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Radical Innate Regulation of Autoimmune Diabetes

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Generation of reactive oxygen and nitrogen species (ROS/RNS) during various immune responses are critically important for innate defenses such as acute inflammation by neutrophils and macrophages for efficient killing of foreign pathogens. On the other hand, accumulation of ROS-producing cells during chronic inflammation often results in tissue damage. It is also becoming increasingly apparent that oxidative stress plays a significant role in the pathogenesis of a number of autoimmune diseases [1].

Oxidative stress is well characterized by an imbalance of either endogenous cellular antioxidant defenses or generation of reactive oxygen and nitrogen species, both of which are locked in a reciprocal cycle of regulatory control. At the same time, it has been difficult to differentiate the cause and the effect relationship of ROS and its role in the pathogenesis of autoimmunity, as chronic inflammation inevitably leads to accumulation of immune cells responsible for oxidant production and subsequent redox imbalance. Nevertheless some organs and associated diseases may be intrinsically more susceptible to redox stress, which may initiate deterioration of tissue function and facilitate progression of autoimmunity, as seen during Type 1 diabetes. Type 1 diabetes (T1D) is a metabolic disease characterized by the inability to produce insulin in response to elevated blood glucose due to autoimmune destruction of pancreatic beta cells that are responsible for production of insulin. Because of the naturally low anti-oxidant defenses in beta cells [2], chronic hyperglycemia has also been linked to ROS production within these cells leading to redox imbalance and beta cell apoptosis which may also be observed during Type 2 diabetes [3]. However, the causative role of oxidative stress in T1D is less well understood. It is thought that genetic and/or environmental factors may lead to initial dysfunction of beta cells involving necrosis and uptake of damaged beta cells by tissue macrophages and their subsequent migration to the pancreatic lymph nodes where generation of auto-reactive T and B cells further amplify the disease process. Likewise, inherent immune cell dysfunction involving T and B cell development and activation also play key roles during autoimmune diabetes pathogenesis. Together, the recruitment of circulating immune cells, including mononuclear myeloid cell populations produce a variety of pro-inflammatory cytokines and ROS that cooperatively exacerbate the development of Type 1 diabetes.

Previous studies using the well characterized mouse T1D model, the non-obese diabetic (NOD) mice, demonstrated that genetic deficiency of NADPH oxidase (NOD. Ncf1^{m1J})

clearly reduced susceptibility to the disease, which was attributed to alterations in T cell polarization from a pathogenic Th1 type to a lesser pathogenic Th17 type [4]. In this issue of SFRBM, Seleme and colleagues put forth an interesting alternative explanation for the redox dependent regulatory effects on experimental autoimmune diabetes [5]. Bone-marrow derived macrophages isolated from NOD. Ncf1^{m1J} mice show reduced production of TNF- α and IFN- β cytokines, which are toxic to beta cells. Moreover, the authors linked this defect with reduced expression of pattern recognition receptor TLR3. What separates this work from other previous publications in the field is that Seleme et al provide compelling evidence suggesting that NADPH oxidase (NOX) may serve a broader role in the immune system than simply contributing to the generation of superoxide. More specifically, that the enzyme can modulate macrophage function as a possible signaling molecule involved in the activation of the transcription factor NF- κ B influencing its translocation into the nucleus and ultimately production of pro-inflammatory cytokines by these cells.

