Comment on Pluskota et al, page 2491

Kindlin ignites a new flame

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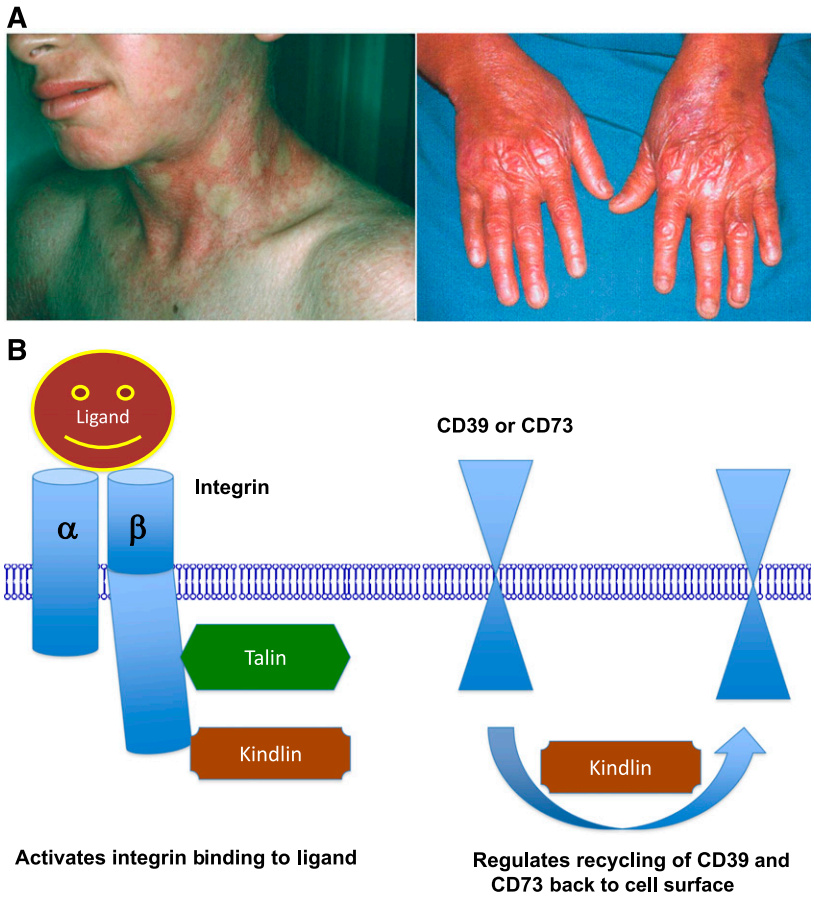
In this issue of *Blood*, Pluskota et al have defined a completely novel role for Kindlin.[1] This protein has been known for decades, but apparently it still has a few new tricks up its sleeve.

Almost 60 years ago, Dr Theresa Kindler described a rare autosomal recessive disorder of hyperkeratosis and hyperpigmentation.[2] Children with this inherited disorder have skin blistering on their extremities that is induced by minimal trauma (see figure). Photosensitivity is often present. As individuals with Kindler syndrome age, the blistering improves, but they develop changes in the pigmentation of their skin and cutaneous atrophy. The disease is associated with frequent cutaneous infections, but intelligence and lifespan are normal.



(A) Mutations within all kindlin family members cause pathology in humans and mice. Patients shown here have Kindler syndrome mutations within Kindlin-1. Kindlin-1 is mutated in Kindler syndrome, a skin-blistering disease caused by impaired actin attachment in basal keratinocytes. Kindlin-1 is expressed in epithelial cells. Kindlin-2 is important in early embryogenesis and is expressed in mesenchymal cells. Kindlin-3 is mutated in leukocyte adhesion deficiency, type III, a disease associated with infections, poor wound healing, and cutaneous bleeding. Kindlin-3 is expressed in hematopoietic cells. (B) Kindlin and Talin bind to the cytoplasmic tail of integrin receptors (left) and induce a conformational change in this receptor that permits the binding to its ligand. Kindlin also regulates the recycling of CD39 and CD73 receptors (right), thereby affecting the expression of these receptors on the cell membrane. The images in panel A were taken from Kloeker et al[3] (left) and Siegel et al[4] (right).

In 2003, work that was performed simultaneously by 2 independent groups demonstrated that mutations within kindlin cause this disorder.[3,4] Because Kindlin turned out to have 2 additional isoforms, this small family of proteins is now referred to as Kindlin-1, -2, and -3. The 3 isoforms of Kindlin have unique tissue expression patterns and therefore play individual roles in biology (see figure). These discrete tissue distributions, along with naturally occurring mutations in humans and engineered mutations in mice, have given us clues about the functions of each of the individual Kindlin isoforms.

Kindlin-1 is only expressed in epithelial cells, and the mutations within the Kindlin-1 gene lead to a disease of skin blistering and, ultimately, cutaneous atrophy. These mutations appear to be attributable to the failure of the cutaneous basal cells to tightly adhere to the lamina densa.

Kindlin-2 is found in the mesenchymal cells, which include the endothelial cells. There are no human mutations known to occur in the Kindlin-2 gene. However, mutations that are engineered into the mouse Kindlin-2 gene cause embryonic lethality in the peri-implantation period. It is notable that endothelial cells that even partially lack the Kindlin-2 have defective cell adhesion.

