of the infections, the comparison between influenza types, the focus on important clinical outcomes, and identification of severity risk factors. The suggestion that high-dose steroids may be beneficial is provocative, but the authors have earlier noted the conflicting data on this point,[7] and randomized trials are clearly needed. Not evaluated in the Choi study was the possible effect of influenza on the subsequent development of noninfectious lung injury or alloreactive syndromes.

Multiple studies have documented substantial morbidity and mortality with seasonal influenza and other community respiratory viruses in HCT recipients. The Choi study emphasizes the importance of early antiviral therapy. Many influenza experts recommend presumptive initiation of therapy for individuals with influenza-like symptoms during times of influenza activity in the community.[8] Consensus guidelines recommend additional measures to protect HCT recipients.[9] Upper respiratory infection (URI) symptoms before start of conditioning should lead one to consider postponement until the URI resolves, if possible, because of the risk for progression to pneumonia. Immunization of patients beyond 6 months after transplant should be performed. Healthcare workers and family members should be immunized to reduce the risk for transmission to the HCT patient. HCT recipients less than 6 months after HCT should receive chemoprophylaxis with neuraminidase inhibitors during community influenza outbreaks. Chemoprophylaxis should be considered for all influenza-exposed HCT recipients during the first 2 years or beyond 2 years if substantially immunocompromised after HCT regardless of vaccination history because of the possibility of suboptimal immunologic response to immunization. Children $<$ 9 years old, less than 6 months after HCT, and receiving their first influenza vaccination, should be given 6 weeks of chemoprophylaxis after the first dose of vaccine. Awareness of drug resistance patterns of circulating influenza strains is advisable and should guide the choice of prophylactic agent.

Seasonal influenza visits every year. What is sobering is how poorly prepared we are for influenza every year. Immunization rates in the general population and among high-risk patients are suboptimal. Failure of many health care workers to get immunized is disappointing. Infection control plans in cancer, leukemia, and HCT inpatient and outpatient facilities are often poorly formulated or not effectively implemented. We clearly can and should do better.[8] The Choi study reminds us of the importance of redoubling our efforts to protect our patients not only for pandemics but for a predictable event every winter.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Choi SM, Boudreault AA, Xie H, Englund JA, Corey L, Boeckh M. Differences in clinical outcomes following 2009 influenza A/H1N1 and seasonal influenza among hematopoietic cell transplant recipients. *Blood*. 2011;117(19):5050-5056.
2. Writing Committee of the WHO Consultation on Clinical Aspects of Pandemic (H1N1) 2009 Influenza. Bautista E, Chotpitayasunondh T, Gao Z, et al. Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. *N Engl J Med*. 2010;362(18):1708-1719.
3. Maines TR, Jayaraman A, Belser JA, et al. Transmission and pathogenesis of swine-origin 2009 A(H1N1) influenza viruses in ferrets and mice. *Science*. 2009;325(5939):484-487.
4. Guarner J, Falcón-Escobedo R. Comparison of the pathology caused by H1N1, H5N1, and H3N2 influenza viruses. *Arch Med Res*. 2009;40(8):655-661.
5. Kasowski EJ, Garten RJ, Bridges CB. Influenza pandemic epidemiologic and virologic diversity: reminding ourselves of the possibilities. *Clin Infect Dis*. 2011;52 (suppl 1):S44-S49.
6. Fowlkes AL, Arguin P, Biggerstaff MS, et al. Epidemiology of 2009 pandemic influenza A (H1N1) deaths in the United States, April-July 2009. *Clin Infect Dis*. 2011;52 (suppl 1):S60-S68.
7. Casper C, Englund J, Boeckh M. How I treat influenza in patients with hematologic malignancies. *Blood*. 2010; 115(7):1331-1342.
8. Wingard JR. Influenza: Preparedness for an inevitable "emergency" for the oncology and BMT unit. *J Natl Compr Canc Netw*. 2008;6(2):215-222.
9. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for Preventing Infectious Complications among Hematopoietic Cell Transplant Recipients: A Global Perspective. *Biol Blood Marrow Transplant*. 2009;15(10):1143-1238.

