



## Blasts from the past

Paul A. Insel, Stuart Kornfeld, Philip W. Majerus, Andrew R. Marks, Paul A. Marks, Arnold S. Relman, Bruce F. Scharschmidt, Thomas P. Stossel, Ajit P. Varki, Stephen J. Weiss, and Jean D. Wilson

**With this issue of the *JCI*, we celebrate the 80th anniversary of the *Journal*. While 80 years is not a century, we still feel it is important to honor what the *JCI* has meant to the biomedical research community for 8 decades. To illustrate why the *JCI* is the leading general-interest translational research journal edited by and for biomedical researchers, we have asked former *JCI* editors-in-chief to reflect on some of the major scientific advances reported in the pages of the *Journal* during their tenures.**

While the look and content of the *JCI* have evolved in many ways over 80 years, there has been at least one constant. The *JCI* has been, and continues to be, the foremost journal focused on publishing important advances in biomedical research. We can now note another important distinction: the *JCI* is the only high-impact general-interest biomedical journal available without barrier online.

In its early days, the *JCI* was *the* journal of record for the most important advances in “clinical science.” This meant that new understandings of human physiology and pathophysiology were published in the pages of the *JCI* along with new techniques for making physiological measurements. This tradition continued into the 1960s and ’70s and included reports of the breakthrough methodology for platelet radiolabeling, elucidation of the physiological role for ketone bodies during starvation, and discovery of the metabolic basis for cholesterol gallstone formation.

In the 1980s, the beginning of the revolution in biomedical research with the advent of increasingly powerful methodolo-

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gies — “DNA technology” — exerted a profound influence on the *JCI*. Initially, the impact pertained to the use of recombinant technology to clone and express human genes, such as the generation of human recombinant tissue plasminogen activator (tPA) to dissolve occlusive thrombi that cause myocardial infarction; and an important series of *JCI* articles elucidating the link between hypertension and kidney disease, which have greatly affected the development of current therapies for hypertension. Within the pages of the *JCI*, the reader will find, throughout the decades, descriptions of state-of-the-art technology that was at the forefront of each field at the time.

Another long-standing theme in the *JCI* has been the discovery of new molecules important in human physiology and/or new

functions for known molecules, including the discovery that endothelium-derived relaxing factor (EDRF), which is linked to blood vessel relaxation, is NO.

During the 1990s the *JCI* assumed a new role as the journal of choice for publishing articles describing animal models (usually

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genetic models in mice) of human diseases. Seminal studies of the pathogenesis of atherosclerosis, based on the use of genetic mouse models, were published in the *Journal*. This theme continues to the present. Within the last decade, the *JCI* has consistently published descriptions of outstanding animal models, providing advances in our understanding of the cellular or molecular basis of key physiologic and pathophysiologic processes and also of novel therapeutics, such as tyrosine kinase inhibitors for the treatment of cancer and oral diabetes therapies. While this is not the sole purview of the *JCI*, it does represent classic “translational” research of our time and in that sense continues according to the original intent of the *Journal*: to provide a venue for the very best clinically relevant research.

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Wherever the future of biomedical research takes us, as techniques change and new challenges arise, after 80 years it is clear that the *JCI* will remain the journal of choice for disseminating (online and barrier free) the most important advances in understanding the basis for human diseases and their treatment.

Andrew R. Marks, Editor-in-Chief

**Nonstandard abbreviations used:** ACD, acid citrate and dextrose solution; ACE, angiotensin I-converting enzyme; AMPK, AMP-activated protein kinase; Apo2L, Apo2 ligand; CDGS, carbohydrate-deficient glycoprotein syndrome; DTT, direct thrombin inhibitor; EDRF, endothelium-derived relaxing factor; FPA, fibrinopeptide A; IκB-α, inhibitor of κB-α; JRA, juvenile rheumatoid arthritis; OxLDL, oxidatively modified LDL; PlGF, placental growth factor; QTL, quantitative trait loci; sFlt1, soluble fms-like tyrosine kinase 1; tPA, tissue plasminogen activator; VPF, vascular permeability factor.

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1962–1967

Arnold S. Relman

*I was editor when clinical research was simple and inexpensive, NIH funds were plentiful, and any bright, hard-working academic physician could make useful scientific contributions without sacrificing a commitment to patients and students. Now, with expensive, sophisticated tools and much better science, funding is harder to find, as are good “triple-threat” people. We need new models for research-oriented clinical departments and new sources of support.*

### Platelet labeling with Cr<sup>51</sup>: ACD better than EDTA

Human platelets were known to survive for between 8 and 11 days, but in 1964 the mechanism of platelet death was a subject of controversy. Was platelet death random or a result of cellular senescence? Researchers were hindered in efforts to solve this mystery by the available methodology. Platelet labeling with the radioisotope Cr<sup>51</sup> using EDTA induced platelet sequestration and the death of two-thirds of the platelet population.

#### *Was platelet death random or a result of cellular senescence?*

Aster and Jandl of Harvard Medical School described a new method for labeling platelets with Cr<sup>51</sup> using an acid citrate and dextrose solution (ACD) that improved platelet viability and eliminated the initial transient sequestration previously noted in platelets prepared with EDTA as an anticoagulant (1). Using ACD, which unlike EDTA is not injurious to platelets, they showed that circulating Cr<sup>51</sup> activity was a true measure of platelet survival. They also showed that platelets are probably removed from the population by a process of senescence and are normally destroyed in the liver.

This was a methodological breakthrough that opened the way for more effective platelet transfusions and made accurate studies of platelet kinetics and turnover possible.

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### Adrenaline arrests insulin secretion

To investigate the mechanism by which epinephrine elevates blood glucose, Porte and colleagues at the University of Washington carried out studies in healthy volunteers in which immunoreactive insulin levels were measured with or without administration of epinephrine, while various biochemical stimuli that normally influence insulin release were administered (2). Prolonged infusion of epinephrine was found to increase plasma glucose but not insulin levels. Infusion of glucose at a rate that produced a lower rise in blood glucose raised insulin levels much more. Administration of glucagon alone raised glucose and insulin levels, and while glucagon plus epinephrine raised glucose lev-

els even more, there was still no observable increase in insulin. Similarly, i.v. administration of the glucose-lowering agent tolbutamide increased insulin levels, but there was less of an increase when tolbutamide was given together with epinephrine.

The authors concluded that their data were consistent with an inhibitory effect of epinephrine upon pancreatic insulin release but did not rule out the possibility of increased insulin removal. In any case, the authors made clear that epinephrine prevents insulin levels from rising in response to biochemical stimuli and that this effect plays a role in epinephrine-induced hyperglycemia.

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### Hormones, fats, fuels, and fasting

A 1996 study by Cahill et al. (3) of Harvard University was designed to obtain information about the metabolic and hormonal response to a 1-week fast in 6 healthy subjects and 2 patients with type 2 diabetes mellitus. The authors showed that the release of peripheral fuels, primarily from fat, and to a lesser degree from protein, as well as the maintenance of blood glucose concentrations, seemed to be related to insulin concentration. Growth hormone levels were apparently not correlated with these changes. The authors concluded that the glucose-insulin feedback mechanism is probably the controlling factor in maintaining viability during prolonged fasting. Their data also suggested that CNS glucose metabolism is decreased as a survival adaptation.

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This study extended the classic earlier work by Benedict (4) on the metabolic adaptation to fasting. It also contributed important new data on the role of hormones (insulin and growth hormone) in the elaborate biochemical adjustments that permit long-term survival when food is withdrawn.



1967–1971

Paul A. Marks

*Being editor-in-chief was a very rewarding and exciting experience. During my tenure, we received many manuscripts, which kept my associate editors and me abreast of the best work in clinical science. It was particularly rewarding to work with the editorial board in what amounted to weekly scientific mini-symposiums. It was also rewarding to see the JCI grow rapidly during our tenure – from a bimonthly to a monthly publication.*

### Food for thought: ketosis mystery solved

Until the late 1960s, studies had suggested that the only significant energy-yielding substrate consumed by the human brain was glucose (5–7). This absolute requirement for glucose in order to sustain brain function calls for the substrate to be derived from limited carbohydrate and protein stores. Herein laid a conundrum: humans are capable of fasting for periods of time beyond which we have utilized all of our carbohydrate stores and muscle for gluconeogenesis in order to maintain blood glucose levels. So how does the brain function during starvation?

