would delete T cells that expressed OVA-specific transgenic T-cell receptors (TCRs). In a widely used OVA transgenic model where OVA was produced in the membrane-bound form, they found that OVA-specific CD4 and CD8 T cells could be deleted as a consequence of Aire expression, which they show partially regulated OVA expression in mTECs. On the contrary, in another OVA transgenic model in which the secreted form of OVA was expressed under the control of the transgenic insulin promoter, they found that OVA expression was not regulated by Aire and as a consequence deletion of OVA-specific OT-I-TCR-transgenic CD8 T cells was not dependent on Aire expression. Interestingly, however, the deletion of OVA-specific OT-II-TCR-transgenic CD4 T cells was dependent on Aire expression but was not directly mediated by mTEC antigen presentation. Instead of mTECs, bone marrow–derived antigen-presenting cells, presumably tDCs, were required for the deletion. Thus, the requirement for the transfer of mTEC-produced self-antigens to tDCs seems dependent on the form of antigens expressed in mTECs. Some forms of mTEC-produced self-antigens need to be transferred to tDCs for presentation to developing T cells, and Aire regulates such an mTEC-to-tDC transfer of self-antigens. Hubert and colleagues further sought the possibility that Aire regulated the expression of chemokines that might bring tDCs and mTECs together. Microarray analysis using mRNAs from wild-type and Aire-deficient mTECs showed that many chemokines were significantly affected by Aire, although Hubert et al did not examine the functions of individual chemokines in the mTEC-tDC interplay. Nonetheless, their study has revealed that a fraction of self-antigens produced by mTECs are transferred to tDCs for the deletion of developing T cells that are reactive to those self-antigens, and that Aire regulates the transfer of the self-antigens from mTECs to tDCs. They suggested that the chemokine-mediated attraction of tDCs to mTECs may help increase the cooperation between mTECs and tDCs in transferring mTEC-produced self-antigens to antigen-presenting tDCs (see figure). These results highlight the idea that the role of Aire in the establishment of self-tolerance is not limited to the ectopic expression of self-antigens in mTECs but includes regulation to facilitate the interplay between mTECs and tDCs. Recent papers have also reported that Aire regulates the expression of several chemokines in mTECs. One of those papers has further shown that mTECs produce the chemokine XCL1 in an Aire-dependent manner and the receptor XCR1 is expressed by tDCs. XCL1-deficient mice are defective in the medullary accumulation of tDCs and the thymic generation of naturally occurring regulatory T (nTreg) cells. Thymocytes from XCL1-deficient mice elicit dacyroadenitis in T cell–deficient nude mice. mTEC expression of XCL1, tDC medullary accumulation, and nTreg cell generation are diminished in Aire-deficient mice, indicating that the XCL1-mediated medullary accumulation of tDCs contributes to the establishment of central tolerance and is regulated by Aire. Thus, XCL1 may participate in the Aire-mediated attraction of tDCs for the efficient transfer of mTEC-produced self-antigens. Further studies of the molecular mechanisms that regulate the interplay between mTECs and tDCs will improve our understanding and aid in the future manipulation of autoimmune diseases.

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Buzz in the dendritic cell synapse

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The immunologic synapse is formed between a dendritic cell (DC) and a T cell and is critical for correct T-cell activation. In this issue of Blood, Bouma and colleagues define that the Wiskott–Aldrich syndrome protein (WASp) regulates assembly and function of the immunologic synapse in DCs.

What makes highly motile T cells stop when they encounter an antigen-presenting cell in lymph nodes? The answer is the immunologic synapse formed at the interface between an antigen-presenting DC and an antigen-specific T cell. The immunologic synapse has been extensively characterized in T cells and is composed of a region of spatially...
and temporally organized adhesion proteins and receptors in supramolecular activation clusters (SMACs; see figure). A full understanding of the immunologic synapse requires analysis of both the T-cell side and the DC side of the synapse. However, the contribution of DCs to immunologic synapse assembly and function is largely unknown.

WASp has emerged as a critical regulator of the actin cytoskeleton in hematopoietic cells and regulates immunologic synapse stability in T cells. The importance of WASp activity for correct function of hematopoietic cells is revealed in patients with Wiskott-Aldrich syndrome who lack expression of WASp and suffer from severe immunodeficiency. Bouma et al investigated the role of WASp and the cell cytoskeleton at the DC side of the synapse. They show that disruption of the cell cytoskeleton and cellular signaling in WASp−/− DCs is enough to destroy the immunologic synapse at both the DC side and T-cell side of the synapse. Their data provide important insight into the active role of DCs for immunologic synapse assembly and function.

