Response to Comment on "Inflammasome Activation of IL-18 Results in Endothelial Progenitor Cell Dysfunction in Systemic Lupus Erythematosus"

Mariana J. Kaplan and J. Michelle Kahlenberg

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Comment on “Inflammasome Activation of IL-18 Results in Endothelial Progenitor Cell Dysfunction in Systemic Lupus Erythematosus”

Recently, Kahlenberg et al. (1) found that inhibition of caspase-1 disrupted IFN-α-mediated endothelial progenitor cell (EPC)/circulating angiogenic cell (CAC) dysfunction in murine and human systems. It lends more information on the role of IFN-α, which might promote atherosclerosis in systemic lupus erythematosus (SLE).

To date, the etiology and pathogenic mechanisms of SLE have not been clearly elucidated. It is well known that SLE was characterized by increased vascular risk due to premature atherosclerosis. Denny et al. (2) discovered that patients displayed aberrant phenotype and function of EPCs/CACs, characterized by significantly decreased circulating EPCs and impairments in the capacity of EPCs/CACs to differentiate into mature endothelial cells, and produced adequate levels of the proangiogenic molecule vascular endothelial growth factor (VEGF). These abnormalities are triggered by IFN-α, which induces EPC and CAC apoptosis and skews myeloid cells toward nonangiogenic phenotypes. Importantly, neutralization of IFN pathways restores a normal EPC/CAC phenotype in lupus. Furthermore, Thacker et al. (3) showed that NZB/W mice manifested reduced numbers and increased apoptosis and impaired function of EPCs. This abnormality was related to significant decreases in endothelium-dependent vasomotor responses and with upregulated type I IFN signature in EPC compartments. On the contrary, B6/lpr mice reflected improvement in endothelium-dependent and endothelium-independent responses, no abnormalities in EPC phenotype or function, and downregulation of type I IFN signatures in EPC compartments. In addition, Thacker et al. (4) demonstrated that IFN-α promoted an antiangiogenic signature in SLE and control EPCs/CACs, characterized by transcriptional repression of IL-1α/β, IL-1R1, and VEGF-A and augmentation of the IL-1R antagonist (IL-1RN) and the decoy receptor IL1-R2. The study found a novel putative pathway by which IFN-α may interfere with vascular repair in SLE through repression of IL-1-dependent pathways.

Collectively, these findings suggest that IFN-α might play an important role in vascular damage and, potentially, atherosclerosis development in SLE. However, more studies are still needed to clarify the role of IFN-α, based on the association with EPCs/CACs in SLE, especially with atherosclerosis.

References

Response to Comment on “Inflammasome Activation of IL-18 Results in Endothelial Progenitor Cell Dysfunction in Systemic Lupus Erythematosus”

We thank Drs. Duan et al. for their comments and for summarizing the observations of several of our recent publications that focus on the putative roles of type I IFNs in the development of premature cardiovascular damage in systemic lupus erythematosus. We agree with them that additional studies are needed to further clarify the role of type I IFNs in the development of premature atherosclerosis in lupus, other autoimmune diseases, and in the general population.

Very recently, we have demonstrated that type I IFNs modulate vascular repair, vasomotor tone, thrombosis, and plaque progression in murine models of lupus and atherosclerosis; these observations provide additional in vivo evidence for a prominent role of these cytokines in vessel damage (1). There is also evidence that other type I IFNs besides IFN-α are proatherogenic in rodents (2). All these observations support the notion that these cytokines should be evaluated as putative biomarkers of vascular risk in various autoimmune diseases associated with endothelial damage, as well as in the general population. Indeed, the pathways leading to atherosclerosis and cardiovascular events are likely heterogeneous with regard to initial inflammatory events that promote endothelial damage and promote plaque progression. We hope that advancing the understanding of the mechanisms that lead to the striking increases in

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cardiovascular risk in lupus will also allow us to further identify important drivers of atherosclerosis in the general population.

