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The innate and adaptive immune response are both involved in drug-induced autoimmunity

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Drug-induced autoimmunity is an intriguing phenomenon that has been described in the medical literature at least since 1945, with a report from the Medical Corps of the Army of the United States describing a case of systemic lupus erythematosus induced by sulfadiazine (1). While an autoantibody response in drug-induced autoimmunity is more universal, the spectrum of clinical autoimmunity is variable and likely influenced by host genetics including genetic variants predisposing to autoimmunity and genetic variants affecting drug metabolism. Indeed, a large proportion of patients who take procainamide or hydralazine develop autoantibodies, but a smaller fraction develops clinical drug-induced autoimmunity, usually characterized by skin involvement, arthritis, serositis, and constitutional symptoms. In some patients, however, a chronic or severe autoimmune disease can develop.

The identification and withdrawal of the offending drug results in abrogation of drug-induced autoimmunity in the majority of cases. Nonetheless, understanding the mechanisms of drug-induced autoimmunity is important as it can help us better understand the pathogenesis of idiopathic autoimmunity. In a pathogenesis paradigm that includes a complex etiology involving interaction between genetic predisposition and environmental exposures, such as in the case of lupus or vasculitis, drug-induced autoimmunity provides a disease model where the extrinsic environmental disease trigger is well defined.

Hydralazine and procainamide are two drugs known to be associated with high risk of drug-induced autoimmunity. Work done by Bruce Richardson's group found that both drugs inhibit DNA methylation in T cells (2), and that adoptive transfer of hydralazine or procainamide treated CD4+ T cells into mice can cause a lupus-like phenotype (3, 4). The same group demonstrated that procainamide directly inhibits the activity of DNA methyltransferase 1 (DNMT1) in T cells, while hydralazine inhibits the extracellular-signal-regulated kinase (ERK) pathway in T cells resulting in reduced DNMT1

expression (5, 6). These critical early observations in drug-induced lupus led to exploring T cell DNA methylation defects in idiopathic lupus, resulting in establishing epigenetic dysregulation and T cell DNA demethylation as a cornerstone in the pathogenesis of this disease (7).

In the last few years, the role of innate immune response in the pathogenesis of autoimmune diseases has been increasingly recognized. Neutrophils constitute the majority of peripheral blood while blood cells and their involvement in lupus has been suspected at least since the initial description of the Lupus Erythematosus (LE) cell phenomenon (neutrophils phagocytosing a whole nucleus or an apoptotic body). Neutrophils might also be capable of producing type I interferons in lupus (particularly interferon alpha), which plays a key role in the pathogenesis of lupus and other autoimmune diseases (8, 9). In addition, the production of anti-neutrophil cytoplasmic antibodies (ANCA) is the hallmark of ANCA-associated vasculitis. Following the description of a unique neutrophil cell death process involving the formation of neutrophil extracellular traps (NETs), called NETosis, studying the role of neutrophils in autoimmunity has received special attention. During NETosis dying neutrophils form NETs to trap, hinder, and defend against invading pathogens. A genetic defect in NET formation, resulting from a predicted deleterious mutation in the neutrophil elastase gene *ELANE*, has been recently reported in a patient with recurrent parvovirus infection associated with chronic inflammatory arthritis (10). NETosis involves the release of autoantigen-rich nuclear material and granular proteins, and is a process dependent upon nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity providing reactive oxygen species, and peptidyl arginine deiminase (PAD) 4 catalyzed histone citrullination which allows for histone unfolding and chromatin externalization. Deficiency in either NADPH oxidase or PAD4 activity is associated with inability of neutrophils to undergo NETosis (11, 12). Neutrophils in lupus and ANCA-associated vasculitis patients are characterized by an increased tendency for NETosis and reduced NETs degradation, resulting in increased and prolonged exposure to autoantigens expelled within NETs (13). NETs have been shown to stimulate the production of interferon-alpha from plasmacytoid dendritic cells in a Toll-like receptor 9 (TLR9) dependent process (14, 15). Mitochondrial DNA, and more specifically oxidized mitochondrial DNA,

extruded within NETs has been recently shown to be a potent interferogenic DNA enhancing neutrophil-dependent type I interferon production in lupus (16-18). Type I interferons also induce NETosis, and autoantibodies particularly against ribonucleoprotein antigens, ANCA, and antiphospholipid antibodies have been demonstrated to stimulate NETosis (13). NETs have been described in vasculitic lesions in ANCA-associated vasculitis, and renal and skin tissue from lupus patients (19, 20). Recent data also suggest that venous thrombosis in an antiphospholipid syndrome mouse model is NETosis-dependent (21).