#### *How does the brain function during starvation?*

In 1967, Owen and colleagues (8) at Harvard Medical School catheterized cerebral vessels in 3 obese patients and demonstrated that under conditions of prolonged (5–6 weeks) starvation, when carbohydrate stores are reduced to just such a low level, the brain could turn to the metabolism of the fat-derived ketones  $\beta$ -hydroxybutyrate and acetoacetate to replace glucose as its primary fuel (by the process of ketosis). Historically, keto-

sis was considered to be a pathological condition associated with diabetes, which resulted in the view that ketone bodies were toxic waste products. This finding triggered a reversal in our way of thinking, and we now know that these two ketone bodies are the only free soluble fats that the body can use.

### Leaving no gallstone unturned

Cholesterol in normal human bile is completely soluble, and cholesterol gallstones in the gall bladder are caused by the precipitation of insoluble cholesterol. What had continued to puzzle researchers in the 1960s was the nature of this precipitation. Was it the result of an increase in cholesterol levels, a decrease in bile salt or lecithin, or a combination of these and/or other factors?

In 1968, Admirand and colleagues (9) at Boston University Medical School compared the bile of healthy subjects and those suffering from gallstones and established for the first time that the relative concentrations of bile salt, lecithin, and cholesterol, but not other constituents of bile, determine the solubility of cholesterol in bile and subsequent cholesterol gallstone formation. Their study became the most frequently cited JCI paper published between 1967 and 1970.



1972–1977

Jean D. Wilson

*Running the JCI was a learning experience. First, my associate editors were a source of scholarly insight, inspiration, and continuing education. Second, it became apparent that the function of peer review is not to accept or reject but to make weak papers good and good papers better. Third, some authors were offended (or offensive), but most were serious participants in the editorial process. Fourth, being editor then involved running a business, as the journal evolved from receiving an annual subsidy from the ASCI to maintaining a positive net balance.*

### Hyperlipidemia and myocardial infarction

It had been known for many years that: (a) atherosclerotic plaques contain cholesterol; (b) raising the serum cholesterol level in animals such as rabbits causes atherosclerosis; (c) coronary heart disease tends to aggregate in certain families; and (d) the rare monogenic disorder idiopathic hypercholesterolemia accelerates development of atherosclerosis and its complications. However, it was not known whether hyperlipidemia was a common factor in ordinary myocardial infarction.

Goldstein and colleagues at the University of Washington analyzed plasma cholesterol and triglyceride levels in 500 survivors

of myocardial infarction and found that 30% had hyperlipidemia and that elevation of triglyceride levels (with or without an associated elevation in cholesterol) was more common than elevation in cholesterol levels alone (10). The authors went on to perform family studies, including segregation analysis, in 2,520 relatives and spouses of 176 survivors of myocardial infarction, including those with and without hyperlipidemia (11). Three distinct lipid disorders – familial hypertriglyceridemia, familial hypercholesterolemia, and familial combined hyperlipidemia – were present in 20% of survivors younger than 60 years. The most common familial disorder in this study – familial com-



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bined hyperlipidemia – was shown to originate from variable expression of an autosomal dominant gene.

Demonstration that hyperlipidemia is a major contributor to the development of myocardial infarction and that a previously unrecognized disorder is the most common cause of hyperlipidemia changed the perception of atherosclerosis and stimulated the development of new therapies for its prevention and treatment.

### Disorders of growth

Because of episodic secretion, rapid turnover in plasma, and wide variability in levels among healthy subjects, the random assay of plasma growth hormone has limited value in the diagnosis of growth disorders. Many of the physiological effects of growth hormone are mediated by secondary, heterogeneous mediators, the somatomedins, which turn over in plasma much more slowly. Measurement of somatomedins had hitherto involved cumbersome bioassay or radio-receptor assays. In 1977, Furnaletto and colleagues at the University of North Carolina succeeded in purifying the major species, somatomedin C, and developed a sensitive and specific radioimmunoassay for its detection in plasma (12). The *JCI* had a tradition of not publishing methods papers, but in this instance, the assay was used to characterize the normal developmental pattern of somatomedin levels from birth to adulthood, to demonstrate its absence in the plasma of hypopituitary children, to define the response of plasma levels following the administration of growth hormone to children deficient in the hormone, and to demonstrate a clear separation in

plasma levels between healthy individuals and subjects with acromegaly (12). This study provided insight into normal growth and changed the way that growth disorders are diagnosed.

### Understanding wall stress and hypertrophy in the human heart

The study of cardiac muscle growth, leading to either physiologic or pathologic hypertrophy, has intrigued clinical and basic scientists for over 100 years. In their 1975 study, Grossman and colleagues at the University of North Carolina examined the relation between wall stress (a surrogate for myocyte force throughout the cardiac cycle) and cardiac hypertrophy (a surrogate for myocyte growth) in patients with different types of hemodynamic overload (13). Their observations led to the hypothesis that increased systolic force at the myocyte level, as seen in sustained-pressure overload of the cardiac ventricle, induces parallel replication of sarcomeres within the myocyte, increased cell *width*, and a thickened ventricle wall but normal chamber size (concentric hypertrophy). In contrast, increased diastolic force at the myocyte level, as seen in sustained-volume overload of the cardiac ventricle, induces series replication of sarcomeres, increased myocyte cell *length*, and an enlarged ventricle chamber but normal wall thickness. Interaction between systolic and diastolic overload adds some complexities that require fine tuning of the model, and these were addressed in subsequent publications by these authors. The cellular mechanisms that transduce these increases in force into biochemical signals have only partially been discovered.



1977–1981

Philip W. Majerus



1981–1982

Stuart Kornfeld

*We were continually impressed with how well the peer review process functioned overall. Referees who were volunteering precious time made great efforts to review the manuscripts in depth and to provide thoughtful critiques. There were numerous instances where the authors thanked the referees for insightful suggestions that strengthened their work. While we're sure we made some errors of judgment concerning priority decisions on papers, the review process served a very useful purpose.*

### Thrombolytic therapy: dissolving blood clots with human tPA

Studies in the 1970s revealed that extrinsic tPA had the potential to be a useful thrombolytic agent because it preferentially activated plasminogen in the presence of fibrin, thereby confining the activation process to the immediate environment of a formed thrombus – in contrast to the systemic activation that occurred following administration of other known thrombolytic agents such as urokinase and streptokinase. However the ability to test this proposal was limited by the small quantities of tPA available at the time. In 1981, Rijken and Collen discovered (14) that relatively large amounts of tPA could be purified from secretions of a human melanoma cell line. Collen and col-

leagues subsequently published 2 papers in the *JCI* in the early 1980s showing that tPA dissolved experimental thrombi in dogs (15) and rabbits (16). The therapy was relatively nontoxic and was most effective if administered soon after the thrombus was formed. They found the thrombolytic effects of tPA to be superior to those of urokinase in these animal models. In 1983, scientists at Genentech Inc. reported the cloning and expression of human tPA. This allowed Collen, Sobel, and their colleagues (17) to follow up these preclinical studies with human trials of tPA for thrombolysis of coronary artery thrombi. By the early 1990s, tPA was a standard therapy for acute myocardial infarction, resulting in a significant reduction in mortality. Thousands of lives have been saved using this therapy.





1982–1986

Thomas P. Stossel

*Serving as editor-in-chief of the JCI was one of the most enjoyable activities of my professional career. It was a privilege to meet weekly with associate editors, leaders in their fields, to process cutting-edge biomedical research. Authors, even of papers we did not publish, were generally responsive and respectful.*



1986–1987

Joseph Avruch

*During the early 1980s, molecular investigation was rapidly becoming the basis of clinically relevant physiological research, and the most frequently cited articles of this period reflect the transitional nature of the era. The manuscripts were remarkably diverse in topic and approach; they were considered with care and respect but without solemnity. The weekly editorial meetings were a pleasure, easily the most enjoyable and informative committee work I've ever done.*

#### **Location, location, location: it's the site of fat, not how much, that proves important**

The paper by Krotkiewski et al. (18) of the University of Goteborg, Sweden, received considerable media attention following its publication in 1983. Obesity had long been viewed as a risk factor for vascular disorders; however, at this time it was viewed in a rather generic way – heavy was heavy, fat was fat. This study presented the astounding finding that the critical factor in the effect of obesity on metabolism in both men and women was the location of adipose tissue. If one's adipose tissue accumulation was peripheral (localized to the arms, legs, and hips), the impact on lipid and carbohydrate metabolism was relatively benign. If adipose tissue

*Not only was the amount of excess body fat important but the location of body fat was critical.*

was centrally distributed (localized to the buttocks and abdomen), then metabolic and clinical consequences were much more severe. The authors also demonstrated that in both obese and nonobese individuals, regional differences existed between the sexes: men and women were found to store their fat in different locations.

