Infection had distinct quality of response. The discrepancies in response to ESAT-6. Of note, the cells from different individuals with TB /H9253 established data from a few active TB patients, we also found that CD4 positive individuals with ESAT-6 resulted in not only the production of cytokines but also the activation and division of memory T cells. These responding T cells, in addition to CD4 T cells, produced IFN-γ in response to M tuberculosis antigens. Immunol Rev. 2007;215:46-58.


Response

Human memory but not naïve γδ T cells from TST-positive individuals respond to M tuberculosis antigen

We thank Casetti et al for their interest in our recent work, “CD4+CD25+ Treg cells inhibit human memory γδ T cells to produce IFN-γ in response to M tuberculosis antigen ESAT-6.”1 In our article, we showed that stimulation of peripheral blood mononuclear cells (PBMCs) from tuberculin skin test (TST)–positive individuals with ESAT-6 resulted in not only the production of cytokines but also the activation and division of memory γδ T cells. These responding γδ T cells displayed the phenotype of memory but not naïve γδ T cells. Most interestingly, CD4+CD25+ Treg cells could inhibit IFN-γ production by γδ T cells.

Casetti et al observed that CD4+ but not γδ T cells from 4 patients with active tuberculosis (TB) disease and 4 subjects with latent TB infection (LTBI) responded to ESAT-6 to express IFN-γ. In accordance with their and others’ observations,2 in our unpublished data from a few active TB patients, we also found that CD4+ T cells, in addition to γδ T cells, produced IFN-γ in response to ESAT-6. Of note, the cells from different individuals with TB infection had distinct quality of response. The discrepancies between their and our results on the response of γδ T cells to ESAT-6 might be influenced by many factors. The concern might be that the source of ESAT-6 we purchased from suppliers was different from that Casetti et al used. The various preparations of recombinant antigens, including cloning, sequences, expression, and purification process from different companies, might have different biological activities. Moreover, differences in the classical and nonclassical major histocompatibility class (MHC) molecules, the affinity to antigenic epitopes, and the distinct biologic features between eastern and western peoples might lead to distinct reactivity to the same antigen. Clearly, it has been reported that γδ T cells from bovines could react to ESAT-6 by IFN-γ production and proliferation.3 In addition, several antigenic epitopes/proteins recognized by human γδ T cells have been identified via CDR3δ peptide–based immunobiochemical strategy.4 These peptides not only bind to γδ T cells but also activate γδ T cells. Moreover, in human chronic human herpesvirus 8 (HHV-8) infection, purified viral proteins resulted in γδ Vδ1 T cell activation.5 Taken together, we agree with Casetti et al that human γδ T cells recognize nonpeptidic phosphoantigens, metabolites of the isoprenoid pathway.6,7 However, the mechanism by which human γδ T cells recognized protein antigens remains unclear currently and needs further investigation.

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Approval was obtained from the Zhongshan School of Medicine, Sun Yat-Sen University institutional review board for these studies. Informed consent was obtained in accordance with the Declaration of Helsinki.

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

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References


To the editor:

Is exclusive Skp2 targeting always beneficial in cancer therapy?

We read with great interest the work published in Blood by Chen et al concerning the therapeutic restoration of p27KIP1 protein levels in cancer after applying the specific Skp2 inhibitor CpdA.1 Skp2 is an E3-ubiquitin ligase that mediates degradation of several cell-cycle
Among its targets are negative cell-cycle regulators, including the kinase inhibitor protein (KIP) family member p27KIP1, and positive ones, such as cyclin E. In addition, p27KIP1 also inhibits cyclin E. Thus, Skp2, p27KIP1, and cyclin E form a tight network controlling S-phase entry.

In various tumors Skp2 is frequently overexpressed and represents a major cause of p27KIP1 protein down-regulation. However, p27KIP1 gene alterations and transcriptional silencing, due to microRNA-dependent repression, promoter methylation, and transcriptional suppressors, is not a rare event, as the authors mention, but a significant source of p27KIP1 inactivation in several malignancies (Table S1, available on the Blood website; see the Supplemental Materials link at the top of the article). Consequently, we cannot exclude the possibility that Skp2 overexpression coexists with transcriptionally silenced and/or mutant p27KIP1. We have observed such a condition with the other KIP member and Skp2 target, p57KIP2.

Based on the above, Skp2 inhibition, in cases with transcriptionally silenced and/or mutant p27KIP1, could result in increased expression of cyclin E with potential deleterious effects. Cyclin E provokes genomic instability, when overexpressed, by producing either DNA damage and/or centrosome amplification. Up-regulation of cyclin E is frequently observed in cancer, and is associated in various malignancies with poor survival.

