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EDITORIAL

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 US Army 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 individuals to autoimmunity and/or 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 other patients, however, a chronic or severe autoimmune disease can develop.

In the majority of cases, the identification and discontinuation of the offending drug results in abrogation of the drug-induced autoimmunity. 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 in which 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. Research performed by Bruce Richardson and colleagues revealed 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 (DNMT-1) in T cells, while hydralazine inhibits the ERK pathway in T cells which results in reduced DNMT-1 expression (5,6). These critical early observations in drug-induced lupus led to exploration of T cell DNA methylation defects in idiopathic lupus, and the establishment of 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 white blood cells, and their involvement in lupus has been suspected at least since the initial description of the lupus erythematosus cell phenomenon (neutrophils phagocytosing a whole nucleus or an apoptotic body) (8). Neutrophils might also be capable of producing type I interferons in lupus (particularly interferon-α [IFNα], which plays a key role in the pathogenesis of lupus and other autoimmune diseases) (9,10). In addition, the production of antineutrophil cytoplasmic antibodies (ANCAs) 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) (11), called NETosis, 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 (12). NETosis involves the release of autoantigen-rich nuclear material and granular proteins, and is a process dependent upon NADPH oxidase activity providing reactive oxygen species (ROS) and peptidylarginine deiminase type 4 (PAD4)–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

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undergo NETosis (13,14). Neutrophils in patients with lupus and/or ANCA-associated vasculitis are characterized by an increased tendency for NETosis and reduced NET degradation, resulting in increased and prolonged exposure to autoantigens expelled within NETs (15). NETs have been shown to stimulate the production of IFNα from plasmacytoid dendritic cells in a Toll-like receptor 9 (TLR-9)–dependent process (16,17). 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 IFN production in lupus (18–20). Type I IFNs also induce NETosis, and autoantibodies particularly against RNP antigens, ANCA, and antiphospholipid antibodies have been demonstrated to stimulate NETosis (15). NETs have been described in vasculitic lesions in ANCA-associated vasculitis, and renal and skin tissue from patients with lupus (21,22). Recent data also suggest that venous thrombosis in an antiphospholipid syndrome mouse model is NETosis dependent (23).

In a study reported in this issue of *Arthritis & Rheumatology*, Irizarry-Caro and colleagues demonstrated that hydralazine and procainamide can induce NETosis in vitro in neutrophils isolated from healthy donors (24). These findings suggest a mechanism for drug-induced autoimmunity involving the innate immune response. Unlike what has been reported in neutrophils isolated from patients with lupus or ANCA-associated vasculitis, in vitro treatment of healthy neutrophils with hydralazine or procainamide did not impair NET degradation. Irizarry-Caro et al showed that NET formation induced by both drugs is dependent upon the formation of ROS, as NET formation was inhibited with exposure to diphenyleneiodonium chloride, which inhibits neutrophil NADPH oxidase and thereby the generation of ROS. Similarly, NET formation was inhibited with a PAD inhibitor, suggesting that hydralazine- and procainamide-induced NETosis is PAD dependent.

Further, the authors demonstrated that hydralazine increases intracellular calcium levels, and that blocking intracellular calcium release inhibits 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 M_1- and M_3-specific muscarinic receptor antagonists. These observations provide potential mechanisms for hydralazine- and procainamide-induced NETosis. Increases in intracellular calcium concentrations and stimulation of muscarinic receptors have been both shown to induce NETosis (25,26).

Irizarry-Caro and colleagues also report possible slight differences in the content of NETs induced by hydralazine and procainamide, probably reflecting the observed differences in the mechanisms used by these two drugs to induce NETosis. Two other drugs sometimes associated with drug-induced autoimmunity, minocycline and clozapine, did not induce NETosis.

The results reported by Irizarry-Caro and colleagues implicate a possible role of the innate immune system in drug-induced autoimmunity. These findings suggest a novel role of 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 costimulation 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 also be needed, with the realization that the short half-life of neutrophils will be a limitation.