#### **A link between hypertension, obesity, and glucose intolerance**

Although hyperinsulinemia was already a well-recognized risk factor for cardiovascular disease, no one had been able to provide a molecular handle to explain the ubiquitous association of glucose intolerance, obesity, and hypertension. In 1985, Modan and colleagues at Tel Aviv University (19) nicely documented that a common characteristic of patients with high blood pressure, obesity, and glucose dysregulation was high insulin levels. Hypertension was found to be associated with insulin response both independent of, and additive to, obesity and glucose intolerance, and hypertension and glucose intolerance appeared to evolve concomitantly, which suggested a common pathogenetic mechanism.

These somewhat counterintuitive findings pointed to altered membrane cation transport in cells controlling insulin secretion. Later studies postulated that insulin resistance and compensatory hyperinsulinemia underlay this clustering, and we now describe this association as the metabolic syndrome – an important cause of atherosclerotic cardiovascular disease.

#### **The rise of antibodies as therapeutics**

Although they were touted as magic bullets at the time of their first creation, by the mid-1980s the word was that monoclonal antibodies, despite their exquisite specificity, could not transcend their utility as diagnostic agents and actually serve therapeutic purposes. Efforts to treat disease, mainly cancer, with these antibodies had all failed. The monoclonal antibody described by Collier et al. of the State University of New York at Stony Brook in two *JCI* papers from 1983 and 1985 (20, 21), initially utilized as a research tool to analyze the function of the platelet integrin (now called  $\alpha_2\beta_3$ ), turned out to be uniquely suited to inhibit the platelet aggregation underlying acute coronary syndromes without the predicted effect of inducing platelet clearance. Eventually developed for human use, this antibody became the proof of concept, demonstrating that in addition to their ability to identify disease pathogens, monoclonal antibodies can be utilized to deliver drugs, and they have since saved many lives by preventing myocardial infarctions and complications of angioplasty.

#### **Leukocyte-endothelial adhesion: let the good times roll**

In the late 19th century, Cohnheim first described the adhesion of leukocytes to postcapillary venules, demonstrating the phenomenon of their rolling along the vessel wall at sites of inflammation (22). This observation even preceded Metchnikoff's discovery of the defensive and inflammatory role of phagocytic leukocytes (23). Just over 100 years later, in 1985, the *JCI* article by Bevilacqua et al. (24) of Harvard Medical School was one of the first to contribute a molecular definition of these phenomena and to show how surprisingly dynamic they are, involving regulated expression of adhesion molecules in response to inflammatory mediators.



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The authors found that the inflammatory-immune mediator IL-1 acted directly and selectively on endothelial cells to alter their surface properties and thereby enhance the adhesion of circulating blood leukocytes to endothelial cells at the site of inflammation.

### High blood pressure contributes to renal injury

Three articles published in the *JCI* in 1985–1986 were a culmination of many years of work by the Brenner laboratory at Harvard Medical School and pointed to the importance of progressive destruction of the nephron due to increased pressure in the glomerular circulation (25–27). Abnormalities in glomerular hemodynamics, particularly elevations in glomerular pressures, had been previously implicated in the development of diabetes-associated glomerulopathy. The first of the 3 studies showed that treatment with the angiotensin I-converting enzyme (ACE), enalapril, maintained blood pressure at normal levels and significantly limited the development of glomerular lesions in a rat model of glomerulopathy (25). The data further supported the premise that glomerular hemodynamic changes mediate renal injury when nephron number is reduced. In a follow-up study, the authors demonstrated the therapeutic advantage of converting enzyme inhibition by arresting progressive renal disease associated with systemic hypertension in rats (26). The study demonstrated for the first time that long-term ACE inhibition in an

animal model of diabetes not only delayed but actually prevented the establishment of diabetic glomerulopathy, primarily by normalizing glomerular capillary pressure (27). That diverse etiologies converged to increase glomerular blood pressure — which is the final common pathway in glomerular deterioration — was impressive. The findings suggested that controlling hypertension might alleviate renal destruction. This concept was eventually shown to hold true in humans, and the approach is now a mainstay of clinical practice.

### IL-1 identified as a proinflammatory cytokine

The revolution in recombinant technology vindicated laborious and persistent efforts to purify and characterize “leukocyte pyrogen,” as specific inflammatory cytokines such as IL-1 and TNF became available for study. The Dinarello laboratory at Tufts University was at the vanguard of this effort, and their 1988 report in the *JCI* arguably established the identity of these cytokines as the long-sought pyrogenic substances that induce hemodynamic and hematological changes typical of septic shock (28). Although their suggestion that cyclooxygenase inhibitors may be of value in patients with IL-1/TNF-mediated shock did not survive as a panacea for preventing the systemic effects of these agents, the research described in the report encouraged the development of specific cytokine inhibitors now in use for the therapy of rheumatoid disorders.



## 1987–1992

**Bruce F.  
Scharschmidt**

*My impression on assuming responsibility for the JCI was that a change in direction was due in order for the journal to maintain its reputation for scientific rigor and become sufficiently competitive for groundbreaking translational work in molecular medicine and other fields. In 1990, we introduced changes in format and operation that collectively triggered an increase in the number and quality of publications that continues to the present. Each of the frequently cited articles published during our tenure and highlighted here significantly impacted current thinking regarding pathophysiology and/or therapy of human disease. Stewardship of the JCI was, for my associate editors and me, a tremendously enriching and rewarding experience.*

### Neutrophil swan song a result of pre-scripted apoptosis

The mechanisms underlying the resolution phase of inflammation, including the tissue clearance of extravasated granulocytes and their potentially histotoxic contents, had received little attention prior to the 1980s. It was assumed that neutrophils met their fate by necrosis, despite the likely deleterious consequences of tissue injury and amplification of inflammation. In contrast, a 1989 *JCI* study by Savill and colleagues (29) at the Royal Postgraduate Medical School, Hammersmith Hospital, London, showed that human neutrophils are constitutively programmed to die by apoptosis, which leads to macrophage phagocytosis of intact senescent cells that retain their complement of granule enzymes. Subsequent work established this process as a paradigm for beneficial deletion of other leukocyte types from inflamed sites and for characterization of the molecular mechanisms and associated anti-inflammatory consequences of phagocyte clearance of apoptotic cells, specific perturbation of which is now known to result in autoimmunity and chronic inflammation. Glucocorticoids and leukotrienes of the

resolvin family can enhance granulocyte apoptosis and/or clearance, raising the prospect that drugs targeted at this process will provide a new “pro-resolution” approach toward therapy for chronic inflammatory disorders.

### All talk and NO action: blocking vascular smooth muscle cell growth

Following the Nobel Prize-winning discovery of EDRF (30), several groups almost simultaneously reported the identification of EDRF as NO. Prior to that time, it had been difficult if not impossible to explore the effects of EDRF that were not directly linked to the relaxation of blood vessels. By 1987, it was already established that damage to the endothelium was associated with vascular remodeling, and there was speculation regarding the agent(s) that could inhibit vascular smooth muscle cell activation. Because EDRF was known to originate in endothelial cells and to target vascular smooth muscle cells, Garg and Hassid of the University of Tennessee, who had already shown that atrial natriuretic factor, which modulates NO production,



decreased mesangial cell proliferation (31), decided to find out whether NO could decrease the proliferation of cultured vascular smooth muscle cells. As reported in their 1988 *JCI* paper (32), using relatively simple techniques such as measurement of DNA synthesis and cell counting, they discovered that both NO and cyclic GMP decreased proliferation of cultured vascular smooth muscle cells without inducing cytotoxicity. Their article was the first to support the hypothesis of a potential interaction between EDRF/NO and vascular smooth muscle cells that is not directly related to vascular relaxation. This work was confirmed and extended by others (33, 34), who reported that NO had similar effects in vivo and could attenuate vascular remodeling and atherosclerosis.

### Selectins stick together in neutrophil-endothelial adhesion

The adherence of human neutrophils to endothelial cells was known to significantly increase following stimulation with different chemotactic factors. The intercellular adhesion molecule ICAM-1 had been shown to be expressed on the endothelial surface; however, little was known about how this molecule interacted with the rolling neutrophil.

The 1989 article by Smith et al. (35) of Baylor College of Medicine on the cooperative interactions of leukocyte function-associated antigen (LFA-1), Mac-1, and ICAM-1 in facilitating adhesion and transendothelial migration of neutrophils was the first of many publications from a number of laboratories defining the concept that the process of leukocyte migration from blood into tissue requires a sequential multistep cascade of adhesive and signaling events. The earliest articles to follow this report introduced shear stress into their model (36), demonstrating that under conditions of flow, the first step in leukocyte migration (37) was the capture of neutrophils by what are now known as selectins. Over the years, the multistep cascade of neutrophil adhesion under flow conditions has been found to be quite complex in terms of the number and variety of adhesion molecules and activation steps, and the process applies to all types of leukocytes with combinatorial determinants selective for specific leukocyte subsets.

