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## MARY ALLEN ENGLE AWARD

# THE GLUE OF LIFE*—A CAREER RETROSPECTIVE

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## ABSTRACT

The author was privileged to be an early contributor to the concept that cell adhesion molecules, the leukocyte ($\beta$2) integrins, play a pivotal role in the acute inflammatory process. For the author, this began with the development of a monoclonal antibody (anti-Mo1) that identified a differentiation antigen on the surface of human myeloid cells (including neutrophils, monocytes, and natural killer (NK) cells). Serendipitously, it was discovered that the Mo1 antigen was the heterodimeric glycoprotein (gp155,95) absent from the surface of neutrophils isolated from patients with adhesion defects *in vitro* and a syndrome characterized by chronic, life-threatening infections *in vivo* (a syndrome now termed leukocyte adhesion deficiency, type 1) (LAD-1). Collaborative efforts with other investigators (including members of the ACCA) revealed that patients with LAD-1 exhibited genetic mutations on chromosome 21 resulting in absent or diminished expression of a class of 4 surface adhesion molecules (now termed CD11a/CD18, CD11b/CD18, CD11c/CD18, and CD11d/CD18) known as the leukocyte or $\beta$2 family of integrins. Knowledge of the role of the $\beta$2 integrins in the acute inflammatory response led to the development of effective gene therapy strategies to treat LAD-1 in preclinical animal models and to the comprehensive testing of anti-integrin antibodies as anti-inflammatory agents to prevent organ damage as a complication of acute inflammation. This retrospective provides one illustration of the potential of bench-to-bedside research to generate new knowledge of clinical significance.

The acute inflammatory response depends upon direct contact and adhesion between leukocytes and the surrounding extracellular milieu including endothelial cells, other leukocytes, subjacent extracellular

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matrix, and pathogenic micro-organisms. We now recognize that leukocyte adhesion is not a nonspecific phenomenon but depends upon defined membrane structures, which include the *selectins* (expressed by leukocytes and endothelial cells), *receptors for chemotactic factors* (such as C5a and IL-8), and the $\beta$2 *integrins* expressed by leukocytes. Neutrophils are attracted to sites of inflammation by chemotactic factors triggered by invasive bacteria and released into the capillary circulation. The first point of neutrophil contact with inflamed endothelium is facilitated by the selectins, which permit loose adherence and rolling of neutrophils along the endothelium. Soluble inflammatory factors activate the $\beta$2 integrins expressed by neutrophils, which engage counter ligands (e.g., intercellular adhesion molecules [ICAMs]) expressed by endothelial cells. Integrin-mediated tight adhesion then permits transendothelial migration along a gradient of chemotactic factors into subendothelial matrix in proximity to pathogenic bacteria. This current model of acute inflammation resulted from an interactive series of clinical and laboratory observations made by many investigators (including members of the ACCA), among them myself and my collaborators. In the paragraphs that follow is a concise, personal account of how we arrived at our current understanding of the role of the $\beta$2 integrins in the acute inflammatory response.

## EARLY OBSERVATIONS

My introduction to the field of leukocyte biology and inflammation occurred when, as a junior faculty member at the Dana-Farber Cancer Center, I developed a murine monoclonal antibody (anti-Mo1) that recognized a membrane glycoprotein expressed by human neutrophils, monocytes, and NK cells (1). On biochemical characterization, this protein had a two-subunit, heterodimeric structure: gp155,95, and seemed to represent an antigen that distinguished myeloid lineage cells from most other leukocytes. The functional significance of Mo1 was unknown until we became aware of the work of a nearby neighbor, Dr. Amin Arnaout, which focused on the characterization of a new disorder of leukocyte function occurring in a child with recurrent, life-threatening infections. As reported in the *New England Journal of Medicine* (2), Dr. Arnaout and his colleagues at the Boston Children's Hospital discovered that the child's neutrophils lacked a membrane protein of approximately 150 kD and that the child's parents expressed reduced levels of this protein relative to normal individuals. By serendipity, these findings led us to quickly test the hypothesis that the "missing p150 protein" was a component of Mo1, and a simple flow-

