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Drugs and autoimmunity – A contemporary review and mechanistic approach

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ABSTRACT

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Drug-induced autoimmunity is an idiosyncratic, non-IgE immune related drug reaction. Interestingly, although many drugs have been reported to induce autoantibodies, only a few have a definitive association with drug-induced autoimmune disease. The prototype disease is drug-induced lupus and the typical drug for drug-induced lupus is minocycline. The production of autoantibodies and the induction of symptoms in drug-induced lupus results from a variety of mechanisms, which can include suppression of central or peripheral tolerance, alteration of gene transcription in T and B cells, abnormal cytokine and/or cytokine receptor balance and function, chromatin structure modification and antigen modification. Multiple mechanisms may apply for different drugs, and understanding the pharmacological actions of these agents helps us decipher the etiology. For example, DNA hypomethylation may occur with hydralazine, which leads to increased transcription, increased LFA-1, the generation of autoreactive T cells and a breakdown in peripheral tolerance. Frequently, more than one pathway may be involved. Interestingly, most patients with newly formed autoantibodies resulting from drugs do not develop clinical disease. Nonetheless, the explosion in the use of biological modifiers has been associated with production of autoantibodies, an observation that illustrates the complex nature of these interactions, in that these agents are frequently used to treat autoimmunity, yet may produce autoimmune diseases themselves.

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1. Introduction

Adverse effects of drugs constitute the primary cause of iatrogenic illness in the world, leading to significant morbidity and mortality. It is estimated that drug reactions may occur in anywhere from 5 to 15 percent of therapeutic courses of medication use and serious drug reactions account for about 100,000 deaths in the United States per year. Drug reactions can be classified into two broad categories, those that are predictable (sometimes referred to as type A, intrinsic, drug-dependent or pharmacologic) and those that are unpredictable (type B, idiosyncratic). Predictable drug reactions result from some pharmacologic action of the drug, which may or may not be for its intended use. These generally cause the same effect in the majority of people, and there is usually a well defined mechanism for the effect and a predictable dose response curve. An example of this is the sedative effect of first generation antihistamines. Idiosyncratic reactions, on the other hand, do not cause side effects in all patients, and even in those patients that experience side effects, they are often quite variable in nature. In these cases, the mechanism of action may be more complex, involving various drug effects or host factors, and frequently a combination of these factors. More specifically, genetic susceptibility, the patient's overall health and the co-existence of other disease

states, interaction with other drugs, and other environmental exposures, such as sunlight, may all play a role in the development of idiosyncratic drug reactions. Many cellular and molecular pathways may be involved, and the reason for the unpredictable nature of these reactions is that our own knowledge of the mechanisms of action of each component leading to the drug reaction is incomplete.

Idiosyncratic adverse drug reactions can be further classified into immune related and non-immune related. Immune related adverse drug reactions can be divided into those that are IgE-mediated and those that are not. IgE-mediated reactions are allergic reactions, or hypersensitivity reactions mediated by a known physiologic pathway, and are relatively easy to understand. Drug-induced autoimmunity is a non-IgE immune related event that can involve the production of autoantibodies, and/or the presence of cell mediated reactions against self. The presence of autoantibodies does not always correlate with the existence of clinical symptoms or signs, and conversely, patients may have features of autoimmunity without detectable autoantibodies.

2. Drug-induced lupus

Over 100 autoimmune diseases affect approximately 5–10% of the world's population [1]. Systemic lupus erythematosus is one of the most common of these diseases. Drug-induced lupus (DIL) accounts for about 10% of all systemic lupus cases in the United States, with the incidence being about 15,000–30,000 cases per year [2]. Indeed,

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drug-induced lupus is the most common form of an iatrogenic induced autoimmune disease. Nearly 100 drugs have been associated with lupus, but those that have a well defined relationship include chlorpromazine, hydralazine, isoniazid, methyldopa, minocycline, procainamide and quinidine (Table 1). Of the many drugs that have been associated with DIL, only two, procainamide and hydralazine, are considered to be high risk. In fact, with the exception of quinidine (which is considered a moderate risk), all the other drugs are either low risk or very low risk. The class of drugs and individual drugs that are associated with DIL, along with their assigned risk levels is shown in Table 2.

There are no well defined criteria for the diagnosis of drug-induced lupus. Nonetheless, the most common clinical features of drug-induced lupus include myalgias and arthralgias, serositis and other constitutional symptoms. In general, symptoms are relatively mild, but renal or central nervous system involvement can occur rarely. There is less of a female predominance compared with idiopathic lupus, and patients tend to be older.

An additional difficulty in diagnosing drug-induced lupus lies in the fact that there is no *in vitro* or *in vivo* test that can definitively establish a causative relationship between drug and disease. Laboratory abnormalities seen in DIL include elevated markers of inflammation, and positive (ANA) antibodies. The induction of anti-nuclear factors by isoniazid and hydralazine was described in C57B1/6 mice in 1968 [3]. Since then, specific antibodies against many nuclear and cytoplasmic components have been described. Because the binding of these antibodies is to chromatin, there is usually a homogeneous staining pattern, although a speckled pattern can sometimes be seen. Chromatin is a nuclear complex consisting of histone proteins, non-histone proteins and DNA. Autoantibodies in drug-induced lupus are usually directed against histones, but in tumor-necrosis factor inhibitor induced lupus, anti-dsDNA can be the more frequently detected antibody. The anti-histone IgG antibodies of drug-induced lupus are directed predominantly against the H2A.H2B–DNA subnucleosome complex [4]. The development of lupus associated glomerulonephritis is associated with the

presence of anti-chromatin antibodies; the predominant isotype found in DIL is IgM.

Drug induced subacute cutaneous lupus erythematosus (SCLE) has also been reported. This manifests primarily on sun exposed areas as photosensitive symmetric papulosquamous or non-scarring annular polycyclic lesions. Autoantibodies include ANA, anti-histone antibodies, anti-SSA or anti-SSB. Many drugs and drug categories have been associated with SCLE [2].

