



- endothelial lineage: a new paradigm in glitazone pleiotropy. *Circulation*. **109**:1392–1400.
14. Lemonnier, D. 1972. Effect of age, sex, and sites on the cellularity of the adipose tissue in mice and rats rendered obese by a high-fat diet. *J. Clin. Invest.* **51**:2907–2915.
 15. Faust, I.M., Johnson, P.R., Stern, J.S., and Hirsch, J. 1978. Diet-induced adipocyte number increase in adult rats: a new model of obesity. *Am. J. Physiol.* **235**:E279–E286.
 16. Ellis, J.R., McDonald, R.B., and Stern, J.S. 1990. A diet high in fat stimulates adipocyte proliferation in older (22 month) rats. *Exp. Gerontol.* **25**:141–148.
 17. Hausman, D.B., DiGirolamo, M., Bartness, T.J., Hausman, G.J., and Martin, R.J. 2001. The biology of white adipocyte proliferation. *Obes. Rev.* **2**:239–254.
 18. Laviola, L., Perrini, S., Cignarelli, A., and Giorgino, F. 2006. Insulin signalling in human adipose tissue. *Arch. Physiol. Biochem.* **112**:82–88.
 19. Prunet-Marcassus, B., et al. 2006. From heterogeneity to plasticity in adipose tissues: site-specific differences. *Exp. Cell Res.* **312**:727–736.
 20. Hausman, G.J., and Richardson, R.L. 2004. Adipose tissue angiogenesis. *J. Anim. Sci.* **82**:925–934.
 21. Fukumura, D., et al. 2003. Paracrine regulation of angiogenesis and adipocyte differentiation during in vivo adipogenesis. *Circ. Res.* **93**:e88–e97.
 22. Kawaguchi, N., et al. 1998. De novo adipogenesis in mice at the site of injection of basement membrane and basic fibroblast growth factor. *Proc. Natl. Acad. Sci. U. S. A.* **95**:1062–1066.
 23. Brakenhielm, E., et al. 2004. Angiogenesis inhibitor, TNP-470, prevents diet-induced and genetic obesity in mice. *Circ. Res.* **94**:1579–1588.
 24. Wright, J.T., and Hausman, G.J. 1990. Adipose tissue development in the fetal pig examined using monoclonal antibodies. *J. Anim. Sci.* **68**:1170–1175.
 25. Hausman, G.J., Wright, J.T., and Thomas, G.B. 1991. Vascular and cellular development in fetal adipose tissue: lectin binding studies and immunocytochemistry for laminin and type IV collagen. *Microvasc. Res.* **41**:111–125.
 26. Hausman, G.J., and Richardson, L.R. 1982. Histochemical and ultrastructural analysis of developing adipocytes in the fetal pig. *Acta Anat. (Basel)*. **114**:228–247.

Selectins revisited: the emerging role of platelets in inflammatory lung disease

Wolfgang M. Kuebler

Institute of Physiology, Charité Universitaetsmedizin Berlin, Campus Benjamin Franklin, and
Department of Anesthesiology, German Heart Institute Berlin, Berlin, Germany.

Neutrophil infiltration into the lung is considered a crucial step in the pathogenesis of acute lung injury, yet data on the underlying mechanisms have been ambiguous: although selectin-mediated leukocyte rolling is absent in lung capillaries, therapeutic strategies targeted at selectin-mediated cell-cell interactions yield partial protection. The study by Zarbock and coworkers in this issue of the *JCI* solves this apparent contradiction by identifying selectin-mediated platelet-neutrophil interaction as a critical step in the mutual activation of leukocytes and endothelial cells (see the related article beginning on page 3211). The emerging role of platelets may be of broad clinical relevance in lung inflammatory disorders, including asthma, chronic obstructive pulmonary disease, and cystic fibrosis.

