inside blood

1 JULY 2010 | VOLUME 115, NUMBER 26

IMMUNOBIOLOGY

Comment on Burns et al, page 5355

Lymphocytes with congenitally active WASP

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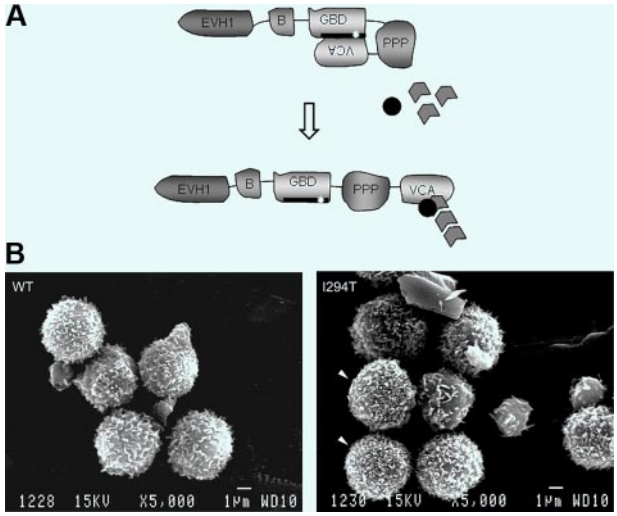
In this issue of *Blood*, Burns and colleagues report that the lymphocytes of a Wiskott-Aldrich syndrome patient with an activating WASP mutation display abnormal L-selectin–dependent adhesion and flow behavior in conjunction with altered cell topography and actin polymerization.



Schematic showing WASP autoinhibition in normal resting cells and conformational activation. The patient's mutation is indicated. Bottom: Lymphocytes of a normal healthy donor and WASP^{I294T} lymphocytes of the patient. See the complete figure by Burns et al beginning on page 5355.

A distinguishing feature of hematopoietic cells is their ability to live free yet be exquisitely competent to make cell-to-cell and cell-to-substratum adhesion contacts when conditions and timing are right. Such properly functioning adhesion contacts are required for cell localization, migration, homing, and also activation, proliferation, cytotoxicity, and phagocytosis. Because all of these processes are affected, Wiskott-Aldrich syndrome (WAS), which was once of interest to only a few clinical immunologists and platelet specialists, has gained broad significance.

WAS is an inherited combined disease involving bleeding caused by thrombocytopenia and immune deficiency of varying severity.[1,2] The spectrum of cell lineages now known to be affected includes not only T and B lymphocytes and platelets, as initially appreciated, but also natural killer (NK) and NKT cells, macrophages and dendritic cells, and to a lesser extent, neutrophils. The affected gene product WAS protein (WASP) is exclusive to hematopoietic cells. Its function is to integrate cell activation signals and, in cooperation with actin-related proteins (Arps), to induce cytoskeletal remodeling. In resting cells, WASP is found in a closed autoinhibited conformation by virtue of binding of the *G*TPase *b*inding *d*omain (GBD) to the C-terminal *v*erprolin homology, *c*ofilin homology, *a*cidic region (VCA domains).[3] On cell activation, the GTP-bound form of the small Rho GTPase cdc42 binds to the WASP-GBD and this, along with other changes, disrupts the autoinhibited structure. Activated WASP with its exposed VCA domain can then interact with Arp2/3 and actin monomers to initiate actin polymerization (figure, see schematic).

In a rare form of the disease, patients inherit one of several mutations in the GBD that disrupt the autoinhibited structure and result in enhanced actin-polymerizing activity.[4-7] These mutations cause a distinct clinical phenotype, called X-linked neutropenia (XLN), involving severe neutropenia and myelocytopenia due to arrest of myelopoiesis in association with abnormalities of actin cytoskeletal structure. In this issue of *Blood*, Burns et al focus on the lymphocytes of a patient with one of these activating mutations, WASP^{I294T}.[8] They report that particularly high densities of microvillus projections were more frequent on patient lymphocytes compared with normal lymphocytes when the two were compared using scanning electron microscopy, and that microvilli were also more dense and were dysmorphic in a cell line model of WASP^{I294T}. The cell line model also showed increased cellular content of F-actin. When examined under flow conditions, primary patient lymphocytes and the WASP^{I294T} expressing model lymphoid cells displayed increased average rolling velocity on the L-selectin ligand sialyl Lewis-X (sLe^x) and showed marked fluctuations in rolling velocity (jerky rolling) compared with counterpart cells expressing normal WASP. The increased average velocity and jerky rolling behavior of lymphoid cells expressing constitutively activated WASP suggest defects in contact between L-selectin and its ligand. The authors were able to eliminate a number of possible abnormalities that