3. Mechanisms of action

Large macromolecules like insulin can bind antibody and initiate an immune or allergic response on their own. But most drugs are small molecules, the majority being less than 1000 Da, and are by themselves, not immunogenic. On the other hand, these smaller molecules can bind to carrier proteins and become immunogenic, a process called haptenization. In allergic reactions to drugs, the target antigen is the drug or haptenized drug. However, the drug may instead elicit an immune response to self, leading to the production of autoantibodies. As early as 1976, it was observed that abnormal tests of lymphocytes were associated with drug-induced autoimmunity [5]. A study of patients with drug-induced autoimmunity secondary to practolol showed that disease activity correlated with depressed lymphocyte responses to mitogens. At this time, autoimmunity was thought to result from a breakdown of a suppressor function mediated by T lymphocytes. Since that time, the field of immunology has greatly expanded, with breakthroughs in the discovery of new agents such as toll-like receptors, new roles for known biological mediators, including cytokines and chemokines, the distinction and characterization of the innate and adaptive immune systems and how they co-relate, all of which help us to understand better the mechanisms of action of immune disorders, such as drug-induced autoimmunity. Despite the large number of drugs reported in case studies, only a small number of drugs or drug classes are known to definitively be associated with autoimmunity. Therefore, the known pharmacologic effects of the drugs may provide clues as to how autoimmunity

Table 1
Drug-induced autoimmunity.

Drug	Approximate date of first report	Incidence/cases reported	Diseases/symptom complexes	Clinical features	Autoantibodies reported	Dose dependent
Procainamide	1962	Approx 20%	DIL	Arthritis, arthralgias, fever	ANA, anti-histone antibodies, anti-histone–DNA complex antibodies	Yes
Minocycline	1992	>60 cases	DIL, autoimmune hepatitis, autoimmune thyroiditis	Rash, arthralgias, arthritis, elevated liver enzymes, jaundice	ANA Anti-ds-DNA pANCA	No
Hydralazine	1968	50% autoantibodies, 5–10% autoimmune disease	DIL, vasculitis	Fever, rash, arthralgias, cutaneous involvement, vasculitis, myalgias, pleuritis, leukopenia	ANA, anti ds-DNA, ANCA	Yes
Sulfasalazine	1977	Case reports	DIL, vasculitis	Inflammatory arthropathy, cutaneous vasculitis	Anti-(H2A-H2B)DNA, anti ds-DNA	No
Aromatase inhibitors [101]	1998	3.5%–27.8%	DIL, sicca syndrome, Sjogren's syndrome, inflammatory arthropathies	Arthralgias	Not reported	No data
Isoniazid	1968	Approx 22%	DIL	Arthralgias, arthritis, anemia, fever, pruritis	Anti-(H2A-H2B)DNA	No
Statins	2001	28 cases	DIL, dermatomyositis, polymyositis, lichen planus pemphigoides	Myalgias, polyarthralgias, polyarthritis, leukopenia, photosensitivity, serositis	ANA, Anti Jo-1, Anti-histone, Anti-dsDNA	No
Cyclosporin	1988	N/A	GVH disease – in rat only, scleroderma – like disease	N/A	N/A	No
Quinidine	1988	7 cases	DIL	Polyarthropathies	ANA, anti-histone antibodies	No
Methyldopa	1968	0.02%	Autoimmune hemolytic anemia	Hemolytic anemia	+ Coombs test	No data

Table 2
Risk levels of drugs associated with DIL.

Class of drug	High risk	Moderate risk	Low risk
Anti-arrhythmic	Procainamide	Quinidine	Acetaminide, Amoprofan, Disopyramide, Propafenone
Antibiotics			Cefuroxime, Isoniazid, Minocycline, Nalidixic acid, Nitrofurantoin, Penicillin, Streptomycin, Sulfadimethoxine, Sulfamethoxypyridazine, Tetracycline
Anti-convulsant			Carbamazepine, Diphenylhydantoin, Ethosuximide, Ethylphenacetamide, Mephenytoin, Phenylethylacetylurea, Phenytoin, Primidone, Trimethadione
Anti-depressants			Lithium carbonate, Normifensine, Phenelzine
Anti-fungal			Griseofulvin
Anti-histaminic			Cimetidine, Cinnarizine, Promethazine, Pyrazinazine
Anti-hypertensive			Clonidine, Guanoxan, Methyldopa, Prazosin
Anti-hypertensives – ACE inhibitors			Captopril, Enalapril
Anti-hypertensives – diuretics	Hydralazine		Chlorthalidone, Spironolactone
Anti-hypertensives – β -blockers			Acebutolol, Atenolol, Labetalol, Metoprolol, Oxyprenolol, Practalol, Prindolol, Propranolol, Timolol, eudrops
Anti-migraine			Methylsergide
Anti-parasitic			Anthiomaline
Anti-parkinson			Levodopa
Anti-psychotics			Chlorpromazine, Chlorprothixene, Levomeprazine, Perazine, Perphenazine
Anti-rheumatic			Reserpine, Thioridazine
Aromatase inhibitor			Penicillamine
Biologics			Aminoglutethimide
Chelating agent			Adalimumab, Etanercept, Infliximab, Interferon- α , Interleukin-2
Cholesterol lowering agent			1,2-dimethyl-3-hydroxy-pyridine-4-1
Hormonal			Atorvastatin, Fluvastatin, Lovastatin, Pravastatin, Simvastatin
NSAID			Danazol, Leuprolide acetate
Other			Benoxaprofen, Diclofenac, Ibuprofen, Mesalazine, Para-amino salicylic acid, Phenylbutazone, Sulindac, Sulfasalazine, Tolmetin
Thyroid medication			Gold salts, Metrizamide (radiocontrast), Minoxidil (hair growth), Oxyphenisatin (laxative), Psoralen (anti-psoriatic), Quinine (anti-malarial), Tetrazine, Tolazamide (anti-diabetes)
Xanthine oxidase inhibitor			Methimazole, Methylthiouracil, Propylthiouracil, Thionamide
			Allopurinol

develops. Elucidating the mechanism of action of one class of drug may help explain how others may induce autoimmunity, and may also help us understand the mechanism of action of idiopathic autoimmunity.