Forty years after its first clinical description, acute lung injury (ALI) and its most severe form, the acute respiratory distress syndrome (ARDS), remain life-threatening conditions with reported age-adjusted incidences as high as 86.2 per 100,000 person-years and in-hospital mortality rates ranging between 31% and 38% (1, 2). The pathogenesis of ALI and ARDS is closely linked to intrapulmonary and systemic inflammatory responses. Neutrophils in particular have been implicated in the onset of both diseases based on the rapid accumulation of these cells observed in histologic lung specimens and bronchoalveolar lavage fluid from affected patients (3,

4). In many experimental models, a role for neutrophils in lung injury was supported by partial protection against injury following neutrophil depletion. Yet the causative role of neutrophils in ALI or ARDS has also been challenged by the clinical observation that even profound neutropenia does not protect patients from ALI and ARDS (5). Although the question of how many neutrophils are required to induce tissue injury remains unanswered, the latter observation suggests that neutrophil accumulation is not imperative for the onset of these diseases and that other inflammatory cells may be involved and compensate for neutropenia.

Neutrophil kinetics in the lung

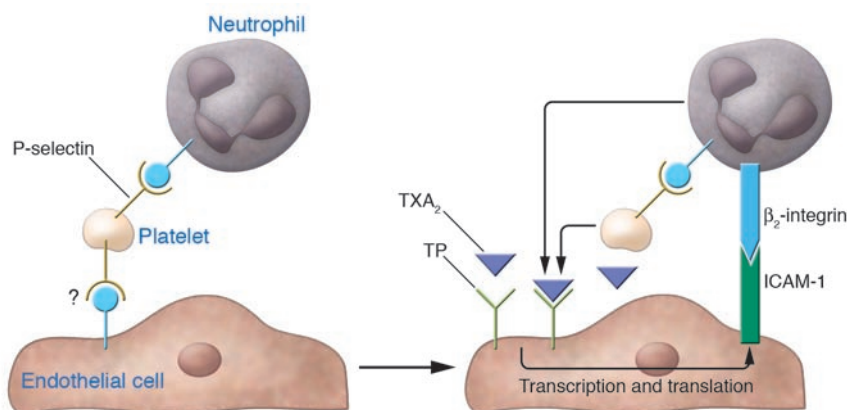
In principle, neutrophil accumulation and subsequent tissue injury are the result of a multistep process comprising the initial tethering of circulating blood cells to the vessel wall and their subsequent rolling

along the wall, followed by firm adherence and finally extravasation. This sequence of events is mediated by consecutive involvement of different families of adhesion molecules; while neutrophil rolling is mediated by selectins interacting with their respective glycoprotein counterligands, firm adherence results from the interaction of neutrophilic β_2 -integrins with ICAMs expressed on the endothelium. In the systemic circulation, this sequence of events is predominantly confined to the venular compartment. In contrast, the prevalent site of leukocyte accumulation and emigration in the lung is the pulmonary capillary bed (6, 7). In lung capillaries, neutrophils do not roll but are temporarily retained at distinct sites of the alveolar capillary network for periods ranging from less than 1 second to more than 20 minutes (8). This phenomenon has been attributed to mechanical retention of circulating neutrophils in the narrow segments of the alveolar capillary network. Following mechanical arrest, the propelling blood flow slowly deforms neutrophils into an elongated shape, ultimately allowing them to continue their passage (9). In accordance with this notion, excessive accumulation of neutrophils in lung capillaries in systemic or pulmonary inflammation has been attributed to increased neutrophil stiffening by polymerization of monomeric to filamentous actin and subsequent firm adhesion to the endothelium via β_2 -integrins (10).

Nonstandard abbreviations used: ALI, acute lung injury; ARDS, acute respiratory distress syndrome; TXA₂, thromboxane A₂.

Conflict of interest: The author has declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **116**:3106–3108 (2006). doi:10.1172/JCI30664.

**Figure 1**

Scheme for the role of platelets in the recruitment of neutrophils to the lung. Initial P-selectin-dependent interaction of neutrophils with platelets, possibly in conjunction with platelet-endothelial interactions (left), results in mutual cell activation and release of the pro-inflammatory eicosanoid TXA₂ (right). Subsequent activation of endothelial TXA₂ receptors (TP) induces de novo expression of ICAM-1, resulting in firm neutrophil adherence via β_2 -integrins.

