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.[6]

Similarly, is exclusive Skp2 targeting efficient in patients with *p27^{KIP1}* haploinsufficiency?[10] In such cases, SKP2 blocking is effective when the levels of the remaining p27^{KIP1} 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 *p27^{KIP1}* 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 cleaved from platelets is responsible for acute transfusion reactions (ATR).[1-3] Ratliff et al[4,5] 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 ($< 10^6$ 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),[8] 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 2×10^4 purified B cells in 200 μL culture medium[9] 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

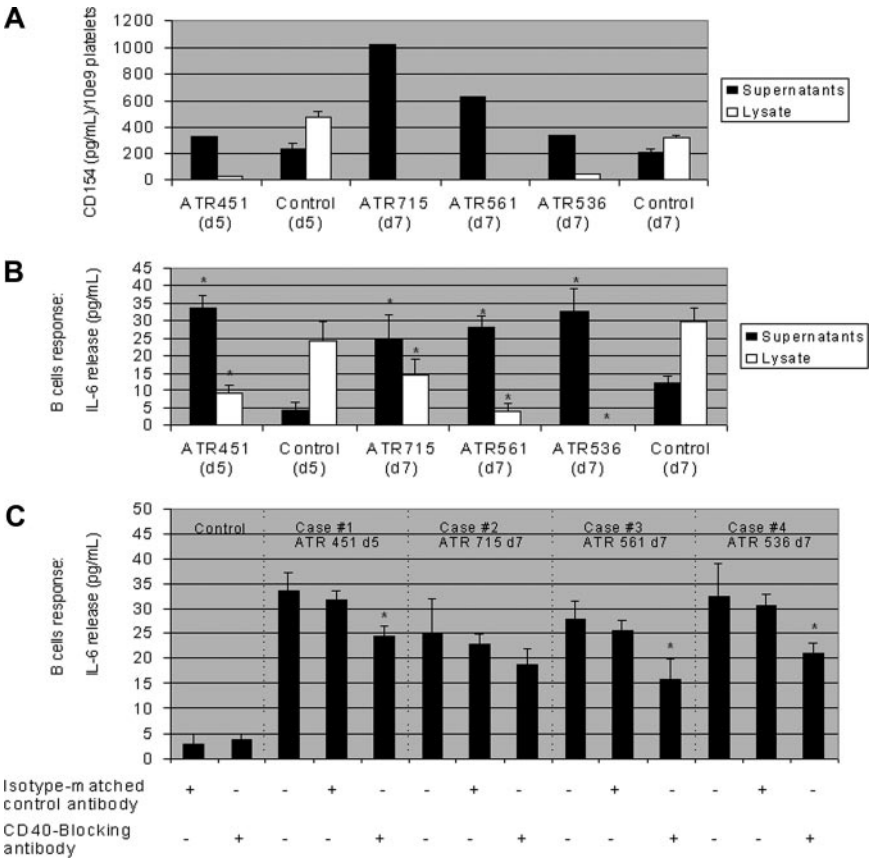

Figure 1. Concentration and physiologic effects of soluble CD40 ligand (sCD40L) in supernatants and lysates of platelet concentrates (PCs) associated with ATR(451,715,561,536) and control PCs. (A) Determination of sCD40L levels in PC supernatants and lysates in platelet components associated with ATR versus control components; data obtained from the clinical ATR occurrence with a day-5 (d5) PC sample and a d7 PC sample. (B) PC supernatants and lysates of individual components associated with reactions were tested with purified blood B cells from healthy blood donors stimulated to secrete IL-6; data obtained from the clinical episodes with one d5 and 3 d7 PC samples, respectively. (C) The consistent decrease of IL-6 secretion in response to stimulation with a high concentration of sCD40L in platelet supernatants after preincubation of B cells with 5 μg/mL CD40-blocking antibody. We observed similar results by preincubation of B cells with 5 μg/mL of another CD40-blocking antibody and with both antibodies together (data not shown). All values (pg/mL) were corrected for background levels. Data are expressed as means plus or minus SD in n = 5 experiments. $*P < .05$ in differences between tested values and respective controls (Wilcoxon paired test).

factors that can be targeted generally or in certain donors or patients to further reduce proinflammatory sCD40L production during collection and storage of platelet components.

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Response

Are PDMVs the biologically active source of CD154 in ATR?

In vivo animal data reporting biologic platelet CD154 function,[1-4] coupled with reports of direct biologic activity on isolated primary human neutrophils and B cells,[5-7] indicate platelet CD154 could have clinically significant function. Indeed, recent reports indicate that acute transfusion reactions (ATRs) may result from soluble CD154 (sCD154) released from platelets during storage.[5,8-10] By demonstrating that only platelet concentrates (PCs) that resulted in clinical ATR can stimulate CD154-specific B cell IL-6 production, Cognasse and colleagues make a compelling case for investigation into improved platelet storage that minimizes platelet release of CD154.

Upon activation, platelets can release microparticles and exosomes[11] (collectively referred to as platelet-derived membrane vesicles [PDMVs]) that can deliver platelet-derived signals in vitro [12-14] or in vivo.[3] This becomes an important consideration