cytometric analysis confirmed the absent expression of Mo1 in this patient (3) and in a separate pediatric patient identified by Dr. Bernard Babior at the Tufts-New England Medical Center (4, 5). In collaboration with Dr. Timothy Springer, also working at Harvard, we subsequently discovered that the patient's leukocytes not only lacked Mo1, but also two other membrane proteins, LFA-1 (gp180,95) and p150,95, each of which is a heterodimer with distinct higher-molecular-weight alpha subunits but sharing a common 95-kD beta subunit (3, 4). This suggested that the underlying molecular defect in the patient's cells was an obstruction in the synthesis of the beta subunit, an hypothesis confirmed by Springer (6) and others.

## CHARACTERIZATION OF LAD-I

In independent work conducted contemporaneously by investigators in Houston [most notably by Dr. Don Anderson (7)], Seattle [Dr. John Harlan and coworkers (8)], and elsewhere, other, similar patients were identified, and a common phenotype began to emerge. All of the patients were children and most demonstrated delayed umbilical cord separation, impaired wound healing, persistent leukocytosis between recurrent bacterial infections, and defective neutrophil mobilization (Table 1) (9). An impairment in neutrophil mobilization in *in vivo* (skin window) testing suggested an underlying problem with leukocyte adherence and migration, a premise supported by parallel *in vitro* experiments. We among others (9) discerned a variety of leukocyte functional abnormalities that depended upon adhesion: neutrophil aggregation; neutrophil and monocyte adherence and spreading on various surfaces, chemotaxis, phagocytosis of opsonized particles, complement receptor type 3 activity including C3-induced degranulation and respiratory burst (despite normal degranulation and burst activity in response to phorbol myristate acetate (PMA); lymphocyte blastogenesis; and cytotoxic T-lymphocyte and NK-cell cytotoxicity (Table 2). These *in vivo* (clinical manifestations) and *in vitro* findings supported the notion that the $\beta$2 integrins play a role in a variety of adherence-

TABLE 1
*Clinical Manifestations of LAD-I*

- Pediatric age group
- Delayed umbilical cord separation
- Impaired wound healing
- Persistent leukocytosis (between infections)
- Defective neutrophil mobilization
- Recurrent bacterial infections

TABLE 2
In Vitro *Leukocyte Abnormalities Associated with LAD-I*

- Neutrophil and monocyte adherence and spreading
- Neutrophil aggregation
- Neutrophil and monocyte chemotaxis
- Neutrophil and monocyte phagocytosis
- Neutrophil and monocyte complement receptor (CR3) activity
- Neutrophil and monocyte C3-induced degranulation and respiratory burst (PMA-induced degranulation and burst normal)
- Lymphocyte blastogenesis
- Cytotoxic T-lymphocyte and NK-cell cytotoxicity

dependent phenomena that may lead to an impairment in the acute inflammatory response to bacterial infection.

## *IN VITRO* CORRELATION WITH *IN VIVO* OBSERVATIONS

To confirm a cause-and-effect relationship between absent or reduced β2 integrin expression and the functional abnormalities observed, we and others performed a series of *in vitro* experiments utilizing monoclonal antibodies specific for the alpha and beta subunits of the β2 integrins (10). Consistent with the role of the β2 integrins in leukocyte adhesion, anti-integrin antibodies were found to block neutrophil aggregation in response to inflammatory stimuli, neutrophil and monocyte adherence and spreading, neutrophil disruption of endothelial-cell monolayers after exposure to PMA, neutrophil degranulation and respiratory burst after contact with C3b-opsonized particles, and lymphocyte blastogenesis, among other adherence-dependent assays of leukocyte function—recapitulating the functional impairments observed in β2 integrin-deficient patient cells (10). These independent clinical and laboratory observations strongly supported the importance of the β2 integrins as leukocyte adhesion molecules that foster the acute inflammatory response.