The role of selectins in neutrophil trafficking in the lungs was frequently considered negligible since the narrow pulmonary capillaries cannot accommodate the typical selectin-mediated rolling phenomenon. Furthermore, selectins did not seem necessary in lung neutrophil sequestration since the deceleration of circulating neutrophils prior to their firm adherence was effectively achieved by their mechanical retention. Yet the notion that neutrophils can accumulate in the lung and mediate ALI and ARDS without the need for selectin-mediated leukocyte rolling was strikingly inconsistent with a large body of experimental data demonstrating that selectin inhibition via the use of blocking antibodies or selectin antagonists or transgenic knockout of 1 or more selectins frequently protected animals from ALI (11). Thus, it seemed as if an important player linking neutrophil sequestration and selectin dependence in ALI still remained to be identified.

Platelet-neutrophil interactions in ALI

In an elegant combination of in vivo and in vitro experiments published in this issue of the *JCI*, Zarbock and coworkers unravel a critical role for platelets in the recruitment of neutrophils to the lung in experimental models of acid aspiration- and sepsis-induced lung injury (12). By platelet depletion, the authors attenuated lung histological changes, reduced protein leakage, and improved alveolar gas exchange in ALI. Importantly, platelet depletion also diminished the accumulation of neutrophils in

all 3 compartments, i.e., the intravascular, the interstitial, and the alveolar spaces of the lung, demonstrating that platelet depletion interfered with an initial step of neutrophil accumulation and establishing a potential mechanistic link between platelets and neutrophils in ALI. P-selectin-dependent platelet-neutrophil interaction was identified as the structural nature of this link. By use of bone marrow chimeric mice, Zarbock et al. identified platelet-derived rather than endothelial-derived P-selectin as the relevant adhesion molecule mediating neutrophil sequestration and lung injury. It remains unclear whether platelet P-selectin interacts only with neutrophils or, in addition, also promotes platelet adhesion on lung endothelial cells, which would allow for “secondary capture” of neutrophils to the vascular wall (Figure 1). Recent intravital microscopic data suggest that activated platelets may attach to lung capillaries via platelet-derived P-selectin (13). This event may even precede the interaction of platelets with neutrophils because intrapulmonary causes of ALI, such as pneumonia or acid aspiration, can be expected to activate the vascular endothelium rather than circulating blood cells (14). Even systemic inflammatory stimuli seem to act primarily on the lung microvascular endothelium, as elegantly shown in experiments by Andonegui and coworkers, demonstrating that endotoxemia-induced neutrophil accumulation is dependent on endothelial but not leukocytic expression of the lipopolysaccharide receptor TLR4 (15).

Following the initiation of neutrophil-platelet interaction, the reciprocal activation of both cells via outside-in signaling mechanisms accounts for the subsequent firm adhesion of neutrophils. In their in vitro experiments, Zarbock and colleagues (12) identified the eicosanoid thromboxane A₂ (TXA₂) as an important proinflammatory signal released by activated platelet-neutrophil aggregates, which mediates firm neutrophil adhesion by inducing the expression of endothelial adhesion molecules such as ICAM-1 (Figure 1). This notion of a critical role for a platelet-derived lipid mediator in ALI is consistent with the observation that transfusion of older, stored platelet concentrates containing bioactive lipids can cause transfusion-related ALI (16). It seems likely that other lipid mediators released from activated platelets, including platelet-activating factor and sphingolipids, contribute to ALI in a redundant or additive fashion. Yet the promising effects of both — a TXA₂ receptor antagonist and a cyclooxygenase inhibitor in the study by Zarbock et al. — render TXA₂ and the eicosanoid pathway key candidates for intervention studies in ALI and ARDS. The failure of a previous clinical trial on the use of the thromboxane synthase inhibitor ketoconazole in ARDS does not conflict with this strategy since ketoconazole also failed to reduce the concentration of the stable TXA₂ metabolite TXB₂ in these patients (17).