To test this hypothesis we mimicked the above scenario by silencing Skp2 alone or in combination with p27KIP1 in A549 cancer cells, which express high Skp2 levels. The experiment showed that sole Skp2 silencing resulted in elevation of p27KIP1, reduction of cyclin E expression (Figure 1A,B), and a decrease in growth, while Skp2/p27KIP1 double knockout, recapitulating the proposed scenario, led to increased levels of cyclin E (Figure 1A,B), centrosome amplification (Figure 1B), abnormal mitoses, micronuclei, lobulated nuclei, and nucleoplasmic bridges (Figure 1C). p53 Ser-15 phosphorylation (Figure 1A), indicating a prominent DNA damage response, provides a mechanistic explanation for the observed genomic instability. Furthermore, the elevated levels of cyclin E comprised not only...
the full-length but also the low-molecular-weight isoforms.5,6 These isoforms are present in both cytoplasm and nucleus, and have increased affinity for cdk2.5,6 They have been associated with genomic instability, resistance to CIP/KIP inhibition, and poor outcome of patients with various malignancies.5

Similarly, is exclusive Skp2 targeting efficient in patients with p27KIP1 haploinsufficiency?10 In such cases, SKP2 blocking is effective when the levels of the remaining p27KIP1 allele are up-regulated above a threshold, able to exert its negative effect on cell-cycle progression. Below this threshold the CIP/KIP molecules will be sequestered by the cyclin D/cdk complexes, further promoting cyclin E/cdk2 activity.11

In conclusion, although the findings of Chen et al are significant and important, our results present an additional point of view, which stresses the impact of defining the transcriptional and/or mutational status of p27KIP1 before applying a therapeutic approach based exclusively on Skp2 inhibition.

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To the editor:

Platelet components associated with acute transfusion reactions: the role of platelet-derived soluble CD40 ligand

Several independent studies indicate that soluble CD40 ligand (sCD40L) derived and cleared from platelets is responsible for acute transfusion reactions (ATR).1-3 Ratliff et al4,5 show in this journal that platelets modulate innate and adaptive immunity in mice away from the site of activation and impact antibody-mediated immune responses. Having shown that platelet-derived sCD40L alters human B-cell responses in vitro,6 we examined whether sCD40L in platelet concentrates (PCs) associated with clinical ATR could mediate B-cell responses as an indication of pathophysiologic function. Apheresed PCs were collected and processed with leukocyte reduction (< 10⁶ per unit); suspended in 35% donor plasma and 65% InterSol platelet additive solution (Fenwal, La Chatre, France); prepared with the amotosalen HCl plus UVA light pathogen inactivation procedure (Intercept; Cerus, Concord, CA); and stored at 22°C with shaking for 5 or 7 days before transfusion.7 An active hemovigilance program evaluated the response to platelet transfusion.7 Reported ATR episodes were investigated using residual platelet components associated with ATR. In the 4 investigated cases of ATR (PCs were older than 3 days; Figure 1),1 2 aliquots from each PC (and, for each aliquot, 10 controls not associated with ATR) were prepared. One aliquot was used to assay supernatant fractions and the other to assay platelet lysates using specific, sensitive ELISAs (R&D Systems Europe, Lille, France) targeting a panel of cytokines and chemokines. IL8, CD62p, and platelet-derived growth factor–AB (PDGF-AB) levels were similar between ATR-associated PCs and PCs without ATR. In ATR-associated PCs, supernatant fractions contained higher levels of sCD40L than the control component, consistent with release; in an inverse correlation, the corresponding platelet lysates contained lower levels of sCD40L, consistent with release during storage (P < .05). To determine whether the released sCD40L (possibly among other costimulators) was biologically active, we incubated purified B cells, isolated from the blood of healthy donors, with PC supernatants and platelet lysates from PCs either associated or not with ATR. We then measured B-cell production of IL-6, on day 2 of the culture, to identify a production plateau (F.C., unpublished data, April 6, 2006). Baseline IL-6 concentrations were consistently less than 5 to 10 pg/mL in each control. The addition of 20 μL 1/20 diluted “ATR” supernatant samples to 2 × 10⁴ purified B cells in 200 μL culture medium⁵ resulted in increased IL-6 production compared with samples from control PCs (P < .05), the corresponding platelet lysates from ATR-associated PCs failed to elicit IL-6 production; recombinant purified sCD40L stimulated IL-6 production (P < .05), a cytokine strongly reactive to B cell stimulation. Preincubation of B cells with 5 μg/mL CD40-blocking antibodies (R&D Systems Europe and ATCC, Manassas, VA) substantially abrogated IL-6 secretion, unlike isotype-matched control. The partial blocking of CD40 binding on CD40⁺ B cells strongly suggests a potentially synergistic role in B cells for cytokines other than sCD40L (under investigation) and indicates a sustained role for PC-derived sCD40L.10

These data prompted us to institute a multicenter collaborative study of a larger series of ATR-associated PCs to determine specific

References

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