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EDITORIAL COMMENT

INKing the Cardiotoxicity Out of Doxorubicin*

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Doxorubicin (Dox) is a widely used anthracycline for a range of adult and childhood cancers including breast cancer, leukemia, lymphoma, osteosarcoma, Ewing sarcoma, and neuroblastoma.[1] Dox significantly improves cancer survival rates, but at the cost of serious cardiovascular complications including the development of heart failure. Dox cardiotoxicity limits its clinical utility, negatively affecting quality of life of cancer survivors.[1] After half a century of efforts by researchers and physicians, the underlying mechanisms of Dox cardiotoxicity remain to be fully elucidated but include the generation of reactive oxygen species, increased apoptosis, mitochondrial impairment, and dysregulation of autophagy, culminating in myocardial atrophy.[1]

One factor that has perhaps been insufficiently recognized is the role of innate and adaptive immunity in Dox cardiotoxicity. Accumulating evidence suggests that Dox induces inflammatory responses via innate immune cells that progressively result in cardiac damage. Yet, few studies have applied the current state-of-the-art high-resolution immune phenotyping to better understand Dox cardiotoxicity.

Invariant natural killer T (iNKT) cells are a unique subset of T lymphocytes that recognize glycolipid antigens presented in the context of the nonpolymorphic molecule CD1d. These cells are innate effector cells that rapidly and robustly release large amounts of effector cytokines and chemokines, such as interferon (IFN)-γ, tumor necrosis factor-alpha, interleukin (IL)-4, and IL-10, and orchestrate tissue inflammation in cardiovascular disease. iNKT cells have an important role in modulating the ensuing immune response. A previous study[2] has demonstrated that activation of cardiac iNKT cells by intraperitoneal injection of α-galactosylceramide (GC) attenuated cardiac fibrosis in the presence of Dox by up-regulating T-helper type 2 cytokines, but the cell-type specific responses and exact molecular mechanisms remain unknown.

In a study reported in this issue of *JACC: Basic to Translational Science*, Sada et al[3] investigated the hypothesis that activation of iNKT cells by α-GC attenuates Dox-induced cardiomyocyte death and explored the underlying molecular mechanisms. To do this, the investigators used α-GC to activate iNKT cells, confirmed its cardioprotective role in Dox cardiotoxicity, and defined the downstream role of IFN-γ-signal transducers and activators of transcription 1 (STAT1)- extracellular signal-regulated kinase (ERK) pathway. After validating α-GC as an activator of iNKT in Dox-treated hearts, Sada et al[3] found that α-GC prevents Dox-induced weight loss and cardiac dysfunction. Using echocardiography, the investigators found that administration of α-GC attenuated Dox-induced cardiac dysfunction, as well as attenuated cardiac atrophy. Second, histologic analysis revealed that α-GC administration remarkably suppressed Dox-induced cytoplasmic vacuolization and myocardial apoptosis using terminal deoxynucleotidyl transferase dUTP nick end labeling staining, without obvious reductions in interstitial fibrosis and infiltration of inflammatory cells. Finally, the authors found that α-GC administration significantly reduced the ratio of Bax to Bcl-2 and cleaved caspase 3 while excluding the potential role of necroptosis by ascertaining the phosphorylation status of MLKL, a marker of necroptosis. Altogether, these

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lines of evidence suggest iNKT cell activation by α-GC attenuates Dox-induced cardiac dysfunction and apoptosis.

In mechanistic studies, Sada et al[3] first evaluated the downstream targets of IFN-γ and found that α-GC administration increased total protein level and phosphorylation of STAT1, a direct downstream of IFN-γ receptor, in the presence or absence of Dox. Interestingly, among multiple STAT1 targets, α-GC increased phosphorylation of ERK and Akt, but not c-Jun N-terminal kinase or p38. These data suggest the protective effects of iNKT cell activation in Dox-induced cardiotoxicity might be mediated by STAT1-ERK/Akt axis specifically. Mass spectrometry in control, Dox, and Dox plus α-GC hearts suggested that α-GC administration alters biomarkers related to energy metabolism and antioxidant capacity, which are intimately involved in cardiomyocyte death. To elucidate the role and importance of IFN-γ in protective effects of iNKT cells in Dox-induced cardiotoxicity, Sada et al[3] administrated neutralizing anti-IFN-γ monoclonal antibody into mice treated with Dox and α-GC. After a series of experiments, the investigators demonstrated that blockage of IFN-γ abolished the cardioprotective effects of iNKT cell activation by α-GC on Dox-induced cardiac dysfunction and apoptosis. In general, these lines of investigation demonstrated that IFN-γ is necessary for the effects of α-GC in models of Dox cardiotoxicity.

Interestingly, prior reports[2] have suggested that iNKT cells attenuate inflammation and fibrosis in Dox cardiotoxicity, which the authors comment could be due to the mode of administration of α-GC. In the context of the broad literature, it is worth noting that Sada et al[3] observed that α-GC also attenuated Dox-induced body weight loss, suggesting systemic effects of α-GC administration that could be evaluated in future studies. The investigators performed 2 independent experiments to test if pretreatment or simultaneous treatment with α-GC would attenuate Dox cardiotoxicity. Interestingly, simultaneous administration of α-GC with Dox did not attenuate cardiotoxicity, a finding that does not limit the potential translational relevance of the findings because pretreatment is reasonable for patients receiving chemotherapy. The authors used both high-dose Dox (20 mg/kg) and lower doses of Dox (6 mg/kg × 3 doses), and suggest that higher doses might lead to more extracardiac manifestations than lower doses. From our vantage point, 20 mg/kg is a very high dose of Dox and single-dose injections cause significant weight loss, mortality, and cardiac atrophy, whereas repetitive injection with a cumulative dose of 15-20 mg/kg also causes atrophy, without significant mortality over the span of 40 days.[4,5] It is definitely a strength of the article that the authors did not rely on the high-dose single injection, which probably should not be used on its own as a model of cardiotoxicity.

In conclusion, the study by Sada et al[3] further confirms the cardioprotective effect of iNKT activation by α-GC administration and provides novel mechanistic insight into the attenuation of Dox-induced cardiotoxicity. Whether cell therapies with cells treated ex vivo with α-GC could be a viable protective strategy against cardiotoxicity should be evaluated in future studies.

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