To the editor:

A growing set of platelet-activating bacterial proteins

We read with interest the paper by Lourbakos et al1 that reports activation of platelets by the Porphyromonas gingivalis proteases HRgpA and RgpB, mediated through the thrombin-responsive protease-activated receptors 1 and 4 (PAR1 and PAR4). While this represents an interesting mechanism of platelet activation by pathogenic bacteria, we feel the authors overlooked a precedent for the paradigm of cellular activation through PARs by a bacterial protein, that of streptokinase-induced, PAR1-mediated platelet activation previously reported in Blood.2

Unlike the P gingivalis–derived gingipains investigated by Lourbakos et al, streptokinase (Sk) has no protease activity itself but binds to plasminogen or plasmin, conferring new functions on the host protein including the ability to activate plasminogen3 and to functionally cleave PAR1 resulting in cell activation.2

Lourbakos et al show that HRgpA is a more potent agonist at PAR4 than thrombin but less potent at PAR1. Thrombin is targeted both to bind to PAR1 and to cleave it at the appropriate site by a hirudinlike region adjacent to the cleavage site.4 However, other proteases reported to cleave PAR1 do so at multiple sites resulting in a combination of receptor-activating and -inactivating scissions.5 HRgpA activates human factors IX and X, as well as prothrombin and protein C, suggesting a thrombinlike substrate specificity.6 In all cases, this activity is dependent on the non–covalently bound adhesin subunit of HrgpA that may modify the relatively open binding site of the gingipains.1

These data mirror our results with Sk. Sk alters the properties of complexed plasmin, changing it from a protease that predominantly inactivates PAR1 by digestion at other sites5,7 into a protease that activates the receptor by cleavage at the appropriate site.2 Lourbakos et al speculate that the adhesin component of HRgpA may also promote binding of that protease to the platelet surface, facilitating PAR cleavage. Sk-induced platelet activation also required an adhesive factor, provided by endogenous anti-Sk antibodies (in plasma) binding to the platelet Fc receptor.2

A similar binding pattern is seen in the presumed teleological role of Sk: harnessing plasmin to allow bacterial invasion across tissue barriers of new body compartments.8 Streptococci express binding sites on their surface for plasminogen and fibrinogen, which form a ternary complex with Sk, generating plasmin that remains cell-surface bound and escapes inhibition by α2-antiplasmin.8 Mammalian cells also localize plasmin to their surface through the coexpression of the urokinase plasminogen activator receptor (uPAR) and binding sites for plasminogen, leading, for instance, to plasmin generation at the leading edge of fibroblasts during wound healing.9 Indeed, on the mouse platelet, thrombin is targeted to PAR4 by the hirudinlike region of PAR3.10 Thus many proteases are regulated through colocalization with their activators or cofactors at cell surfaces where they evade soluble inhibitors. This may contribute to thrombosis when platelets’ cell-surface protease-activated receptors are cleaved by gingipains1 or Sk-plasmin.2

James P. McRedmond and Desmond J. Fitzgerald
Correspondence: Desmond J. Fitzgerald, Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, 123 St Stephen’s Green, Dublin 2, Ireland; e-mail: dfitzgerald@rcsi.ie

References


Response:

Platelet-activating bacterial proteins: continuing the emergence of a paradigm

We have read the letter by McRedmond and Fitzgerald with great interest. We were aware of their work on streptokinase-plasminogen–induced activation of platelets,1 but at the time of writing our article on gingipain activation of platelets through the PARs, the relationship of their work to bacterial pathogenesis was not described because their article was set in the context of the side effects experienced when streptokinase is used in the therapeutic context of treating myocardial infarction. The streptokinase-plasminogen-antistreptokinase antibody complex is also obviously a far more complex interaction than that required for gingipain activation of mammalian cells. It should also be noted here that, as far as we are aware, the first precedent for bacterial proteins activating mammalian cells through the PARs was in fact our work reported in 1998,2 in which we first showed that RgpB (which does
not contain associated adhesin domains in contrast to HrgpA was able to activate human neutrophils through the PAR-2 receptor. Thrombin does not activate PAR-2, and thus, combining this with other data on the specificity of the gingipains, we cannot entirely agree with the contention of McRedmond and Fitzgerald that the gingipains appear to have a thrombinlike specificity.

McRedmond and Fitzgerald now make the case for similarities between the action of gingipains on the PARs and that seen with the overall streptokinase-plasminogen-antistreptokinase complex. In particular, the authors focus upon the similar function of the associated adhesins of HRgpA in aiding the cleavage of the PARs, much as the streptokinase-plasminogen complex is guided to the platelet surface by the binding of the antistreptokinase antibodies to the platelet Fc receptor, as described in their work. Recent work in our laboratory has shown that HRgpA and RgpB in fact have some differences in their specificity for peptide substrates that may relate to small differences in their active site architecture induced by a limited number of point mutations (N. Ally et al, manuscript in preparation). We cannot rule out that the adhesins of HrgpA play some role in enhancing functions such as cleavage of coagulation factors and PARs, but it would certainly appear that we can no longer be sure that it is only these adhesins that are modifying the action of the larger protein. Certainly, it must be noted that RgpB can activate the PARs directly and therefore the adhesin domains are not as vital in this process as in the streptokinase scenario.