Last, the third isoform of Kindlin, called Kindlin-3, is exclusively expressed in hematopoietic cells. Mutations within the Kindlin-3 gene lead to a type of the human disease called leukocyte adhesion deficiency type III. This is a disorder of both white blood cells and platelets that is characterized by infections and bleeding. Blood cells lacking Kindlin-3 also have a defect in adhesion.

The diseases that are associated with Kindlin mutations all have defects in cell adhesion. This is because Kindlin is required to regulate a family of adhesion receptors, which are called integrins. Integrins are a specific family of adhesion receptors that are deemed to be "integral" to life. Integrins not only allow cells to adhere to substrates or to other cells, but are also indirectly essential for diverse biologic processes that include cell motility, cytoskeletal organization, cell survival, gene transcription, and cell proliferation.

Integrins do not efficiently bind their extracellular substrates unless intracellular

kindlin is stuck to the cytoplasmic tail of the integrin (see figure). The binding of Kindlin to the integrin induces a conformational change in the integrin tail that facilitates the integrin to bind to another protein called Talin. Together, the binding of Kindlin and Talin to an integrin induces changes that allow the integrin to bind to its ligand. Until now, it appeared that the Kindlin-associated diseases—Kindler syndrome and leukocyte adhesion deficiency type III—were due to an inability of integrin adhesion receptors to do their job.

Pluskota et al have now uncovered another role for kindlins. When they analyzed mice heterozygous for the Kindlin-2 knockout mutation, they observed that the adhesion of the their platelets to the endothelium was impaired. This finding is just as one would predict because this association is an integrin-dependent phenomenon. What was not predicted was that this defective adhesion did not have anything to do with the activation of integrins. Instead, it was due to the increased surface expression on endothelial cells of 2 enzymes that are involved in adenine nucleotide degradation: adenosine triphosphate diphosphohydrolase (CD39) and ecto-5′-endonucleotidase (CD73). This surprising finding was due to the binding of Kindlin-2 to a component of the clathrin complex. This binding mechanism enabled Kindlin-2 to regulate the endocytosis and recycling of CD39 and CD73 on the endothelial cell surface (see figure).

Kindlin family members are certainly critical components of integrin receptor activation, and mutations within the Kindlins gene can cause a variety of human diseases. The work presented in this issue of *Blood* demonstrates that Kindlins have an additional function that does not involve integrins, but instead regulates endocytosis and the recycling of cell-surface receptors. Based on this work, it would be worthwhile to determine whether patients with kindlin mutations have defects in endocytosis. It will also be interesting to determine whether there are even more functions for this fascinating family of proteins.

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

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Comment on Haarberg et al, page 2500

Doubling down on PKC benefits allogeneic BMT

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In this issue of *Blood*, using genetically deficient donor T cells or posttransplant pharmacologic inhibition, Haarberg et al have conclusively demonstrated that protein kinase C (PKC)-θ and PKC-α each contribute to alloreactivity after experimental allogeneic bone marrow transplantation (BMT).[1]

These PKC isoforms, which belong to a broader family of PKC proteins that transduce extracellular signals that dictate key cellular functions such as proliferation and differentiation, act within T cells downstream to T-cell receptor and CD28 activation. Relative to recipients of single PKC-θ–deficient donor T cells, Haarberg et al found that recipients of dual PKC-θ/PKC-α–deficient T cells had improved protection against graft-versus-host disease (GVHD) but still benefitted from a graft-versus-leukemia (GVL) effect. Remarkably, post-BMT therapy with a drug (R524) that potently inhibits PKC-θ/PKC-α while largely sparing other kinases mimicked the gene knockout results for an improved balance of GVHD and GVL effects.

The current results therefore advance an understanding of the PKC signaling pathway after experimental allogeneic BMT, which previously focused on the role of PKC-θ in balancing GVHD and GVL effects.[2] It has been previously shown that PKC-θ and PKC-α make both unique and overlapping contributions to T-cell signaling and to the capacity of murine hosts to reject cardiac allografts.[3] The study by Haarberg et al, therefore, has successfully addressed the key question of whether similar cooperativity exists between these PKC isoforms after allogeneic BMT.

Dual targeting of PKC-θ and PKC-α led to marked inhibition of inflammatory cytokines post-BMT, with relative preservation of allogeneic T-cell cytolytic function. It is possible, as the authors have proposed, that differential modulation of cytolytic vs cytokine function may help account for an ability of dual PKC inhibition to potently prevent GVHD while permitting a GVL effect (see figure); additional experiments using T cells deficient in cytolytic molecules may further clarify this potential mechanism of action. In light of the known role of the PKC pathway in diverse tumors including melanoma[4] and diffuse large B-cell lymphoma,[5] it may also be possible that the GVL effect achieved during PKC inhibition might relate to a direct effect of the PKC inhibitor on the tumor cells; the investigators went to substantial lengths to evaluate this possibility and found no evidence for a direct antitumor effect in vitro or in vivo after syngeneic BMT. However, PKC inhibition can lower the apoptotic threshold of chronic lymphocytic leukemia cells to various cell death triggers[6]; as such, it seems possible that PKC modulation of tumor cell biology, when combined with allogeneic cytotoxic T cells, might synergize for the mediation of GVL effects.

Although it will be interesting to further explore the mechanisms accounting for the ability of PKC inhibition to separate