### Endothelial glycoprotein emerges as an adhesion

GMP-140, also called PADGEM, was a membrane glycoprotein in search of a function. McEver and colleagues at the University of Oklahoma Medical Science Center knew that platelets expressed GMP-140 in  $\alpha$ -granules and that platelet activation induced rapid redistribution of GMP-140 to the plasma membrane. In their 1989 *JCI* article, they demonstrated that GMP-140 was also present in endothelial cells, mostly in postcapillary venules (38). Human endothelial cells synthesized GMP-140 and stored it in membranes of Weibel-Palade bodies, where it was rapidly mobilized to the cell surface after cell activation. These data pointed to an important role for GMP-140 at injured or inflamed sites, where activated endothelial cells and platelets would be present. Concurrently with the *JCI* study, the authors cloned the cDNA for GMP-140 (39), which revealed a modular protein with an N-terminal lectin domain that was remarkably similar to 2 leukocyte adhesion receptors that were reported simultaneously: endothelial leukocyte adhesion molecule 1 (ELAM-1) (40) and a lymphocyte homing receptor (41, 42). This made it obvious that GMP-140 was an adhesion receptor for

leukocytes, which was soon documented (43–45). By binding to cell-surface glycoconjugates, these molecules, now called P-, E-, and L-selectin, mediate rolling of leukocytes on vascular surfaces during inflammation, thrombosis, and immune responses (46). Since the 1989 *JCI* study by McEver et al., thousands of papers have addressed the biochemical, biophysical, physiological, and pathological properties of selectins.

### The many faces of IL-8

IL-8 has interesting properties: it was the first identified selective attractant for neutrophils, was inducible in leukocytes and tissue cells, and in 1989 was the subject of much attention because of the implication of its role in the pathology of many diseases. Throughout a decade of mining for the mediators of leukocyte trafficking, human chemokines surfaced as a mega-family of 50 ligands and 20 receptors, all involved in this process. Important? Viruses continue to think so. They learned to use chemokine receptors to enter leukocytes and to express chemokines, receptors, antagonists, and even chemokine-binding proteins to control leukocyte traffic. Baggiolini, Walz, and Kunkel at the University of Bern and University of Michigan explored the role of IL-8 in leukocyte trafficking when it was still a novel cytokine known to activate neutrophils. In their review article (47), the authors emphasized that IL-8 was “a product of many cells” (as originally discovered in Kunkel’s laboratory; ref. 48) and was thus ready to act ubiquitously in the body. They anticipated its role in host defense and in inflammatory diseases and suggested receptor blockade as a new approach for anti-inflammatory therapy. It was subsequently found that chemokines regulate lymphocyte homing, and IL-8 was proposed to function in hematopoiesis, morphogenesis, angiogenesis, and metastasis formation, but its primary role was linked to inflammation. Clark-Lewis and others proved that chemokine antagonists have anti-inflammatory and HIV-suppressive activities (49–51), and the discovery of low molecular weight antagonists (52, 53) has since initiated a promising, major effort in the development of new anti-inflammatory drugs.

### Vessel blocker found: oxidized LDL present in human atherosclerotic lesions

This 1989 article by Ylä-Herttuala, Witztum, and colleagues (54) at the University of California, San Diego, demonstrated that atherosclerotic lesions, in both rabbits and humans, contained oxidatively modified LDL (OxLDL). Although some evidence – from the authors’ laboratory and others – already supported the hypothesis that oxidation of LDL was important in atherogenesis in animals, the occurrence of OxLDL in vivo had not been definitively demonstrated previously. In this study, lipoproteins in the lesions were gently eluted from fresh specimens obtained from both rabbits and human organ donors. The presence of in vivo OxLDL that had all the properties of OxLDL generated in vitro was demonstrated using physical, chemical, immunological, and cell biology techniques.

This article considerably strengthened the case for a pathogenic role of OxLDL in animal and human disease and encouraged both basic research and subsequent clinical trials to test whether antioxidants might inhibit atherogenesis in humans, as it had in animals. To date, clinical trials in humans with natural antioxidants have been largely negative, possibly due to the use of antioxidants inappropriate to the task or other trial design reasons.





Further clinical trials are underway with new and stronger agents. This paper was pivotal in supporting the oxidative modification hypothesis of atherogenesis, which continues to stimulate both basic and clinical research.

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#### **A novel target for cancer treatment**

Daniel Connolly's first assignment after joining Monsanto Co. was to purify tumor angiogenesis factors. Monsanto Co. had been primarily a chemical company; however, this was a new direction consistent with the company's strategic decision to invest in more highly valued pharmaceutical research. As part of this effort, collaborations were established with leading researchers including Harold Dvorak of Harvard University. Dvorak and his colleague, Donald Senger, had identified a potent protein from tumors that had the unusual ability to induce vascular leakage when administered subcutaneously, and it was hence given the name vascular permeability factor (VPF) (55). In their 1989 *JCI* paper (56), Connolly and colleagues reported, to their surprise, that when endothelial cells in culture were exposed to highly purified VPF, VPF was also highly mitogenic. More importantly, VPF stimulated new blood vessel growth when administered onto the cornea or into bone. This same group subsequently cloned the gene for VPF, and their article was published in *Science* back-to-back with similar work from a group at Genentech Inc. (57, 58). The Genentech group had termed the protein vascular endothelial growth factor (VEGF), a name that quickly displaced VPF. In the years that followed this early research, VEGF has been the subject of approximately 11,000 publications and has become an important and validated therapeutic target (59). Inhibitors of VEGF are used for treatment of cancer, while VEGF itself holds promise for treatment of ischemic heart disease.

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#### **Clot-bound thrombin thwarts heparin inhibition**

Heparin, which catalyzes antithrombin-mediated inhibition of the protease thrombin, attenuates blood clotting. Although heparin is widely used for treatment of acute coronary syndromes caused by coronary thrombi, recurrent ischemic events can occur despite heparin therapy. In their 1990 *JCI* report, Weitz and colleagues at McMaster University set out to determine whether clot-bound thrombin is protected from inhibition by the heparin-antithrombin complex (60). The authors demonstrated that plasma clots harbor thrombin and induce progressive fibrinopeptide A (FPA) generation, consistent with unopposed thrombin activity. In contrast, with free thrombin, FPA generation rapidly reaches a plateau as throm-

bin is complexed by its inhibitors. These data support the concept that clot-bound thrombin is enzymatically active and resistant to inactivation by fluid-phase inhibitors. The authors further demonstrated that clot-bound thrombin is protected from inhibition by the heparin-antithrombin complex because heparin concentrations that completely blocked FPA generation by free thrombin had minimal effects on clot-induced FPA generation.

In contrast, direct thrombin inhibitors (DTIs), such as hirudin, blocked FPA generation by clot-bound and free thrombin equally well. The capacity of DTIs to inactivate clot-bound thrombin subsequently prompted clinical trials comparing hirudin with heparin and set the stage for development of small-molecule DTIs, such as argatroban, bivalirudin, and ximelagatran, a prodrug of melagatran. For thromboprophylaxis after hip replacement surgery, hirudin was superior to heparin or low-molecular-weight heparin (61). In acute coronary syndromes, a meta-analysis of studies comparing DTIs with heparin demonstrated a greater reduction in death and myocardial infarction with DTI therapy (62). Although it is uncertain whether the superiority of DTIs reflects their capacity to inhibit clot-bound thrombin, 3 DTIs are now licensed for limited indications, and ximelagatran, an orally active agent in this class, has the potential to be the first new oral anticoagulant since the introduction of coumarins 60 years ago (63).

#### **NO and endothelin have opposing effects on vascular tone**

By 1990, vascular endothelial cells were known to release both NO and the novel 21-amino-acid peptide endothelin. Endothelin is a potent vasoconstrictor, which has deleterious effects including fibrosis, vascular hypertrophy, and inflammation, while NO, a potent vasodilator, exhibits vasculoprotective effects. At this time, a study by Boulanger and Lüscher (64) of University Hospital, Basel, showed for the first time that a significant part of the vascular protective effects of NO comes from its ability to downregulate endothelin production. In their *JCI* report, the basal endothelium-dependent release of endothelin was increased by several different agonists. Agonist-induced endothelin release was augmented by inhibitors of the NO pathway, but reduced by superoxide dismutase (a superoxide scavenger that increases the bioavailability of NO) and a cyclic GMP analog. These results demonstrated that in intact blood vessels, endothelium-derived NO inhibits the subsequent production of endothelin via a cyclic GMP-dependent pathway. This remains one of the most important physiological mechanisms for moderating endothelin's effect and was later confirmed in intact animals. Ever since this discovery, the interaction between NO and endothelin has become an important yin-yang principle in vascular homeostasis. Furthermore, this study provided new pharmacological targets for NO-donating compounds as well as novel phosphodiesterase inhibitors and cyclic GMP activators.