The letter by McRedmond and Fitzgerald now amplifies upon the role of streptokinase-induced cellular activation in bacterial pathogenesis. The scenario posed by them is certainly a fascinating one and, together with our recently published work on the activation of PARs on epithelial cells by the gingipains and the resulting induction of interleukin-6 secretion, the data obtained with streptokinase certainly aids in establishing our proposal that PAR-mediated activation of mammalian cells by bacterial proteins is an intriguing new mechanism to be investigated in bacterial pathogenesis. The importance of PAR-mediated platelet activation by bacterial proteins also needs to be established further, which will provide further intriguing glimpses into the possibility that cardiovascular diseases might, in some cases, have an underlying chronic bacterial disease as the cause.

Robert N. Pike, Afroditte Lourbakos, James Travis, and Jan Potempa

Correspondence: Robert N. Pike, Department of Biochemistry and Molecular Biology, Monash University, PO Box 13D, Clayton, Victoria 3800, Australia; e-mail: rob.pike@med.monash.edu.au

References


To the editor:

Terminal plasmocytoid differentiation of malignant B cells induced by autotumor-reactive CD4+ T cells in one case of splenic marginal zone B-cell lymphoma

In lymphoid organs invaded by malignant B-cell lymphomas, the development of reactive CD4+ tumor-infiltrating T cells (TIL-T) at the contact of tumor cells is now firmly established.1,2 It has been reported that lymphoma B cells are capable of proliferating in response to various recombinant signals usually provided by CD4+ T cells, such as interleukin-4 (IL-4), or CD40 ligand.3 However, the exact nature of the functional relationships between CD4+ TIL-T and autologous non-Hodgkin lymphoma (B-NHL) cells remains largely unknown mainly because this question has not yet been extensively investigated in autologous CD4+ T/malignant B-cell coculture systems in vitro. We report here evidence that CD4+ TIL-T have the potential to drive autologous lymphoma B cells toward a terminal differentiated state, in one case of splenic low-grade, marginal zone B-cell lymphoma.

In May 1998, a 67-year-old man presented with a low-grade lymphoma (nonfollicular small-cell lymphoma) with involvement of spleen, blood, periaortic lymph nodes, liver, and bone marrow. The patient underwent splenectomy in July 1998. Histologic, cytologic, and immunophenotypic features were compatible with splenic marginal-zone B-cell lymphoma.4 All malignant B cells were surface IgM+, kappa+, CD19+, CD24+, CD40+, and were negative for IgD, CD23, and CD5. Less than 1% of malignant cells were CD38+ or CD138+. The percentage of CD3+ TIL-T was 9% with 5% CD4+ and 4% CD8+.

A population containing both CD4+ T cells and malignant B cells was negatively selected from total spleen cells by depleting CD8+ T cells, residual NK cells, monocytes, and normal B cells by one round of immunomagnetic bead depletion. Purity was assessed by flow cytometry and CD4+ T/malignant B-cell preparations usually contained 8% to 10% CD4+ T cells and 88% to 90% CD19+ kappa+ B cells. Residual cells not stained by CD3, CD4, CD19, or kappa antibodies were always less than 1%. Cocultures were then performed in the presence of recombinant IL-2 (rIL-2) at 10 UI/mL. Control cultures consisted of purified malignant B cells cultured with rIL-2 but without the presence of CD4+ T cells.

A representative experiment of CD4+ T/malignant B-cell cocultures is given in Figure 1. After 7, 14, and 21 days of coculture, cells were harvested, triple stained with anti-CD4, CD19, or kappa antibodies were always less than 1%. Cocultures revealed the spontaneous formation of conjugates between T cells and malignant B cells. Residual conjugates were negative for CD4, CD19, or kappa antibodies were always less than 1%. In the course of the coculture, T cells and residual NK cells, monocytes, and normal B cells by one round of immunomagnetic bead depletion. Purity was assessed by flow cytometry and CD4+ T/malignant B-cell preparations usually contained 8% to 10% CD4+ T cells and 88% to 90% CD19+ kappa+ B cells. Residual cells not stained by CD3, CD4, CD19, or kappa antibodies were always less than 1%. Cocultures were then performed in the presence of recombinant IL-2 (rIL-2) at 10 UI/mL. Control cultures consisted of purified malignant B cells cultured with rIL-2 but without the presence of CD4+ T cells. A representative experiment of CD4+ T/malignant B-cell cocultures is given in Figure 1. After 7, 14, and 21 days of coculture, cells were harvested, triple stained with anti-CD4–PE-Cy5, kappa–PE, and CD138–FITC antibodies (Abs) and analyzed by flow cytometry. CD138+ (Syndecan-1) is a transmembrane heparan sulfate proteoglycan expressed in Ig-producing, normal and malignant mature plasma cells.5 At analysis, according to a multicolor gating/painting strategy, CD138+ cells were gated and colored in orange, and CD138− cells were colored in gray. At the initiation of the coculture (day 0), the spontaneous formation of conjugates between T cells and malignant B cells was negligible. At the contact of malignant B cells, autotumor-reactive CD4+ T cells became activated, expanded, and formed stable conjugates with malignant B cells. Between day 7 and day 21 of the coculture, the percentage of T/B-cell conjugates gradually increased (5% at

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James P. McRedmond and Desmond J. Fitzgerald