Correspondence

Bovine Thrombin and Systemic Autoimmunity

To the Editor-in-Chief:

In a recent issue of *The American Journal of Pathology*, Schoenecker and colleagues¹ reported that topical application of bovine thrombin leads to the development of an illness resembling systemic lupus erythematosus (SLE) in mice. This observation is surprising. Bovine thrombin has been used in the clinic for hemostasis for over 40 years and there are no reports of the development of SLE in patients who have received this therapy (see results using the Medline search words "thrombin" and "systemic lupus erythematosus").

However, the central contention of this study, that bovine thrombin may induce SLE-like syndrome, has not really been addressed in the work. Very high doses of a single, relatively impure preparation of thrombin were used to induce the autoimmune syndrome. Another less impure preparation of thrombin, Thrombin-JMI, was not tested with respect to its capacity to induce autoimmunity in this study. Moreover, since very high doses of Thrombogen were used, it is unclear whether the observations made are relevant to the clinical use of this product.

The central hypothesis of the work of Schoenecker et al,¹ that bovine thrombin is responsible for the development of autoimmunity, is difficult to understand given the antigenic diversity of the antibodies that developed, including the development of antibodies to unrelated murine clotting factors, which were reported in this study. These findings suggest an alternative hypothesis. Thrombin is a proteolytic enzyme, which in high concentration may act on other substrates including activation of a group of proteolytically activated receptors (PARs) belonging to the large family of G protein-coupled receptors involved in the process of inflammation.^(cf²) In addition, a variety of other potentially inflammatory proteins and peptides may be generated during the clotting process (including fibrin-derived peptides³ and kinins). The hypothesis that thrombin's proteolytic activity, regardless of the source, may be responsible for the development of autoimmunity, suggests that exposure of mice to large quantities of murine thrombin might have resulted in a similar pattern of autoimmunity. Thus, a more appropriate test of the hypothesis that administration of bovine thrombin induces autoimmunity should have included studies of the effects of murine and/or recombinant human thrombin.

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Note: Dr. Cronstein has acted as a consultant to King Pharmaceuticals, which manufactures one of the bovine thrombin preparations. He has also acted as a consultant to or has been supported as a speaker by Merck, Aventis, Amgen, Bristol-Myers-Squibb, and Pfizer.

References

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Authors' Reply:

Thank you for the opportunity to respond to Dr. Cronstein's letter regarding our manuscript. When we first submitted our paper,¹ we set forth to study two mechanisms that may lead to the autoimmunity observed in mice exposed to bovine thrombin. The first mechanism was based on "molecular mimicry." Under this model, exposure to similar, but not homologous, antigens such as coagulation proteins from another species could lead to the development of autoimmunity by breaking tolerance. The second mechanism that we began to explore was that the proteolytic activity of coagulation enzymes could activate protease-activated receptors (PAR) on immune cells and lead to a generalized inflammatory and immune response.

At the time of publication, molecular mimicry was an accepted mechanism capable of breaking self-tolerance, leading to autoimmunity. However, the role of PAR in the adaptive immune response had not been demonstrated. Therefore, we deemed it premature to suggest that PAR may play a role in our observations.

However, through the use of PAR deficient mice, we have recently identified a possible mechanism by which

coagulation protease(s) may initiate an adaptive immune response by activating dendritic cells.²

Dendritic cells represent a heterogeneous population of potent antigen-presenting cells that govern the effector cell response of the immune system.^{3,4} Although it has been suggested that “danger” signals released from damaged or necrotic cells induce dendritic cell maturation, these signals and their receptors have not been well characterized.⁵ Serine proteases are not expressed, or are rapidly inhibited, in the extracellular space of healthy tissue. However, during many disease processes they are released by necrotic cells, secreted by cells of the innate immune system, and are activated from zymogens.⁶ In this manner, serine proteases are ideal “danger” signals. We found that developing dendritic cells express PAR-2 and that activation of this receptor induces dendritic cell maturation. The ability of proteases to influence dendritic cell development represents a novel pathway of affecting immune responses. Thus, we concur with Dr. Cronstein’s comments and thank him for his insight into our work.

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