● ● ● IMMUNOBIOLOGY

Comment on Sierra-Filardi et al, page 5092

The yin and yang of Activin A

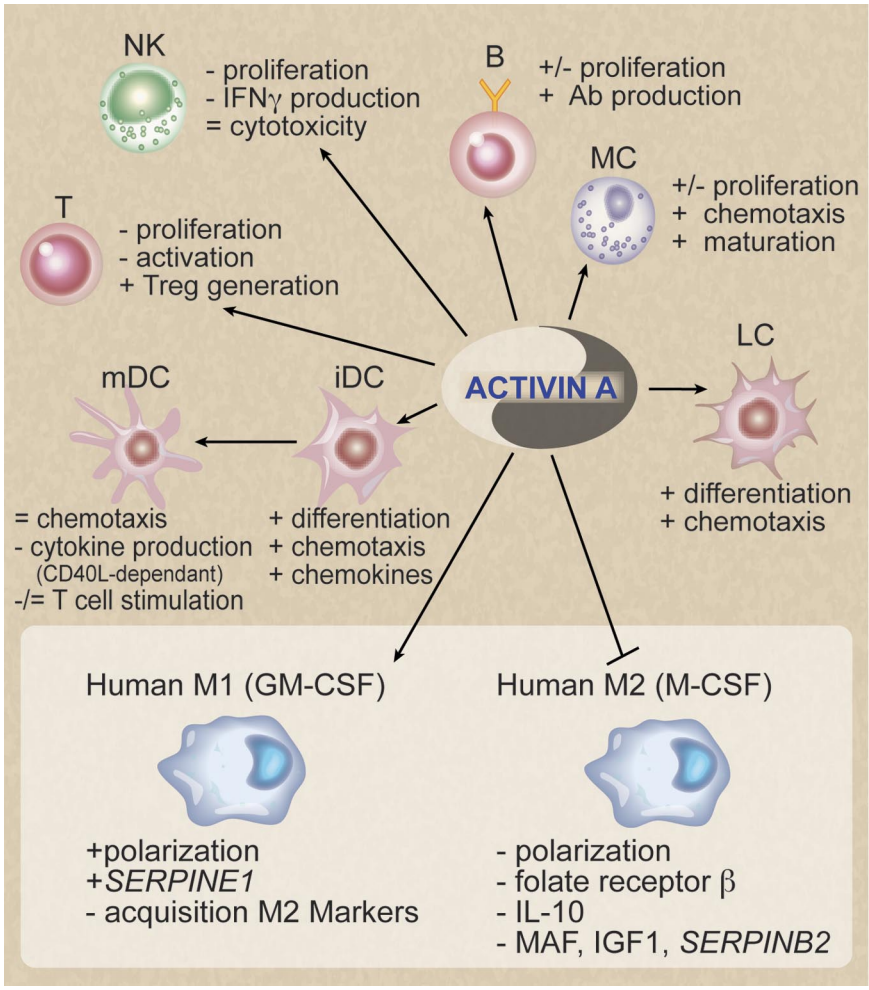
Silvano Sozzani and Tiziana Musso UNIVERSITY OF BRESCIA; UNIVERSITY OF TURIN

In this issue of *Blood*, Sierra-Filardi and colleagues shed new light on the still poorly understood proinflammatory role of Activin A in macrophage polarization.[1]

Activin A is a member of the TGFβ family first identified in late 1980s as an inducer of follicle-stimulating hormone. Similarly to other members of the family, Activin A is highly conserved in evolution and throughout the animal kingdom and regulates a variety of biologic processes including cell proliferation, hematopoiesis, wound healing, and fibrosis. Activin A signals through the activin type I (Alk2, 4, or 7) and type II (ActRII or ActRIIB) receptors and shares with TGFβ the activation of the Smad cascade.[2,3] In the past few years the crucial role of TGFβ in the regulation of both innate and adaptive immunity has been thoroughly investigated. In contrast, the role of other members of the TGFβ family in immunity is more elusive and only recently Activin A has attracted the attention of immunologists for the ability to modulate innate and adaptive immune responses.

