answered before we can proceed with this concept. (1) Will the age cutoff be validated by other investigators, and if so, would it apply to the newly diagnosed patients? (2) The use of thalidomide as a single agent or in combination with steroids in smoldering and active newly diagnosed myeloma patients has not shown an improved result when compared with results for the drug in relapsed refractory patients (Weber et al, J Clin Oncol. 2003;21:16-19; Rajkumar et al, J Clin Oncol. 2002;20:4319-4323), and long-term effects are yet to be determined; is it worth foregoing an active agent early in the course of the disease and continuing to use standard chemotherapy that has not shown a significant impact on the overall survival since the introduction of melphalan and prednisone (Attal et al, N Engl J Med. 1996;335:91)? (3) Considering its immunomodulatory effects that involve integrin modulation and the resultant unfastening of the myeloma cell from the stroma, could this increase the incidence of extramedullary relapses, especially when used in the absence of an active chemotherapeutic agent? These issues will be addressed with further study and longer follow-up of the current trials. The introduction of new, less toxic interferons may allow us to revisit the question of synergism of thalidomide and other immunomodulatory agents (Borden et al, Semin Cancer Biol. 2000;10:125-144).

—Mohamad A. Hussein
Cleveland Clinic Myeloma Research Program

Platelets are anticoagulants?

Effective control of blood coagulation requires that hemostasis proceed at the wound site without progressing to thrombosis. To accomplish this, it is presumably necessary for the anticoagulant mechanisms to respond more vigorously as the procoagulant stimulus increases. The protein C anticoagulant pathway provides one such response since, from infusion studies, the generation of activated protein C (APC) appears to be roughly proportional to the thrombin concentration infused (Hanson et al, J Clin Invest. 1993;92:2003-2012), and the APC can then prevent clot extension. Protein C activation occurs when thrombin binds to thrombomodulin on the surface of the endothelium and then rapidly converts protein C bound to the endothelial cell protein C receptor to APC.

In this issue, Slungaard and colleagues (page 146) have identified a novel mechanism for further stimulation of protein C activation near platelet-rich thrombi. Through studies on both endothelial cells in culture and thrombin infusion experiments in monkeys, they demonstrate that platelet factor 4 (PF4) stimulates protein C activation substantially. In vivo, the coinfusion of PF4 and thrombin increased APC levels about 2-fold, compared with thrombin plus vehicle. Thus, the net effect of the PF4 is likely antithrombotic. This is quite surprising. PF4 is known to have many effects, but one dominant hypothesis was that it played a role in preventing heparin stimulation of antithrombin inhibition of thrombin. This would normally be considered procoagulant. But the inhibition of thrombin neutralization would lead to increased deposition of thrombin on thrombomodulin, thus increasing protein C activation. While this likely contributes to some of the increase in APC formation, another, possibly more important, mechanism involves direct stimulation of protein C activation by the thrombin-thrombomodulin complex.

The implications of the stimulated APC formation are many. APC has anti-inflammatory activity, reducing a variety of cytokines and adhesion molecules induced on cells primarily through NFκB-induced pathways. Stimulated APC formation in arterial beds could help decrease inflammation in atherosclerotic areas or following angioplasty. Consistent with a dominant role of the pathway in arterial injury, experiments from Woo’s group (Waugh et al, Circulation. 2000;102:332-337) demonstrated that increasing thrombomodulin expression in injured arteries reduced thrombosis, leukocyte infiltration, and vessel thickening. Some of these activities may be due to direct anti-inflammatory activities of thrombomodulin itself (Conway et al, J Exp Med. 2002;196:565-577), but others are probably enhanced by the increased protein C activation, some of which would be further enhanced by PF4.

Another interesting finding from this paper was that the ability to enhance protein C activation varied markedly among cultured endothelium derived from different sources. Some of these differences are probably due to differences in attaching the chondroitin sulfate to thrombomodulin since, in purified systems, the chondroitin sulfate augments (but is not required for) the stimulation of protein C activation by PF4. Of major interest is the observation that PF4 has no effect on protein C activation by blood outgrowth endothelial cells. This suggests that there is yet another factor that participates in protein C activation and that it is missing from these endothelial cells. Since small changes in protein C activation can have a major effect on the coagulant and inflammatory responses, identification of this putative new member of the protein C activation complex could provide new insights into the regulation of both coagulation and inflammation.

—Charles T. Esmon
Oklahoma Medical Research Foundation

PECAM-1/CD31 provides survival signals to suppress apoptosis

In this issue, Gao and colleagues (page 169) report their interesting findings that the absence of platelet endothelial cell adhesion molecule 1 (PECAM-1) leads to enhanced susceptibility of murine endothelial cells and human T lymphocytes to apoptosis following x-ray irradiation or staurosporine treatment. Specifically, the cytoprotective effects of PECAM-1 were shown to suppress the intrinsic mitochondrial-dependent Bax-induced cytochrome c release, caspase activation, and nuclear fragmentation. The ability of PECAM-1 to deliver survival signals to suppress Bax-induced apoptosis required the outside-in signaling properties.
of PECAM-1, including homophilic contact binding, intact immunoreceptor tyrosine-based inhibitory motifs (ITIMs), and, in part, recruitment and activation of the protein-tyrosine phosphatase, SHP-2. The authors propose that the formation of the PECAM-1/SHP-2 signaling complex may affect signal transduction pathways that modulate either the location and/or activation state of pre-existing pro- and antiapoptotic components of the cell-death pathway.

