inside blood

Clotting in whole blood: analysis of a biochemical reaction network

The coagulation cascade is a complex biochemical network replete with regulatory reactions and cellular contributions. The behavior of the system cannot necessarily be predicted intuitively from the existing state-of-the-art biochemical knowledge of the individual reactions. Knowledge of the reaction kinetics of the components in this complex system has major implications for an understanding of the regulation of coagulation and its effective control by therapeutic agents.

Brummel and colleagues (page 148) describe an analysis of thrombin formation and function following the addition of tissue factor to initiate coagulation in whole blood in which the contact pathway is inhibited. They present results observed in samples from 20 healthy individuals describing the time course of thrombin formation as well as its procoagulant activation products: activated platelets, fibrinopeptides A and B, factor Va, and factor XIIIa. Each of these products accumulate in an initial slow phase (initiation) followed by an explosive phase of product formation (propagation). They observe clot formation to coincide with the onset of the propagation phase for thrombin–antithrombin III formation. But the transition points for the other thrombin activation products occur much earlier at very low concentrations of thrombin. Crucial activation of the different procoagulant thrombin substrates occurs in a graded fashion before the clot is visible and is catalyzed by 0.2% or less of the possible thrombin that can be formed. The bulk of thrombin formation (96%) occurs well after overt clot formation.

Their quantitative analyses raise interesting questions regarding the significance of the high concentrations of thrombin available after the clot has already formed. Experimental and computational approaches pioneered by the Mann laboratory that also incorporate the protein C pathway might shed light on this interesting finding. From a clinical standpoint, their findings also highlight the complexities of using direct thrombin inhibitors as antithrombotic agents. Such therapeutics would need to effectively inhibit the low levels of thrombin activity necessary for the initiation phase even when the maximum concentrations of thrombin can vastly exceed those necessary for the activation of its procoagulant substrates.

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Radiosensitization of recipient stem cells promotes engraftment

For a long time it has been accepted that engraftment of stem cells requires formation of "space" in the recipient marrow. In the past few years, this concept has been challenged by observations showing that significant donor chimerism can be obtained in mice without induction of hypoplasia. Even in nonmyeloablated or unprepared hosts, donor engraftment can be achieved provided that large numbers of stem cells are transplanted (Stewart et al, Blood. 1993;81:2283-2289). This has resulted in the dogma of stem cell competition as a major factor determining stem cell engraftment. This concept has been applied in allogeneic nonmyeloablative transplantations in humans, wherein high numbers of stem cells are transplanted following a conditioning regimen consisting of short-lasting intensive immune suppression without myeloablation.

Noach and colleagues (page 312) elegantly demonstrate that growth factor treatment of recipient mice prior to receiving low-dose irradiation and syngeneic stem cell transplantation results in increased long-term donor engraftment levels. They present evidence that this is related to depletion of remaining recipient stem cells. In their study they applied 3 different schedules of growth factor treatment that were administered for 1 or 7 days. One-day growth factor treatment resulted in reduced radiosensitivity of recipient stem cells, whereas treatment for 7 days resulted in enhanced radiosensitivity and a significant decrease in the number of recipient stem cells. After treatment with growth factors for one week, a marked enhancement of engraftment was observed. In recipients pretreated for one day with combinations of growth factors, initial engraftment was decreased in comparison with controls. These data suggest that short-term growth factor treatment mediates radioprotection, whereas longer treatment results in radiosensitization. These observations are reminiscent of earlier studies with other cytokines (ie, interleukin-1) that mediate protection from lethal radiation (Neta et al, J Immunol. 1986;136:2483-2485). Although most of the data are compatible with the hypothesis that engraftment levels are determined by stem cell competition, some of the data are unexplained by this hypothesis. In animals receiving a short-term treatment with SCF/IL-11 or SCF/FL, an increase in primitive stem cells (CAFCs, day 35) was observed after TBI in comparison with controls not receiving growth factor treatment. The hypothesis predicts decreased levels of engraftment in these recipients, but instead increased levels were observed.

The mechanisms underlying the observed stem cell depletion and enhancement of engraftment remain unclear from the present study. It has been suggested that sensitivity to irradiation is dependent on the cell-cycle status of stem cells, but this has not been addressed. A direct effect of growth factor treatment on engraftment is also not excluded. Furthermore, engraftment levels changed in time, possibly as a result of secondary effects of irradiation on the recipient marrow stroma.

An important question for the clinical application of this approach is whether a similar effect occurs in an allogeneic setting. In nonmyeloablative transplantations, where high numbers of donor stem cells compete with residual host stem cells, depletion of recipient stem cells could help to promote donor chimerism. However, this first requires additional studies addressing the effect of growth factor treatment on T cells and NK cells that are involved in graft rejection.

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