The work from Seleme et al adds to an increasing body of evidence that supports an important role of reactive oxygen and nitrogen species in regulation of both innate and adaptive immunity. In rheumatoid arthritis (RA), T cells isolated from the synovial fluid were found to have reduced levels of intracellular glutathione (GSH) and proliferated less vigorously than T cells isolated from the peripheral blood (PB) [6]. Interestingly, although addition of N-acetyl-L-cysteine (NAC) partially restored T cell responses, they were still well below of those isolated from the PB. Similar findings were reported for patients with systemic lupus erythematosus (SLE) [7]. Depletion of GSH in T cells has been also linked with mitochondrial hyperpolarization and necrotic cell death [8]. Uptake of necrotic cells by dendritic cells stimulated their maturation and activation of the immune responses instead of tolerance, which results from uptake of apoptotic cells [9,10]. It has been postulated that nitric oxide (NO) produced by activated macrophages via inducible NO synthase (iNOS) is known to regulate proliferation of T cells: at low levels (nanomolar to low micromolar) NO enhances T cell proliferation, while at higher levels (high micromolar) potentially suppresses T cell proliferation ([11]; Ostanin DV, unpublished observations). Production of reactive oxygen and nitrogen species by myeloid-derived suppressor cells (MDSCs) inhibit T cell function through nitrosative modifications of T cell receptors/CD8 molecules resulting in the inability to bind peptide-MHC complex and induction of non-responsiveness [12]. Moreover, NO-dependent mechanisms for T cell suppression have been reported for myeloid cells isolated from mice with various autoimmune conditions [13, 14]. In addition to the effects of NO on T cells, expression of functional NOX in T cells has been demonstrated [15]. Interestingly, in addition to the evidence that NOX may be involved in T cell polarization [15], this molecule has also been implicated in the cytokine-dependent T cell homeostasis, as gp91-deficient T cells showed improved survival and better recall responses after immunization in vivo [16]. Data by Seleme et al suggest that NOX protein expression **and** superoxide are both necessary for efficient innate immune sensing and inflammatory cytokine production. Their findings substantiate recently published results by Thayer et al who attributed lack of disease in NOD. Ncf1^{m1J} mice to a dysfunction of, presumably, antigen presentation by macrophages [17] and went on to show that depletion of macrophages and neutrophils with anti-Gr-1 antibody afforded significant protection from diabetes in NOD mice.

Nevertheless additional questions remain. Since neutrophils represent the most abundant cell type of the inflammatory infiltrate characteristic of chronic inflammation, it would be important to determine if NADPH is also involved in NF- κ B-mediated signaling and production of cytokine and chemokines by these cells. Although authors show increased MHC class II expression by bone marrow-derived macrophages from NOD.Ncf1^{m1J} mice, it is essential to understand the importance of this observation in the context of attenuated disease demonstrated by these mice. Given the central role that CD4 T cells play in the pathogenesis of T1D [16] might there be a higher incidence of T cell anergy in these mice? Another important question would be to determine whether the current finding with in vitro-generated macrophages translates to in vivo settings: do macrophages and/or DC isolated from these mice also show enhanced expression of MHC-II? Although data from Seleme et al report decreased expression of TNF- α , reduced NF- κ B translocation is most certainly not the sole explanation as other NF- κ B-dependent cytokines (IL-6, IL-1 β) were not altered. Similarly, while IFN- β levels were significantly reduced, this did not affect IFN- α production by these cells. Thus, a deeper understanding of the signaling mechanisms that may be altered by NOX deficiency is clearly needed and may reveal novel regulatory pathways controlling cytokine expression aside from what is currently understood.

Looking into the future, the paper by Salame et al highlights the importance for a better understanding of various redox dependent mechanisms responsible for the development as well as protection from T1D on a cellular and biochemical level. On the whole, little effort is currently devoted to uncovering redox mechanisms operating in the immune system, especially the role of redox signaling in shaping the adaptive immunity. While Tse et al reported that lack of NADPH oxidase skews polarization of T cells from Th1 to a Th17 phenotype [4], it is important to expand this and other observations to autoimmune disease models to obtain clearer insight on the importance of NOX modulation of immune dysfunction. What is clear is that in diabetes, and possibly other autoimmune diseases, acute/innate inflammatory responses can trigger positive feedback responses that may “fuel” recruitment of immune cells that quickly and progressively deteriorates to chronic autoreactive inflammation. With that in mind, it is tempting to speculate that identification of individuals more susceptible to oxidative stress through the genetic analyses of various antioxidant pathways may reveal interesting new information regarding disease susceptibility, especially in persons with known Idd diabetic loci. Future results from these and other similar studies might one day identify a unique redox dependent pathway that may provide useful therapeutic targets for this dreadful disease with few new approaches on the horizon.

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