#### **The beginning of the angiotensin I-converting enzyme gene saga**

ACE converts the peptide angiotensin to its activated form (angiotensin II), causing blood vessels to narrow and blood pressure to rise. By the mid-1980s, the measurement of plasma ACE levels had been used as an indicator for some diseases; however, significant variability in these levels, independent of blood pressure, suggested differences in ACE gene expression due to genetically determined regulatory mechanisms operating in somatic





tissues. A report published elsewhere in 1988 had demonstrated that plasma ACE levels were indeed genetically determined (65), and the *ACE* gene was cloned soon thereafter (66). In a subsequent effort to identify the alleles exerting a genetic effect on this quantitative trait (known as quantitative trait loci; QTL), Rigat, Soubrier, and colleagues from INSERM, Paris, searched for DNA polymorphisms at the *ACE* gene locus in a simple and relatively small association study (67). In their 1990 *JCI* paper, the authors identified an insertion/deletion polymorphism at this locus and used this as a marker genotype to demonstrate that the *ACE* gene locus appeared to be the major locus determining serum ACE concentration.

A subsequent study by Villard et al. (68), which showed the existence of a major QTL on the 3' side of the *ACE* gene and a minor QTL on the 5' side was confirmed by independent studies that mapped the major variant between two recombination breakpoints within the *ACE* gene (69, 70). Soubrier and colleagues later identified a mutation of the *ACE* gene in families

of Dutch origin that leads to a higher secretion rate of ACE and very high plasma ACE levels. However, this mutation differed from the more common genetic variation that results in elevated plasma ACE (71). Surprisingly, by studying West Africans, in whom the haplotype diversity is greater than that in white people, Cox et al. eliminated the insertion/deletion polymorphism previously identified as the functional variant (72). Due to the importance of the enzyme in the processing of a variety of peptides, several studies have continued to explore the relationship between this genetic variation and disease. The relative risk conferred by the D allele of the insertion/deletion polymorphism, associated with high levels of plasma ACE, for cardiovascular and renal diseases remains uncertain and, if it exists, seems small. More recently, the I allele, associated with lower plasma ACE levels, was found repeatedly to confer a moderately increased risk of late-onset Alzheimer disease in whites and Asians (73, 74). The functional variant of ACE has still not been identified, and the saga continues.



1992–1996

Ajit P. Varki

*Along with primary responsibility for the entire operation came a remarkable degree of freedom, allowing one to do whatever might be best for the JCI and the community. Quality and consistency were assured by the weekly editorial board meeting of actively working scientists, who also served as an outstanding group of policy advisers. Among other achievements, we made the journal available on the internet for the first time, with a model to always keep it freely available to everyone — thereby conceiving “open access” long before it became popular.*

### Atherosclerosis in genetically modified mice

While the *JCI* gives preference to studies on humans, it has a long tradition of publishing articles on animal models that teach us about the human condition. Three articles published between 1993 and 1996 from the University of Texas Southwestern Medical Center serve to highlight the fact that raising plasma cholesterol levels by manipulating one gene can lead to massive atherosclerosis, even in the mouse, a species relatively resistant to developing this common human disease.

The first 2 articles (75, 76) confirmed that *LDLR*, the LDL receptor gene, was chiefly responsible for regulating LDL levels, and the first study even presented an early attempt at a gene-therapy approach for correction of LDL receptor deficiency. The third article (77) affirmed the *in vivo* role of the SREBPs, which are cholesterol-regulated transcription factors that control and integrate nearly all aspects of hepatic lipid metabolism. Transgenic mice overexpressed a truncated version of human SREBP-1a that entered the nucleus without a requirement for proteolysis and therefore could not be downregulated. Arguably, the SREBP work is fundamentally even more important than the Nobel Prize-winning discovery of the LDL receptor pathway, as the latter is just one of the genes and pathways regulated by the SREBPs.

### Oxidative stress plays a key role in vascular disease

The 1993 article by Ohara and colleagues (78) of Emory University was part of a series of studies aimed at understanding why endothelium-

dependent vasodilatation is impaired in hypercholesterolemia. The authors showed a 3-fold increase in superoxide production in aortas of rabbits fed a high-cholesterol diet, and almost all of this reactive oxygen species was shown to originate within the endothelium. Since then, the idea that many common risk factors for atherosclerosis increase vascular superoxide production has become widely recognized as important not only for regulation of vasomotor tone, but also in modulation of other redox-sensitive pathophysiological events. Altered endothelium-dependent vasodilatation had also been observed in a variety of disorders other than hypercholesterolemia, including hypertension. Earlier work by one of these authors had shown that cultured vascular smooth muscle cells possessed an NADPH oxidase that was responsive to angiotensin II (79), but the role of this enzyme in hypertension was unclear. In their 1996 article, chronic angiotensin II infusion was shown to stimulate activity of the NADPH oxidase (80). This in turn increased vascular superoxide production, altered endothelium-dependent vasodilatation, and contributed to the hypertension caused by angiotensin II. The study was the first to demonstrate that the NADPH oxidase was regulated *in vivo* and provided an understanding of how a stimulus such as angiotensin II could cause oxidative stress. Since then, there has been much interest in the NADPH oxidase and how it contributes to vascular disease.

### Eotaxin attraction: recruiting eosinophils at sites of allergic inflammation

The existence of chemoattractants for eosinophils and other allergy-associated leukocytes was long suspected, but by the early



## 1924–2004

1990s, none had been found that could account for the marked accumulation of eosinophils within certain types of inflammatory lesions, such as allergic airways or sites of parasitic infections. In a 1996 report by Ponath and colleagues (81) of LeukoSite Inc. the authors cloned human eotaxin and established several important concepts: (a) that eotaxin is the high-affinity ligand for a receptor that was expressed at high levels by eosinophils and not neutrophils or other leukocyte types; (b) that it selectively attracts eosinophils in vitro better than any other chemokine, as well as in vivo when injected into the skin; and (c) that eotaxin is expressed abundantly at sites of allergic inflammation. One reason this article is so widely cited is that elucidation of the actions of chemokines, in this case eotaxin and its receptor CCR3 specifically, greatly assisted our understanding of Th2 type inflammatory mechanisms and the way in which allergic-type leukocytes are recruited to tissues.

It has now become widely accepted that the binding of eotaxin and related chemokines to CCR3 is the principal mechanism for the selective recruitment of eosinophils to inflammatory lesions. Eotaxin and CCR3 have thus also become interesting targets for drug development.

### A spoonful of sugar can rescue some glycosylation disorders

More than 1% of human genes are dedicated to the production and metabolism of the complex sugar chains that extensively decorate cell surface and secreted glycoproteins and glycolipids. Over

the years, many human genetic defects in lysosomal catabolism of these glycoconjugates had been well recognized. However, until the 1990s, human mutants in the biosynthesis of glycans were vanishingly rare. The clinical definition of carbohydrate-deficient glycoprotein syndromes (CDGSs, now renamed congenital disorders of glycosylation) in the early 1990s led to the realization that there were many such defects that had mostly gone unrecognized, because of their multisystem pleiotropic clinical manifestations. This early study of type I CDGS by Panneerselvam and Freeze (82) of the La Jolla Cancer Research Foundation (now the Burnham Institute) analyzed fibroblasts from patients in whom entire N-linked glycan chains failed to add to glycoproteins. Even before defining the precise genetic defect, the authors were able to show diminished incorporation of tritiated mannose into N-linked glycoproteins and into the lipid-linked oligosaccharide precursor of N-glycans. Remarkably, addition of exogenous mannose, but not glucose, corrected the defects. The authors predicted correctly that patients with some types of CDGS would benefit from simply including mannose in their regular diets. Less than a decade later there are more than a dozen well-defined human defects in N-glycosylation with protean clinical manifestations, and a few can be treated simply by supplementing the diet with the appropriate monosaccharide. An unfortunate side effect has been the spawning of unregulated, non-FDA-approved, scientifically unproven claims by some “health food” manufacturers that healthy humans will also benefit from dietary supplementation of monosaccharides such as mannose.



