[1]*University of Virginia, Charlottesville, Virginia, USA and* [2]*Instituto de Biotechnolgia, UNAM, Cuernavaca, Mexico.*
E-mail: jwf8x@virginia.edu

REFERENCES

Ballou L, Laulederkind S, Rosloniec E, Raghow R (1996) Ceramide signaling and the immune response. *Biochim Biophys Acta* 1301:273–87

Bauerle PA, Henkel T (1994) Function and activation of NF-κB in immune system. *Annu Rev Immunol* 12:141–79

Desai A, Lankford HA, Warren JS (2000) *Loxosceles deserta* spider venom induces the expression of vascular endothelial growth factor (VEGF) in keratinocytes. *Inflammation* 24:1–9

Desai A, Miller MJ, Gomez HF, Warren JS (1999) *Loxosceles deserta* spider venom induces NF-κB-dependent chemokine production by endothelial cells. *Clin Toxicol* 37:447–56

Elston D, Eggers J, Schmidt W, Storrow A, Doe R, McGlasson D *et al.* (2000) Histological findings after brown recluse spider envenomation. *Am J Dermatopathol* 22:242–6

Futrell J (1992) Loxoscelism. *Am J Med Sci* 304:261–7

Gomez HF, Greenfield DM, Miller MJ, Warren JS (2001) Direct correlation between diffusion of *Loxosceles recluse* and the extent of dermal inflammation. *Acad Emerg Med* 8:309–14

Hogan C, Barbaro K, Winkel K (2004) Loxoscelism: old obstacles, new directions. *Ann Emerg Med* 44:608–24

Jones S (2005) Directing transition from innate to acquired immunity: defining a role for IL-6. *J Immunol* 175:3463–8

Kaplanski G, Marin V, Montero-Julian F, Alberto Mantovani A, Farnarier C (2003) IL-6: a regulator of the transition from neutrophil to monocyte recruitment during inflammation. *Trends Immunol* 24:25–9

Kurpiewski G, Forrester LJ, Barrett JT, Campbell J (1981) Platelet aggregation and sphingomyelinase D activity of a purified toxin from the venom of *Loxosceles reclusa*. *Biochim Biophys Acta* 678:467–76

Matsusaka T, Fujikawa K, Nishio Y, Mukaida N, Matsushima K, Kishimoto T *et al.* (1993) Transcription factors NF-IL6 and NF-κB synergistically activate transcription of the inflammatory cytokines, interleukin 6 and interleukin 8. *Proc Natl Acad Sci USA* 90:10193–7

Olvera A, Ramos-Cerrillo B, Estevez J, Clement H, Roodt A, Paniagua-Solıs J *et al.* (2006) North and south American *Loxosceles* spiders: development of a polyvalent antivenom with recombinant sphingomyelinases D as antigens. *Toxicon* 48:64–74

Patel KD, Modur V, Zimmerman GA (1994) The necrotic venom of the brown recluse spider induces dysregulated endothelial cell-dependent neutrophil activation: differential induction of GM-CSF, IL-8, and E-selectin expression. *J Clin Invest* 94:631–42

Pedrosa MFF, Azevedo ILMJ, Goncalves-de-Andrade RM, van den Berg CW, Ramos CRR, Ho PL *et al.* (2002) Molecular cloning and expression of a functional dermonecrotic and haemolytic factor from *Loxosceles laeta* venom. *Biochem Biophys Res Commun* 298:638–45

Ramos-Cerrillo B, Olvera A, Odell G, Zamudio F, Paniagua-Solís J, Alagón A *et al.* (2004) Genetic and enzymatic characterization of sphingomyelinase D isoforms from the North American fiddleback spiders *Loxosceles boneti* and *Loxosceles recluse*. *Toxicon* 44:507–14

Silva PH, Silveira RB, Appel MH, Mangili OC, Gremski W, Veiga SS (2004) Brown spiders and loxoscelism. *Toxicon* 44:693–709

Tambourgi DV, Magnoli FC, van den Berg CW, Morgan BP, de Araujo PS, Alves EW *et al.* (1998) Sphingomyelinases in the venom of the spider *Loxosceles* intermedia are responsible for both dermonecrosis and complement dependent hemolysis. *Biochem Biophys Res Commun* 251:366–73

Tambourgi DV, Paixao-Cavalcante D, Goncalves de Andrade RM, Fernandes-Pedrosa MF, Magnoli FC, Morgan BP *et al.* (2005) *Loxosceles* sphingomyelinase induces complement-dependent dermonecrosis, neutrophil infiltration, and endogenous gelatinase expression. *J Invest Dermatol* 124: 725–31

Upregulation of Cytokine Expression in Fibroblasts Exposed to *Loxosceles* Sphingomyelinase D: What is the Trigger?