The development of autoantibodies as a result of exposure to a target antigen is often explained by the concepts of central and peripheral tolerance. The inability to develop tolerance to self antigens is a component of autoimmunity. On one hand, the drug may not bear any similarity to the antigen to which the autoantibody is directed. But the drug may somehow affect the mechanisms by which we develop tolerance. Sometimes the drug must be metabolized in order for it to exert such an effect. The drug may interact with T cells in the thymus, altering central tolerance, or alternately, may act peripherally. The same drug may act at different sites, and may have more than one mechanism of action for inhibiting tolerance. An alternative mechanism for the development of autoantibodies is based on the concept of cross-reactivity. In this case, the production of antibodies against self occurs because the antigenic determinant of a drug or haptenized drug is similar in secondary and tertiary structure to a self protein. There must also be a distinction made between the production of autoantibodies and the development of an autoimmune disease, since the incidence of autoantibodies is much higher than clinical disease. More recently, the expanded use of biologics has been accompanied by an observation that many patients on these new drugs develop autoantibodies. This is intriguing because many of these drugs are used in the treatment of autoimmune diseases themselves. This has opened up new hypotheses about the mechanism of drug-induced autoimmunity, and it is possible that these drugs may actually have an immunostimulatory role under one set of conditions, while acting in an immunoregulatory manner in a different genetic and environmental circumstance. A number of different mechanisms of action have been proposed, and multiple pathways may be in play at the same time (see Fig. 1).

4. Abrogation of central tolerance by metabolites of procainamide

Procainamide induced lupus was first described in 1962. Since then, this has been the prototype drug for drug-induced autoimmunity. Procainamide treatment for cardiac arrhythmias in 52 patients, who had no previous evidence of lupus or connective tissue disease, was associated with the formation of anti-nuclear antibodies in 43/52 of these patients [6]. Autoantibodies to histones were present in 34/52 patients, with the most commonly detected target being the H2A.H2B dimer. There appeared to be a dose response relationship with the development of these antibodies. Only one of these patients subsequently developed drug-related lupus.

The metabolism of procainamide, as in the case of many drugs, occurs via the action of reactive oxygen species and myeloperoxidase on the drug in an extracellular environment, leading to unstable oxygen rich intermediates with potential autoimmune activity when coming in contact with specific target tissues, such as the lymphoid compartment or the thymus. The generation of these reactive oxygen species occurs as a result of neutrophil activation [7]. The involvement of the thymus and of T cells in the abrogation of central tolerance by pharmacologic agents has been studied by Rubin, who administered two injections of the metabolite of procainamide, procainamide-hydroxylamine (PAHA), into the thymus of normal adult mice [8]. IgM antidenatured DNA antibodies appeared promptly, and this was followed 3 weeks after the second injection by the presence of serum IgG anti-chromatin antibodies. These anti-chromatin antibodies were directed against the (H2A.H2B) DNA histone complex. PAHA has also been found to disrupt the induction of anergy in mature T lymphocytes *in vitro*. It is suggested that PAHA interferes with the development of self tolerance by hampering positive selection in the thymus, leading to an inability of thymocytes to develop tolerance to low affinity self antigens [9,10]. Procainamide may also act via another mechanism, as it also has DNA demethylation activity [11] (see below).

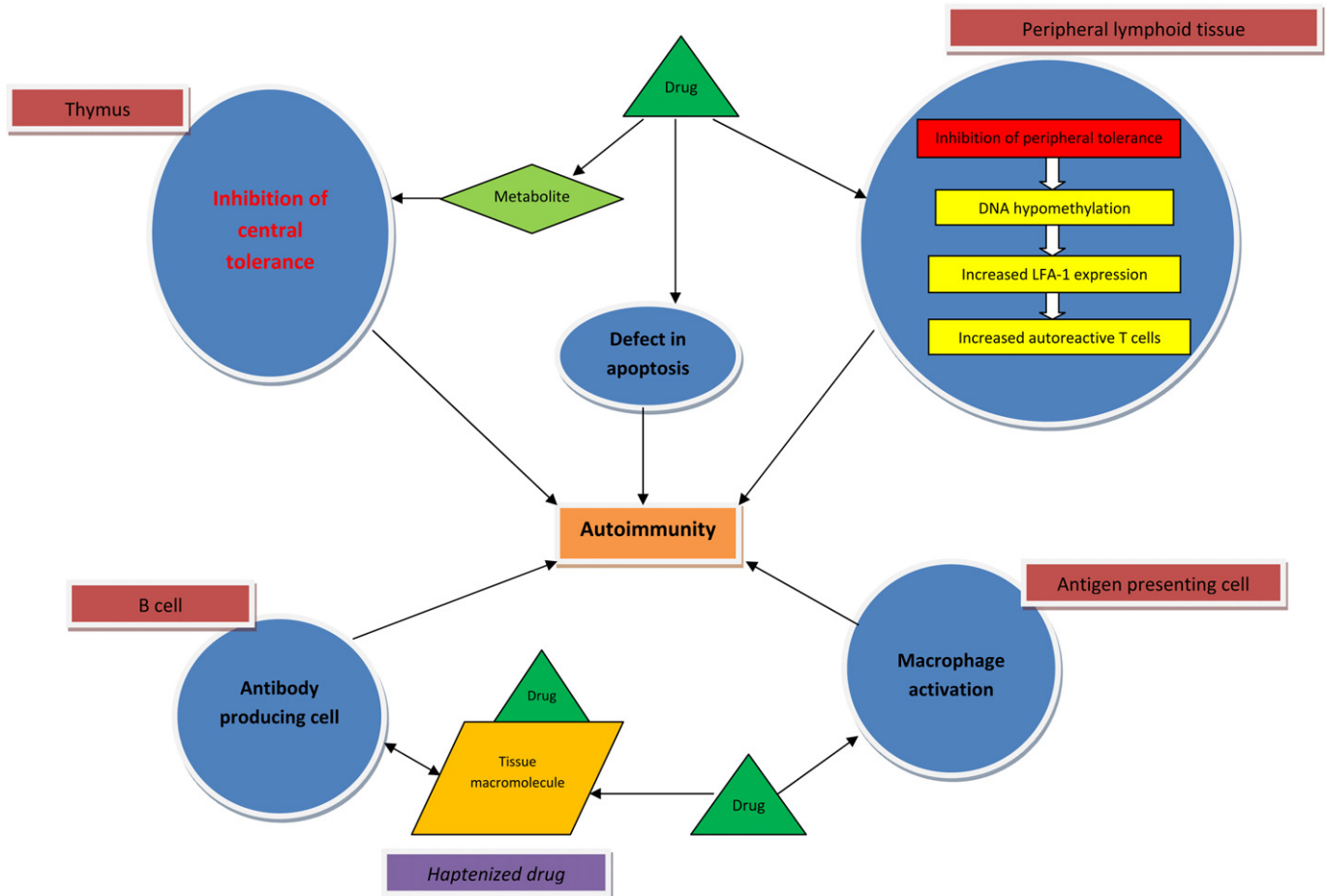


Fig. 1. Mechanism of action of drug-induced autoimmunity.