## 1996–1997

Paul A. Insel



Martin F. Kagnoff

*The period when we served as coeditors-in-chief was a time of considerable activity. The growth in number of submissions, especially from outside the United States, coupled with page limitations, forced the group of 18 associate editors and editors to make often-difficult decisions regarding acceptances. Publication of the journal — including editorials and perspectives — every other week made for a continuous series of deadlines, but we look back with pride at what appeared in the JCI at that time.*

### Targets for leptin

The identification of leptin in the mid-1990s as an endocrine signal from adipose tissue that regulates the body fat depot was a major discovery in endocrinology/metabolism. Several papers in the *JCI* in 1996–97 defined properties of leptin and its potential contribution to obesity and disease. Schwartz et al. (83) of the University of Washington found that rats respond to intra cerebroventricularly administered leptin with decreased food intake, decreased mRNA expression for neuropeptide Y (which stimulates food intake) in the arcuate nucleus of the hypothalamus, and increased mRNA expression for corticotrophin releasing hormone (an inhibitor of food intake) in the hypothalamic paraventricular nucleus. The mRNA changes did not occur in obese Zucker (*fa/fa*) rats that had been administered leptin. The

authors also found high expression of leptin receptor mRNA in the arcuate nucleus with lower levels in other hypothalamic nuclei and brain regions involved in energy balance. The data provided new information implicating particular hypothalamic nuclei and neuropeptides in mediation of the ability of leptin to regulate food intake and energy balance. Subsequent work suggests that leptin acts at additional sites in the brain not generally associated with energy homeostasis (84). Since publication of their 1997 *JCI* article, the Seattle group has actively pursued the role of leptin and other regulators of energy intake (85), but they are not alone: searching “leptin” in PubMed, one identifies more than 8,000 papers. Leptin, though, is but one of the endocrine signals now recognized to derive from adipocytes (86, 87).



1997–2002

Stephen J. Weiss

*We always felt that the JCI's unique philosophy would speak for itself — with an editorial board of peer-scientists who meet each week to review all submitted works, to arbitrate upon sometimes opposing opinions, and, most importantly, to act as author-advocates for interesting contributions. To take each submitted work and attempt to apply a common standard of excellence independent of the subject material or its "timeliness" was certainly a challenge. But with a "14-headed beast" huddling over each work, we knew that checks and balances were applied that allowed us to publish with the authoritative voice that has always distinguished the JCI.*

### IL-6: friend or foe in inflammatory disease?

IL-6, a cytokine produced by both hematopoietic and mesenchymal cells, had been reported previously to exert both proinflammatory and anti-inflammatory actions. In this widely cited 1998 *JCI* paper, Xing et al. (88) of McMaster University, Hamilton, Ontario, demonstrated that IL-6 acted primarily as an anti-inflammatory agent in a mouse model of acute lung inflammation or following the administration of intraperitoneal endotoxin. Shortly thereafter, however, Fishman and colleagues (89) of University College London Medical School published results of an analysis of the 5' flanking region of the *IL-6* gene in patients with systemic onset juvenile rheumatoid arthritis (JRA). Interestingly, they found a reduced frequency of an allele, termed the C-allele, which associates with lower levels of IL-6 in healthy subjects. This finding is potentially relevant to systemic-onset JRA, since IL-6 levels are elevated in this disease and correlate with febrile episodes.

Is IL-6, therefore, a friend or foe in human inflammatory disease? Differences among species, model systems, organs, inflammatory stimuli, and acute versus chronic inflammatory states might not allow this question to be answered in a simple way. Nevertheless, important clinical evidence has emerged since 1998 that clearly establishes IL-6 as a central proinflammatory mediator in several human diseases. Indeed, development of an anti-IL-6 receptor antibody has been followed by careful clinical studies of rheumatoid arthritis, in which efficacy has been shown. Moreover, in systemic onset JRA, dramatic clinical results have been obtained with this antibody in patients with especially severe and refractory disease. Thus, the work of Fishman et al. (89) helped point the way toward a clinically relevant molecular target for treatment of systemic JRA. As the use of the anti-IL-6 receptor antibody is extended to a wider range of human autoimmune diseases, it should become clear whether IL-6 is indeed anti-inflammatory in other pathologic states. Precedent for opposite effects of cytokine neutralization in diverse human autoimmune diseases has been well established with TNF blockade, which effectively treats rheumatoid arthritis and Crohn disease but exacerbates lupus and multiple sclerosis. A fuller understanding of the biological roles played by IL-6 will certainly arise as a consequence of these early studies and the broadening efforts to use biologics that neutralize IL-6 actions in human disease states.

### Phagocytosis of apoptotic cells inhibits macrophage production of proinflammatory factors

Physiological cell death is a process designed to eliminate functionally inappropriate cell populations in a manner that does not elicit an inflammatory response. Indeed, selective cell death plays a role

in the homeostasis of mature tissues. The cell death process generally assures both that cells destined to die will cease to function and that they will be cleared in an orderly fashion. Cells that undergo apoptosis are typically removed rapidly by phagocytic cells, primarily macrophages. In contrast to phagocytosis mediated by distinct receptors on the macrophage surface that normally trigger an inflammatory response (e.g., Fc receptors), physiological cell death leads to the targeted elimination of apoptotic cells via a phosphatidylserine receptor-dependent process. Consistent with this theme, the 1998 article by Fadok et al. (90) demonstrated that ingestion of apoptotic cells suppressed the ability of macrophages to express a number of cytokines and other products associated with inflammation (e.g., IL-1 $\beta$ , IL-8, TNF- $\alpha$ , GM-CSF, leukotriene C<sub>4</sub>, and thromboxane B<sub>2</sub>). Of particular note, however, was the demonstration that the anti-inflammatory effects exerted by apoptotic cells constituted more than a passive avoidance of stimulation but rather occurred as a consequence of an active suppressive mechanism involving the autocrine/paracrine action of TGF- $\beta$ , PGE<sub>2</sub>, and platelet activating factor. These important results suggest that this autoregulatory process is likely critical not only for the efficient clearing of apoptotic debris, but also for initiating the resolution of proinflammatory events.

### How withdrawal of VEGF reduces tumor vasculature and mass

The tumor neovasculature is an attractive target for suppressing the formation, proliferation, and metastasis of cancer cells. Not only does the tumor rely on newly recruited blood vessels to meet its metabolic needs, but it also depends on this vasculature to deliver various growth and survival factors. In their earlier studies of the developing retinal vasculature, Keshet and colleagues at Hebrew University, Jerusalem, demonstrated that newly formed blood vessels are dependent on the endothelial cell growth factor VEGF in order to remain structurally intact, while mature vessels no longer depend on the growth factor for survival (91). Because the maturation process corresponded with the investment of pericytes in the endothelial cell tubules constituting the vessel wall, these investigators reasoned that the dynamic turnover of tumor-associated blood vessels might be biased toward a neovasculature enriched in pericyte-deficient, and hence VEGF-dependent, structures. In their 1999 article (92) the investigators demonstrated that, consistent with this hypothesis, the blood vessels recruited by the growing tumor mass contained large numbers of "naked" (pericyte-poor) endothelial cell tubules. More important, in a series of patients with prostate cancer, they found that hormone ablation therapy decreased the local expression of VEGF





and induced the rapid regression of the pericyte-poor, immature tumor vasculature. While more recent studies suggest that the number of tumor blood vessels that are pericyte deficient is highly variable depending on the specific type of cancer, endothelial cell–pericyte interactions are nonetheless frequently abnormal (93–95). Indeed, new studies suggest that therapeutic interventions simultaneously directed against growth factor receptors and/or ligands on endothelial cells as well as pericytes can discriminate between the normal host vasculature and the abnormal network of tumor-associated blood vessels (93). Undoubtedly,

*Undoubtedly, future studies will continue to search for chinks in the cancer cell “armor,” but the work of Keshet and colleagues provided one of the first mechanistic insights into the dysfunctional status of the tumor vasculature and identified a potential Achilles’ heel toward which therapeutic intervention could be directed.*

future studies will continue to search for chinks in the cancer cell “armor,” but the work of Keshet and colleagues (92) provided one of the first mechanistic insights into the dysfunctional status of the tumor vasculature and identified a potential Achilles’ heel toward which therapeutic intervention could be directed.

