no proof that low VEGF levels contribute to neurodegeneration. It is possible that low levels of VEGF are neuroprotective, while high levels cause neuronal sheath edema and ischemic polyneuropathy.[3] VEGF's effects on the endothelial cells are more complicated. VEGF increases vascular permeability and promotes angiogenesis by stimulating endothelial-cell growth.[4] Extensive apoptosis of endothelial cells as suggested by Straume et al could have catastrophic consequences such as bleeding, a known side effect of bevacizumab. Although the data are limited, we hypothesize that endothelial damage stimulates platelet aggregation and release of VEGF that is stored at high levels in the platelets, thus maintaining adequate VEGF levels locally to preserve the newly formed blood vessels. It is worth noting that inhibition of platelet function has resulted in decreased VEGF levels and clinical improvement in POEMS.[5]

Understating the cause and source of VEGF overproduction in POEMS will help define the role of bevacizumab, if any, in the treatment of this disorder. In the absence of clinical trials, bevacizumab should be carefully considered in selected POEMS patients as adjuvant to conventional chemotherapy, and when used, patients must be monitored carefully for potential toxicities.

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To the editor:

Does the serpin PI-9 protect tumor cells?

The report of Godal et al[1] raises an important issue on the effectiveness of apoptosis inhibitors in preventing death induced by cytotoxic lymphocytes (CLs) in clinically relevant settings. A large variety of tumors express apoptosis inhibitors such as Bcl-2 and PI-9, which protect cells from granzyme B (GrB)–induced apoptosis in in vitro settings,[2–4] and were thus proposed to induce immune escape of tumors. Based on in vitro assays Godal et al[1] now claim that expression of these apoptotic regulators is irrelevant for CL sensitivity of lymphomas and therefore unlikely to affect immunotherapy of lymphomas. However, a major limitation of this conclusion is that it is based on in vitro analysis, and current data suggest that this doesn't necessarily represent the in vivo situation.

CL degranulation induces apoptosis mainly via GrB, while other granzymes bring about different modes of cell death. As PI-9 inhibits GrB, it effectively prevents apoptosis, but not these other deaths. In agreement, Godal et al show that in vitro cytotoxicity of lymphomas does not depend on PI-9 or Bcl-2 expression, which then warrants the conclusion that these do not protect lymphomas. Although relevant, this conclusion is not supported by data from other groups. For instance, Classen et al[5] concluded that PI-9 expression in pediatric acute lymphoblastic leukemias is correlated with protection in vitro. Similarly, PI-9 expression in MCF-7 cells prevents death induced by long-term activated natural killer (NK) cells (expressing little GrB), while it is ineffective against shortly activated NK cells (expressing high levels of GrB).[2] These findings suggest that PI-9 efficacy depend on the conditions used. Although this may appear trivial, Godal et al[1] assume that cytotoxicity measured in their in vitro assays is identical to the in vivo situation. This strongly contrasts with current knowledge, as it is clear that CLs isolated from blood contain less granzymes than most CL lines. For instance, granzyme M, which induces an alternative death pathway, is not detected in activated cytotoxic T lymphocytes (CTLs), while it is present in CTL lines.[6,7] Similarly, NK cells have little GrB, while lymphokine-activated killer (LAK) cells contain enormous amounts.[6] It is therefore likely that the level of CL activation, and thus the diversity of granzymes expressed, determines the efficacy of inhibition by PI-9. In agreement, we have shown that expression of SPI-6 (mouse PI-9) in T lymphomas does not protect these cells against cytotoxicity induced by a CL in vitro, but does convey a level of protection in vivo.[8] Also, the clinical data available support this notion. The group of Oudejans and Kummer provided compelling evidence that PI-9 expression is associated with poor prognosis in anaplastic large B-cell lymphoma.[9] More importantly, they showed that PI-9 expression is an important determinant in disease-free survival time of melanoma patients following immunotherapy.[10]

In conclusion, measuring cytotoxicity using highly activated CL in vitro may over-exaggerate the effectiveness of these cells and thus underestimate the protective capacity of antiapoptotic molecules. Indeed, the experimental evidence pictures PI-9 as a crucial determinant in the outcome of immunotherapeutic approaches in cancer.

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Response:

Is there any evidence supporting a critical role of ectopic PI-9 expression in tumor immune escape?

The authors appreciate the interest of Bots et al in our recent paper regarding the role of PI-9 in CTL- and NK-cell–mediated lysis of lymphoma cells.[1] The hypothesis that the granzyme B inhibitor PI-9 expressed in certain human malignancies may prevent their clearance by the immune system has been controversial for many years.[2] We think, however, that the argumentation of Bots et al is debatable. The study by Classen et al,[3] which Bots et al cite as not supporting our results, has investigated leukemic blasts from 2 patients only. The notion that "cytotoxic lymphocytes isolated from peripheral blood contain less granzymes than most cytotoxic lymphocyte lines," referring to the study by Sayers et al,[4] is partially true for granzyme M, but not for granzyme B, which is the only granzyme inhibited by PI-9. Sayers et al clearly show that the 2 major granzymes A and B are highly expressed in freshly isolated $CD8^+$ T cells at a level similar to cytotoxic lymphocyte lines. In their own study, Medema et al[5] have actually shown that indeed, transfection of SPI-6 (mouse PI-9) into tumor cells conveyed a level of protection from granzyme B–induced apoptosis, but did not inhibit CTL-induced cytolysis. This finding is in accordance with various more recent studies showing that other granzymes with strong antitumor activity can substitute granzyme B in vitro and in vivo, as reviewed by Lieberman.[6] In the 2 studies mentioned by Bots et al, which linked PI-9 expression in tumor tissues to clinical outcome, there was no evidence of a clinically effective immune response to tumor in which granzyme B resistance might play a role.[7,8]

In our opinion, the most convincing in vivo studies regarding the potential role of granzyme B and perforin in cancer are those in knock-out mice reported by Trapani's group.[9,10] Lymphoma cells grew efficiently in perforin-deficient mice, whereas granzyme A– and/or B–deficient mice rejected large tumor doses as avidly as wild-type mice, indicating that granzyme B was completely dispensable in lymphoma eradication.[9] These studies also clearly show a strong correlation between cytolytic activity of granzyme B knockout CTLs in vitro and in vivo.[10] Taken together, the studies by the groups of Trapani and Lieberman[6,9–10] are in line with our studies in human lymphoma cells as they provide strong evidence that granzyme B is not critical for antitumor effector functions in vitro and in vivo. We therefore believe that the results from the murine knock-out models as well as our human in vitro experiments justify the conclusion considering lymphoma to be sensitive to perforin-dependent pathways despite PI-9 expression. Of course, we fully agree with Bots et al that the final answer regarding the role of PI-9 in immune rejection of human tumors can only be provided in vivo, which would require clinical studies based on immunotherapy with proven clinical efficacy.

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To the editor:

Does modulation of P-glycoprotein have clinical relevance in pediatric acute myeloid leukemia?

Becton et al report the results of a trial in children with acute myeloid leukemia (AML) who were randomized to receive consolidation therapy with or without cyclosporine-A (CsA).[1] The authors measured P-glycoprotein (P-gp) expression in vitro by flow cytometry, using MRK16 antibody staining. P-gp positivity was defined as more than 5% of cells staining MRK16 positive, which