

## The Role of Factor XII in Contact System Activation

To the Editor:

Although the excellent review on the contact system by Colman and Schmaier<sup>1</sup> that appeared recently in *Blood* examines the subject in great detail, there are some points that deserve further discussion.

A major item in that review is the novel observation, described recently in *Blood*,<sup>2</sup> that prekallikrein (PK) becomes activated when it is bound to high molecular weight kininogen (HK) on endothelial cells, independently of factor XII. This activation is mediated by an ill-defined cell-associated thiol-protease and is said to be regulated by HK, because increasing concentrations of HK upregulate this unknown protease. The investigators speculate that this novel mechanism may be the predominant activation pathway for the contact system in vivo, because it does not require an artificial surface. However, some considerations need to be made. First, the stimulus for this novel activation mechanism of PK is unclear. The investigators mention that increasing HK concentrations trigger the activation of PK by upregulating the thiol-protease. In the original study describing this novel pathway, activation of PK was observed at concentrations of HK of 20 nmol/L,<sup>2</sup> which is more than 30-fold lower than the plasma concentration of HK.<sup>1</sup> So, this factor XII-independent activation of PK should be turned on under physiological conditions and may explain why normal persons have higher levels of kallikrein-C1-inhibitor than of factor XIIa-C1-inhibitor.<sup>3</sup> However, as it is now, this pathway does not provide an explanation for enhanced contact activation under pathophysiological conditions, because under these conditions HK levels do not increase markedly. For example, we have observed activation of PK (and factor XII) in patients with insect sting-induced angioedema and shock, which was accompanied by decreasing rather than increasing concentrations of HK.<sup>4</sup> Second, there is in vivo evidence for a factor XII-dependent activation of PK. We have observed activation of PK in healthy persons upon intravenous injection of desamino D-arginine vasopressin (DDAVP).<sup>5</sup> This activation was not observed in factor XII-deficient persons,<sup>5</sup> indicating the existence of a factor XII-dependent activation of PK in vivo (and, notably, not triggered by an artificial surface). In our experience, activation of PK in clinical situations is often, if not always, accompanied by activation of factor XII, which is not expected in case of a factor XII-independent activation of PK. Therefore, factor XII-dependent activation of PK likely predominates contact activation under pathophysiological conditions.

A second comment concerns the fibrinolytic activities of the contact system. In their review, Colman and Schmaier<sup>1</sup> propose that factor XII-independent activation of prekallikrein on endothelial cells is involved in two pathways for fibrinolysis, one involving the release of tissue-type plasminogen activator induced by bradykinin and another involving the activation of pro-urokinase by kallikrein. They do not mention the existence of another fibrinolytic pathway involving the contact system, ie, factor XII-dependent activation of plasminogen. There is in vivo evidence for the existence of this pathway, which is independent of kallikrein and urokinase.<sup>5</sup> Direct activation of plasminogen by factor XIIa is often considered to be an unlikely scenario for this contact pathway, because plasminogen is a too poor substrate for this contact

enzyme. For example,  $\beta$ -factor XIIa is about 300,000 times less active than urokinase in activating plasminogen. However, we have recently observed that the interaction of factor XII with plasminogen can be considerably potentiated by the presence of dextran sulphate, yielding conditions that urokinase is 10,000 and 1,500 times more active than factor XII in activating Glu- and Lys-plasminogen, respectively (Ravon et al, unpublished observations). Thus, considering the relative plasma concentrations of factor XII and urokinase (that of factor XII is 4 orders of magnitude higher), factor XII may be equally potent as urokinase in activating plasminogen, as indeed has been found in vivo.<sup>5</sup> Notably, the theme of a plasminogen-like molecule being activated by a factor XII-like molecule has been used more often by nature, as is illustrated by hepatocyte growth factor (scatter factor), which is homologous to plasminogen, and its activating protease, which is homologous to factor XII.<sup>6</sup> The observation that genetic deficiencies of factor XII, rather than those of PK or HK, are associated with thromboembolic disorders underscores the importance of factor XII-dependent activation of plasminogen for in vivo fibrinolysis.