5. The effects of inhibition of DNA methylation on peripheral tolerance

Early studies pointed to a defect in DNA methylation as a possible mechanism of action for the mitigation of peripheral tolerance, although the cellular effects of DNA methylation were not completely clear at the time. We now know that DNA methylation is a mechanism for regulating gene expression. DNA methylation takes place at the cytosine residues in CpG dinucleotides, and involves the formation of 5-methylcytosines on the fifth carbon of the pyrimidinyl ring. In human DNA, most CpG dinucleotides are methylated. Hypomethylation leads to activation of transcription, while hypermethylation has a suppressive effect; therefore methylation is a natural means to regulate the immune system. Abnormalities in T-cell DNA methylation has been previously implicated in the pathogenesis of systemic lupus erythematosus, and it seemed logical that drugs with the ability to inhibit DNA methylation could also produce a lupus-like illness [12]. It is now clear that drugs that can cause inhibition of DNA methylation may do so via different mechanisms. Procainamide is a competitive inhibitor of DNA methyltransferase (DNMT) [13]. On the other hand, hydralazine does not inhibit DNMT activity. Instead, hydralazine inhibits DNMT expression *in vivo* by inhibiting the ERK pathway signaling, producing an effect similar to that of idiopathic lupus [14]. Inhibition of the T-cell ERK pathway signaling with a mitogen activated protein kinase kinase (MEK) inhibitor can induce anti-dsDNA autoantibodies in mice [15]. The defect leading to the inhibition of ERK pathway signaling in

idiopathic and hydralazine induce lupus has been reported to be an impairment in protein kinase C δ phosphorylation [16]. Many drugs, including procainamide and hydralazine, also upregulate poly(ADP-ribose) polymerase, a chromatin-bound enzyme that may influence both DNA methylation and apoptosis [17,18]. Decreases in mRNA coding for poly (ADP-ribose) polymerase have been observed to occur in lymphocytes of systemic lupus erythematosus patients [19].

Whatever the mechanism for DNA hypomethylation in T cells, this leads to overexpression of LFA-1 (CD11a/CD18), which leads to T cell autoreactivity and produces a lupus-like disease [20]. LFA overexpression was induced on cloned murine Th2 cells by transfection. Upon transfer of these cells into syngeneic recipients, a lupus-like illness ensued, with both production of anti-dsDNA antibodies and pulmonary and glomerular disease [20]. Additional effects of DNA hypomethylation are perforin overexpression and increased CD70 expression. Increased perforin expression leads to increased cytotoxicity of autoreactive CD4 $^{+}$ T cells, and increased CD70 expression leads to B cell overstimulation. Overexpression of LFA-1 [21], CD70 [22] and perforin [23] genes and demethylation of the corresponding regulatory sequences are found in both CD4 $^{+}$ T cells from lupus patients and T cells treated with inhibitors of DNA methylation. This mechanism of action can explain drug-induced lupus caused by DNA methylation inhibitors, procainamide, hydralazine and 5-azacytidine. The end result of these anomalies in T cell signaling is a disruption of normal homeostatic mechanisms leading to the production of autoreactive T cells and abolition of tolerance.

6. Cross-reactivity – possible mechanism of action of minocycline-induced autoimmunity

Minocycline is a semisynthetic tetracycline antibiotic also used in the treatment of acne vulgaris. The first report of minocycline-associated lupus appeared in 1992 [24]. Since then, multiple reports of minocycline-induced lupus have been reported, and minocycline has also been associated with autoimmune hepatitis [25]. A study of 60 minocycline-induced lupus patients and 24 minocycline-induced autoimmune hepatitis patients revealed that 13 of the patients studied had both conditions. In these patients, the typical characteristics included symmetric polyarthralgias or polyarthritis, positive anti-nuclear antibodies and elevated liver enzymes [26]. It is of interest to note that while all of the patients had a positive ANA, the majority of these patients did not have anti-histone antibodies, and some had perinuclear anti-neutrophil cytoplasmic antibodies. Another series of 23 patients with minocycline-induced lupus showed the presence of anti-nuclear antibodies, anti-dsDNA, pANCA, anti-cardiolipin IgG antibodies, but no anti-histone antibodies. 10 of these patients were later rechallenged with minocycline with reoccurrence of symptoms, confirming the association between minocycline and lupus [27]. One possible mechanism of minocycline-induced lupus is that reactive metabolites of minocycline leads to the generation of antibodies against cross-reacting microsomal cytochromes, generating an immune response which ultimately results in degradation of chromatin [26]. The role of genetic susceptibility was suggested by a series of 13 patients with minocycline-induced lupus who were either HLA-DR4 and HLA-DR2 positive and all of whom had an HLA-DQB1 allele with a tyrosine at position 30 of the first domain [28]. Of interest is that all patients had anti-neutrophil cytoplasmic antibodies (11 with antimyeloperoxidase and 10 with antielastase antibodies), suggesting that pANCA can be used as a marker for development of minocycline-induced lupus in genetically susceptible patients.