Irizarry-Caro and colleagues in this issue of *Arthritis & Rheumatology* demonstrate that hydralazine and procainamide can induce NETosis *in vitro* in neutrophils isolated from healthy donors (insert A&R paper to be published reference). These findings suggest a mechanism for drug-induced autoimmunity involving the innate immune response. Unlike what has been reported in neutrophils isolated from lupus or ANCA-associated vasculitis patients, *in vitro* treatment of healthy neutrophils with hydralazine or procainamide did not impair NET degradation. They show that NET formation induced by both drugs is dependent upon the formation of reactive oxygen species, as NET formation was inhibited with exposure to diphenyleneiodonium chloride, which inhibits neutrophil NADPH oxidase and thereby the generation of reactive oxygen species. Similarly, NET formation was inhibited with a PAD inhibitor, suggesting that hydralazine and procainamide induced NETosis is PAD dependent. Further, they demonstrate that hydralazine increases intracellular calcium levels, and that blocking intracellular calcium release inhibited hydralazine-induced NET formation. Procainamide did not significantly alter intracellular calcium concentrations. Unlike hydralazine, procainamide-induced NETosis was inhibited by the muscarinic receptor antagonist atropine and by M1 and M3 specific muscarinic receptor antagonists. These observations provide potential mechanisms for hydralazine and procainamide induced NETosis. Increase in intracellular calcium concentrations and stimulation of muscarinic receptors have been both shown to induce NETosis (22, 23). Irizarry-Caro and colleagues also demonstrate possible slight differences in the content of NETs induced by hydralazine and procainamide, probably reflecting the observed differences in the mechanisms employed by these two drugs to induce NETosis. Two other drugs sometimes

associated with drug-induced autoimmunity, minocycline and clozapine, did not induce NETosis.

The work by Irizarry-Caro and colleagues implicates a possible role for the innate immune system in drug-induced autoimmunity. The findings suggest a novel role for neutrophils in hydralazine and procainamide-induced autoimmunity, expanding their possible pathogenic mechanism beyond their role as T cell DNA methylation inhibitors (**Figure 1**). It is also possible that both mechanisms act synergistically. Increased NETosis provides a source of autoantigens, and demethylated T cells induced by these drugs are characterized by increased capacity for autoreactivity, inducing B cell co-stimulation and an autoimmune response (**Figure 1**). Future studies performed on neutrophils isolated from patients with drug-induced autoimmunity will help to confirm the role of enhanced NETosis in hydralazine and procainamide induced autoimmunity. To prove a causal relationship, animal studies using hydralazine and procainamide treated neutrophils will be needed, with the realization that neutrophil short half-life will be a limitation. Integrating the findings of this report with previously known mechanisms for drug-induced autoimmunity will be important to consider in future studies. For example, as mentioned above, hydralazine inhibits ERK signaling in T cells resulting in reduced expression of DNMT1, while procainamide directly inhibits DNMT1. Previous work suggested that ERK signaling is activated during NETosis, and that inhibiting ERK signaling inhibits NET formation (24). Is it possible that increased intracellular calcium induced by hydralazine by-passes any possible effect of hydralazine on neutrophil ERK signaling? Or perhaps hydralazine does not affect ERK signaling in neutrophils? Or is it possible that both increased and decreased ERK signaling can induce NETosis utilizing different mechanisms that might or might not involve changes in intracellular calcium concentrations, as multiple diverse pathways have been recently shown to induce NETosis? Lupus neutrophils are demethylated compared to neutrophils from healthy matched controls (25). Procainamide is a DNA methylation inhibitor, so does inhibiting DNA methylation in neutrophils by procainamide or other DNA methylation inhibitors contribute to enhanced NETosis? It is also tempting to explore the possibility that hydralazine and procainamide both inhibit neutrophil DNA methylation in addition to inducing NETosis, thereby exerting a dual pathogenic effect by inducing NETosis and at

the same time enhancing the immunogenic and interferogenic properties of released NETs through an augmented TLR9 stimulation, as TLR9 is sensitive to demethylated DNA (**Figure 1**).