#### **IL-13: a key player in the inflammatory immune response**

Key advances in the field of cytokine biology have underscored the importance of cytokine phenotypes, which not only characterize particular disease states, but also serve to direct the evolution of the associated pathologic states. While specific cytokines appear to be associated with specific immune reactions, the full spectrum of their biologic activity is far from understood. In their 1999 article, Zhu and colleagues (96) provided key insights into the activity of an important cytokine, IL-13, that contributes to the progression and pathology of a Th2-polarized inflammatory response. Using an in vivo model in which IL-13 was overexpressed specifically in the lungs of transgenic mice, these investigators demonstrated that the cytokine triggered a localized inflammatory response wherein mononuclear leukocytes and eosinophils infiltrated both the small and large airways. The

*These studies established an important precedent, namely, that the expression of specific cytokines can contribute to the manifestation of specific pathologies in defined disease states, and raised the intriguing possibility that IL-13 may play an important role in asthma.*

lung pathology displayed in these mice included hypertrophy of airway epithelial cells, metaplasia of mucus-producing cells, hyperproduction of neutral and acidic mucus, and subepithelial airway fibrosis. These pathologic changes likely contributed to physiologic alterations of the affected lungs, as the IL-13 transgenic mice displayed an increase in baseline airway resistance and airway hyperresponsiveness. Hence, these studies established an important precedent, namely, that the expression of

specific cytokines can contribute to the manifestation of specific pathologies in defined disease states, and raised the intriguing possibility that IL-13 may play an important role in asthma as well as other Th2-polarized disease states.

#### **Mutations in *ABC1* in Tangier disease and HDL deficiency**

From its initial report in 1961, Tangier disease was viewed as a mysterious and puzzling genetic disorder that held an important key to the understanding of cholesterol metabolism. Patients with the disease have a peculiar constellation of characteristics associated with massive tissue deposition of cholesteryl esters, together with a nearly complete absence of HDL. Because of the importance of HDL in atherosclerosis pathogenesis, Tangier disease became the subject of a massive gene hunt by a number of laboratories, despite the small number of patients affected with this rare disorder. In their 1999 *JCI* article, Richard Lawn and coworkers identified the *ABC1* transporter as the gene responsible for Tangier disease, using a combined strategy of gene expression microarray analysis and genetic mapping (97). Independently, 3 other groups arrived at the same conclusion through a purely positional-cloning approach, publishing their studies in the same issue of *Nature Genetics* (98–100). Lawn and coworkers at CV Therapeutics Inc. started with fibroblasts from skin explants from healthy and Tangier subjects and identified a number of genes that were underexpressed in Tangier cells compared with normal cells. One of these genes, dubbed *ABC1*, localized to the large genetic interval to which the Tangier disease had previously been mapped on the long arm of chromosome 9. DNA sequencing identified candidate missense mutations in 2 of 3 patients. The investigators also showed that reduced *ABC1* expression inhibited lipid efflux from cultured cells, whereas increased expression enhanced it. These results provided the first functional characterization of the *ABC1* gene product and represent one of the first successful uses of RNA expression profiling to identify candidate genes for a human disease.

#### **On the TRAIL of anti-cancer drugs**

The Apo2 ligand (Apo2L; also known as TNF-related, apoptosis-inducing ligand, or TRAIL) was uncovered because of sequence similarity to other members of the TNF family of proteins, including TNF and Fas ligand. Like other TNF family members, Apo2L can induce programmed cell death, or apoptosis, and does so via interactions with the transmembrane death receptors DR4 and DR5. Death receptor agonists such as TNF previously captured the attention of the cancer therapy field because of their potent activity against cancer cells. However, toxicity and safety issues, such as the apoptosis-inducing activity of TNF in normal cells, have curbed enthusiasm about the potential clinical value of most death receptor agonists. Nonetheless, the discovery of relative resistance of normal cells to Apo2L, in some cases due to expression of “decoy receptors” that compete for binding to Apo2L, increased interest in the potential of Apo2L in cancer therapy. While some prior attempts to express and purify recombinant soluble Apo2L had yielded encouraging data, the 1999 *JCI* report by Ashkenazi and colleagues (101) at Genentech Inc. described the first recombinant purified Apo2L protein that lacked any exogenous sequences and that readily formed active Apo2L homotrimers. The authors then used an array of systems and approaches to demonstrate the safety and lack of obvious toxicity when cultured primate cells and even intact animals were exposed to





physiologically relevant doses of Apo2L. Finally, they demonstrated the potent activity of recombinant soluble Apo2L against many human cancer cell lines growing in tissue culture or in nude mouse xenograft models. While a challenge to the authors' results and conclusions was later presented, a full accounting for the potential toxicity issues of Apo2L by Jo et al. (102) appears to have been offered (103, 104), bolstering hopes that recombinant soluble Apo2L may indeed prove to be a promising anticancer agent.

### A novel therapy using PPAR $\gamma$ for inflammatory bowel disease

PPARs are a subfamily of nuclear hormone receptors that were originally cloned and characterized as a consequence of their ability to induce gene transcription in response to drugs that stimulate peroxisome proliferation. Subsequently, PPAR $\gamma$  was discovered to play central roles in adipocyte differentiation and glucose homeostasis (e.g., the insulin-sensitizing drugs thiazolidinediones are PPAR $\gamma$  ligands). With the passage of time, PPAR $\gamma$  also has been shown to be intimately involved in various inflammatory processes. In their 1999 report, Su and colleagues (105) at the University of Pennsylvania demonstrated that PPAR $\gamma$  is expressed in the normal colon, primarily by epithelial cells located in the proliferative crypts. They found that in intestinal cell lines, TNF- $\alpha$  and IL-1 $\beta$  induce the expression of various proinflammatory

*The key observations made by Su and colleagues not only identified a new role for PPAR $\gamma$  ligands in regulating the immune response, but also provide a rationale for the development of these ligands in the treatment of inflammatory bowel disease.*

cytokines, such as IL-8. This occurs because IL-1 $\beta$  causes the rapid degradation of inhibitor of  $\kappa$ B- $\alpha$  (I $\kappa$ B- $\alpha$ ), and the subsequent activation of NF- $\kappa$ B, which then binds to, and activates, the IL-8 promoter. The authors found that PPAR $\gamma$  ligands prevent IL-1 $\beta$  from degrading I $\kappa$ B- $\alpha$ , thus blocking NF- $\kappa$ B activation and the induction of IL-8. Further, Su et al. proved that this potent anti-inflammatory effect also operated in vivo by demonstrating that PPAR $\gamma$  ligands dramatically inhibited inflammation in a mouse model of colitis. Subsequent studies by other groups have demonstrated that PPAR $\gamma$  heterozygous null mice have an increased inflammatory response in several models of colonic inflammation (106) and that adenoviral-induced overexpression of PPAR $\gamma$  enhances the ability of PPAR $\gamma$  ligands to reduce colonic inflammation in vivo (107). Taken together, the key observations made by Su and colleagues (105) not only identified a new role for PPAR $\gamma$  ligands in regulating the immune response, but also provided a rationale for the development of these ligands in the treatment of inflammatory bowel disease.

### Understanding the action of metformin in type 2 diabetes therapy

Metformin, a biguanide originally isolated from the French lilac, is one of the most commonly prescribed oral medications for diabetes mellitus. For many years, it was known that metformin reduced insulin resistance and hepatic gluconeogenesis as well

as increasing fatty acid oxidation, but its mechanism of action remained undefined. The 2001 report by Zhou and coworkers of Merck and Harvard Medical School (108) provided the first evidence that metformin exerts its palliative effects by activating the AMP-activated protein kinase (AMPK). AMPK is a serine/threonine kinase that is stimulated in response to cellular energy deprivation by a coordinate rise in the intracellular AMP concentration. Once activated, AMPK has pleiotropic effects that result in an increase in cellular energy production by affecting glucose uptake and fatty acid oxidation. Thus, this article provided a unified explanation for many of its cellular effects. As metformin does not activate AMPK directly, its mechanism of action remains to be determined, including possible effects on either upstream modifiers that increase AMPK activity or downstream phosphatases that inhibit the activity of the kinase. Further work, spurred by this landmark article, should lead to new insights into the control of AMPK as well as the development of improved drugs that target this critical kinase in diabetic states.