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References


Comment on “Expression of Helios in Peripherally Induced Foxp3 Regulatory T Cells”

Helios distinguishes thymic-derived natural Tregs (nTregs) from periphery-induced Tregs (iTregs) (1). However, concerns have been raised and Helios expression was induced in Foxp3 T cells (2–4). More recently, a strong Helios expression in iTregs was shown, excluding its value as a marker of nTregs (5). Without the generation of a reporter mouse for Helios, it would be impossible to determine the exact origin and function of Helios Tregs (6). Regardless of this debate, there are important conclusions that can be made.

Helios in peripheral and tumor-infiltrating Foxp3 Tregs is of particular interest. Expanded Foxp3 Tregs in cancer patients express Helios (7). Tumor-infiltrating Foxp3 Tregs are largely positive for Helios (Refs. 4, 8, 9 and E. Elkord, S. Sharma, D. J. Burt, and R.E. Hawkins, unpublished observations). The increased Tregs in some tumors could be derived from nTregs, but this should be interpreted carefully. Helios is a marker of T cell activation (2), and Foxp3 Helios Tregs do not secrete effector cytokines (10), indicating they are bona fide Tregs (11). Interestingly, Helios Tregs express higher levels of TGF-ß, proliferate more in vivo, while the suppressive activity correlates with the absolute number of Helios T cells (4). Therefore, Foxp3 Helios Tregs represent a functional subset with more suppressive characteristics, compared to Foxp3 Helios Tregs.

Several questions need to be answered. Do higher levels of Foxp3 Helios Tregs correlate with poorer prognoses in cancer patients? Could Foxp3 Helios be the optimal subpopulation for biotherapy of autoimmune diseases? What are the main phenotypic/functional differences between Foxp3 Helios and Foxp3 Helios Tregs? What is the exact role of Helios in Foxp3 T cells?

Response to Comment on “Expression of Helios in Peripherally Induced Foxp3 Regulatory T Cells”

We recently reported in the *The Journal of Immunology* that Helios is expressed in peripherally induced T regulatory cells (Treg), precluding the use of this transcription factor as a marker of thymically-derived natural Treg (1). We noted differences in the optimal conditions for Helios and Foxp3 induction in vitro, and in the kinetics of their expression in vivo. Following a single dose of i.v. peptide, the robust Helios expression described in our study was more rapid than induction of Foxp3, but began to decrease 2–3 wk after stimulation without a second administration of Ag.

While Helios does not discriminate thymic Treg from those that develop in the periphery, we agree that Treg expression of Helios remains of significant interest and potential therapeutic importance. The expression of effector cytokines by Helios-negative Treg from human peripheral blood (2) is of partic-
ular interest, as it suggests that Helios expression may correlate with distinct Treg function, rather than simply degree of activation. While Helios-deficient Treg were able to suppress proliferation in vitro (2), characterization of Helios-deficient Treg function during a physiological immune response should yield significant insight into the specific role of this transcription factor.

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Comment on “Class B Scavenger Receptor Types I and II and CD36 Targeting Improves Sepsis Survival and Acute Outcomes in Mice”

We have several concerns regarding the article by Leelahavanichkul et al. (1). 1) Inappropriate use of control mice. The article stated that the SR-BI−/− mice were in a C57BL/6 (B6) background, but their cholesterol levels (∼280 mg/dl) were abnormally high compared with the 10×B6/SR-BI−/− mice in our hands. According to Dr. Bocharov (personal communication), the mice were in 5xB6N, rather than 10× backcrossed. It is inappropriate to use B6N mice as controls, given the profound survival advantage of mice in a mixed background. 2) Problematic data. It is impossible to get 55% survival for CD36−/− (n = 17), 17% for WT (n = 17) (Fig. 6A in Ref. 1), 48% for SR-BI−/−+/S (n = 9), and 5% for WT+S (n = 10) (Fig. 6B in Ref. 1) given the sample sizes. For example, the 5% survival would imply that 0.5 mice out of 10 survived. 3) Pretreatment with steroids 24 h before cecal ligation and puncture (CLP). This approach does not seem justified, since SR-BI−/− mice do not lack steroids in physiological conditions. Furthermore, steroid pretreatment suppresses both innate and adaptive immunity, leading to an immunocompromised status before CLP (2) and completely eliminates SR-BI expression in adrenal glands, leading to a lack of endogenous steroid production in SR-BI−/− mice during CLP (3).