A role in inflammation is supported by the observation that Activin A is rapidly induced after intravenous injection of LPS with a kinetics that precedes those of primary proinflammatory cytokines, like TNFα, IL-1β, and IL-6. Increased circulating levels of Activin A were reported in certain inflammatory conditions, such as inflammatory bowel disease and rheumatoid arthritis and in humans during bacterial septicemia, hepatitis C infection, and trauma conditions. The role of Activin A in inflammation is further supported by the finding that the administration of follistatin, a natural receptor antagonist, inhibits the onset of the inflammatory cytokine cascade. However, the pure proinflammatory nature of Activin A was challenged in other pathologic situations. In atherosclerosis, Activin A behaves more like an anti-inflammatory mediator being able to inhibit the formation of foam cells. Similarly, in lung hypersensitivity, the systemic administration of Activin A resulted in a protective effect; although in the same experimental model, it acted as a proinflammatory mediator when administered locally.[2,3]

Many leukocytes, including monocytes, macrophages, dendritic cells, Th2, and B lymphocytes, produce Activin A, with Toll-like receptors being a main pathway of activation.[2,4,5] Consistent with a positive action on immune response, Activin A was shown to



Schematic representation of the pleiotropic effects of Activin A on immune cells. At the bottom of the figure (in the box) the data on macrophage polarization reported by Sierra-Filardi et al are summarized.[1] LC indicates Langerhans cells; iDC, immature dendritic cells; mDC, mature dendritic cells; MC, mast cells; +, stimulation; −, inhibition; and =, no effect. Professional illustration by Debra Dartez.

stimulate the production of proinflammatory cytokines, iNOS, and MMP-2 in myeloid cells. Activin A also promoted in vitro and ex vivo the differentiation of Langerhans cells and the migration of myeloid dendritic cells, Langerhans cells, and mast cells.[6-8] Finally, Activin A promoted IgG, and indirectly, IgE production by B cells. In contrast to these pro-inflammatory effects, Activin A was also reported to inhibit cytokine production in human monocytes/macrophages and to attenuate, in an autocrine manner, cytokine production by CD40L-, but not LPS- or *Escherichia coli*–activated dendritic cells.[4] In the mouse, Activin A was also shown to inhibit phagocytosis and NO production in LPS-stimulated macrophages.[2,3] The dichotomy between the pro- and anti-inflammatory actions of this cytokine is further underscored by the finding that Activin A can control Th1 and Th2 responses through the induction of antigen-specific regulatory T cells.[9] Finally, Activin A was reported to suppress cytokine (IFNγ IL-6, TNFα, GM-CSF, and IL-1β) and chemokine (MIP-1β, MIP-1α, IL-8, and IP-10) production by activated NK cells, with no effect on their cytotoxic activity.[10]

In this issue of *Blood*, Sierra-Filardi and colleagues[1] contribute new data to the complex scenario of Activin A regulation of the immune response. Using an in vitro protocol of human blood monocyte polarization to M1 and M2 macrophages, the authors found that M1 cell supernatant is able to inhibit the acquisition of M2 markers by M-CSF–stimulated cells (see figure). Searching for the factor responsible for this effect, the authors identified Activin A as the responsible molecule. Activin A was exclusively released by macrophages during M1 polarization and the presence of Activin A blocking antibodies inhibited the acquisition of M1 markers by GM-CSF–stimulated cells. M1 and M2 macrophages represent the extremes of a broad range of macrophage functional states. M1 macrophages release high levels of IL-12 and IL-23, are characterized by a high production of reactive toxic oxygen species, and mediate resistance against intracellular pathogens. Conversely, M2 macrophages are characterized by the low production of proinflammatory cytokines and play a role in resistance against parasites and in tissue remodeling.[11] As discussed by Sierra-Filardi and colleagues, this report identifies a role for Activin A in the regulation of the transcriptome of M1 versus M2 macrophages and thus in the acquisition of the macrophage proinflammatory phenotype.

