subset. Mast cell–deficient Kit^Wts^ or Kit^W-ak^/W-ak^ mice carry mutations of the c-Kit gene, which obviously do not affect basophil biology. On the other hand, basophil–deficient Mctp8–DTR or basoph8 mice carry a transgenic receptor that mediates toxin–mediated cell death and mast cells in these mice remain intact. In mice deficient in IL-3, both mast cell and basophil generation are severely impaired only after parasite infection.  Therefore, Cpa3-Cre; Mcl-1^W^/W^ mice will prove a useful animal model from which both redundant and nonredundant functions of mast cells and basophils in vivo can be unveiled.

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Comment on Choi et al, page 6963

A cofactor for factor XI activation

David Galli VANDERBILT UNIVERSITY

During formation of a blood clot, the key enzyme thrombin is formed through the coordinated activities of a group of plasma proteases (factors VIIa, IXa, Xa, and Xla) that facilitate the activation and/or activity of the plasma proteases on membranes of platelets and tissues. In this issue of Blood, Choi and colleagues present intriguing results showing that a nonprotein, inorganic polyphosphate (PolyP), can enhance thrombin generation by serving as a cofactor for formation of factor Xla. 1

Factor XI, the precursor of factor Xla, differs structurally in several important respect from the vitamin K–dependent (VKD) coagulation protease zymogens (the thrombin precursor prothrombin and factors VII, IX, and X). 2 For example, factor XI lacks the Glu domain through which the VKD proteases bind to phospholipid membranes. In addition, factor Xla does not appear to require a cofactor to mediate its primary hemostatic function, activation of factor IX. These properties reflect the different natural histories of factor Xla and the VKD proteases.

The VKD proteases and their cofactors form the core of an ancient hemostatic mechanism that is common to all vertebrate organisms. Factor XI, in contrast, is a relative newcomer, appearing during mammalian evolution as the result of a duplication of the gene for plasma prekallikrein. 3 The protease zymogens prekallikrein and factor XII, along with high molecular weight kinogen, comprise the plasma kallikrein–kinin system (KKS). The KKS participates in a number of homeostatic and host-defense functions, including the innate immune response to invading microorganisms. 4,5 KKS components assemble and are activated on the surface of microorganisms, generating antimicrobial peptides and contributing to complement activation. 5 The capacity of the KKS to bind to surfaces is also important for initiating blood coagulation in vitro in the activated partial thromboplastin time assay (aPTT) used in clinical practice. In the aPTT, anionic substances such as purified earths trigger reciprocal activation of factor XII and prekallikrein in a process called contact activation. Activated factor XII (factor XIIa) then propagates clotting by activating factor XI. Given its close ties to the KKS, then, it is not surprising that factor XI activation by factor XIIa is enhanced in vitro by a variety of polymers, including the bacterial product dextran sulfate and glycocalicinoglycans such as heparin. The importance of factor XIIa–mediated factor XI activation to hemostasis has been questioned, justifiably, because of the absence of a bleeding disorder in persons lacking factor XII. Other proteases including various forms of thrombin activate factor XI and may be more physiologically relevant activators. 6 However, regardless of the activating protease, factor XI activation proceeds slowly in the absence of a polyanion, strongly suggesting that a cofactor (perhaps with features of a polyanion) is required to promote the reaction.

Choi et al have now convincingly demonstrated that PolyP secreted from activated platelets is a potent enhancer of factor XI activation by the α and β forms of thrombin. 7 Previously, PolyP has been shown to influence blood coagulation by (1) induction of factor XII activation (contact activation), (2) acceleration of factor V activation by factor Xa, and (3) enhancing fibrin fibril thickness. 1,6,7 PolyP is a linear polymer of inorganic phosphate groups linked by high-energy phosphoanhydride bonds. A minimum PolyP chain length is required to support factor XI activation by thrombin, suggesting a template mechanism in which factor XI and thrombin bind to the polymer in proximity to each other. PolyP also supports factor XI autoactivation, consistent with previous descriptions of the effects of polyanions such as dextran sulfate and heparin on factor XI in solution.

In 1972, Walsh observed that collagen–stimulated platelets possessed a procoagulant activity that required factor XI but not factor XII, and proposed that this activity could explain why factor XII deficiency is not associated with a bleeding disorder. 8 Subsequent work from several groups showed that activated platelets support factor XI activation by factor XII–dependent and –independent mechanisms. These findings are entirely consistent with the properties of PolyP presented by Choi et al, and with their observation that activated platelets and platelet releasates support factor XI activation in a manner that is blocked by a PolyP specific binding protein. 9 Taken as a whole, this work
strongly supports the hypothesis that platelet PolyP can function as a physiologic cofactor for factor XI activation.