WASp−/− DCs have decreased migration and adhesion responses and reduced capacity to activate naïve T cells. In the current investigation, Bouma et al show that WASp−/− DCs form less stable interactions with antigen-specific wild-type CD4+ T cells in lymph nodes. To exclude the migratory defect of WASp−/− DCs, the authors analyzed the DC-T synapse in vitro using time-lapse microscopy. They show that WASp−/− DCs form less stable interactions with antigen-specific wild-type CD4+ T cells also in vitro, resulting in decreased T-cell activation and proliferation. Polarization of the microtubule organizing center toward the immunologic synapse is thought to be required for release of certain cytokines in the synapse interface. One such cytokine is IL-12, and a recent report showed that polarized secretion of IL-12 by DCs to the synapse is regulated by the WASp interacting protein Cdc42 and required for normal T-cell activation. WASp−/− DCs had normal polarization of the microtubule organizing center to the synapse, but reduced production of IL-12. It is tempting to speculate that WASp−/− DCs may fail to correctly activate T cells due to failure of targeting IL-12 secretion to the immunologic synapse.

Bouma et al next investigated the molecular structure of the synapse formed between WASp−/− DCs and wild-type T cells. Antigen-specific CD4+ T cells in contact with wild-type DCs showed the classic bull’s-eye appearance of the synapse with large adhesion molecules such as CD45 and LFA-1 more distal and TCR in the central SMAC (see figure). In contrast, wild-type DCs in contact with WASp−/− DCs failed to assemble the bull’s-eye synapse and had decreased localized tyrosine phosphorylation, a sign of diminished signaling at the synapse. To investigate the molecular structure at the DC side of the synapse, polarization of the LFA-1 adhesion partner I-CAM to the synapse was examined. Wild-type DCs in contact with antigen-specific CD4+ T cells had organized localization of I-CAM to the peripheral SMAC. Strikingly, WASp−/− DCs showed only diffuse I-CAM localization at the synapse interface.

The activity of WASp is regulated by its structural conformation and phosphorylation of a critical tyrosine residue (Y293). To determine how WASp regulates the DC immunologic synapse, Bouma et al investigate a WASp mutant strain that has abrogated phosphorylation of tyrosine-293 (Y293F). Similar to WASp−/− DCs, WASp-Y293F DCs showed diffuse polarization of I-CAM to the synapse interface, providing evidence that tyrosine phosphorylation of WASp is required for normal immunologic synapse assembly in DCs. Finally, Bouma and colleagues translate their findings using murine WASp−/− DCs to DCs from a patient with Wiskott-Aldrich syndrome. The authors show that human WASp−/− DCs have reduced capacity to prime T cells as measured by IFN-γ secretion.

Activation of antigen-specific T cells during an immune response depends on orchestral cycles of T-cell migration and stable immunologic synapse assembly with antigen-presenting DCs. On each T-cell migration phase, WASp regulates reassembly of synapse symmetry and stability in T cells. The present investigation by Bouma et al provides evidence that WASp serves a similar function in DCs. Future studies will define whether WASp regulates reassembly of the immunologic synapse in DCs after rounds of migration to find antigen-specific T cells. Bouma and colleagues provide new and important information about the myeloid deficiency in Wiskott-Aldrich syndrome. The reduced capacity of WASp−/− DCs to form a functional immunologic synapse with T cells has implications for the treatment of Wiskott-Aldrich syndrome patients and raises concerns for those patient who have limited myeloid reconstitution after bone marrow transplantation and gene therapy.

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Clots vs bugs: who’s ahead?

Russell F. Doolittle

Whether or not vertebrate blood clotting has a major role in defending against microbial infection has long been a matter of debate. Apart from the mere physical barrier that a fibrin clot presents against external invaders when plugging a wound, is there a strategy specifically targeted against the pathogen?

In this issue of Blood, Loof et al have achieved a major step toward answering that question by definitively showing that the bacterium Streptococcus pyogenes not only provokes coagulation but when it subsequently becomes entrapped in the fibrin clot, the two entities becoming covalently cross-linked by the action of coagulation factor XIIIa.
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