7. The role of macrophages in the development of autoimmunity – D-penicillamine

D-Penicillamine has been associated with lupus and a variety of other autoimmune diseases, including myasthenia gravis, pemphigus,

pemphigoid, membranous glomerulonephritis, polymyositis and dermatomyositis. An animal model for D-penicillamine-induced autoimmunity developed in Brown Norway rats suggests that the activation of macrophages occurs via the interaction of penicillamine with an aldehyde groups on the macrophage [29]. The same aldehyde group on the macrophage is involved in reversible Schiff base formation with an amine on T cells and is a potential basis for T cell–macrophage interaction. However, in the case of penicillamine, this covalent bonding is irreversible, and the interaction leads to activation of the aldehyde-containing signaling molecule on macrophages. It is postulated that a similar mechanism may occur in hydralazine or isoniazid induced lupus, because both hydralazine and isoniazid can interact with aldehydes, forming hydrazones. The binding of aldehydes on macrophages could be the initial event preceding autoimmunity. The T cell inhibitor tacrolimus can prevent disease onset, reverse ongoing disease, and prevent disease relapse upon re-exposure to D-penicillamine in this mouse model, emphasizing the importance of a T cell role in the mechanism of disease. Experimental depletion of macrophages significantly decreases the incidence of the disease, so both cell types and probably their interaction are important in inducing drug-related autoimmune disease [30]. Enhanced macrophage activation, and increased expression of allele 3 of the solute carrier family 11 member a1 (SLC11A1), has been shown to be associated with autoimmune disease [31]. A downstream consequence of macrophage activation is the increase in IL-15 and IL-1 β production, which leads to NK cell proliferation and the secretion of interferon- γ and GM-CSF, two cytokines that play a role in the further activation of macrophages, suggesting a positive feedback loop between macrophages and NK cells. The activation of macrophages leads to the release of TNF α , IL-6 and IL-23, proinflammatory cytokines that play a role in the development of autoimmunity.

8. Anti-TNF drugs and apoptosis

The recent development of immunomodulatory drugs used in the treatment of rheumatologic diseases and cancer has led to the observation that autoantibodies can develop in conjunction with the use of these drugs (Table 3) [32]. The most widely used immunomodulatory agents include the TNF inhibitors. Tumor-necrosis factor

Table 3
Biologics and drug-induced autoimmunity.

Drug	Classification	Indications	Newly induced autoantibodies	Clinical features/diseases	Possible mechanism of action
Infliximab (Remicade)	Anti-TNF α monoclonal antibody	Psoriasis, Crohn's disease, AS, PA, RA, UC	Anti-dsDNA, nucleosomes, ANA, Anti-cardiolipin	Fever, skin rash, arthralgias, malaise/Leukocytoclastic vasculitis, DIL	Apoptosis, alteration of cytokine profile
Etanercept (Enbrel)	TNF α soluble receptor-fusion protein	RA, JIA, PA, AS, moderate to severe plaque psoriasis	Anti-dsDNA, nucleosomes, ANA, Anti-cardiolipin	Fever, skin rash, arthralgias, malaise/Leukocytoclastic vasculitis, DIL	Apoptosis, alteration of cytokine profile
Adalimumab (Humira)	Anti-TNF α monoclonal antibody	RA, PA, AS, Crohn's disease, moderate to severe chronic psoriasis, JIA	Anti-dsDNA, nucleosomes, ANA, Anti-cardiolipin	Fever, skin rash, arthralgias, malaise/Leukocytoclastic vasculitis, DIL	Apoptosis, alteration of cytokine profile
Certolizumab (Cimzia)	PEGylated Fab' fragment of a humanized monoclonal antibody	Crohn's disease	Not known	Not known	Apoptosis, alteration of cytokine profile
Interferon- α -2b	Pegolated interferon	Hepatitis B & C, melanoma, other malignancies	Antithyroid antibodies, microsomal thyroid antigen, thyroglobulin, ANA, anti-smooth muscle	Autoimmune thyroid disease	Affecting Th1/Th2 balance, modulation of cytokine profile
IL2	Recombinant IL-2	Metastatic cancer	RF, ANA	Inflammatory arthritis	Enhancement of expression of HLA class II antigen

RA = rheumatoid arthritis, UC = ulcerative colitis, PA = psoriatic arthritis, AS = ankylosing spondylitis, JIA = juvenile idiopathic arthritis, ANA = anti-nuclear antibodies, RF = rheumatoid factor.

is a proinflammatory cytokine that is involved in systemic inflammation. Overproduction of TNF α leads to promotion of the inflammatory response that is seen in autoimmune diseases such as rheumatoid arthritis, ankylosing spondylitis, Crohn's disease, psoriasis and even refractory asthma. However, it is now known that TNF α has more than just a stimulatory role, and that it is a major regulator of the immune system as well (a kind of "Master Regulator"). It is the role of TNF α as a regulatory protein that may explain why the use of TNF α inhibitors is associated with the production of autoantibodies [33].

Anti-nuclear antibodies are frequently detectable in patients on TNF inhibitors, but the incidence varies greatly. Most ANA activity is directed against chromatin, as in most other drug-induced lupus syndromes, but in the case of TNF inhibitors, the more common target is ds-DNA, and not histones. A study of 156 rheumatoid arthritis patients receiving infliximab and 37 patients receiving placebo infusions demonstrated the development of anti-dsDNA antibodies in 22 patients on infliximab, but only 1 developed a self-limiting clinical lupus syndrome. Most of the antibodies were of the IgM isotype [34]. In a series of 125 consecutive Crohn's disease patients receiving infliximab, the incidence of positive ANA was 56.8% (71 patients), with greater than 75% developing autoantibodies after fewer than three infusions [35]. Anti-dsDNA, anti-ssDNA and anti-histone antibodies were the most frequent antibodies seen in 43 of the 71 patients with positive ANA who were subtyped. Only 2 of the 71 seropositive patients developed clinical symptoms of lupus. Other autoantibodies described in infliximab induced lupus include anti-cardiolipin IgM and IgG [36] and antibodies to histones [37].

While infliximab, like adalimumab, are monoclonal antibodies directed against TNF α , etanercept is a soluble receptor-fusion protein. While the data is inconclusive, most studies have shown that infliximab causes a higher incidence of autoantibodies than does etanercept. However, autoantibodies have been detected in etanercept treated patients. A comparison study of infliximab and etanercept demonstrated positive ANAs in 62% of spondyloarthropathy patients on infliximab compared with 15% on etanercept. Patients with rheumatoid arthritis treated with infliximab showed a 41% rate of newly developed ANAs, compared to 10% for etanercept. None of the etanercept patients developed anti-dsDNA antibodies, compared to 71% and 49% in the infliximab treated spondyloarthropathy and rheumatoid arthritis groups respectively. Both etanercept and infliximab have also been reported to be associated with leukocytoclastic vasculitis [38].

