hypothesis that this feature could contribute to explain Treg enrichment in cancer tissues, where increased levels of oxidative stress occur, we also wish to briefly discuss their observations in the light of other potentially relevant findings.

We previously reported that human Tregs contain high levels of the catecholamines (CA) dopamine, norepinephrine, and epinephrine.\(^2\) CA autooxidation occurs spontaneously, leading to formation of oxidative moieties, a process extensively investigated in neurodegeneration.\(^3\) In human lymphocytes, CA are synthesized and stored into the cells upon activation with mitogenic stimuli, and pharmacologic inhibition of their production results in reduced activation-induced apoptosis,\(^4\) in line with their cytotoxic potential. It is therefore not surprising that Tregs, which contain high amounts of CA,\(^5\) are also endowed with high levels of thiols,\(^6\) conferring increased resistance to oxidative stress.

CA, however, also provide lymphocytes with an array of transmitters which can act in autocrine/paracrine fashion on cells bearing dopaminergic and/or adrenergic receptors. Indeed, we showed that in human lymphocytes, and in particular in Tregs, CA may be released upon appropriate treatments, eg with the CA-releasing agent reserpine\(^2\) or with type I interferons (IFNs).\(^3\) In Tregs, released CA (and in particular dopamine) act upon dopaminergic D1-like (possibly D5) receptors and subserve a feed-back loop leading to functional suppression of these cells.\(^2\) CA may play opposite roles in tumor growth: dopamine exerts antitumor effects, possibly through dopaminergic D2-like receptor-dependent inhibition of angiogenesis,\(^6\) whereas norepinephrine and epinephrine, acting through \(\beta\)-adrenoceptors, promote tumor growth and angiogenesis.\(^7\)

Tumor-infiltrating Tregs may thus at the same time represent a source of endogenous CA and a target for exogenous drugs acting on CA receptors. Treatment with dopaminergic agents could result in reduction of both tumor neovascularization and of Treg-dependent local suppression of the immune response. CA release from Tregs themselves, triggered by use of a CA-releasing agent such as reserpine, type I IFNs, or possibly other drugs such as bupropion,\(^8\) could provide an additional local source of dopamine, while inclusion of appropriate \(\beta\)-adrenoceptor antagonists (\(\beta\)-blockers) could block the potentially detrimental effects of norepinephrine and epinephrine released from sympathetic-nervous-nerve endings and adrenals, as well as from tumor-infiltrating Tregs.

In summary, increased resistance of Tregs against oxidative stress\(^1\) is in line with the high content of CA which occurs in these cells.\(^2\) CA, together with their receptors, may indeed represent a convenient target for novel immunomodulating and anticancer therapies, also in view of the wide array of dopaminergic and adrenergic agents in clinical use for different indications (eg, neurology, neuropsychiatry, cardiology) and of their usually good tolerability profile.

**Response**

**Resistance of naturally occurring regulatory T cells toward oxidative stress: possible link with intracellular catecholamine content and implications for cancer therapy**

Dr Cosentino et al in their letter to the editor discuss our previous report demonstrating increased resistance of regulatory T cells (Tregs) toward oxidative stress\(^1\) in the context of their findings on a catecholamine (CA) dependent inhibitory functional loop in Tregs.\(^2\) Their results demonstrate that Tregs contain higher amounts of CA compared with conventional T cells. As CA is a potential source of endogenous oxidative stress, Tregs would require a greater antioxidative capacity, previously demonstrated by us. We appreciate the insightful comments and wish to highlight 2 important associations: first, the emerging role of the neuroimmunologic axis in cancer; and second, the effects of oxidative stress on CA signaling and vice versa.

A large number of studies in diverse tumor models suggest that stress can promote tumor recurrence and metastasis accompanied by impairment of cellular immunity.\(^3\) Based on the established immunosuppressive properties of Tregs, several neurotransmitters such as dopamine, epinephrine, norepinephrine, serotonin and substance P, have been tested in this context for their impact on Tregs. Dopamine was shown to mediate a reduction of suppressive activity, adhesive and migratory capacity in Tregs via the dopaminergic D1-like receptors (D1 and D3 receptors).\(^2,4\) Using serum-derived cortisol and metanephrine as the stress surrogates, a recent study on lymphocyte subpopulations in patients after stroke,
found increased CRP-induced integrin preparation, we have previously reported that platelets, we have repeated our experiments using the platelet also occurred in indomethacin-treated platelets. To address whether the differences between our studies reflect differences in platelet activation, we have repeated our experiments using the platelet preparation method described by Nagy et al, and treated the platelets with indomethacin. However, under these conditions we still find enhanced CRP-induced dense granule secretion in PKCζ platelets (Figure 1A).

Nagy et al4 supported their data from mouse platelets with experiments using a peptide (V1-1; CGLSNFD) predicted to inhibit PKCζ’s interaction with its RACK adaptor protein, coupled to a TAT peptide (CYGRKKRRQRRR) to allow its entry into cells. To the best of our knowledge, this is the first published report of V01-1-TAT. Mochly-Rosen and colleagues (Stanford University) have kindly provided us with the same peptide. We found that CRP-induced ATP secretion was enhanced by V01-1-TAT pretreatment (1 μM) in wild-type (WT) platelets (Figure 1B), similar to the

To the editor:

PKCζ in platelet activation

Protein kinase C (PKC) is a central regulator of platelet activation, and individual PKC isoforms are likely to have distinct roles.1 We and others had previously reported roles for the novel PKC isoform, PKCζ, in integrin signaling2 and platelet function.3 The recent paper by Nagy et al,4 which attempts to characterize further the importance of PKCζ, is valuable in this regard. However, their data conflict with other published data in several respects, and we are unable to repeat some of the findings of Nagy et al despite preparing platelets in the manner they describe.

While Nagy et al4 report that granule secretion in response to the glycoprotein VI collagen-related peptide (GPVI) agonist CRP is reduced in PKCζ−/− platelets, we have previously reported that CRP-induced secretion is enhanced in these cells.5 Similarly, we found increased CRP-induced integrin αIIbβ3 activation and thrombus formation under flow in vitro,5 whereas Nagy et al reported a decrease in integrin activation. Nagy et al6 indicate that their effects are independent of their proposed role for PKCζ in thromboxane synthesis, as they report that decreased aggregation and secretion also occurred in indomethacin-treated platelets. To address whether

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