### VCAM-1, but not ICAM-1, at play in atherosclerosis

The vascular biologist's view of the initiation and perpetuation of the atherosclerotic lesion has long included implicit mechanistic roles for receptor-counter-receptor pairs that mediate leukocyte-endothelial cell adhesion. A prominent proatherotic contribution by VCAM-1, an immunoglobulin-like molecule that binds to  $\alpha_4\beta_1$  integrin on monocytes and other leukocytes, had been assumed in part because endothelial VCAM-1 expression is localized primarily to regions predisposed to lesion formation and to the lesions themselves. Studies that used adhesion blocking anti- $\alpha_4$  integrin antibodies or peptides lent further, if indirect, support to the notion that VCAM-1 is pivotal to atherogenesis. However, efforts to establish definitive evidence for VCAM-1-dependent pathogenic complicity, via the creation and analysis of VCAM-1-null mice on a proatherogenic background, had been confounded by the fact that VCAM-1-null mice (and  $\alpha_4$  integrin-null mice) die during embryogenesis. Cybulsky et al. of the University of Toronto (109) circumvented this obstacle by creating mice homozygous for a mutant allele of VCAM-1 capable of expressing just one of the normal pair of  $\alpha_4$  binding sites. The resulting *Vcam1<sup>D4D/D4D</sup>* mice are largely healthy as adults and express low levels of the altered form of VCAM-1 with reduced  $\alpha_4$  binding activity. Analysis of these mice, bred on an Ldl receptor-null background and subjected to a high-cholesterol diet, disclosed a 40% reduction in the surface area of macrophage foam cell-rich fatty streaks that can mature into more sinister, and oftentimes lethal, occlusive and proembolic lesions. This paper not only provided the first direct evidence that VCAM-1 is pathogenic in early atherosclerotic lesions, but also suggests that the partial blockade of VCAM-1 function that might be achieved with pharmacological intervention could exert significant antiatherogenic effects in humans as well. Indeed, a correlation between protection from atherosclerosis and the "dose" of VCAM-1 expression in a subsequent study with the *Vcam1<sup>D4D/D4D</sup>* mice (110) predicts that even modest reductions in VCAM-1-dependent adhesive activity may afford protection from the atherosclerotic process. These studies may thus give confidence to those considering programs to develop VCAM-1-specific atherosclerotic therapies and provide new opportunities for generating mechanistic insights into the molecular processes underlying neointima formation.



## 2002– present

Andrew R. Marks

*Serving as editor-in-chief of the JCI has been one of the most challenging and rewarding experiences of my career. One of the greatest challenges has been helping the Journal evolve during a period when biomedical publishing is undergoing rapid changes. I am proud that the JCI has been able to preserve free, online access while remaining fiscally sound. Working with my co-editors, from whom I have learned a great deal, has helped my own science in many ways.*

### Better to target 2 tyrosine kinases than 1: new paradigms for cancer therapy

In their 2003 article, Bergers et al. (93) from University of California, San Francisco, used a mouse model of human pancreatic cancer to test angiogenesis inhibitors (receptor tyrosine kinase inhibitors) against the distinctive stages of tumorigenesis. They reported that a drug (SU6668) that inhibits PDGF receptors is effective against all stages of islet carcinogenesis and is particularly effective on late-stage tumors, whereas a VEGF receptor inhibitor, SU5416, does not affect late-stage tumors. They showed that combination of SU5416 and SU6668 improved efficacy over either single agent. Moreover, the combination of Gleevec (Novartis Pharma AG, Basel, Switzerland) (which also inhibits the PDGF receptor) with the SU5416 was most effective against large tumors. A striking finding in this study was that treatment with either of the PDGF receptor inhibitors (Gleevec or SU6668) caused selective dissociation of pericytes from the endothelial tubes in tumors but not normal tissues, which indicated that the pericytes (support cells for endothelial cells) are functionally important for sustaining vascularity and angiogenesis in well-established solid tumors. From this study the concept emerged that Gleevec, an approved agent, should be tested in combination with experimental VEGF inhibitors (e.g., Avastin; Genentech Inc., San Francisco, California, USA) in order to improve efficacy against late-stage human cancers. Moreover, we also gained the insight that small molecule inhibitors of VEGFR and PDGFR are not specific for their intended target, and this paper provided key insights as to why single-agent small-molecule inhibitors do not perform well in the clinic. This was an important paper with significant implications for preclinical and clinical trials.

### Bone marrow: an extrapancreatic hideout for the elusive pancreatic stem cell?

In their 2003 study, Ianus and colleagues at New York University addressed the possibility of a new strategy for the cell-based treatment of diabetes mellitus (111). The authors showed that an extrapancreatic source of pancreatic  $\beta$  cells may play a role in  $\beta$  cell turnover and possibly the adaptation of islet mass in response to physiological and environmental stimuli. In this elegant study, bone marrow cells were transplanted into lethally irradiated recipient mice and gave rise to insulin-producing cells in pancreatic islets. The bone marrow-derived cells expressed marker genes that identified them as differentiated  $\beta$  cells. The cells were functional, and the authors ruled out cell fusion as the mechanism. Although the study raised many important questions about how bone marrow cells can contribute to pancreatic islet mass, based on this study we now know that the use of bone marrow as a source of pancreatic  $\beta$  cell progenitors is a valuable strategy to pursue for cell-based therapy for type 1 and possibly some forms of type 2 diabetes mellitus.

### The irresistible biology of resistin

In early 2003, Rajala and colleagues (112) at Albert Einstein College of Medicine reported on the metabolic effects of 2 novel circulating proteins, resistin and the resistin-like molecule RELM $\beta$ , which play an important role in linking obesity to insulin-dependent diabetes. These cysteine-rich proteins share considerable homology and are exclusively produced by adipose cells and epithelial intestinal cells, respectively. The authors generated highly purified recombinant proteins and used them to demonstrate that both resistin and RELM $\beta$  rapidly induce severe hepatic insulin resistance in conscious rats. These effects were demonstrated under well-controlled experimental conditions (pancreatic/insulin clamp) and in the presence of either basal or high levels of circulating insulin. The importance of inter-organ communication to the regulation of nutrient absorption, metabolism, storage, and disposal has been recently supported by the identification of novel glucoregulatory hormones synthesized and secreted by distinct organs such as adipose tissue and gut. This important JCI study provided clarification of the biological functions of this family of proteins. The authors demonstrated that administration of recombinant resistin and RELM $\beta$  to rats results in acutely impaired hepatic insulin sensitivity and glucose metabolism. The primary pathway underlying changes in hepatic glucose metabolism appeared to be increased glucose production. Interestingly, no effect was observed on peripheral glucose disposal under the clamp conditions, effectively ruling out a role for resistin in this part of the insulin action, at least under the experimental paradigms used in the study. This study revealed that resistin and the closely related RELM $\beta$  may act to establish links among adipose tissue, the intestine, and the liver and made a huge step toward clarifying the confusing picture concerning the physiological role of resistin in linking obesity to insulin resistance and diabetes.

### Soluble VEGF receptor Flt1: the elusive preeclampsia factor discovered?

Maynard et al. (113) of Beth Israel Deaconess Medical Center reported on the mechanisms of preeclampsia, a condition estimated to affect 5–10% of all pregnancies worldwide and one of the leading causes of maternal and perinatal mortality. Generalized endothelial dysfunction has been reported in preeclampsia; however, the exact mechanisms are unclear. None of the factors linked to preeclampsia have been shown to cause the clinical phenotype of severe proteinuria, glomerular endotheliosis, and hypertension. In this 2003 JCI study, the authors reported elevated levels of a circulating, soluble fms-like tyrosine kinase 1 (sFlt1), which captures free VEGF and placental growth factor (PlGF) in patients with preeclampsia. They demonstrated that excess sFlt1 in the serum of these patients



causes endothelial dysfunction in vitro by antagonizing VEGF and PlGF. Furthermore, they showed that exogenous sFlt1 therapy in rats produced a syndrome of nephrotic range proteinuria, hypertension, and glomerular endotheliosis, strongly suggesting that sFlt1 plays an important causal role in preeclampsia. These data changed the current understanding of diagnostic and therapeutic options for patients with preeclampsia.

### Tetrahydrobiopterin can reduce high blood pressure

In their 2003 study, Landmesser et al. (114) of Emory University, the NIH, and the University of Texas reported on a novel pathway involving oxidation of a vascular factor that may be involved in causing vascular complications of one of the most common abnormalities – hypertension. The authors showed that tetrahydrobiopterin, a critical cofactor of eNOS, is oxidized in hypertension involving activation of NAD(P)H oxidase. This results in eNOS “uncoupling,” i.e., increased superoxide and reduced NO production by the enzyme, which may represent a fundamental abnormality in hypertensive vessels that promotes vascular damage and

lesion formation. The authors concluded that tetrahydrobiopterin oxidation may represent an important abnormality in hypertension. From this study we learned that treatment strategies that increase tetrahydrobiopterin or prevent its oxidation may prove to be a novel and useful approach to preventing vascular complications of hypertension.

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Address correspondence to: The Editors, *Journal of Clinical Investigation*, 630 West 168th Street, New York, New York 10032, USA. Phone: (212) 342-0497; Fax: (212) 342-0499; E-mail: editors@the-jci.org.

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