A recent review of the literature evaluated reported cases of TNF α induced DIL, and found 53 cases in 27 papers. After exclusion of 20 cases that either only had cutaneous manifestations or had overlap syndromes, they found that 21 cases were related to infliximab, 10 to etanercept and 2 to adalimumab. They noted a higher percentage of cases with antibodies to ds-DNA, rash and hypocomplementemia and renal disease compared to DIL resulting from other drugs [39].

While the incidence of autoantibodies appears to be quite different for infliximab versus etanercept, clinical manifestation of lupus has been reported to occur with similar frequencies with both drugs. Shakoor et al. describes four patients who developed clinical signs and symptoms of systemic lupus erythematosus while receiving etanercept for the treatment of rheumatoid arthritis [40]. The diagnosis was established by a combination of clinical presentation and the development of autoantibodies, which were either against ds-DNA or histones. In each of these patients, withdrawal of etanercept led to symptom resolution within 6 weeks. A MEDLINE search analysis of 233 cases of patients who developed autoimmune diseases while on anti-TNF drugs for a variety of conditions revealed that 105 had been on infliximab, 96 on etanercept and 21 on adalimumab [41]. Of the 92 who developed drug-induced lupus, 40 had been on infliximab, 37 on etanercept and 15 on adalimumab.

The mechanism of action of TNF-inhibitor induced lupus is unknown. Several theories exist [42]. It is possible that TNF inhibitors, as well as other drugs, interfere with normal apoptosis, leading to a decreased clearance of autoreactive T and B cells [43]. In addition, without apoptosis, cell death is unregulated and haphazard, and may be associated with impaired clearance of cellular waste, including nuclear material [44]. Accumulation of nucleosomes and their breakdown products in a genetically susceptible host may result in the development of autoantibodies. TNF inhibitors are not the only drugs in which apoptosis may be a contributing mechanism to autoimmunity. Minocycline, for example, has been implicated as an inhibitor of caspase-dependent and caspase-independent apoptosis, and the failed apoptosis of damaged keratinocytes leads to the development of autoimmunity [45].

The effect of TNF α and interferon- α on the Th1/Th2 balance may also prove to be a factor in determining the quantity and activity of the different cytokines. Blockers of TNF α increase production of Th2 cytokines that favor antibody production, such as IL-4, IL-5, IL-6, IL-10 and IL-13. TNF blockers may also increase expression of interferon- α , an effect that may produce contrasting effects. Type I interferons are classically thought to be promoters of Th1 inflammation, but they can also inhibit Th1 and Th17 mediated inflammation. What exactly occurs may vary with different drugs and may also depend on the disease being treated.

Another factor to consider in the discussion of TNF inhibitors is that frequently patients on these drugs developed increased infections [46]. There is evidence that infections can induce autoimmune diseases [47,48], as in the case of autoimmune thyroiditis, rheumatic fever or Guillian–Barre syndrome [49,50]. It has been shown that unmethylated CpG motifs in bacterial DNA can stimulate B cell activation. Other effects of CpG include activation of CD86 expression, and the induction of interleukin-6 (IL-6) synthesis by B cells, and interferon- γ by Th1 cells and NK cells. In 8 rheumatoid arthritis patients treated with etanercept 25 mg twice weekly for 85 weeks, anti-cardiolipin IgG and anti-DNA were found to be present when the subjects experienced a nasal or bronchial infection due to *Staphylococcus aureus* or a urinary tract infection due to *Escherichia coli*. These levels decreased to normal levels after antibiotic treatment. In a control group of 39 patients treated with other DMARDs, anti-cardiolipin antibodies were also seen, but the frequency of infections was less than that of the etanercept group [51].

9. Mechanism of action of other biologics – effects of cytokine profile changes

Cytokine profiles and cytokine levels can also play a role in drug-induced autoimmunity. These effects can affect signaling pathways. A study on neonatal exposure in a Sjogren's mouse model (NFS/sld mice) to low-dose 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which has been shown to influence immune responses, demonstrated the appearance of autoimmune lesions in the salivary glands and other organs [52]. An increase in the production of IL-2 and IFN- γ from splenic T cells was also observed. Autoantibodies relevant to Sjogren's syndrome were also found in the sera of the mice exposed to TCDD, demonstrating that TCDD upregulated expression of aryl hydrocarbon receptor in the thymus of neonatal mice.

Treatment with interleukin-2 and lymphokine-activated killer cells in 34 patients with advanced neoplasms was associated with the development of goiter, hypothyroidism and autoimmune thyroiditis [53]. The development of inflammatory arthritis in 3 patients who were receiving IL-2 for metastatic cancer has been reported as well. The mechanism is unknown, but IL-2 is a cytokine produced by activated helper T cells than can enhance NK cell activity and antigen-specific cytotoxic T cell activity, and it appears that autoimmunity correlates with regression of the cancer.

The success of treatment of melanoma with interferon α -2b seems to correlate with the appearance of autoantibodies or the development of clinical manifestations of autoimmune diseases [54]. Of 200 patients with advanced melanoma, those who had autoimmunity had a significantly longer relapse free survival and overall survival. A similar effect has been demonstrated to occur with anti-cytotoxic T lymphocyte antigen 4 (CTLA-4) antibodies for the treatment of advanced melanoma [55]. This coupling of autoantibodies and treatment success suggests that blocking T cell regulatory functions may lead to the development of autoantibodies and/or autoimmune reactions, but also increases tumorlytic activity at the same time. In another study of 135 patients with carcinoid tumors treated with interferon- α , newly diagnosed autoimmune disorders, mostly thyroid disease, occurred in 25 patients after a median of 9 months of therapy [56]. However, in this case, there was no correlation between the development of autoimmunity and tumor response to interferon- α .