Platelets in inflammatory lung disease

Over recent years, we have come to recognize the relevance of platelets not only in hemostasis, but also in numerous inflammatory processes, including atherosclerosis, ischemia-reperfusion injury, and sepsis. Neutrophil-platelet interactions promote mutual cell activation, and platelet-endothelial interactions facilitate the secondary capture of neutrophils and other leukocytes. Zarbock and colleagues (12) have implemented this concept convincingly into the pathophysiology of ALI, but it may extend even further to other inflammatory respiratory diseases. Considerable numbers of circulating platelet-leukocyte aggregates can be found in patients with allergic asthma or cystic fibrosis (18, 19), and a critical role for platelet P-selectin in the recruitment of eosinophils and lymphocytes was recently demonstrated in an experimental model of allergic lung disease (20). Whether platelets alone may



suffice to mediate inflammatory lung disease remains to be elucidated, but platelet-derived mediators may underlie or contribute to the development of ARDS in neutropenic patients.

The notion of platelet-neutrophilic interactions closes a previous conceptual gap in the pathogenesis of ALI and ARDS: it explains the critical relevance of selectins in a setting where the initial retention of leukocytes is predominantly attributable to mechanical factors. Thus, a new role for selectins, not as mediators of leukocyte-endothelial cell interaction, but as amplifiers of platelet and leukocyte activation, has emerged in inflammatory lung disease.

Address correspondence to: Wolfgang M. Kuebler, Institute of Physiology, Charité Universitaetsmedizin Berlin, Campus Benjamin Franklin, Arnimallee 22, 14195 Berlin, Germany. Phone: 49-0-30-8445-1648; Fax: 49-0-30-8445-1634; E-mail: wolfgang.kuebler@charite.de.

1. The Acute Respiratory Distress Syndrome Network. 2000. Ventilation with lower tidal volumes as compared with traditional tidal volumes for

acute lung injury and the acute respiratory distress syndrome. *N. Engl. J. Med.* **342**:1301–1308.

2. Rubenfeld, G.D., et al. 2005. Incidence and outcomes of acute lung injury. *N. Engl. J. Med.* **353**:1685–1693.

3. Bachofen, M., and Weibel, E.R. 1977. Alterations of the gas exchange apparatus in adult respiratory insufficiency associated with septicemia. *Am. Rev. Respir. Dis.* **116**:589–615.

4. Pittet, J.F., Mackerzie, R.C., Martin, T.R., and Matthay, M.A. 1997. Biological markers of acute lung injury: prognostic and pathogenetic significance. *Am. J. Respir. Crit. Care Med.* **155**:1187–1205.

5. Laufe, M.D., Simon, R.H., Flint, A., and Keller, J.B. 1986. Adult respiratory distress syndrome in neutropenic patients. *Am. J. Med.* **80**:1022–1026.

6. Downey, G.P., Worthen, G.S., Henson, P.M., and Hyde, D.M. 1993. Neutrophil sequestration and migration in localized pulmonary inflammation. Capillary localization and migration across the interalveolar septum. *Am. Rev. Respir. Dis.* **147**:168–176.

7. Lien, D.C., et al. 1987. Physiological neutrophil sequestration in the lung: visual evidence for localization in capillaries. *J. Appl. Physiol.* **62**:1236–1243.

8. Kuebler, W.M., Kuhnle, G.E., Groh, J., and Goetz, A.E. 1994. Leukocyte kinetics in pulmonary microcirculation: intravital fluorescence microscopic study. *J. Appl. Physiol.* **76**:65–71.

9. Gebb, S.A., et al. 1995. Sites of leukocyte sequestration in the pulmonary microcirculation. *J. Appl. Physiol.* **79**:493–497.

10. Doerschuk, C.M. 2001. Mechanisms of leukocyte sequestration in inflamed lungs. *Microcirculation.* **8**:71–88.

11. Bock, D., Aydt, E.M., Kuebler, W.M., and Wolff, G. 2006. The role of selectins during lung inflam-

mation and their potential impact for innovative therapeutic strategies. *Current Respiratory Medicine Reviews.* **2**:339–354.