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## Response

We appreciate the opportunity to respond to Dr Hack's comments because it provides a forum to discuss the implications of our work.<sup>1,2</sup> Dr Hack's questions focus on the novel mechanism for prekallikrein

(PK) activation presented in recent publications.<sup>2,3</sup> We agree that we do not know yet what regulates the membrane-associated PK activating cysteine protease. However, we propose that this PK activation

mechanism is the first physiologic pathway by which the kallikrein/kinin system can be activated *in vivo*. We do not believe that this PK activation system is constitutively active *in vivo*. The amount of high molecular weight kininogen (HK) necessary for maximal PK activation is limited by the number of kininogen receptors on cells and not the ambient plasma concentration. How HK modulates the activity of this enzyme needs to be examined further. Furthermore, HK is not the single regulator of activation of this pathway. In a study now in press, we show that activation of this pathway also is dependent on an optimal free zinc ion concentration.<sup>4</sup> In the absence of an optimal free zinc ion concentration, the system is quiescent. These data suggest that the local liberation of zinc ion may be the immediate regulator of activation of this system. Furthermore, we show that on endothelial cells, factor XII (FXII) does not autoactivate in any reasonable period of time (<1 hour) and that FXII activation is dependent on PK activation and not vice versa.<sup>4</sup> Activated FXII is then able to reciprocally activate more plasma PK.

We agree that our proposed pathway for contact system activation is not a substitute for the role of anionic surfaces (dirt, cardiopulmonary bypass tubing, bacteria, etc) in FXII activation in nonphysiologic states. Furthermore, we agree that Dr Hack's DDAVP infusion experiments suggest that, in response to endothelial cell agonists, FXII activation can initiate PK activation.<sup>5</sup> It was an unintended oversight not to refer to the FXII-dependent pathway of plasminogen activation described by Levi et al.<sup>5</sup> As described by Dr Hack, the presence of an artificial surface potentiates FXIIa's activation of plasminogen. This pathway, which may become operative in nonphysiologic states, can be conjoined with the proposed physiologic contact system pathways for fibrinolysis mediated by bradykinin-induced tPA liberation and kallikrein activation of single-chain urokinase.<sup>2,3,6</sup> Last, it is not rigorously proven that FXII-deficient patients are at increased risk for thrombosis. Furthermore, because PK and HK deficiencies are so rare, there have not been sufficient number of individuals described with these defects to deter-

mine if they have less of a risk for thrombosis than FXII-deficient patients.

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## The Prothrombin G20210A Mutation and Factor V Leiden Mutation in Patients With Cerebrovascular Disease

To the Editor:

Genetic variants leading to a persistent hypercoagulable state may predispose to thrombotic events. A recently discovered G to A mutation at position 1691 of the factor V gene (factor V Leiden mutation), occurring in 3% to 5% of the normal Caucasian population, has emerged as a major genetic risk factor of venous thrombosis.<sup>1</sup> The role of this mutation for arterial vascular events, in particular cerebrovascular disease, is still under discussion.<sup>2-6</sup> Another recently described mutation (G to A at position 20210 in the 3'-untranslated region of the prothrombin gene) has been associated with a threefold increased risk of venous thrombosis in heterozygous carriers of the mutation.<sup>7</sup> The mutation is as frequent as 1% to 2% in the population and also seems to represent an important genetic risk factor of venous thrombosis. Currently, the role of the prothrombin variation for arterial vascular disease is unclear. In a recent study, 5.1% heterozygous carriers of the mutation among young women with myocardial infarction were found, compared with 1.6% heterozygous carriers among a control population.<sup>8</sup> The age-adjusted odds-ratio for MI was 4.0 and was particularly high in the presence of other vascular risk factors, eg, smoking. In another recent study, the G20210A prothrombin mutation was found in 5.1% of 98 patients with coronary heart disease as compared with 1.96% among healthy newborns.<sup>9</sup> On the other hand,

three studies did not find a significantly increased prevalence of the mutation in patients with cerebrovascular disease.<sup>10-12</sup> Only in one of these studies were cerebrovascular risk factors detailed,<sup>10</sup> and only one study also analyzed the presence of the factor V Leiden mutation in age- and sex-matched control subjects.<sup>12</sup> Furthermore, patients were highly selected in one of these studies,<sup>11</sup> with 30 of 125 patients suffering from arterial dissection as the cause of cerebral infarction. Another study found no increased prevalence of the prothrombin G20210A mutation in patients with cerebrovascular disease<sup>13</sup>; however, a synergistic role of the prothrombin mutation and the factor V Leiden mutation in the development of thrombosis was observed. To date, no study has evaluated the effect of the factor V Leiden mutation and the G20210A prothrombin mutation in patients with cerebrovascular disease and age- and sex-matched control subjects under particular consideration of other vascular risk factors in patients with and without the prothrombin G20210A mutation.

We investigated the prevalence of the factor V Leiden mutation and the G20210A prothrombin mutation in 96 patients with transient ischemic attacks (TIA) or minor strokes (MS; 58 men and 38 women; age [x ± s], 64.4 ± 13.1 years; range, 28 to 91 years) and in 96 age- (±10 years) and sex-matched control subjects free of clinically manifest vascular disease. Furthermore, we compared history, clinical data, and the prevalence of risk factors for stroke between patients with