
10:40	**BREAK**	
10:55	Aging and the Adaptive Immune System	**Donna Murasko, Ph.D.** *Drexel University*
11:15	*Discussion*	

11:20	Adipocytes, Inflammatory Responses and Metabolic Regulation	**Gokhan S. Hotamisligil, M.D., Ph.D**. *Harvard School of Public Health*
11:40	*Session Discussion*	
12 noon	**LUNCH**	

Mediators of Inflammation as Risk Factors for Morbidity and Mortality
Session Leaders: Jeremy Walston, M.D., and Sherry Sherman, Ph.D.

1:00 p.m.	Inflammation Reflects Molecular Processes in Fat and Multiple Organ Systems	**Tamara Harris, M.D.** *National Institute on Aging*
1:25	*Discussion*	
1:30	Inflammation, Homeostasis and Frailty	**Luigi Ferrucci, M.D., Ph.D.** *National Institute on Aging*
1:55	*Discussion*	
2:00	Inflammatory Cytokines and the Anemia of Frailty	**William B. Ershler, M.D.** *Institute for Advanced Studies in Aging and National Institute on Aging*
2:20	*Discussion: Cytokines and Frailty*	
2:30	Periodontal Disease, Inflammation, and Cardiovascular Outcomes	**Steven Offenbacher, D.D.S., Ph.D.** *University of North Carolina Chapel Hill School of Dentistry*
2:50	*Discussion*	
2:55	Chronic Stress, the HPA Axis, and Inflammation	**Janice Kiecolt-Glaser, Ph.D.** *Ohio State University College of Medicine*
3:15	*Discussion*	
3:25	**BREAK**	

Integrative Physiology
Session Chairs: Caleb E. Finch, Ph.D. and Jill Carrington, Ph.D.

3:40	Caloric Restriction, Inflammation and Aging	**Richard Weindruch, Ph.D.** *University of Wisconsin*
4:00	*Discussion*	
4:05	Inflammatory Processes in Brain Aging	**Caleb E. Finch, Ph.D.** *Andrews Gerontology Center University of Southern California*
4:25	*Discussion*	

4:30	Inflammatory Cells, Cytokines, and Cognition in Aging	**Steven F. Maier, Ph.D.** *University of Colorado*
4:50	*Discussion*	
5:00	**ADJOURN**	

THURSDAY, SEPTEMBER 2

8:30 a.m.	Inflammatory Cytokines and the Cardiovascular System	**Jeffrey Bender, M.D.** *Yale University School of Medicine*
8:50	*Discussion*	
8:55	Muscle Effects Related to the Modulation of Cytokine Action by Aging, Diet, and Physical Activity	**Helle Bruunsgaard, M.D., Ph.D.** *University of Copenhagen, Denmark*
9:15	*Discussion*	
9:20	Inflammatory Cells, Cytokines, Estrogen Effects and Bone	**Sundeep Khosla, M.D.** *Mayo Clinic, Rochester, MN*
9:40	*Discussion*	
9:45	Reproductive Hormones, Inflammatory Cytokines, and Inflammatory Markers in the Onset of X-Ray Defined Osteoarthritis of the Knee	**MaryFran Sowers, Ph.D.** *University of Michigan*
10:05	*General Discussion: Caleb E. Finch, Ph.D.*	
10:25	**BREAK**	

Implications of Interventions Which Modulate Cytokine Action

10:45	Inflammation and Aging: Lessons Learned from Medical Intervention Studies	**Russell Tracy, Ph.D.** *University of Vermont*
11:15	*Discussion*	

Future Opportunities for Research

11:30 Innovative Strategies for Basic Research
Discussion Leader: *Elizabeth J. Kovacs, Ph.D., Loyola University, Chicago*

11:50 Methodological Issues and New Studies for Investigating Rick Factors Related to Inflammation
Discussion Leader: *Marco Pahor, M.D., Wake Forest University, School of Medicine*

12:10 Integrative Physiology: New Perspectives on Tissue Interactions
Discussion Leader: *Ronald Glaser, Ph.D., Ohio State University*

12:30 **ADJOURN**

APPENDIX 2

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