Journal of Investigative Dermatology (2007) **127,** 1266–1267. doi:10.1038/sj.jid.5700745; published online 8 March 2007

TO THE EDITOR

In a recent report, Dragulev *et al.* (2006) tested the hypothesis that stromal fibroblasts may participate in the pathophysiology of *Loxosceles* spider envenomation, which is characterized by local skin injury as well as systemic toxicity including severe inflammation. To this end, the authors determined the fibroblast gene expression response to sphingomyelinase D (SMD), the causative agent of *Loxosceles* venom. They found that SMD upregulates the expression of proinflammatory cytokines and chemokines, such as IL-6, IL-8, CXCL1, and CXCL2. On the basis of these results, the authors hypothesized that ceramide-1-phosphate (C1P), the product of sphingomyelin (SM) hydrolysis in the outer leaflet of the plasma membrane, is responsible for the observed upregulation of proinflammatory genes. However, although the conversion of SM to C1P in the outer leaflet may modulate ion channel activity (Ramu *et al.*, 2006), C1P is not obviously a signaling molecule. How then does SMD trigger an inflammatory response in its target cells?

What the authors do not mention is the fact that SMD has intrinsic lysophospholipase D activity to generate the lipid mediator lysophosphatidic acid (LPA) from lysophosphatidylcholine (van Meeteren *et al.*, 2004; Lee and Lynch, 2005). Through activation of its cognate G protein-coupled receptors, LPA exerts numerous biological and pathophysiological responses in many different cell types (Moolenaar *et al.*, 2004). By producing bioactive LPA, SMD can activate LPA receptor-mediated signaling pathways that may impinge on inflammatory gene expres-

Abbreviations: SMD, sphingomyelinase D; LPA, lysophosphatidic acid; C1P, ceramide-1-phosphate

sion; indeed, LPA induces the expression of various cytokines, including IL-6, IL-8, CXCL1, and CCL2 (e.g., Palmetshofer *et al.*, 1999; Fang *et al.*, 2004; Klemm *et al.*, 2007; C. Stortelers, unpublished results). That LPA is a key mediator of SMD activity is demonstrated by the failure of SMD to evoke biological effects in LPA receptor-negative cells (van Meeteren *et al.*, 2004). Thus, LPA rather than C1P is the likely trigger of the observed inflammatory response to *Loxosceles* SMD. Specific LPA antagonists could be useful tools for the treatment of bites by *Loxosceles* spiders.

CONFLICT OF INTEREST

The authors state no conflict of interest.

Laurens A. van Meeteren[1], Catelijne Stortelers[1] and Wouter H. Moolenaar[1]

Division of Cellular Biochemistry, The Netherlands Cancer Institute, Plesmanlaan 121, Amsterdam, The Netherlands.
E-mail: w.moolenaar@nki.nl

REFERENCES

Dragulev B, Bao Y, Ramos-Cerrillo B, Vazquez H, Olvera A, Stock R *et al.* (2006) Upregulation of IL-6, IL-8, CXCL1, and CXCL2 dominates gene expression in human fibroblast cells exposed to Loxosceles reclusa sphingomyelinase D: insights into spider venom dermonecrosis. *J Invest Dermatol.* doi:10.1038/sj.jid.5700644

Fang X, Yu S, Bast RC, Liu S, Xu HJ, Hu SX *et al.* (2004) Mechanisms for lysophosphatidic acid-induced cytokine production in ovarian cancer cells. *J Biol Chem* 279: 9653–61

Klemm S, Zimmermann S, Peschel C, Mak TW, Ruland J (2007) Bcl10 and Malt1 control lysophosphatidic acid-induced NF(kappa)B activation and cytokine production. *Proc Natl Acad Sci USA* 104:134–8

