Leukocyte Adhesion Deficiency IV
Monocyte Integrin Activation Deficiency in Cystic Fibrosis

Leukocyte adhesion plays key roles in immune responses and inflammation. Leukocyte adhesion requires an orchestrated set of coordinated events, starting with selectin-mediated rolling, followed by β_2 integrin–dependent arrest (1). Leukocyte adhesion deficiency (LAD) syndromes are genetic disorders of adhesion molecules that affect leukocyte adhesion (2). In this issue of the *Journal*, Sorio and colleagues (pp. 1123–1133) show that gene mutations of cystic fibrosis transmembrane conductance regulator (*CFTR*) found in patients with cystic fibrosis (CF) can lead to a monocyte-specific adhesion deficiency (3).

More than 30 years ago, the first LAD was discovered (4) and later called LAD-I. Patients with LAD-I have widespread bacterial infections as a result of defective leukocyte adhesion functions caused by defective expression of β_2 integrins (CD18) (5), which are key molecules in leukocyte arrest and migration. A second LAD syndrome, LAD-II, is caused by inherited defects in the gene encoding guanosine diphosphate (GDP)-fucose transporter 1 (6), which is a key player in fucose metabolism. The GDP-fucose transporter 1 defect results in the absence of sialyl Lewis X (7) and other structurally related fucosylated glycans, which make up the carbohydrate ligands for selectins (8). During the last decade, a rare, autosomal recessive LAD syndrome distinct from LAD-I and LAD-II has been reported (originally called LAD-I variant, now called LAD-III) (9). Similar to LAD-I and LAD-II, patients with LAD-III exhibit severe recurrent infections and marked leukocytosis, accompanied by a bleeding tendency, which is not seen in LAD-I and LAD-II. The expression of β_2 integrins is normal, and leukocyte rolling is also intact in these patients, but the activation of leukocyte (and platelet) integrins is severely impaired, and lymphocytes fail to arrest. LAD-III is caused by mutations in the Kindlin-3 gene (10).

Similar to LAD-I, LAD-II, and LAD-III, CF is also a genetic disease. CF is a life-threatening disease that causes persistent lung infections and progressively limits the ability to breathe. In people with CF, a defective *CFTR* gene (11) causes a buildup of thick mucus in the lungs and intestines. The most common mutation is F508del, which is present in 90% of patients with CF. This mutation and several other mutations (N1303K, G85E, and G91R) lead to a misfolded CFTR protein that is prematurely degraded. About 5–10% of *CFTR* mutations (e.g., G542X) are a result of premature truncation or nonsense alleles. Other *CFTR* mutations (e.g., G551D and A455E) encode properly processed, full-length CFTR protein that lacks normal ion channel activity (12). Before the current paper, disrupted mucociliary transport resulting from defective CFTR in epithelial cells was thought to be the main mechanism of CF and cause abnormal mucociliary clearance, and dehydration of periciliary liquid. CF lung disease shows exaggerated neutrophil infiltration even before bacterial infection. The present study (3) suggests that a severe monocyte adhesion defect may play an important role in CF.

This is the first study that links the *CFTR* defect (probably all genotypes) to integrin function on leukocytes. Monocytes from patients with CF show an impressive defect (~80%) in adhesion to intercellular adhesion molecule-1 (a ligand for the β_2 integrins lymphocyte function-associated antigen-1 [LFA-1] and macrophage-1 antigen [Mac-1]), fibrinogen (a ligand for Mac-1), and vascular cell adhesion molecule-1 (a ligand for $\alpha_4\beta_1$ integrin). A minimal defect is seen in lymphocyte adhesion, and no defect in neutrophil adhesion, suggesting that the CFTR-dependent leukocyte adhesion deficiency (LAD-IV) is monocyte specific. Unlike LAD-I, LAD-IV appears to affect both β_2 and $\alpha_4\beta_1$ integrins, whereas integrin expression on the monocyte surface is normal. Formyl methionyl leucyl phenylalanine–triggered chemotaxis was also impaired in CF monocytes assessed by *in vitro* migration assay. CFTR-correcting drugs VRT325 and VX809, which can recover the CFTR expression on monocytes from patients with F508del mutation CF, reconstituted monocyte adhesion. Conversely, CFTR inhibitors reduced adhesion of monocytes from healthy donors. *In vivo* evidence for this adhesion defect is provided by defective CFTR F508 del monocyte accumulation in mice. The mutant