Immunologic effects of interferon- α that could potentially lead to autoimmune disease include the inhibition of regulatory T cell function, activation of antigen presenting cells leading to the production of cytokines and other mediators, enhancement of immunoglobulin production, the development of anti-interferon antibodies that may result in circulating immune complex, and also the enhancement of expression of major histocompatibility complex class I and II antigens which may react with autoreactive T or B cell clones. One should keep in mind that these changes have not been observed in patients who developed autoimmune diseases after interferon- α treatment, and these mechanisms, though proposed nearly two decades ago, are merely speculative at this time [57].

10. Mechanism of action of other drugs associated with autoimmunity – isoniazid, chlorpromazine, methyldopa, quinidine – is there a common mechanism?

Quinidine has been implicated in the development of autoimmune polyarthropathies attributed to a drug-induced lupus syndrome [58]. Not all patients with polyarthropathy had autoantibodies. The lack of autoantibodies was associated with a milder form of the disease. Rechallenge with quinidine causes recurrence of the disease. Anti-histone IgG antibodies to H1, H2A.H2B complex, and H3.H4 complex were detected but not to the core histones. Antiphospholipid antibodies have also been detected in a case of quinidine induced hypoprothrombinemia [59]. Quinidine, as well as procainamide, has also been found to affect the way macrophages handle apoptotic and necrotic thymocytes [60]. Recent evidence has suggested that the autoimmune response in systemic lupus erythematosus involves an inability of macrophages to clear apoptotic cell particles, in particular breakdown products of nucleosomes, thus facilitating development of autoantibodies to these particles [61]. The alteration in this aspect of macrophage function may also contribute to the development of autoimmunity.

Reports of an association between sulfasalazine and drug-induced lupus in two patients demonstrated the presence of IgG antibodies against the H2A-H2B DNA complex [62]. Several other case reports of sulfasalazine induced lupus have been reported over the years. In another study, four out of forty-one rheumatoid arthritis patients on sulfasalazine developed rashes and/or positive ANA [63]. These four patients seemed to have higher baseline serum IL-10 levels compared to control RA patients who did not develop autoantibodies or lupus-related symptom, but the serum IL-10 level decreased upon initiation of sulfasalazine. It was also found that increased IL-10 levels correlated with SLE associated HLA-haplotypes, suggesting a genetic susceptibility for drug-induced lupus-related side effects. Nonetheless, the mechanism of sulfasalazine induced lupus is unknown, and while sulfasalazine has been observed to be related to reductions in proinflammatory

cytokines such as TNF α , IL-1 and IL-6, the relationship of cytokines to the development of drug-induced autoimmunity remains speculative.

11. Statins and autoimmunity

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, also known as statins, are used for primary and secondary prevention of atherosclerosis associated cardiovascular events. A recent 2007 review revealed 28 cases of statin related autoimmunity [64]. Ten cases were systemic lupus erythematosus, 3 were subacute cutaneous lupus erythematosus, and there were seven cases each of polymyositis and dermatomyositis. The remaining case was one of lichen planus pemphigoides. A proposed mechanism involves the proapoptotic effects of statins [65], leading to the release of nuclear antigens which can induce the production of autoantibodies. This mechanism is analogous to the effect of ultraviolet light as a triggering factor for exacerbations of skin lesions of dermatomyositis and lupus [66,67]. Statins may also have direct effects on T cells, shifting the balance of T-helper cells from a Th1 to a Th2 bias, and thereby, through previously discussed pathways, leading to the production of autoantibodies and clinical autoimmunity. On the other hand, it is interesting to note that a therapeutic role of statins in the treatment of autoimmunity has also been suggested, based on the putative role of cholesterol homeostasis in the maintenance of key cell membrane structures such as lipid rafts. This has led some to propose that statins can have disruptive influences on those processes that utilize these structures, such as T cell signaling pathways [68], and thereby reduce the disease activity.

12. Other agents and autoimmunity

Chlorpromazine induced lupus erythematosus has also been described. It has been found that chlorpromazine induces apoptosis in human lymphoblasts by stimulating proapoptotic signaling pathways [69]. This is in contrast to the mechanism proposed for TNF α induced autoimmunity, in which case a defect in apoptosis leads to the failure to eliminate autoreactive B and T cells, leading to a breakdown of central or peripheral tolerance. Further research on the death receptor, and the regulation of apoptosis could potentially help sort out these apparently contrasting theories, and improve our understanding of autoimmunity.

Cyclosporin-induced autoimmunity has been described in rats, and is a thymus dependent, T cell mediated animal model for human scleroderma [70]. Cyclosporin is an immunosuppressive agent used in the treatment of autoimmune diseases and cancer, and acts by blocking translocation of NF-AT from the cytoplasm to the nucleus in the pathway for T cell signaling after T cell receptor ligation. One would expect that cyclosporin would therefore prevent the development of autoimmunity. In fact, cyclosporin, like so many biological modulators, has multiple effects on the thymus and on *de novo* T cell development. It is postulated that cyclosporin inhibits apoptosis of thymocytes that would normally have undergone negative selection. However, the subsequent increased in thymus derived autoreactive T cells has been observed to be insufficient to produce autoimmunity, unless accompanied by UV irradiation in the periphery. In fact, it has been suggested that in the absence of UV radiation, cyclosporin has a protective or regulatory effect on the generation of autoimmunity, which will last until cyclosporin administration is discontinued.

Autoimmune cytopenias have developed in association with a number of drugs including chemotherapeutic agents. Cyclophosphamide has been reported to negatively affect function of T reg cells and has been associated with autoimmune disease in [71].

Table 4
Possible mechanisms of action of drug-induced autoimmunity.

