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,[2] are also endowed with high levels of thiols,[1] 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).[5] 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 β-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 a CA-releasing agent such as reserpine, type I IFNs, or possibly other drugs such as buproprione,[8] could provide an additional local source of dopamine, while inclusion of appropriate β-adrenoceptor antagonists (β-blockers) could block the potentially detrimental effects of norepinephrine and epinephrine released from sympathoadrenergic 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 (in, eg, neurology, neuropsychiatry, cardiology) and of their usually good tolerability profile.

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Conflict-of-interest disclosure: The authors declare no competing financial interests.

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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 D_1-like receptors (D_1 and D_5 receptors).[2,4] Using serum-derived cortisol and metanephrine as the stress surrogates, a recent study on lymphocyte subpopulations in patients after stroke,

demonstrated that Tregs were less affected upon hormonal stimuli compared with other T-cell subpopulations.[5] These observations underlie the complex balance of the effects exerted by CA in the organism. We are therefore in consensus with Cosentino and colleagues that dopaminergic agents as well as β-adrenoreceptor antagonists should be used in targeted therapy approaches.

In their recent publication Cosentino et al demonstrate that despite the expression of D_1-like receptors on conventional T cells, only Tregs are capable of an effective receptor–second messenger coupling resulting in increased intracellular cAMP.[2] One potential factor contributing to this phenomenon could be the increased basal levels of intracellular oxidative stress seen in conventional T cells compared with Tregs.[1] Hydrogen peroxide and subsequent lipid peroxidation leads to an uncoupling of the D_1-like receptor/G-protein unit with a loss of signal transduction.[6] Treatment with antioxidants restored the coupling, indicating the importance of the redox state. It would be of great relevance to evaluate the effects on D_1-like receptor signaling in conventional T cells treated with antioxidants. Furthermore, low doses of dopamine acting on D_1-like receptors is known to decrease the levels of oxidative stress through phospholipase-D (PLD) signaling,[7] suggesting an additional mechanism potentially contributing to the distinct redox states in Tregs and conventional T cells, as PLD is also regulated by cAMP.

In addition to the therapeutic approach based on dopaminergic signaling as suggested by Cosentino and coworkers, targeting potential key enzymes responsible for the increased antioxidative capacity in Tregs may be a suitable option. A link between antioxidative enzymes and immunosuppression has already been established in mesenchymal stem cells.[8] Furthermore, there are indications in the murine system that FOXP3, the master regulator of Tregs, may control the expression of important antioxidative molecules like glutaredoxin.[9] This hypothesis is currently under investigation and endeavors are directed at abrogating the survival advantage of Tregs in an environment with high levels of oxidative stress.

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To the editor:

PKCθ 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, PKCθ, in integrin signaling[2] and platelet function.[3] The recent paper by Nagy et al,[4] which attempts to characterize further the importance of PKCθ, 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 al[4] report that granule secretion in response to the glycoprotein VI collagen-related peptide (GPVI) agonist CRP is reduced in PKCθ$^{-/-}$ platelets, we have previously reported that CRP-induced secretion is enhanced in these cells.[3] Similarly, we found increased CRP-induced integrin $\alpha_{IIb}\beta_3$ activation and thrombus formation under flow in vitro,[3] whereas Nagy et al reported a decrease in integrin activation. Nagy et al[4] indicate that their effects are independent of their proposed role for PKCθ in thromboxane synthesis, as they report that decreased aggregation and secretion also occurred in indomethacin-treated platelets. To address whether the differences between our studies reflect differences in platelet preparation, we have repeated our experiments using the platelet preparation method described by Nagy et al,[4] and treated the platelets with indomethacin. However, under these conditions we still find enhanced CRP-induced dense granule secretion in PKCθ platelets (Figure 1A).

Nagy et al[4] supported their data from mouse platelets with experiments using a peptide (Vθ1-1; *C*GLSNFD) predicted to inhibit PKCθ's interaction with its RACK adaptor protein, coupled to a TAT peptide (*C*YGRKKRRQRRR) to allow its entry into cells. To the best of our knowledge, this is the first published report of Vθ1-1-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 Vθ1-1-TAT pretreatment (1 μM) in wild-type (WT) platelets (Figure 1B), similar to the