This report opens new perspectives but also raises new questions. Macrophages undergo different forms of M1 and M2 polarization according to their in vitro cytokine milieu. For instance, different types of M2 polarization are obtained using IL-4/IL-13, IL-10/glucocorticoids, or LPS/immunocomplexis.[11] At the moment it is unclear whether the action of Activin A observed here in M-CSF–induced M2 macrophages might also be extended to other types of M2 macrophages. A second open issue is the analogy between human and mouse macrophages. Using mouse macrophages, Ogawa et al reported that Activin A increased the expression of arginase, a marker of mouse but not human M2 macrophages, and inhibited IFNγ-induced expression of iNOS, that is again a marker of mouse but not human M1 macrophages.[8] Although both studies highlight a role for Activin A in macrophage polarization, the reason for this apparent discrepancy is unclear. It is possible that the different actions of Activin A should be interpreted in light of the high degree of plasticity that this cytokine shows in relation to the nature of the target cell, the nature of the agonist, and the duration and intensity of the response.[8] In this context it is interesting to note that plasticity and multifaced responses seem to be common characteristics of other highly evolutionary conserved proteins, such as pentraxins[12] and TGFβ itself, and possibly reflect a strategy to finely tune immune functions with a limited number of effector proteins.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

REFERENCES

1. Sierra-Filardi E, Puig-Kröger A, Blanco FJ, et al. Activin A skews macrophage polarization by promoting a pro-inflammatory phenotype and inhibiting the acquisition of

anti-inflammatory macrophage markers. *Blood*. 2011;117(19):5092-5101.

2. Phillips DJ, de Kretser DM, Hedger MP. Activin and related proteins in inflammation: not just interested bystanders. *Cytokine Growth Factor Rev*. 2009;20(2):153-164.

3. Werner S, Alzheimer C. Roles of activin in tissue repair, fibrosis, and inflammatory disease. *Cytokine Growth Factor Rev*. 2006;17(3):157-171.

4. Robson NC, Phillips DJ, McAlpine T, et al. Activin-A: a novel dendritic cell-derived cytokine that potently attenuates CD40 ligand-specific cytokine and chemokine production. *Blood*. 2008;111(5):2733-2743.

5. Scutera S, Riboldi E, Daniele R, et al. Production and function of activin A in human dendritic cells. *Eur Cytokine Netw*. 2008;19(1):60-68.

6. Musso T, Scutera S, Vermi W, et al. Activin A induces Langerhans cell differentiation in vitro and in human skin explants. *PLoS ONE*. 2008;3(9):e3271.

7. Salogni L, Musso T, Bosisio D, et al. Activin A induces dendritic cell migration through the polarized release of CXC chemokine ligands 12 and 14. *Blood*. 2009;113(23):5848-5856.

8. Ogawa K, Funaba M. Activin in humoral immune responses. *Vitam Horm*. 2011;85:235-253.

9. Semitekolou M, Alissafi T, Aggelakopoulou M, et al. Activin-A induces regulatory T cells that suppress T helper cell immune responses and protect from allergic airway disease. *J Exp Med*. 2009;206(8):1769-1785.

10. Robson NC, Wei H, McAlpine T, Kirkpatrick N, Cebon J, Maraskovsky E. Activin-A attenuates several human natural killer cell functions. *Blood*. 2009;113(14):3218-3225.

11. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol*. 2002;23(11):549-555.

12. Bottazzi B, Garlanda C, Cotena A, et al. The long pentraxin PTX3 as a prototypic humoral pattern recognition receptor: interplay with cellular innate immunity. *Immunol Rev*. 2009;227(1):9-18.

