molecules are known as truly specific for Tregs, and, in accordance with this association was stated for the CD4+/CD25+ Tregs. Thus, it was clear that authors referred to Tregs.

In one study, stimulation of latently infected immortalized cells can induce remissions is unknown. The mechanism of viral inhibition is likely to influence the efficacy of an antiviral drug. Ganciclovir is effective in reducing the frequent viraemia and Kaposi’s sarcoma progression in two patients with AIDS. AIDS. 2001;15:2061-2062.

Response:

The role of antiviral agents in the treatment of multicentric Castleman disease

We appreciate the interest in our article, “Remission of HHV-8 and HIV-associated multicentric Castleman disease with ganciclovir treatment,” and the thoughtful description of 5 patients with multicentric Castleman disease (MCD) by Berezne et al. Their letter characterizes 5 patients with “chemo-dependent” MCD and shows that neither treatment with vinblastine, etoposide, or cidoflovir, nor splenectomy, was effective in reducing the frequent flares of MCD.

Of the findings, 2 appeared similar between our study and that of Berezne et al. First, it is notable that the treatment of patient 2 with etoposide had negligible effects, as we observed with 1 patient in our series. Additionally, as first described by Oksenhendler et al., two studies found that remission of clinical symptoms is consistently accompanied by declines in human herpesvirus 8 (HHV-8) viremia, although causality is difficult to infer.

As the pathogenesis of MCD is unclear, especially its relationship to ongoing HHV-8 replication, the role antivirals play in inducing remissions is unknown. The mechanism of viral inhibition is likely to influence the efficacy of an antiviral drug. Ganciclovir and cidoflovir both inhibit the secretion of HHV-8 virions after stimulation of latently infected immortalized cells. In one study, however, cidoflovir failed to block the production of early-lytic HHV-8 antigens.

To the editor:

Only genuine CD4+CD25+ Tregs may be friends or foes

In their recent paper, Stanzani et al. sought to ascertain whether the relationship between donor CD4+CD25+ regulatory T cells (Tregs) and graft-versus-host disease (GVHD) severity, found in murine transplantation, would hold up in humans. With this aim, they measured CD4+ and CD8+ T cells coexpressing CD25 within 60 donor grafts infused into matched siblings, reaching the unexpected conclusion that higher numbers of donor CD4+CD25+ (as well as CD8+CD25+) T cells correlated with an increased risk for GVHD. A similar association was stated for the CD4+CD25high subset, but data were not shown. Thus, it was clear that authors referred to Tregs.

If we had thought to demonstrate a similar hypothesis, we would have tried to more appropriately identify the subset of CD4+CD25+ Tregs. As stated by the authors, we agree that currently no surface molecules are known as truly specific for Tregs and, in accordance with convention, the intensity of CD25 expression on CD4+ T cells is used to point out Tregs; but CD25 expression may be induced potently and persistently by several stimuli on conventional T cells. Consequently, the lack of expression of a phenotype of recently activated T cells should be always assessed on presumed Treg subset. Indeed, isolated murine and human CD4+CD25+ Tregs are known to be hyporesponsive to allogeneic or polyclonal activation, to suppress the proliferation and cytokine secretion by activated CD4+CD25+ responder T cells, and to express Foxp3, as recently demonstrated in both murine and human Tregs.

Almost all papers so far published, except that of Stanzani et al, following the identification of a population of nonactivated CD4+CD25+ T cells, provide in vitro evidence that these cells are endowed with immunoregulatory properties. Taking this into consideration, we, and likely all people working with Tregs, agree with the authors’ sentence asserting that “...the coexpression of CD4 and CD25 may be insufficient to identify regulatory T cells in humans.” But, whether
CD25+ cells were activated conventional T cells, genuine Treg, or a mixture cannot be determined in this study. Moreover, we wonder why the authors did not assess markers of activation on their subset, since they suggest that the association observed could depend on the presence of activated T cells, due to mobilization or collection procedure. As well, we are dubious about the reason for evaluating the CD8+CD25+ cells, unless authors imagined the presence of a regulatory subset in the donor graft on the basis of the recent paper of Cosmi et al in human thymus. Probably it is not so, since this point was never discussed.