There is an important contrast that can be realized between the initial work implicating DNA demethylation in T cells (adaptive immunity) and the current work implicating increased NETosis (innate immunity) in drug-induced autoimmunity. In the former, the implicated mechanism (T cell DNA demethylation) was first described and characterized in drug-induced autoimmunity and then extended to help understand idiopathic autoimmunity, while in the latter a role for NETosis was initially described in idiopathic autoimmunity. In both cases, however, drug-induced autoimmunity provides an excellent disease model to further understand idiopathic disease. Additional exploration and characterization of drug-induced NETosis and the autoantigen 'cargo' carried within released NETs will help us better understand autoimmune mechanisms. Whether other environmental triggers either previously known to be involved in autoimmunity (such as Epstein-Barr virus infection) or triggers yet to be discovered can contribute to disease pathogenesis, progression, or flares via mechanisms resulting in increased NETosis is something to be considered. Studies to uncover the diverse mechanisms behind drug-induced autoimmunity will collectively provide a better understanding of the role of environmental triggers in idiopathic autoimmune diseases and potentially identify novel therapeutic targets.

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Figure 1

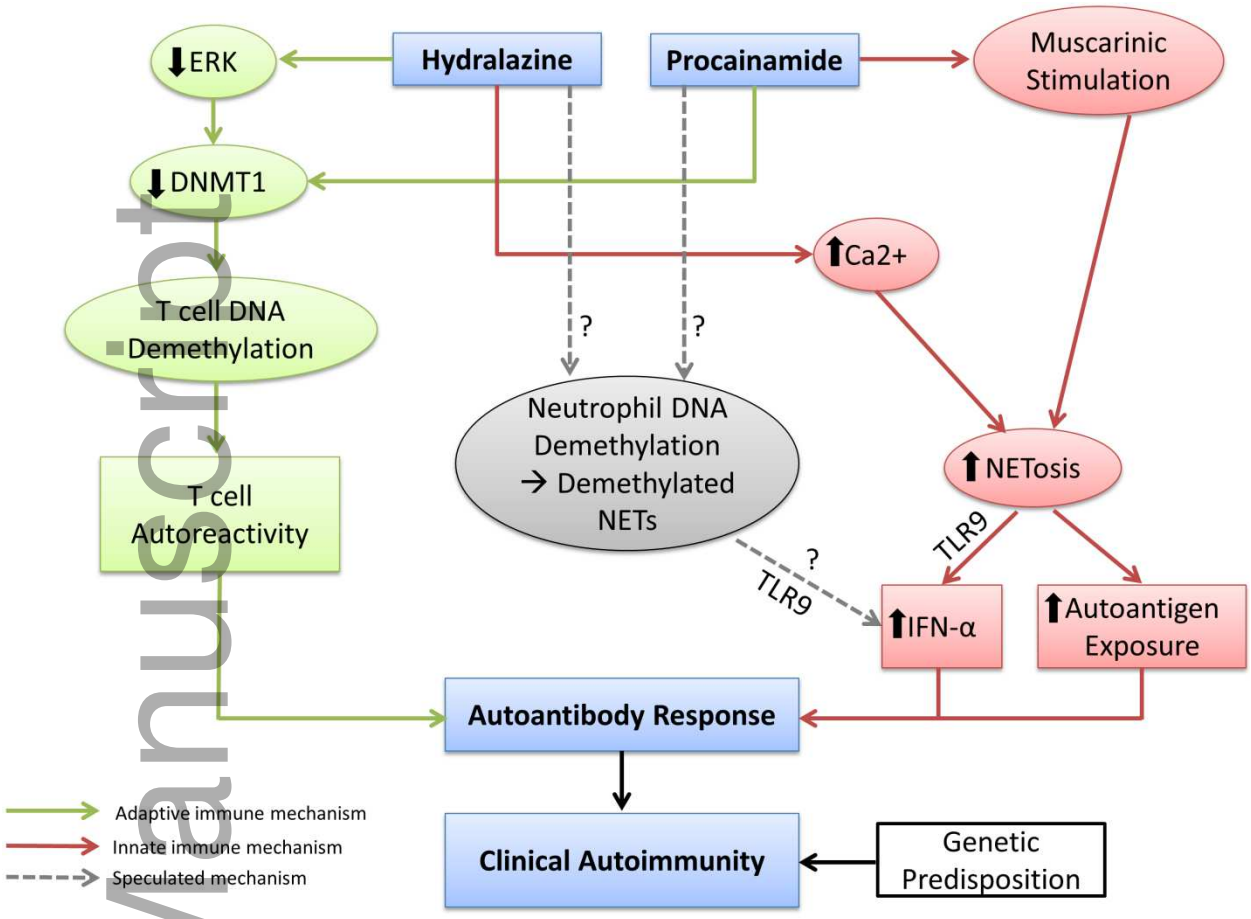