could cause this phenomenon, including L-selectin surface density, its localization to microvilli, and activation-induced ectodomain shedding, and also expression and activation state of the cytoskeletal anchorage proteins ezrin-radixin-moesin because none of these differed between $WASP^{I294T}$ and $WASP^{WT}$ lymphoid cells. Finally, the authors used time-lapse interference reflectance microscopy to monitor persistence of lymphoid cell–substratum adhesion contacts. This was done under static conditions because of the extremely transient nature of adhesion contacts under flow conditions. They found that $WASP^{I294T}$ lymphoid cells had a higher percentage of relatively long-lived adhesions, that is, they had decreased turnover (decreased dynamics) of contact adhesions, compared with $WASP^{WT}$ cells. This was true when the cells were plated on sLe^x, but contact adhesion dynamics were similar for $WASP^{I294T}$ and $WASP^{WT}$ cells when plated on the nonspecific adhesion substrate poly-L-lysine.

The presented data are the first extensive study of lymphocyte defects in XLN; they substantially extend the earlier finding that B lymphocytes with this mutation showed increased migration toward CXCL13.[9] Relating these defects to the clinical impact of WASP-activating GBD mutations on lymphocytes is not straightforward because of the rarity of these patients, the variability of their clinical status, and the coexistence of severe neutropenia. Nonetheless, patients with XLN are thought to have attenuated lymphocyte dysfunction. Within the larger framework of WASP function, abnormalities of microvillus structures are not novel. Indeed, the reciprocal finding, decreased density along with structural abnormalities of microvillus projections on lymphocytes[10] and lymphoid cell lines[11] of patients with classical WAS, was arguably the first structural defect directly linked to the WAS disease gene, which at the time was still unidentified.[11] The increase of microvilli on lymphocytes bearing the WASP autoactivating mutation[8] verifies that WASP plays a role in lymphocyte microvillus formation, even in the resting state, and renders the current findings directly relevant to classical WAS and to WASP function in normal lymphocytes. The novelty and importance of the study lie in the connection of autoactivated lymphocyte WASP to increased cellular F-actin, dysmorphic nature and increased density of microvillus structures, altered dynamics of adhesion contact turnover, and defective rolling behavior on L-selectin ligand. For better understanding, the question of whether or not these connections are direct will need to be addressed, and the apparent role of WASP in regulating lymphocyte adhesion contact dynamics also deserves further exploration. Finally, it will be important to learn whether WASP's role in regulating adhesion contact dynamics is limited to lymphocytes and L-selectin ligand, or is a more general property of cell-substratum and cell-cell adhesion contacts of hematopoietic cells. The diversity of cell lineages and cell functions affected in WAS strongly suggests a broader involvement.

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

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TRANSPLANTATION

Comment on Shono et al, page 5401

Hematopoietic niches: targets of GVHD

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In this issue of *Blood*, Shono and colleagues provide clear evidence that the disrupted hematopoiesis associated with graft-versus-host disease is not caused by the direct elimination of hematopoietic precursors, but instead is the product of an attack to the osteoblastic cell compartment by alloreactive $CD4^+$ T cells. This destruction is mediated by Fas-Fas ligand interactions and results in the loss of integrity of the microenvironment able to support hematopoiesis.[1]

Graft-versus-host disease (GVHD) is one of the main barriers to the success of allogeneic bone marrow transplantation. The main manifestation of GVHD is the immune attack to multiple organs, with the gastrointestinal tract, lungs, liver, and skin being the preferential targets.[2] Although not usually described with the classic symptoms, marrow suppression with compromised hematopoiesis and poor immune reconstitution has long been appreciated in clinical GVHD. The cause of this phenomenon has not been carefully studied and up until now, it was not known whether this was the product of destruction of hematopoietic progenitors or an indirect effect of damage to the bone marrow microenvironment.

Using murine models of GVHD, Shono et al in this issue of *Blood* confirm the bone marrow (BM) suppression effect. After transplantation of allogeneic T cells, in a fully major histocompatibility complex (MHC)–mismatched BM model, there was a decrease in hematopoietic components in bone marrow and periphery. In bone marrow, these included compromise of hematopoietic stem cells, rapid decrease of early erythropoiesis, and a drastic loss of B-cell progenitors. Interestingly, the development of all these cells and their lineage progressions are dependent on the integrity of bone marrow microenvironment.

To identify the cell types responsible for mediating these alterations, the experimental