Lee S, Lynch KR (2005) Brown recluse spider (Loxosceles reclusa) venom phospholipase D (PLD) generates lysophosphatidic acid (LPA). *Biochem J* 391:317–23

Moolenaar WH, van Meeteren LA, Giepmans BN (2004) The ins and outs of lysophosphatidic acid signaling. *Bioessays* 26:870–81

Palmetshofer A, Robson SC, Nehls V (1999) Lysophosphatidic acid activates nuclear factor kappa B and induces proinflammatory gene expression in endothelial cells. *Thromb Haemost* 82:1532–7

Ramu Y, Xu Y, Lu Z (2006) Enzymatic activation of voltage-gated potassium channels. *Nature* 442:696–9

van Meeteren LA, Frederiks F, Giepmans BN, Pedrosa MF, Billington SJ, Jost BH *et al.* (2004) Spider and bacterial sphingomyelinases D target cellular lysophosphatidic acid receptors by hydrolyzing lysophosphatidylcholine. *J Biol Chem* 279:10833–6

Response to Moolenaar *et al.*

Journal of Investigative Dermatology (2007) **127,** 1267–1268. doi:10.1038/sj.jid.5700812; published online 8 March 2007

TO THE EDITOR

We thank the authors for presenting these valuable comments and for giving us the opportunity to extend the discussion limited by the format of the paper. van Meeteren *et al.* (2004) confirmed that recombinant SMDs from Loxosceles laeta and *Corynebacterium pseudotuberculosis* possessed intrinsic lysophospholipase D activity to generate bioactive lysophosphatidic acid. They demonstrated that SMD did not activate mitogen-activated protein kinase (ERK1/2) in receptor-deficient B103 neuroblastoma cells, whereas both SMDs were activating mitogen-activated protein kinase when the same cells expressed lysophosphatidic $acid_1$ receptors. They also showed that bacterial and *Loxosceles* SMDs triggered receptor internalization in HEK293 cells only when preincubated with albumin-LPC. Lee and Lynch (2005) extended the findings by demonstrating that recombinant *L. reclusa* SMD hydrolzses various lysophospholipids and identified specific histidine residues that are essential for the enzyme activity.

Pettus *et al.* (2003, 2004) in two consequential investigations provided evidence that ceramide-1-phosphate (C1P) interacts directly with cytosolic phospholipase A_2 ($cPLA_2$) acting as an activator of $cPLA_2$ and subsequent inflammatory response. They determined that in A549 lung adenocarcinoma cells, natural and endogenous (produced by SMD) C1Ps were potent and specific inducers of arachidonic acid and prostanoid synthesis. The treatment of A549 cells with SMD resulted in a threefold increase in arachidonic acid release. Using RNA-mediated interference technology, Pettus *et al.* confirmed in A549 and in J774.1 microphages that $cPLA_2$ was downstream of C1P. It was also found that in A549 cells, C1P caused translocation of $cPLA_2$ to membranes. Their *in vitro* binding studies disclosed that C1P directly binds and activate with full-length $cPLA_2$. Other publications regarding biological activities of C1P are reviewed in details by Gomez-Munoz (2004).

The focus of our investigation was the expression pattern of human fibroblasts treated with recombinant SMD to gain insight into cellular mechanisms of loxoscelism pathology. We observed a dose- and time-dependent upregulation of several cytokines (data not shown). The pattern of continuous increase was present up to 18 hours of treatment (data not shown). Although we recognize the evidence that lysophospholipase D activity of SMD is obviously an important factor in loxoscelism, we are not convinced that the sphingomyelin–ceramide pathway involvement in the *L. reclusa* pathology can be ignored. More than one mechanism of action is also possible in this pathology and further investigation would benefit the understanding of the complex immunological response following spider envenomation.

CONFLICT OF INTEREST

The authors state no conflict of interest.

Bojan Dragulev[1], Yongde Bao[1], Blanca Ramos-Cerrillo[2], Hilda Vazquez[2], Alejandro Olvera[2], Roberto P. Stock[2], Alejandro Alagon[2] and Jay W. Fox[1]

Abbreviation: EP, epidermis