Certainly, Treg are like the mythical Hydra, as suggested by Solomon, and, taking into consideration data from literature and some recent findings obtained by our group, we agree that Treg may be friends as we observed in lung transplants or foes as seen in lung tumors (A.F. and F.M., unpublished data, January 2004). However, it is commending to unambiguously identify Treg before giving rise or decapitating a head of this monster.

Anna Fietta and Federica Meloni

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Response:

CD4+CD25+ T cells: friends, foes, and the future

While we agree with many of the points raised by Fietta and Meloni, we also fear that they misinterpret some of our conclusions. Prior murine studies suggested that coexpression of CD4 and CD25 alone could demarcate a regulatory T-cell (Treg) subset capable of suppressing graft-versus-host disease (GVHD) after mismatched stem cell transplantation (SCT). In contrast to findings of murine studies, we found that increased frequencies of CD4+CD25+ or CD8+CD25+ donor graft T cells were positively associated with recipient GVHD in humans. We quantitated CD25 expression on donor CD8+ T cells as a control population, and not because we expected regulatory activity to be associated with the CD8+CD25+ subset. Because we found that the infusion of increased numbers of CD25+ T cells was positively associated with the risk of GVHD, we concluded that CD25 expression might have reflected activation within donor T cells. These data confirmed that the CD4+CD25+ phenotype should not be equated with Treg function. However, our results in no way diminish the important work of others who have demonstrated the very real potential of Treg to modulate human immune responses in a number of disease settings.

Fietta and Meloni cite a study by Ng et al as evidence that “the lack of expression of a phenotype of recently activated T cells should be always assessed on presumed Treg subset.” While Ng et al demonstrated that cells stimulated with phytohemagglutinin for short periods expressed higher levels of CD69 than “naturally occurring CD4+CD25+ cells” in healthy human donors, it is notable that they also demonstrated that cells isolated purely on the basis of a CD4+CD25+ phenotype acted as functional Treg, remaining hyporesponsive to mitogens and proving capable of suppressing activation in CD4+CD25− T cells. While CD69 expression is closely associated with cytokine production after short-term stimulation and might help us to identify the subset of CD4+CD25+ T cells that are activated, persistently activated T cells may down-regulate surface CD69 expression, suggesting that the lack of CD69 expression might not be sufficient to demarcate Treg. While it has been suggested that the intensity of CD25 expression may discriminate activated versus regulatory CD4+CD25+ T cells, we could not confirm this in our study, as we found that increased frequencies of CD4+CD25+ cells within donor grafts were also associated with recipient GVHD.

Fietta and Meloni suggest that they would have conducted our study differently, by examining additional phenotypic or functional markers of Treg, or by assessing Foxp3 expression within these grafts. Many of the studies they suggest (eg, functional studies demonstrating the ability of CD4+CD25+ T cells to suppress activation of CD4+CD25− cells) could not have been performed given the limited number of total cells available within the convenience samples we used for our analysis. We began our investigation prior to recent studies establishing Foxp3 as a molecular marker of Treg. Even now, there are no standard methods for quantitating Treg on the basis of Foxp3 expression within a heterogeneous cellular population. In an essay that is nearly 35 years old, Ziman argued that “a typical scientific paper has never pretended to be more than another little piece in a larger jigsaw—not significant in itself but as an element in a grander scheme.” In this spirit, we hope that our study inspires other investigations that will leave off where we began, so that we may better understand the cellular allies and adversaries influencing both positive and negative posttransplantation outcomes.

Marta Stanzani and Krishna V. Komanduri

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To the editor:

On the roles of cGMP and glycoprotein Ib in platelet activation

In a recent publication, Marshall et al reported that they were unable to confirm our findings that cyclic guanosine monophos-
phate (cGMP) and the cGMP-enhancing drug sidenafl promote platelet activation induced by von Willebrand factor (VWF) and low-dose thrombin. They also contradict our findings on the role of extracellular signal-responsive kinase (ERK) in platelet activation. We would like to highlight some differences between their studies and ours that may explain this controversy.