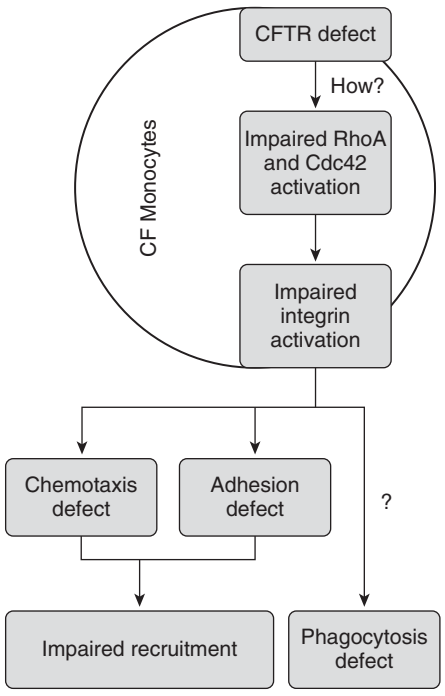

Figure 1. Effects of cystic fibrosis transmembrane conductance regulator (CFTR) defect on integrin activation. Absence or mutations in CFTR cause reduced Ras homolog gene family member A (RhoA) and cell division control protein 42 (Cdc42) activation, resulting in impaired α_4 and β_2 integrin activation and monocyte function. CF = cystic fibrosis.

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Table 1. Leukocyte Adhesion Deficiencies Syndrome

	Discovered	Frequency	Mutated Molecules	Molecular Defect	Major Defect in
LAD-I (3)	1980	Rare (~300 cases)	CD18 (integrin β_2)	Reduced or altered expression of CD18	Neutrophils
LAD-II (6)	1992	Very rare (11 cases)	GFTP	No functional selectin ligands	Neutrophils
LAD-III (8)	1997	Rare (27 cases)	Kindlin-3	Impaired β_2 and β_3 integrin activation	Neutrophils, platelets
LAD-IV	2015	Common (~70,000 cases; ~1,000 cases/yr)	CFTR	Impaired α_4 and β_2 integrin activation	Monocytes

Definition of abbreviations: CFTR = cystic fibrosis transmembrane conductance regulator; GFTP = GDP-fucose transporter 1; LAD = leukocyte adhesion deficiencies.

monocytes accumulate in the lung, but not the bronchoalveolar lavage.

The activation of Ras homolog gene family member A (RhoA) and cell division control protein 42 (CDC42), which are both small rho GTPases and involved in integrin inside-out activation (13), are impaired in CF monocytes. This suggests that integrin activation may be defective in LAD-IV, but the details of the mechanism remain to be investigated (Figure 1). Consistent with an integrin activation defect, monoclonal antibodies KIM127 and 327C, which report β_2 integrin activated conformations, fail to bind CF monocytes. Thus, similar to kindlin-3 deficiency in patients with LAD-III, CFTR deficiency appears to impair the activation, but not expression, of integrin. Different from LAD-III, only monocytes showed the adhesion defect in patients with CF.

Taken together, these findings show a severe monocyte adhesion defect in patients with CF. This may explain the excessive neutrophil infiltration. In addition to the mucociliary transport defect, patients with CF clearly suffer from a new leukocyte adhesion deficiency, tentatively called LAD-IV. CFTR is upstream of small rho GTPases in integrin inside-out activation. At this time, it is not known how CFTR influences Rho and Cdc42 activation, and thus integrin inside-out activation. LAD-IV is the first LAD that affects only monocytes (Table 1). It is also the first LAD that affects both β_2 and α_4 integrins. The β_2 defect was shown for LFA-1 and Mac-1, but not the other two family members, $\alpha_X\beta_2$ and $\alpha_D\beta_2$. The α_4 defect was shown for $\alpha_4\beta_1$, but not $\alpha_4\beta_7$, which may also be affected.