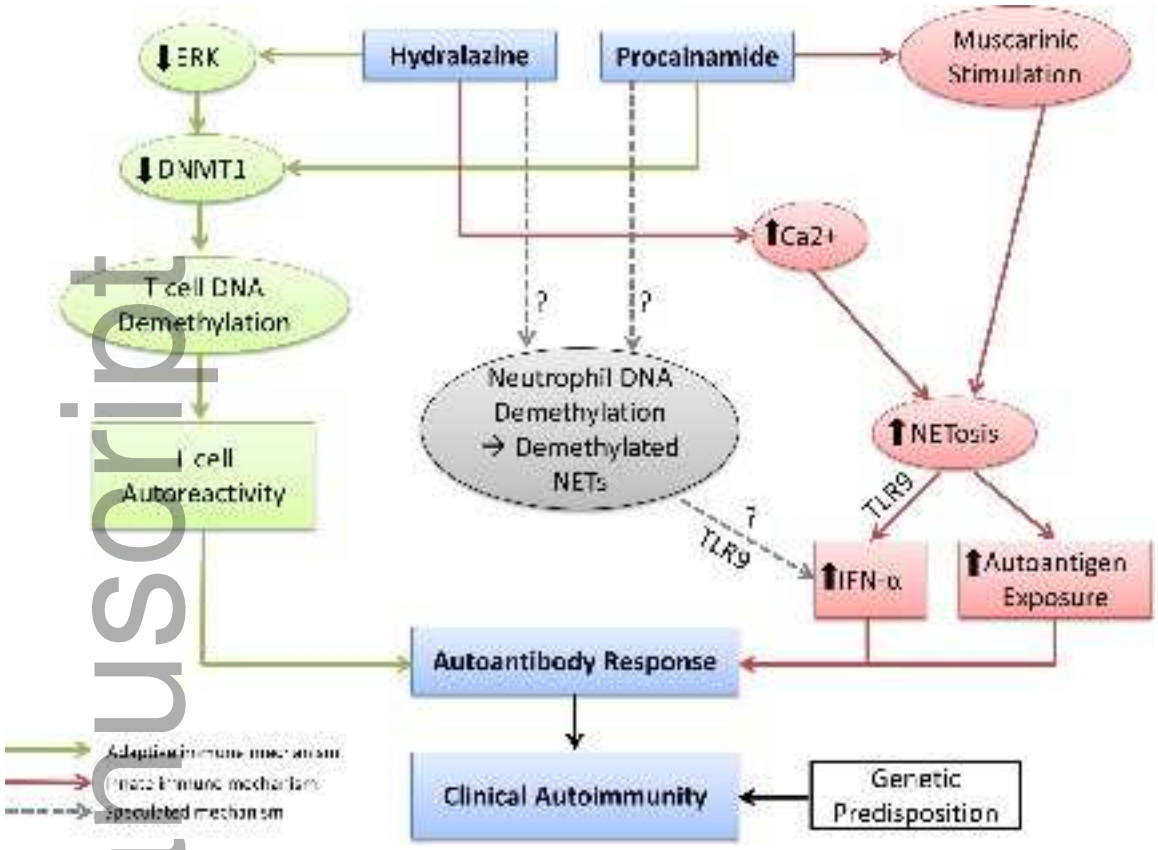


Figure Legend

Figure 1: Drug-induced autoimmunity involves both adaptive and innate immune mechanisms. Both procainamide and hydralazine have been previously shown to inhibit T cell DNA methylation. Hydralazine inhibits T cell ERK signaling pathway which

regulates the expression of DNMT1, while procainamide is a direct inhibitor of DNMT1. Both hydralazine and procainamide induce T cell demethylation, T cell autoreactivity *in vitro*, and T cell-mediated autoimmunity *in vivo*. The present study suggests that both drugs can induce NETosis, implicating the innate immune response in drug-induced autoimmunity. Hydralazine increases intracellular calcium concentrations, while procainamide induced NETosis through stimulation of neutrophil muscarinic receptors (particularly M1 and M3 receptors). NETosis is known to stimulate type I interferon production from plasmacytoid dendritic cells through TLR9 stimulation, and provides a source for autoantigens released within NETs. It remains to be seen if procainamide and hydralazine also inhibit DNA methylation in neutrophils, similar to what has been described in T cells, and if this might induce neutrophil DNA demethylation, similar to what has been described in neutrophils in idiopathic lupus. Demethylated DNA released within NETs might result in enhanced TLR9 stimulation, as TLR9 is sensitive to demethylated DNA.



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