(1) Marshall et al questioned the physiologic significance of the platelet VWF receptor, glycoprotein Ib-IX (GPIb-IX), in mediating signaling, mainly because VWF induces “weaker” signals compared with agonists such as collagen. However, whether a signal pathway is important cannot be determined by the strength of the signals but only by the outcome of the signals. In fact, it is well documented that GPIb-IX signaling is required for stable platelet adhesion and spreading on VWF under both flow and static conditions, and that it also induces integrin activation, platelet secretion, and aggregation. Thus, GPIb-IX signaling is physiologically significant.

(2) Marshall et al failed to induce full-scale platelet aggregation with 0.1 U/mL thrombin, which was thus referred to as “subthreshold.” However, in our studies, platelets fully aggregated in response to a much lower dose of thrombin (0.025 U/mL). This difference suggests that the platelets used in their study were desensitized. One possible desensitization mechanism is activation of the platelets during their isolation (point no. 3). In our study, protein kinase G (PKG) knock-out mouse platelets showed a reduced aggregation response to low-dose thrombin (0.025 U/mL) compared with full-scale aggregation of wild-type platelets. Platelet aggregation in response to high concentrations of thrombin was not significantly affected. Thus, the inability of wild-type platelets to respond to low-dose thrombin may represent a possible cause for the inability of Marshall et al to repeat our finding that PKG knock-out platelets had reduced platelet aggregation in response to low-dose thrombin.

(3) The inability of the platelets prepared by Marshall et al to respond normally to GPIb-IX-dependent agonists is also documented in another recent paper published by the same group, in which VWF failed to induce functional responses in washed platelets, in contradiction to other investigators in the field. The different responsiveness of platelets to VWF stimulation in their studies thus provides a possible explanation why Marshall et al failed to show cGMP elevation, ERK phosphorylation, and stimulatory effects of cGMP in VWF-stimulated platelets. It is not clear why platelets prepared by Marshall et al respond differently from platelets prepared by other investigators. However, we note that the basal platelet cGMP level determined by Marshall et al is dramatically higher than that reported by us using the same detection method and by other investigators (Table 1). It is therefore possible that the cGMP pathway was inadvertently activated during platelet preparation in their study, which may explain why no further elevation in cGMP levels was observable in response to VWF. We have observed that the effects of cGMP on platelet activation are biphasic. If cGMP is already elevated, exogenous cGMP or cGMP-enhancing drugs would not further increase platelet activation but would rather exert the secondary phase inhibitory effect.

(4) Marshall et al show that PKG inhibitors failed to inhibit thrombin-induced ERK phosphorylation. This result contradicts not only our data but also data from their companion paper by Gambaryan et al, in which PKG inhibitors attenuated thrombin-induced ERK phosphorylation and VWF caused a “variable” increase in platelet cGMP levels. Despite the differences in data interpretation and conclusions, Gambaryan et al clearly show that PKG inhibitors attenuate platelet aggregation induced by ristocetin and thrombin, and inhibit thrombin-induced ERK phosphorylation, which is consistent with our data.

Table 1. Basal cGMP concentrations in human platelets (pmol/10^8 platelets)

<table>
<thead>
<tr>
<th>Basal cGMP</th>
<th>Method</th>
<th>Reference no.</th>
</tr>
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<tbody>
<tr>
<td>Approximately 180</td>
<td>EIA</td>
<td>Marshall et al</td>
</tr>
<tr>
<td>0.63</td>
<td>EIA</td>
<td>Li et al(2)</td>
</tr>
<tr>
<td>0.2 ± 0.06</td>
<td>RIA</td>
<td>Eigenthaler et al(13)</td>
</tr>
<tr>
<td>0.9 ± 0.2</td>
<td>RIA</td>
<td>Radomski et al(14)</td>
</tr>
<tr>
<td>0.14 ± 0.06</td>
<td>EIA</td>
<td>Moro et al(15)</td>
</tr>
<tr>
<td>0.09 ± 0.01</td>
<td>Prelabeling technique</td>
<td>Jiang et al(16)</td>
</tr>
<tr>
<td>1 ± 0.3</td>
<td>RIA</td>
<td>Mullershausen et al(17)</td>
</tr>
</tbody>
</table>

All samples used were washed platelets. EIA indicates enzyme immunoassay; and RIA, radioimmunoassay.

References


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Only genuine CD4+CD25+ TregS may be friends or foes

Anna Fietta and Federica Meloni