12. Zarbock, A., Singbartl, K., and Ley, K. 2006. Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. *J. Clin. Invest.* **116**:3211–3219. doi:10.1172/JCI29499.

13. Kiefmann, R., Heckel, K., Schenkat, S., Dorgier, M., and Goetz, A.E. 2006. Role of P-selectin in platelet sequestration in pulmonary capillaries during endotoxemia. *J. Vasc. Res.* **43**:473–481.

14. Kuebler, W.M., Parthasarathi, K., Wang, P.M., and Bhattacharya, J. 2000. A novel signaling mechanism between gas and blood compartments of the lung. *J. Clin. Invest.* **105**:905–913.

15. Andonegui, G., et al. 2003. Endothelium-derived Toll-like receptor-4 is the key molecule in LPS-induced neutrophil sequestration into lungs. *J. Clin. Invest.* **111**:1011–1020. doi:10.1172/JCI200316510.

16. Silliman, C.C., et al. 2003. Plasma and lipids from stored platelets cause acute lung injury in an animal model. *Transfusion.* **43**:633–640.

17. The ARDS Network. 2000. Ketoconazole for early treatment of acute lung injury and acute respiratory distress syndrome: a randomized controlled trial. *JAMA.* **283**:1995–2002.

18. Pitchford, S.C., et al. 2003. Platelets are essential for leukocyte recruitment in allergic inflammation. *J. Allergy Clin. Immunol.* **112**:109–118.

19. O'Sullivan, B.P., et al. 2005. Platelet activation in cystic fibrosis. *Blood.* **105**:4635–4641.

20. Pitchford, S.C., et al. 2005. Platelet P-selectin is required for pulmonary eosinophil and lymphocyte recruitment in a murine model of allergic inflammation. *Blood.* **105**:2074–2081.

Proinsulin: a unique autoantigen triggering autoimmune diabetes

Sylvaine You and Lucienne Chatenoud

Université René Descartes Paris 5, INSERM U580, Hôpital Necker-Enfants Malades, Paris, France.

In healthy individuals the immune system does not react aggressively toward host cells, a phenomenon defined as self tolerance. If self tolerance is broken autoimmune disease can develop, during which autoreactive lymphocytes are directed to a variety of autoantigenic epitopes. However, researchers have yet to determine whether immune responses to multiple autoantigens develop independently of each other or are the result of the response “spreading” from one autoantigen to another. In a study of NOD mice in this issue of the JCI, Krishnamurthy et al. show that the autoreactive T cell response to the autoantigen proinsulin lies upstream of that to islet-specific glucose-6-phosphatase catalytic subunit–related protein, suggesting that the pathogenic autoimmune response to proinsulin subsequently spreads to other antigens (see the related article beginning on page 3258). These data support the current view that this pancreatic β cell hormone is the first autoantigen targeted by the immune response in autoimmune diabetes.

Nonstandard abbreviations used: IGRP, islet-specific glucose-6-phosphatase catalytic subunit–related protein.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **116**:3108–3110 (2006). doi:10.1172/JCI30760.

Pathologic autoimmunity is characterized by an aberrant, self-perpetuating, immune-mediated, inflammatory response. It is the uncontrolled chronicity of this response that eventually leads to irreversible destruction of the target tissue. Among the major mechanisms under-

lying this chronicity is the diversification of the pathogenic autoimmune response, also termed *epitope spreading*.

The concept of epitope spreading was initially described by Eli Sercarz in the early 1990s in autoantigen-induced EAE, which is a model for multiple sclerosis (1). This term was used to describe how a self-directed immune response induced by a single peptide (or epitope) could spread to include other peptides (or epitopes) not only on the same autoantigen (i.e., intramolecular spreading), but also on other self molecules clustered in close vicinity within the target cell (i.e., intermolecular spreading). Thereafter, several studies confirmed the crucial role of epitope spreading in EAE (2–4) and also in demyelinating diseases of the central nervous system that follow some viral infections (e.g., Theiler’s murine encephalomyelitis; ref. 5) and IDDM, also known as type 1 diabetes (6, 7).