Potential mechanism	Pathway	Effect	Drugs for which this may apply
DNA demethylation	ERK pathway inhibition, increase in gene transcription, LFA-1 overexpression, increased autoreactivity	Interferes with peripheral tolerance	Hydralazine, procainamide, 5-azacytidine
Blocking T-cell signal transduction	Prevents thymic deletion during negative selection	Interferes with central tolerance	Cyclosporin A
Adenosine diphosphate ribosylation	DNA demethylation, apoptosis	Interferes with peripheral tolerance	Procainamide, hydralazine
Histone deacetylase inhibition	Increased acetylation of histones leading to increased gene expression	Interferes with peripheral tolerance	Sodium butyrate, trichostatin A
Macrophage activation	Covalent bonding of drug and aldehyde-containing signaling molecules, interaction between T cell and macrophage	Inhibits peripheral tolerance	D-penicillamine, hydralazine, isoniazid
Antigen modification/haptenization	Binding of drug to tissue macromolecules, rendering it immunogenic	Cross-reactivity or production of neoantigens	Minocycline
Defective <i>de novo</i> T cell development	Increase in Th1 T cells and decrease in regulatory T cells	Preponderance of Th1 over Th2 cells	Cyclosporin A
Apoptosis	Increase in autoreactive T and B cells, increase in nuclear debris	Generation of autoantibodies to nuclear matter	Chlorpromazine, TNF inhibitors, minocycline

Autoimmune hemolytic anemia has been reported to occur with methyl dopa therapy [72]. Evan's syndrome is a rare condition in which autoantibodies are directed against red blood cells and platelets. Thus it is a combination of autoimmune hemolytic anemia and autoimmune thrombocytopenic purpura. Patients with Evan's syndrome have a decreased T4 and increased T8 cells with a decreased T4/T8 ratio. These patients have CD4⁺/CD8⁺ T cells which is a predictor of autoimmune lymphoproliferative syndrome. A handful of drug-induced Evan's syndrome patients have been described, related to the drugs oxaliplatin [73] (for advanced colorectal cancer), fludarabine [74] (for chronic lymphocytic leukemia), diclofenac [75] and ramipril [76].

In addition to traditional drugs, one must not forget that other environmental agents such as heavy metals have been associated with autoimmunity. The most well characterized of these is mercury [77]. In genetically susceptible mice, mercury exposure has been shown to induce an autoimmune disease characterized by hypergammaglobulinemia, lymphoproliferation and the presence of IgG antinuclear antibodies. It was shown, using a mouse strain that is deficient for the Fcγ-chain or FcγRIII, that Fc-receptors can be important in the determining activation or inhibition of immune responses [78]. Others substances associated with autoimmunity include iodine [79,80], vinyl chloride [81], canavanine [82], organic solvents [83], L-tryptophan [79], anilides [84], vaccines, microbials, and a variety of pollutants including particulate silica [85], airborne particulate pollution, ozone [86], and ultraviolet radiation [87,88]. The current swine flu pandemic has renewed interest in the role of vaccine induced autoimmunity [89], as we recollect the high association between the influenza vaccine used in the last swine flu epidemic and Guillain Barre syndrome [90]. A report of SLE and the development of autoantibodies to Smith antigen and the U1 nuclear ribonucleoproteins (nRNPs) following the administration of EBV nuclear antigen EBNA-1 in mice and rabbits, further illustrates the link between vaccines and autoimmunity.

Understanding why there is difference in gender predominance between idiopathic and drug-induced autoimmunity will improve our knowledge of the mechanisms of these diseases [91,92]. Epidemiological studies can help identify patients who are at risk for developing drug-induced autoimmunity, as well as other autoimmune diseases [93]. Delineating the relative contributions of genetic susceptibility [94,95] and environmental triggers [96–98] will help identify patients who are at risk for receiving certain drugs or therapeutic agents, and hopefully will enable us to make choices of medications for individual patients based on a better understanding of these mechanisms.

13. Diagnosis and treatment of drug-induced autoimmunity

Until we understand better the mechanisms leading to disease, our treatment of drug-induced autoimmune disease is limited. Correct diagnosis is critical, part of which involves establishing a temporal relationship between the onset of drug use and disease. Unfortunately, this may not be possible because features of DIL may take up to several months to develop following exposure to the drug. Moreover, there does not seem to be any correlation between drug dosage and incidence or severity of the disease, except in the case of procainamide and hydralazine.

Unfortunately, precise diagnostic criteria for drug-induced autoimmunity have not been established, and clinical signs and symptoms may vary widely from patient to patient. Moreover, since many of these drugs are sometimes used in patients with autoimmune diseases, the diagnosis of a new autoimmune disease against the backdrop of existing autoimmunity is sometimes challenging. Luckily, removal of the offending drug frequently will reverse the clinical course of the disease, but is has been demonstrated that autoantibodies can persist for a long time afterwards. Corticosteroids remain the mainstay of treatment, but this is not without inherent risks itself. Nonetheless, if in doubt, stop the drug.

Recently, micro RNAs (miRNAs) have been found to play a role in the post-transcriptional regulation of gene expression by virtue of its action on the 3'-untranslated region (UTR) of specific messenger RNAs [99]. Whether or not this form of emerging therapy will become useful in drug-induced autoimmunity or even idiopathic autoimmunity remains to be seen [100].

14. Summary

The mechanism of action of drug-induced immunity is different for various classes of medications (4) (Table 4). In the end, the production of autoantibodies results from a loss of tolerance to self antigens. Signaling pathways in T and B cells, activation of macrophages, direct effects of metabolites of the drugs leading to the production of neoantigens, effects on cytokine profiles and cytokine levels, haptenization and the development of cross-reacting antigens can all play a role in the development of drug-induced autoimmunity. These effects may act in concert or by themselves, but it is clear that the generation of autoantibodies is not sufficient to cause clinical disease. As we decipher the mechanism of action of systemic lupus erythematosus, this may likely be applicable to drug-induced autoimmunity as well. Cellular targets for the development or autoimmunity that may be common for the

idiopathic and drug-induced forms of the disease include mediators of apoptosis including Fas, Fas ligand, B cell survival factors such as Bcl2/BclX or BAFF and Poly ADP ribose polymerase (PARP). Other targets may be regulatory proteins such as the cytokines, especially TNF α , interferon- γ and interferon- α , but may include compounds and pathways of the innate immune system such as Toll-like receptors and the Jak-STAT pathway. All of these factors and cells interact in a complicated manner in immune system homeostasis, so it is not difficult to see why the development of drug-induced autoimmunity is likely to be a complex process involving genetic, epigenetic and environmental factors. Further research is needed to clarify and develop the precise cellular mechanisms for the pathogenesis of drug-induced autoimmunity. As we learn more about these drugs, host factors and their interaction, we may one day reach the goal of being able to predict which patients will develop autoimmune diseases when exposed to certain drugs [97].

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