IN THE SPOTLIGHT

CNS Endothelial Cell ActivationEmerges as a Driver of CAR T Cell–Associated Neurotoxicity

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Summary: Central nervous system (CNS) toxicity associated with chimeric antigen receptor–based therapeutics has emerged as a significant cause of morbidity and mortality, and insights into the pathophysiology of this syndrome have been lacking. A new study provides evidence that cytokine-induced CNS endothelial cell activation leading to disruption of the blood–brain barrier plays an early and critical role in this phenomenon. These insights provide new opportunities for targeted therapeutic interventions to modulate endothelial cell activation.

See related article by Gust et al., p. 1404 (9).

CD19-chimeric antigen receptor (CAR) T cells induce impressive antitumor effects in a high fraction of patients treated for B-cell malignancies, but cytokine release syndrome (CRS) and CAR T cell–associated neurotoxicity have emerged as significant toxicities, necessitating treatment in an intensive care unit in at least one quarter of patients in most series (1–6). The pathophysiology of CRS is largely understood to result from inflammatory cytokines derived directly or indirectly from CAR T-cell activation, and targeted therapeutic interventions targeting the IL6/IL6R axis, with or without corticosteroids, have decreased CRS morbidity and mortality (7, 8). In contrast, the pathophysiology of CAR T cell–associated neurotoxicity, recently coined CAR T–related encephalopathy syndrome (CRES; ref. 8), has remained enigmatic, and treatment options remain limited.

In this issue of Cancer Discovery (9), Gust and colleagues present data from a retrospective analysis of 133 adult patients receiving CD19. BB.1 CAR T cells for B-cell malignancies (diffuse large B-cell lymphoma, chronic lymphocytic leukemia, and B-cell acute lymphoblastic leukemia) following a lymphodepleting regimen. Forty percent experienced grade 2 (Common Terminology Criteria for Adverse Events v4.03) or above neurotoxicity, and all of these patients had concomitant CRS. The most common symptoms were delirium with preserved alertness and headache, but language disturbance, seizures, focal deficits, and diminished consciousness including coma also occurred. Consistent with previous reports, the vast majority of patients experienced complete resolution within 28 days; however, death occurred in 4 patients. CRES severity tracked largely with CRS severity, which correlated with CAR T-cell expansion in vivo. Not surprisingly, therefore, risk factors for neurotoxicity in multivariate analyses included higher disease burden, higher dose of CAR T cells, and a cyclophosphamide/fludarabine–based preparative regimen. Severe CRS was a major risk factor for severe (grade 3) CRES, and IL6 levels ≥501 pg/mL within 6 days of CAR T-cell infusion were associated with grade 4 neurotoxicity in 100% of patients. Although severe neurotoxicity grade 4 occurred in 5% of patients overall in this series, this rate was largely attributed to patients who received a dose of CAR T cells subsequently determined to be above the MTD, with a 1.3% risk of grade 4...
Inflammation-mediated activation of CNS ECs leads to a breakdown of the BBB that drives CD19-CAR–associated neurotoxicity. Profound activation of CNS ECs in the setting of CD19-CAR–associated CRS drives release of ANG2 and von Willebrand Factor (vWF) from EC Weibel–Palade bodies. ANG2 displaces ANG1 and inhibits TIE2 signaling, which drives EC permeability. Sequestration of high molecular weight vWF by activated ECs contributes to coagulopathy. Leakage of cells and cytokines across the disrupted BBB barrier results in pericyte activation, which further activates ECs, resulting in a feedforward loop. The model predicts that agents that restore a normal ANG1:ANG2 ratio would restore CNS EC homeostasis and restore the BBB.

neurotoxicity at doses equal to or below the MTD. This observation raises the prospect that standardization of CD19-CAR dosing as experience with these therapeutics increases may provide an important advance, in and of itself, by diminishing the risk of severe neurotoxicity. Together, consistent observations from this and previous reports (1–3) that enhanced CAR T-cell expansion is associated with both CRS and CRES provide strong circumstantial evidence that the pathophysiology of these two distinct clinical syndromes is intertwined.

The authors also observed that severe neurotoxicity was associated with vascular leak and coagulopathy, namely disseminated intravascular coagulation, raising the prospect that endothelial activation may be an important component of the syndrome. To test this hypothesis, they measured circulating levels of angiopoietin-1 (ANG1) and angiopoietin-2 (ANG2), regulators of EC activation. In healthy conditions, ANG1 constitutively signals ECs to maintain a quiescent state, whereas ANG2, a high-affinity TIE2 antagonist, remains sequestered in EC Weibel–Palade bodies (12). Patients with severe neurotoxicity (≥grade 4) manifested higher ANG2 levels and higher ANG2:ANG1 ratios, which would be predicted to induce EC activation through interruption of signaling of ANG1 via TIE2 (13). The data demonstrating that elevated pretreatment ANG2:ANG1 ratios and early rises in ANG2 levels following CAR T-cell infusion were associated with severe CAR-associated neurotoxicity provided further evidence to support EC activation as an early, initiating event. Further evidence of EC activation was provided by the finding of elevated levels of von Willebrand Factor (vWF) and diminished levels of ADAMTS13, a vWF cleaving enzyme, a scenario predicted to diminish the bioactivity of vWF and contribute to the coagulopathic state. Finally, the authors also demonstrated elevated inflammatory cytokines in the cerebrospinal fluid and infiltrating CAR T cells within the CNS of autopsy samples obtained following fatal neurotoxicity, leading to the hypothesis that leakage of inflammatory cytokines across the BBB combined with local production of inflammatory cytokines from T cells within the CNS further drive the pathophysiology.