While the concept that homophilic engagement of PECAM-1 can result in the transduction of survival signals in vascular endothelial cells and in macrophage phagocytosis has been previously demonstrated (Noble et al, J Immunol. 1999;162:1376-1383; Brown et al, Nature. 2002;418:200-203), these workers attempt to define the complex molecular mechanisms by which PECAM-1 may exert cytoprotective effects in suppression of apoptosis. They show that PECAM-1 can negatively regulate intrinsic mitochondrial-dependent Bax-mediated apoptosis by preventing a post–Bax-translocation event, but not extrinsic Fas-mediated apoptosis. This cytoprotective effect of the PECAM-1/SHP-2 signaling pathway does not appear to involve phosphatidylinositol-3 (PI-3) kinase–serine/threonine kinase (Akt), or integrin activation. Further studies will be required to unravel the importance of PECAM-1 ITIM (inhibitory) and ITSM (switch)–signaling properties, the spatial-temporal organization of signaling complexes, and subcellular compartmentalization that lead to modulation of pre-existing signaling circuits, including the cell-death machinery.

—Denise E. Jackson
Austin Research Institute

\gamma\delta T cells in cancer immunotherapy

The largest subset of human \gamma\delta T cells is the V\gamma2 (alternate V\gamma9) V\delta2 subset, comprising 2% to 5% of peripheral blood T cells. These V\gamma2V\delta2 T cells uniformly recognize nonpeptide alkylamine, nitrogen-containing bisphosphonate, and organophosphate antigens in a T cell receptor (TCR)–dependent fashion. Such recognition enhances \gamma\delta T cell–mediated cytotoxicity, and secretion of IFN-\gamma and TNF-\alpha, which are important antitumor effector mechanisms (Morita et al, Springer Sem Immunopath. 2000;22:191-217).

Mouse models provide strong evidence for \gamma\delta T cell–mediated resistance to tumors and infection. In humans, certain lymphoma and myeloma cells display cell-surface antigens that are recognized in a V\gamma2V\delta2 TCR-dependent manner. Others display nonclassical major histocompatibility complex (MHC) class I–related proteins such as MHC class I–related chain A (MICA) and UL16 binding proteins (ULBPs) that can be recognized by NKG2D receptors on activated \gamma\delta T cells. Cells from common cancers metastatic to bone, such as those from breast cancer and prostate cancer, can be exposed to large concentrations of bone-avid nitrogen-containing bisphosphonates, such as pamidronate and risedronate. Such exposure may kill these cells directly, in several days, but \gamma\delta T cells can kill these sensitized cells in a matter of minutes. Thus, bisphosphonates can at once activate \gamma\delta T cells and sensitize tumor cells for elimination by \gamma\delta T cells (Das et al, Blood. 2001;98:1616-1618). Treatment of multiple myeloma with pamidronate has increased survival and decreased the incidence of metastatic lesions and pathologic fractures (Berenson et al, J Clin Oncol. 1998;16:593-602).

In this issue, Wilhelm and colleagues (page 200) report successful treatment of refractory lymphoma and myeloma with pamidronate and interleukin 2 (IL-2). Importantly, objective clinical responses correlated with proliferation of \gamma\delta T cells in vivo, strongly suggesting a role for \gamma\delta T cells in mediating the response. As more potent \gamma\delta T-cell antigens are coupled with treatments earlier in disease, we can soon expect even better results. These data represent an important initial step in manipulating \gamma\delta T cells in vivo to treat tumors and, perhaps, to treat or prevent infections that accompany them.

—Jack F. Bukowski
Brigham and Women’s Hospital

Resistance to imatinib: more and more mutations

In the short time since the introduction of imatinib in 2001, investigators have already defined the primary mechanisms of drug resistance: mutations in the \(BCR-ABL\) gene that affect drug binding or an overall increase in the level of \(BCR-ABL\) protein due to gene amplification. In this issue, Branford and colleagues (page 276) report the first comprehensive mutation analysis of an unselected population of chronic myeloid leukemia (CML) patients treated with imatinib. The key finding is a very tight correlation between detection of a mutation and relapse, dispelling any remaining doubt about the causal role that mutations play in imatinib resistance.

A few additional points are worth noting. First, the authors find that mutations in the adenosine triphosphate (ATP)–binding loop confer a worse prognosis than other mutations, raising the possibility that early detection would mandate a change in treatment. Second, new clinical mutations are described here that were also picked up in a cleverly designed in vitro screen for imatinib resistance (Azam et al, Cell. 2003;112:831-843). Finally, the probability for finding a mutation increases with disease duration.

This last point is particularly important because it provides the first epidemiologic support for a clonal expansion model of imatinib resistance arising from pre-existing mutant subclones (Shah et al, Cancer Cell. 2002;2:117-125). The theory goes as follows. The CML clone makes sequence errors during DNA replication, some of which affect \(BCR-ABL\). Over time, increasing clonal diversity raises the likelihood of generating imatinib-resistant subclones that expand in the setting of imatinib treatment. This model is based on data from patients who were treated with imatinib several years after their initial CML diagnosis. Will early imatinib treatment of newly diagnosed CML patients prevent this