We found that SR-BI protects against sepsis regardless of the presence of steroids (Fig. 1B) or antibiotics (Fig. 1C). The increased survival of SR-BI−/− mice observed by the authors is likely caused by the survival advantage of the mixed B6/129 background of the mice versus B6N controls and by altered immunity due to steroid pretreatment.

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FIGURE 1. SR-BI protects against septic death. 8- to 12-wk-old SR-BI−/− and SR-BI+/+ littermates were treated with CLP, and survival was observed for 5 d. The survival assay was analyzed by log-rank x2 test using SAS software. The data were expressed as the percentage of surviving mice at indicated times. (A) CLP without steroids/antibiotics, 22G full ligation; (B) CLP with steroids (0.1 mg dexamethasone/0.1 mg fludrocortisone in 1 ml PBS, i.p. following CLP), 27G full ligation; (C) CLP with steroids and antibiotics (steroids following CLP; and 1 mg sulfamethoxazole/0.2 mg trimethoprim administered by oral gavage 5 h following CLP and every 12 h afterward), 22G full ligation.
Response to Comment on “Class B Scavenger Receptor Types I and II and CD36 Targeting Improves Sepsis Survival and Acute Outcomes in Mice”

We commend Dr. Li and colleagues for critical analysis of our work (1) and for attempting to reconcile the results we reported that apparently conflict with the Li group’s previously published results (2). By adjusting their model to include steroid supplementation and antibiotic treatment, they observed that the net effect of SR-BI is still qualitatively protective, although quantitatively closer to our result of SR-BI contributing significantly to mortality. However, several differences remain between the models, and it is clear from our collective data that the context provided by each animal model can reveal dramatically different roles for SR-BI in sepsis. While we do not know which factor(s) provide the tipping point, remaining differences include 1) a dramatic difference in model severity (100% survival [full length cecum ligated and punctured by 22/27 gauge needle] versus 10% survival [12 mm cecum ligated and punctured by 21 gauge needle]), 2) mouse age (8–12 wk versus 24–30 wk), 3) antibiotic type/route (sulfamethoxazol/trimethoprim by oral gavage versus imipenem/cilastin s.c.), 4) fluid administration, 5) steroid dose/route/timing (0.1 mg/mouse or 4 mg/kg i.p. after cecal ligation and puncture (CLP) versus 20 uM in drinking water or ∼2.25 mg/kg/day before and after CLP, 6) gender and background (male/female C57BL6/F10 backcross versus male C57BL6/F10 backcross versus male C57BL6/F5 backcross versus male C57BL6/F5 backcross), or 7) potential environmental factors such as bedding (3) or microbiome. Two of these differences warrant additional elaboration. First, we agree with the Li group that adrenocorticotropic hormone-mediated feedback of steroidogenesis is a clinically relevant confounding factor that should be carefully considered in future studies. Any supplementation strategy to replenish glucocorticoids in SR-BI–deficient mice will require carefully monitoring the time course of serum steroid levels after CLP (4) to ensure that the nonsteroidogenic functions of SR-BI are not overwhelmed by pharmacological effects of supplementation. Our nonfasting cholesterol values may further make comparisons difficult between our models. Second, the differences in model severity (100 versus 10% mortality) could be even more important, as the risk/harm balance for several therapeutics, including glucocorticoids, depends on sepsis severity in patients and animal models (5). We hope that further experiments will explain why SR-BI can have such widely divergent roles in sepsis; understanding the mechanism(s) would be crucial before properly applying SR-BI–based therapies to a diverse sepsis patient population. Discrepant results often provide opportunities for profound insights, and these data represent a scientific opportunity that should be pursued.

We thank the Li group for pointing out arithmetic errors in our paper (Fig. 6A: CD36+/−, 9/17 = 53%, not 55%; WT, 3/17 = 18%, not 17%; Fig. 6B: WT+ S, 1/10 = 10%, not 5%; Fig. 6B SR-BI+/− + S, 4/9 = 44%, not 48%).

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