## β2 INTEGRINS AS RECEPTORS AND THE GENETIC BASIS OF LAD-I

Fast-forwarding to the present, based on the cumulative efforts of many investigators, we now know that the β2 integrins consist of a family of 4 (not 3) heterodimeric membrane glycoproteins, which have since been termed CD11b/CD18 (the original Mo1), CD11a/CD18 (LFA-1), CD11c/CD18 (p150,95), and CD11d/CD18. Each functions as a multispecific receptor (Table 3), that, upon activation by inflammatory stimuli, recognizes a set of ligands expressed by cellular and non-cellular constit-

TABLE 3
*Leukocyte Integrins are Receptors*

| Integrin | | | Ligands |
|---|---|---|---|
| Common Names | CD Nomenclature | Subunit Structure | |
| Mo1/Mac-1 | CD11b/CD18 | $\alpha M\beta 2$ | More than 30, including ICAMs-1, -2, -4; VCAM-1, C3bi, fibrinogen, Factor X, $\beta$-glucan; multiple exracellular matrix proteins (fibronectin, vitronectin, laminin, collagens, thrombospondin); multiple proteases (elastase, plasminogen, MMP-9, kininogen); uPAR, *Leishmania* gp63, *Candida albicans,* LPS, NIF |
| LFA-1 | CD11a/CD18 | $\alpha L\beta 2$ | ICAMs-1, -2, -3, -4. -5; JAM-1 |
| P150,95 | CD11c/CD18 | $\alpha X\beta 2$ | iC3b, ICAM-1, fibrinogen, LPS |
| | CD11d/CD18 | $\alpha D\beta 2$ | Similar to CD11b/CD18 including ICAM-3, VCAM-1, various ECM proteins, plasminogen |

Abbreviations: ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule; ECM, extracellular matrix molecules; MMP, matrix metaloproteinase; uPAR, urokinase plasminogen activator receptor; LPS, lipopolysaccharide; NIF, neutrophil inhibitory factor; JAM, junctional adhesion molecule.

uents of the inflammatory milieu (endothelial cells, other leukocytes, certain bacteria and their products, extracellular matrix proteins, various proteases, and complement components) (11–13). Through the work of Tim Springer and others, we also know that pediatric patients with reduced or absent expression of the $\beta 2$ integrins express mutations in the gene on chromosome 21 that encodes the common beta (CD18) subunit (14), which in turn leads to an impairment in the synthesis of all 4 intact surface receptors. This genetic syndrome is what is now termed leukocyte adhesion deficiency, type 1, or LAD-1. Patients with mutations that cause a severe deficiency in $\beta 2$ integrin expression demonstrate a worse clinical phenotype (often resulting in death from overwhelming infection), whereas mutations that permit a limited degree of $\beta 2$ integrin expression are associated with a less severe phenotype and a better prognosis (15).

## THE POSSIBILITY OF GENE THERAPY FOR LAD-1

It occurred to us and to our colleagues working in this field that our new found knowledge about the role of the $\beta 2$ integrins in the acute inflammatory response could have clinical applications. The first potential application was the concept of a gene-therapy approach to the