Monocytes play an essential role in inflammation, resolution of inflammation, and protective immunity (14). Thus, the recruitment defect of monocytes in the lung of patients with CF may cause abnormal cytokine/chemokine homeostasis, impaired resolution of inflammation, and impaired pathogen capture. Beyond adhesion, integrins play vital roles in most other monocyte functions, including phagocytosis, which is also important in pathogen clearance, antigen presentation, and neutrophil clearance during inflammation resolution. All these may contribute to the pathogenesis of CF. The most exciting aspect of this paper is that it not only identifies LAD-IV but also introduces a new aspect of pathophysiology that may affect the care for and improve the lives of patients with CF. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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The Year of Ozone

Ozone was rivaled only by climate change as the key environmental issue of 2015 in the United States. On October 1, 2015, after a flurry of lobbying and predictions of doom, the U.S. Environmental Protection Agency (EPA), ending (perhaps) a process begun under the Bush Administration, issued a final rule setting the National Ambient Air Quality Standard for ozone at 70 ppb for an 8-hour average, down from 75 ppb. This was met not merely by the usual lawsuits but by a legislative assault leading to riders that overturned the rule, which were only dropped in hard negotiations, against the background of shutting down the government. They will be back as independent bills in 2016. Why all the fuss? And why did this process take so long?

The last decade has witnessed a collision between developing science on the health effects of ozone and a wrenching change in industrial technology in the United States. Historically, EPA estimates of health benefits of ozone reduction were focused on elegant exposure chamber studies showing reversible reductions in lung function and increases in symptoms after exposure compared with filtered air. Although these results were clear, chamber studies necessarily cannot capture more severe effects of both short-term and long-term exposure to ozone. It became harder to justify tighter and more expensive standards based on such short-term, modest effects. However, even in the 1980s, there were signs that more serious effects occurred, such as increased hospital admission for respiratory disease (1). More recently, studies of long-term exposures of monkeys to ozone demonstrated permanent changes in lung morphology (2).

For effects of short-term exposure, these epidemiologic results soon became a flood. Studies from all over the world reported associations of ozone exposure with hospital admissions for respiratory disease (3–9) and with mortality (10–17). Moreover, a large multicity study found no evidence of a threshold down to about 20 ppb (18). Other studies reported associations with biomarkers that make those findings plausible (19–23). A chamber study this year reported that 100 ppb of ozone for 4 hours resulted in an increase in C-reactive protein during the next 24 hours and a shift to more sympathetic tone in heart rate variability, indicating that cardiovascular as well as respiratory effects are plausible (24). So if ozone at current concentrations is killing people and putting them in the hospital, why has it taken so long to tighten the standards?

The fuss comes from the nature of ozone formation. In most of the United States, that formation depends on emissions of nitrogen oxides, which in turn come from combustion sources, primarily motor vehicles and large industrial and utility coal boilers. Motor vehicles have been subject to increasingly stringent controls of nitrogen oxides since 1980. Further tightening will occur with the Tier III standards scheduled to begin in 2017. Given the half-life of cars, these reductions will phase in quickly. For coal boilers, many of which are more than 50 years old, turnover is slow, and the "grandfather" policy of not requiring retrofits has resulted in old plants without controls. At the same time, inexpensive natural gas has become a cost-effective competitor with coal for these processes, and the "grandfathered" status a regulatory subsidy that keeps them from being replaced by cleaner facilities. The tougher ozone standard, therefore, threatens the existence of coal as a cost-competitive fuel for industrial and utility boilers. Internal combustion engines for nonautomotive use may also require additional controls. Hence, the controversy and the crucial role that the identification of more serious health effects has played in justifying these standards. As the controversy has grown, the need for such understanding of ozone's health effects has likewise grown.

What EPA did not rely on in setting or defending its ozone standards is the developing evidence on the effects of long-term exposure. In this issue of the *Journal*, Turner and colleagues (pp. 1134–1142) indicate that the evidence for chronic effects is growing (25). Jerrett and coworkers (26), using the Cancer Prevention Study II cohort, reported a significant effect of long-term ozone exposure on respiratory mortality rates, but no significant findings for cardiovascular mortality rates. Turner and colleagues followed the same cohort, but with additional years of follow-up and twice as many deaths. In addition, they used greatly improved exposure estimates based on combinations of land use regression and chemical transport models. They report an association (95% confidence interval) of annual ozone and all-cause mortality with a hazard ratio of 1.02 (1.01–1.04), and with cardiovascular mortality of 1.03 (1.01–1.05) in models controlling for particulate matter with a diameter smaller than 2.5 μm and NO_2. Moreover, this comes on the heels of another study, the CanCHEC study (27), looking at a cohort of 2.5 million Canadians, which reported essentially identical associations with all-cause mortality and somewhat larger effects for cardiovascular mortality.

Turner and colleagues' article also includes extensive sensitivity analyses showing no evidence that the ozone effects