Together, the work by Gust and colleagues provides data consistent with a model wherein CNS EC activation is a central, early feature of CRES. Confirmation in other series is required, including in patients receiving alternative CD19-CAR constructs, such as those incorporating a CD28 costimulatory domain. Nonetheless, the model is attractive given that numerous other clinical syndromes that share this pathophysiology and/or clinical symptomatology provide insights into possible therapeutic interventions. For instance, EC activation plays a major role in the pathophysiology of sepsis, which shows many similarities with CRS, including widespread immune activation, hypotension, vascular leak, and coagulopathy. ANG2 also serves as a biomarker for sepsis severity, acute respiratory distress syndrome, and toxic shock syndrome, and ADAMTS13 is also reduced in sepsis (13). Notwithstanding these similarities, it remains unclear why EC activation in CRES is dominated by neurologic
symptomatology, whereas neurotoxicity in sepsis is rarely seen in the absence of concomitant dysfunction in other organs, such as the lungs, kidneys, or liver. Another clinical setting that bears hallmarks of the emerging CRES pathophysiology is thrombotic thrombocytopenic purpura (TTP), a syndrome associated with pathologic EC activation and coagulopathy, wherein neurologic dysfunction is often the predominant clinical manifestation. Like the patients with CRES reported here, patients with TTP demonstrate evidence for EC activation, diminished serum levels of high molecular weight vWF multimers, and an altered ADAMTS13:vWF ratio. Although renal dysfunction and microangiopathic hemolytic anemia are not major features of CRES, the data presented here suggest that targeted interventions demonstrated to be efficacious in TTP (e.g., plasmapheresis), might also provide therapeutic benefit for CRES. Such studies will likely be tested in future clinical trials.

Plasmodium falciparum malaria provides another clinical scenario associated with EC activation and neurologic dysfunction. Reduced ANG1 levels leading to CNS microvascular dysfunction have been implicated in the pathophysiology of cerebral malaria in children, and recombinant BowANG1 improved outcomes in an animal model of cerebral malaria (12). If displacement of ANG1 by ANG2 is an essential primary trigger for CRES, recombinant BowANG1 could represent a targeted therapeutic that would not be expected to diminish the antitumor effects of CAR T-cell therapy. Interestingly, cerebral malaria is also sometimes associated with hemophagocytic lymphohistiocytosis, which several groups have observed following CD19-CAR therapeutics (3, 8).

The association of EC dysfunction and cerebral edema also raises the prospect that the pathophysiology of CRES may overlap with that of posterior reversible encephalopathy syndrome (PRES). Endothelial damage is an essential component of PRES pathophysiology, and although the syndrome most often occurs in the setting of hypertension, inflammatory events can also induce PRES in the absence of hypertension (14). Papilledema and MRI findings of edema, although noted to be distinct from vasogenic edema in this report, suggest overlying pathophysiologies between CRES and PRES. Finally, the observation that severe clinical symptoms in PRES typically fully reverse in a relatively short period with supportive care alone is reminiscent of the clinical course for patients with severe CRES, most of whom recover fully without evidence of neurologic sequelae.

In summary, the report by Gust and colleagues provides much-needed insights into the pathophysiology of CAR-associated neurotoxicity. The data are consistent with a large body of previous work implicating CRS and CRES as overlapping off-target toxicities derived from excessive T-cell activation, although formal proof that the CD19 antigen is not involved will require an observation of similar neurotoxicity using CARs targeting antigens other than CD19. Despite the overlying pathophysiologies between CRS and CRES, clinical experience has demonstrated that targeted interventions, such as IL6R blockade, which are typically highly effective treatment for CRS, do not rapidly reverse CRES nor reliably prevent progression of the syndrome. The notion that targeted therapies specifically aimed at modulating CNS EC activation might be more effective in this regard is provocative and will compel clinical interventions in appropriate populations in the coming months to years.

Disclosure of Potential Conflicts of Interest
C.L. Mackall reports receiving a commercial research grant from Bluebird Bio, has ownership interest (including patents) in Juno Therapeutics, and is a consultant/advisory board member for Adaptimmune LLC and Unum Therapeutics. D.B. Miklos reports receiving commercial research support from Kite Pharma and is a consultant/advisory board member for Kite Pharma and Novartis.

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