treatment of patients with LAD-I. The rationale for a gene-therapy strategy was that LAD-1 comes from a single gene defect, severe forms of LAD-1 are often fatal and are curable only with allogeneic bone marrow transplantation, and restoration of normal levels of gene expression is not required for clinical benefit. The proof-of-concept of gene therapy for LAD-1 came from experiments performed in collaboration with Dr. James Wilson (Michigan) and Don Anderson (Baylor College of Medicine). An LAD-1 lymphocyte line established by Anderson was exposed to a retroviral vector containing the normal CD18 gene and subsequently screened by flow cytometry for CD11a/CD18 expression. A small subpopulation of lymphocytes exhibited successful transfection, with normal levels of expression of the heterodimer, and was purified to homogeneity by cell sorting (16). The functional significance of a restoration of normal CD11a/CD18 expression in LAD-1 lymphocytes was shown in an *in vitro* aggregation assay in which restored expression was associated with the ability of CD18-transfected (but not sham-transfected) lymphocytes to aggregate in response to PMA, and that this adherence response could be blocked by antibodies specific for the alpha (CD11a) and beta (CD18) subunits (16). The results of these experiments, as well as similar independent findings of Springer and co-workers, (17) provided basic proof-of-principle to establish the genetic basis for gene therapy for LAD-1.

We went on to show that in a murine model, CD18 gene transfer into hematopoietic stem cells could result in human integrin subunit expression in circulating leukocytes of the mice (18, 19). This work set the stage for a gene-therapy approach targeted to larger animal models. This work was facilitated by our observation that LAD-1 is not only seen in humans, but also in certain other species, including dogs. In a study performed in collaboration with Drs. Urs Giger (University of Pennsylvania) and Larry Boxer (Michigan), we identified an Irish setter with the same phenotype as human patients with LAD-1 (20). Dr. Dennis Hickstein (NIH) was successful in developing a colony of dogs with canine LAD-1 (CLAD-1) and used them as subjects of a CD18 gene transfer strategy. Using a foamy virus vector to transfect hematopoietic stem cells, followed by autologous transplantation of transfected cells, he was able to demonstrate significant and sustained levels of $\beta 2$ integrin expression in the circulating lymphocytes, monocytes, and neutrophils of 4 of 5 transplanted CLAD-1 dogs (21). Most importantly, these 4 animals exhibited prolonged survival (beyond 2 years at the time of publication) relative to controls transplanted with sham-transfected cells (all of whom died within 200 days of transplantation). Hickstein's positive findings have clearly set the stage for a potential human clinical trial of LAD-1 gene

therapy, which awaits further methodological refinements to ensure the safety of gene therapy in humans.

## ANTI-INTEGRIN ANTIBODIES AS ANTI-INFLAMMATORY AGENTS?

Whereas LAD-1 demonstrates the critical role of the β2 integrins in supporting the acute inflammatory response, there is a "dark side of the force," namely that leukocyte adhesion can also promote inflammatory responses that are deleterious to the host. Specifically, there are a large number of human non-infectious diseases in which the symptoms and tissue damage may be the result of neutrophils "running amok." These include gout, rheumatoid arthritis, immune vasculitis, glomerulonephritis, psoriasis, inflammatory bowel disease, acute respiratory distress syndrome (ARDS), stroke, and myocardial infarction, among several others (22). Because, as we and others had observed, monoclonal anti-integrin antibodies can effectively block adhesion-dependent leukocyte interactions *in vitro,* we wondered if they could inhibit these interactions *in vivo*. That is, could monoclonal anti-integrin antibodies be used effectively as anti-inflammatory agents? To test this hypothesis, many investigators working in this field set out to determine what effect antibody treatment would have in preventing organ damage in animal models of inflammation in which the neutrophil was implicated. In support of the hypothesis, systemic administration of antibodies specific for murine, rat, and canine β2 integrins was indeed found to reduce or prevent tissue injury in models of myocardial infarction, stroke, intestinal ischemia, multi-organ failure resulting from shock, frostbite, and bacterial meningitis (Table 4) (23, 24). At the University of Michigan (where I had moved from Boston), most of our efforts in this regard were performed with Drs.