Integrating the findings of Irizarry-Caro and colleagues' study 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 DNMT-1, while procainamide directly inhibits DNMT-1. Previous work suggested that ERK signaling is activated during NETosis, and that inhibiting ERK signaling inhibits NET formation (27). Is it possible that increased intracellular calcium induced by hydralazine bypasses any possible effect of hydralazine on neutrophil ERK signaling? Or perhaps hydralazine does not affect ERK signaling in neutrophils? Or, given that multiple diverse pathways have been recently shown to induce NETosis, is it possible that both increased and decreased ERK signaling can induce NETosis, utilizing different mechanisms that may or may not involve changes in intracellular calcium concentrations?

Moreover, lupus neutrophils are demethylated compared to neutrophils from healthy matched controls (28), and procainamide is a DNA methylation inhibitor, so does inhibition of 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

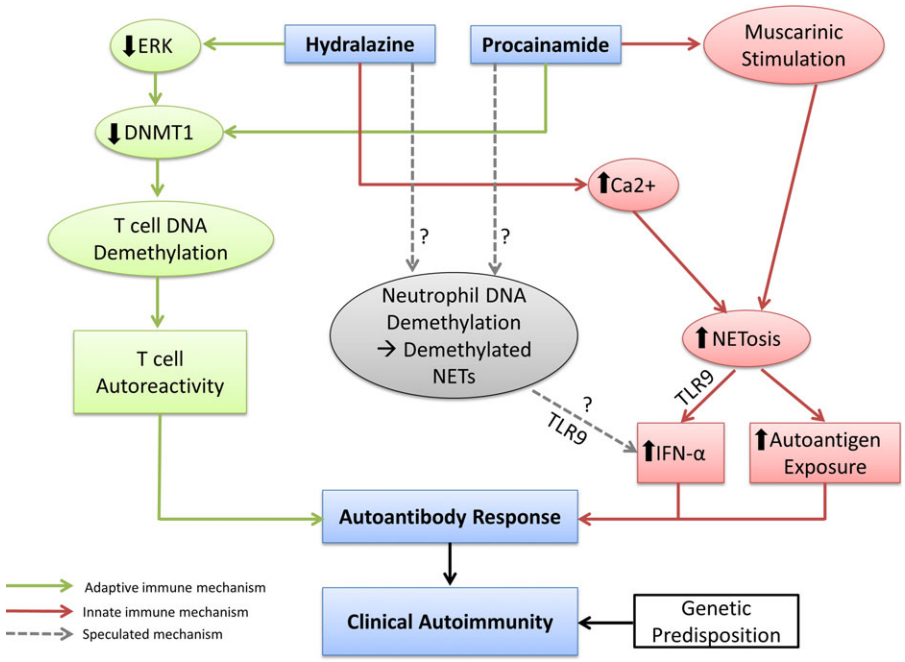

Figure 1. Involvement of adaptive and innate immune mechanisms in drug-induced autoimmunity. Both procainamide and hydralazine have been previously shown to inhibit T cell DNA methylation. Hydralazine inhibits the T cell ERK signaling pathway which regulates the expression of DNA methyltransferase 1 (DNMT-1), while procainamide is a direct inhibitor of DNMT-1. Both hydralazine and procainamide induce T cell demethylation, T cell autoreactivity in vitro, and T cell–mediated autoimmunity in vivo. The results presented by Irizarry-Caro et al (24) suggest that both drugs can induce NETosis, implicating the innate immune response in drug-induced autoimmunity. Hydralazine increased intracellular calcium concentrations, while procainamide induced NETosis through stimulation of neutrophil muscarinic receptors (particularly M_1 and M_3 receptors). NETosis is known to stimulate type I interferon (IFN) production from plasmacytoid dendritic cells through Toll-like receptor 9 (TLR-9) stimulation, and provides a source for autoantigens released within neutrophil extracellular traps (NETs). It remains to be determined whether procainamide and hydralazine also inhibit DNA methylation in neutrophils, similar to what has been described in T cells, and whether 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 TLR-9 stimulation, as TLR-9 is sensitive to demethylated DNA.

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 TLR-9 stimulation, as TLR-9 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 (24). 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 of 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 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 elucidate 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.

AUTHOR CONTRIBUTIONS

Dr. Sawalha drafted the article, revised it critically for important intellectual content, and approved the final version to be published.

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