THROMBOSIS & HEMOSTASIS

Comment on Wang et al, page 5231

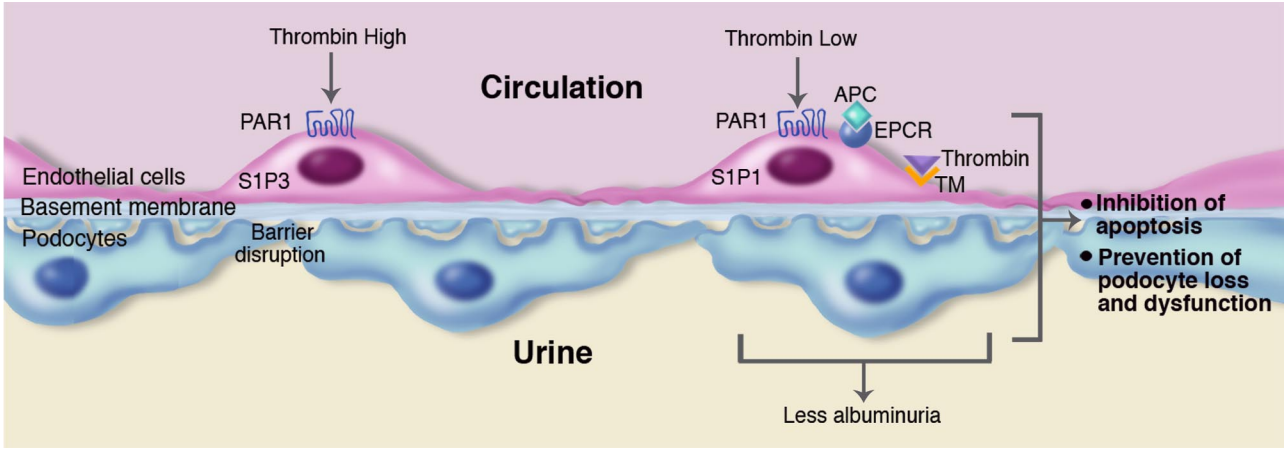
Thrombin and diabetic nephropathy

Tom van der Poll CENTER OF INFECTION AND IMMUNITY AMSTERDAM

The coagulation system is regulated by several anticoagulant mechanisms.[1] The Protein C system provides important control of coagulation via the capacity of activated protein C (APC) to proteolytically inactivate the coagulation cofactors Va and VIIIa.

Protein C is converted into APC by thrombin bound to thrombomodulin, a receptor present on the vascular endothelium, in a process strongly augmented by the presence of the endothelial protein C receptor (EPCR). The factor V Leiden (FVL) mutation is a missense mutation in FV gene replacing arginine at position 506 with glutamine, which results in resistance of activated FV to inactivation by APC and causes increased thrombin formation and, thereby, an elevated risk of (mainly venous) thrombotic events.[2] The high prevalence of this mutation (4%–6% in Caucasians) despite its prothrombotic effects has prompted speculation that the mutation might be subject to positive selection pressure.[3] In the current issue of *Blood*, Wang et al report that one such evolutionary advantage of the FVL mutation may be protection against diabetic nephropathy (see figure).[4]

Diabetic nephropathy is a leading cause of end-stage renal failure in the developed world, with albuminuria, a key abnormality, caused by loss and dysfunction of podocytes. Wang and colleagues established that the FVL mutation reduces the extent of diabetic nephropathy using an approach that elegantly combines mouse and human studies.[4] Relative to normal wild-type mice, heterozygous and homozygous FVL mice displayed reduced albuminuria after 26 weeks of persistent hyperglycemia induced by intraperitoneal administration of streptozotocin. Other manifestations of diabetic nephropathy were also attenuated in FVL mice, including extracellular matrix accumulation, the number of apoptotic cells in glomeruli, and podocyte loss and dysfunction. These findings were corroborated by the observation that type 1 and type 2 diabetes patients heterozygous for the FVL mutation showed reduced albuminuria compared with diabetes patients without FVL who were similar with regard to other risk factors for diabetic nephropathy such as glucose control



Role of thrombin in diabetic nephropathy. Sustained hyperglycemia results in diabetic nephropathy characterized by apoptosis of glomerular cells and loss and dysfunction of podocytes and albuminuria. Left side: High thrombin concentrations can induce disruption of the physiologic vascular barrier by an effect on protease activated receptor (PAR)1-dependent signaling via sphingosine 1 phosphate S1P receptor 3 (S1P3). High thrombin levels can also cause enhanced apoptosis of podocytes (not depicted in the figure). Right side: Low thrombin concentrations and the presence of activated protein C (APC) cause barrier protective effects via PAR1-dependent activation of another S1P receptor, S1P1. Both low thrombin levels and APC inhibit apoptosis of endothelial cells, as well as apoptosis and loss and dysfunction of podocytes, resulting in reduced albuminuria. Thrombin bound to thrombomodulin (TM) is essential for APC generation, a process augmented by the presence of the endothelial cell protein C receptor (EPCR). In this of *Blood*, Wang and colleagues[4] show that the Factor V Leiden mutation, which is associated with modestly elevated thrombin generation, protects against diabetic nephropathy by mechanisms indicated in the right panel of the figure. Professional illustration by Marie Dauenheimer.