TABLE 4
*Inflammatory Conditions in Which Tissue Damage is Reduced by the Administration of Monoclonal Anti-β2 Integrin Antibodies*

| |
|---|
| **Animal Models** |
| • Myocardial ischemia-reperfusion injury |
| • Brain ischemia-reperfusion injury |
| • Intestinal ischemia-reperfusion injury |
| • Multi-organ failure after hypovolemic shock |
| • Frostbite |
| • Bacterial meningitis |
| **Human Conditions** |
| • Plaque psoriasis (anti-CD11a) |

Benedict Lucchesi (25, 26) and Peter Ward (27–32). A particularly promising set of experiments involved the administration of a monoclonal anti-CD11b antibody to dogs at the time of coronary artery reperfusion after a period of cardiac ischemia (to mimic the setting of reperfusion after coronary artery thrombosis as a cause of myocardial infarction). When the hearts of antibody-treated dogs subjected to ischemia and reperfusion were compared with those of sham-treated controls, the magnitude of left ventricular damage was reduced by nearly 50%, independent of the duration of reperfusion from 4–24 hours) (25, 26).

## HUMAN CLINICAL TRIALS OF ANTI-INTEGRIN THERAPY

The fairly consistent positive results in multiple animal models of neutrophil-mediated inflammatory damage created a sufficient incentive for the pharmaceutical industry to invest in and sponsor human clinical trials of anti-integrin therapy aimed at asthma, multiple sclerosis, myocardial infarction, psoriasis, psoriatic arthritis, rheumatoid arthritis, stroke, transplant rejection and traumatic shock (33). By and large, most of these human clinical trials failed to prevent or reduce organ damage. These negative studies are exemplified by the results of two large randomized, placebo-controlled trials, LIMIT AMI (34) and HALT-MI (35), to assess the impact of anti-CD18 administration at the time of coronary reperfusion (by either thrombolytic therapy in LIMIT AMI or angioplasty in HALT-MI) on subsequent estimations of myocardial infarct size, complications of infarction, or death in patients with myocardial infarction marked by segment ST-elevation in the electrocardiogram. Despite the trials being adequately powered to detect relatively small differences in infarct size, there were no statistically-significant benefits that could be attributed to the antibody administration (34, 35).

In contrast to the negative results of most anti-integrin antibody clinical trials, a randomized, placebo-controlled, phase III trial to assess the potential benefit of a humanized anti-CD11a monoclonal antibody in the treatment of plaque psoriasis was clearly positive (Table 4). In this trial, weekly, long-term, subcutaneous administration of the antibody (Efalizumab) resulted in a significant improvement in a quantitative measure of disease severity (36), which led to its FDA approval for patients with moderately severe, plaque-forming psoriasis. Unfortunately, several treated patients developed life-threatening infections, which first led to a black-box warning, and ultimately to a withdrawal of FDA approval when other patients developed progressive multi-focal leukoencephalopathy (37, 38). The

pharmacokinetics of the antibody administration to treat a chronic condition may have inadvertently led to the development of a state of immune compromise analogous to LAD.

Why the concept of anti-integrin, anti-inflammatory therapy "worked" in animal models of human disease, but generally failed in human clinical trials, is open to speculation [and has been addressed by John Harlan (33)]. However, the lack of success to date in treating human inflammatory conditions does not diminish the impact of what we have learned about the $\beta$2 integrins as "players" in the pathophysiology of the acute inflammatory response. The complex interplay among the $\beta$2 integrins, members of other integrin "families," the selectins, endothelial counter-receptors, and bacteria and their inflammatory products should provide an opportunity for further investigation, which may lead to new avenues for therapy.

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## DISCUSSION

**Rich, Birmingham:** What can you tell us about the pharmacodynamics of the anti-$\beta$2 integrin in human studies, which is to say, specifically, the duration of the inherent defect that you induced as measured either *in vivo* or *in vitro*?