PolyP ranging in size from several to more than 1000 phosphate units can be found across all taxonomic kingdoms of organisms.9,10 Interestingly, PolyP in bacteria are often > 200 phosphate units long, a size that efficiently supports activation of the KKS. Platelet PolyP, on the other hand, is of a somewhat smaller size (~ 75 units) that facilitates factor XI activation, while having less effect on the KKS. It is tempting to speculate that the size of phosphate polymers in platelet granules is optimized to promote factor XI activation during hemostasis without excessive activation of the KKS. It will be interesting to determine whether dysregulation of PolyP synthesis (either amount or polymer size) is associated with pathologic consequences such as bleeding or an increased risk of thrombosis.

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Comment on Amir et al, page 6733

TCR-MHC-peptide(s): in vivo veritas

Paul J. Martin  FRED HUTCHINSON CANCER RESEARCH CENTER

In this issue of Blood, Amir et al report how donor T cells recognize major histocompatibility complex (MHC) alloantigens of the recipient in a patient with graft-versus-host disease (GVHD).1

During development in the thymus, T cells are selected to survive when the T-cell receptor (TCR) has weak affinity for self-MHC molecules, so that in the periphery, immune responses are triggered when foreign antigen-derived peptides are inserted into the groove between the α-helices of self-MHC molecules and strengthen the interaction with the TCR above a critical threshold. In this way, T cells are “biased” toward recognition of peptide antigens presented by self-MHC molecules. This understanding raised questions of whether T cells respond to MHC alloantigens primarily by recognizing polymorphic differences between allo-MHC molecules per se, with relatively little contribution from the peptide, or by recognizing foreign peptides in a highly restricted way that somehow reflects the bias toward self-MHC molecules.2

A previous study showed that a human T-cell clone specific for an Epstein-Barr virus–derived peptide in conjunction with self–HLA-B8 molecules could also recognize as many as 4 distinct human–derived peptides in conjunction with HLA-B35, suggesting that MHC-polymorphism has a dominant role in allo–MHC recognition.4 In another study, however, 3 human T-cell clones specific for HLA-A*0201 each recognized unique, single, distinct peptide peaks in experiments where T-cell recognition was reconstituted with fractions isolated by reverse-phase, high-performance liquid chromatography (HPLC), suggesting that the peptide plays a critical role in allo-MHC recognition.4 A more recent study of murine T-cell clones specific for an MHC–class II alloantigen showed mixed results: 3 clones responded to 2 or 3 different peptides into the groove between the α-helices, whereas 6 clones appeared to be specific for a single peptide.5

Amir et al isolated 56 T–cells clones from a patient with GVHD after hematopoietic cell transplantation from a related donor.1 The related donor and recipient were HLA genotypically identical except at HLA–A. Because of a recombination between HLA–A and –B, the recipient was HLA–A*0201–positive, whereas the donor was HLA–A*0201–negative, thereby creating a situation in which the donor T–cell response was driven by recognition of HLA–A*0201 in the recipient. Fifty of the clones recognized HLA–A2 (see figure). Thirty–two of these clones were tested to determine whether T–cell recognition could be reconstituted by loading HPLC fractions of peptides isolated from HLA–A*0201 molecules into HLA–A*0201–positive human T2 cells that are not able to insert peptides into MHC class 1 molecules through a transporter associated with antigen processing or by loading HPLC fractions into HLA–A*0201–positive insect cells that lack peptides derived from human molecules other than HLA–A*0201. In 27 of the 28 informative assays, the clones each responded to a single unique peptide, and only 1 clone responded to 2 distinct peptides. Six of the single peptides recognized by a clone and both peptides recognized by the clone with dual specificity were identified by mass spectrometry. Experiments with silencing short hairpin RNA sequences that suppressed expression of specific genes encoding 6 of the 8 identified peptides confirmed that these T–cell clones recognized no other peptides in conjunction with HLA–A*0201.

These results show that unlike minor histocompatibility antigens composed of a single specific peptide presented by a specific MHC molecule, major histocompatibility antigens represent large families of single specific peptides that can all be presented to T cells by a specific MHC molecule. The response to a single MHC alloantigen has been estimated to be approximately 3 orders of magnitude stronger than the response to any single minor histocompatibility antigen. These results also show why the response to an MHC alloantigen overwhelms any concurrent response against minor histocompatibility antigens that would otherwise have been generated in the absence of an MHC mismatch.

Amir et al also showed that the T–cell clones did not recognize a panel of virus-
A cofactor for factor XI activation

David Gailani