**Todd, Houston:** In the human clinical trials of anti-integrin therapy, pharmacokinetic studies were conducted: In the LeukArrest (anti-CD18) HALT-MI study, for example, the humanized antibody was used at doses that produced at least 80% receptor saturation for 24 to 48 hours; in the efalizumab (anti-CD11a) psoriasis study, the dose of humanized antibody was sufficient to produce down-regulation of T-cell CD11a for the 1-week interval between doses. Although I didn't have time to speak to this in my presentation, the latter "positive" study was associated with a severe complication in some psoriasis patients receiving the anti-CD11a antibody: after initial FDA approval of efalizumab for the treatment of patients with severe plaque-forming psoriasis, several patients subsequently developed progressive multifocal leukoencephalopathy, which seems to have been due to reactivation of the BK virus. Unfortunately, by using chronic, weekly administration of this agent, we've recapitulated, to an extent, some of the manifestations of immune deficiency seen in LAD patients. This subsequently led to the withdrawal of the anti-CD11a antibody and points to the danger of applying this anti-integrin therapy for chronic conditions.

**Hasday, Baltimore:** Great talk about one of my favorite molecules! In terms of the function of the $\beta$2 integrins, in addition to their effect in promoting adhesion to the endothelium and triggering signaling in the leukocyte, they also trigger signaling of the endothelial cells through cross-linking with ICAMs. What happens if you stimulate

the endothelium in a patient with LAD? Do you get any partial restoration of leukocyte transmigration?

**Todd, Houston:** I am not aware that those experiments have been done. So I just can't comment.

**Benz, Boston:** I've heard some work described in patients with sickle cell anemia who are in crisis or have an infection. When you examine their leukocytes, almost every cell is coated with at least a few platelets. The question I have for you is whether the difference between the efficacy of the antibodies in blocking the integrin proteins *in vitro* (or even in certain animal models), as compared to the failure of these antibodies to prevent inflammatory damage in human clinical trials, relates to a platelet-associated bypass mechanism for leukocyte adhesion that operates *in vivo* but is not observed *in vitro?*

**Todd, Houston:** That's a possible mechanism to explain the negative findings that were observed in most of the human studies. However, beyond the obvious explanation that people really are different than dogs, mice, and rats, there could also be a very narrow window of opportunity to prevent the adherence mechanisms mediated by the $\beta 2$ integrins during the inflammatory process. In most of the human clinical trials, we simply didn't have the ability to intervene at the appropriate time point before $\beta 2$ integrin-dependent adhesion events occur. Also, as you suggest, other compensatory adhesion events may drive the inflammatory process.

**Fagin, New York:** CD11b is also expressed by monocytes and macrophages. I was wondering, first, whether the monoclonal antibody you developed, or any other, has interfered with macrophage function, and specifically, if this strategy has been used to interfere with tumor-associated macrophages, about which there's a lot of interest in their role in tumorigenesis right now.

**Todd, Houston:** Well, it definitely does interfere with monocyte adhesive function, including adherence to substrates, phagocytosis, and other integrin-dependent processes. Most of the defects we've seen in neutrophil function in LAD patients or in anti-integrin-treated normal neutrophils are recapitulated in the monocyte. In terms of macrophage killing of tumor cells, integrins may have an accessory function.

**Silverstein, Cleveland:** That was a terrific talk. As Ed said, one of the things you see *in vivo* is this association between the endothelium, the macrophage or the monocyte, and the platelet. One of the interesting and unique things about Mac-1 is its promiscuity and how many different antigens and co-partners it will bind to. There are some interesting data that suggest that blocking the Mac-1–platelet interaction has a very potent anti-inflammatory effect. Do you think that this might be a way around the adverse events and risk of infection, by just blocking that one part of the interaction rather than knocking the whole thing out?

**Todd, Houston:** Well, that's what we were hoping for. Several of the clinical trials were performed using an antibody specific for the common $\beta 2$ subunit, with the thought that this would inactivate the function of all 3 integrin glycoproteins. However, as a result of our work, we produced and licensed an antibody specific for CD11b with the hope that it might show some selective advantage in preventing the early events in acute inflammation. Despite initial interest by the biotechnology and pharmaceutical industries (and dreams of royalties flowing in!), the antibody never made it to clinical trial—no cigar!