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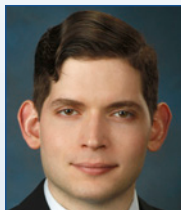
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## Newer Treatment Strategies for Autoimmune Diseases

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In contemporary parlance, autoimmune disease has been used as a designation for a variety of chronic inflammatory disorders that are characterized by the presence of autoantibodies. This rubric has been applied to diseases whose etiologies are not infectious, neoplastic or degenerative in nature. The autoantibody profile may simply include a positive antinuclear antibody (ANA) test or it may be further defined by autoantibodies with specific identifiable autoantigens. By this description, autoimmune disease encompasses a wide variety of disorders manifesting as chronic inflammation confined to a tissue or organ or as a systemic inflammatory disease. Although, this nosology may serve to define syndromes that may be amenable to specific anti-inflammatory or immunosuppressive treatments, it has not served to advance our understanding of the etiology of these diseases, nor does it imply that the autoantibodies themselves directly participate in the pathogenesis of the disease. While these diseases may be “immunologically-mediated”, there is often very little evidence that these autoantibodies actually are involved in the disease pathogenesis.

A stricter definition of autoimmune disease would include the stipulation, that not only should autoantibodies be present, but that there is evidence to support the notion that they actually participate in the etiopathogenesis of the disorder. This definition adds a more rational framework for understanding autoimmune disease. It also allows for a better characterizing of the immunopathogenesis of these

syndromes and can more readily allow for immunotherapy directed at specific pathways (“targeted therapies”).

In this issue of the *Rhode Island Medical Journal*, we review four inflammatory syndromes traditionally considered to be autoimmune in nature. By the above definition, two of these (SLE, pemphigus) would be viewed as autoantibody-mediated but the other two (cytopenias, CIDP) both likely fit the criteria even though the demonstration of specific autoantibodies in these disorders has been elusive. Other immunologically-mediated diseases (rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, myasthenia gravis, and autoimmune thyroid and liver disease) should also be viewed as autoimmune in nature. There have been significant developments in immunomodulatory therapy as reviewed in the introductory article by Lefebvre and McAuliffe. There is convincing evidence that the diseases listed in this review article are characterized by antigen-driven T-Cell activation and subsequent pro-inflammatory cytokine generation. However, effective strategies to abrogate T-cell activation and block resultant cytokines or cytokine receptors have outpaced the ability to identify specific triggering antigens or subsequent autoantibodies that are pathogenic. Nonetheless, the advent of such sophisticated targeted therapies will undoubtedly improve management and outcomes for immunologically-mediated diseases, some, but not all, of which should be considered auto-immune in nature.

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# Targeted Immunomodulatory Therapy: An Overview

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## ABSTRACT

Monoclonal antibodies and other biologic response modifiers have allowed for targeted drug therapy in managing various autoimmune diseases. A number of immune pathways have been exploited in the development of targeted immunomodulatory therapies, including cytokine-directed therapies such as tumor necrosis factor-alpha and interleukins, integrins, B-cells, and co-stimulation modulators. With new targeted therapies in the pipeline, more options are becoming available for treatment of autoimmune diseases.

**KEYWORDS:** monoclonal antibodies, biologic response modifiers, immunomodulatory therapy

## INTRODUCTION

With major advances in genetic sequencing and biomedical research, targeted therapy with monoclonal antibodies (mABs) has emerged as a successful strategy for managing autoimmune diseases. Treatment with mABs has the advantage of modifying specific immune pathways as opposed to other non-specific therapies. The first mAB (muromonab CD3) was developed from mice and approved in 1986 to prevent rejection of a kidney transplant.<sup>1</sup> However, this first generation of mABs was not well-tolerated due to foreign recognition of the murine components by the patient's immune system.<sup>2</sup>

Since then, different approaches to producing chimeric (part mouse, part human) and fully humanized mABs have been discovered, rendering mABs less immunogenic. One such approach to producing mABs is from hybridomas, formed from the fusion of B-lymphocytes and immortal myeloma cells.<sup>1</sup> The B-lymphocytes are obtained from the spleens of mice after they have been immunized against a specific antigenic determinant, or epitope.<sup>1</sup> The hybridomas are cultured, leading to the generation of polyclonal antibodies. The polyclonal culture is screened for the desired antibody activity and then cloned.<sup>1</sup>

The World Health Organization has policies for nomenclature of mABs.<sup>3</sup> The structure is composed of four parts: the prefix, substem-A, substem-B, and suffix. Substem-A indicates the nature of the target of the mAB, such as tumor or cardiovascular. Substem-B indicates the originating

species of the mAB (i.e. human, mouse, chimeric, etc.). The suffix "-mab" is common to most mABs.<sup>3</sup>

## CYTOKINE-DIRECTED THERAPIES

### TNF $\alpha$ Inhibitors

Tumor necrosis factor-alpha (TNF $\alpha$ ) is a cell-signaling protein, or cytokine, that induces cell proliferation and differentiation through its interaction with TNF receptors on cell surfaces. TNF $\alpha$  plays a role early in many inflammatory immune processes. It is produced primarily by macrophages, but also by monocytes, B-cells, and other tissues. Activation of TNF $\alpha$  also leads to the secretion of interleukin (IL)-1 and IL-6, both proinflammatory cytokines. Dysregulation of TNF $\alpha$  can lead to the development of various autoimmune diseases such as rheumatoid arthritis (RA), inflammatory bowel disease (IBD), ankylosing spondylitis, and psoriasis.<sup>4</sup> For instance, in IBD, TNF $\alpha$  secretion leads to the stimulation of endothelial cells to express adhesion molecules, facilitating migration of various white blood cells into inflamed tissue.<sup>5</sup>

TNF $\alpha$  inhibitor therapies are recombinant IgG mABs that essentially serve as decoy TNF receptors. They bind to TNF $\alpha$  molecules and prevent their interaction with TNF receptors, ultimately leading to suppression of the immune system and inflammatory responses. Examples of TNF $\alpha$  inhibitors are adalimumab and etanercept, with the latter possessing a longer half-life due to its dimeric nature. The goal of treatment with TNF $\alpha$  inhibitor therapy is to reduce inflammation and severity of symptoms, with the hope of achieving improved quality of life.

### Interleukins

Interleukins are a large class of cytokines responsible for various immune responses, including inflammatory response mediation, lymphocyte growth and differentiation, and immune cell chemotaxis, which can be implicated in autoimmune diseases. Interleukin-17A (IL-17A), produced largely by T-helper 17 cells (Th17), acts directly on keratinocytes to stimulate various pro-inflammatory processes in plaque psoriasis.<sup>6</sup> Interleukin-12 (IL-12) and interleukin-23 (IL-23) have been implicated in the production and development of Th17 cells, leading to psoriatic plaques and joint inflammation in psoriatic arthritis.<sup>7</sup> In RA, IL-6 is released directly by synovial cells and macrophages into the synovium causing inflammation and destruction.<sup>8</sup>

Monoclonal antibodies targeting interleukins are directed at cytokines involved in the production of interleukin, the interleukin itself, or receptors at which interleukin exerts its effect. Ustekinumab was developed against the p40 subunit of both IL-12 and -23, both important in differentiation of naïve T-cells to Th17 cells that produce IL-17A. Ustekinumab binds the p40 subunit of IL-12 and -23, resulting in reduced levels of Th17 cells.<sup>9</sup> Secukinumab, an anti-IL-17A mAB, has been approved for treatment of plaque psoriasis. Inhibition of IL-17A prevents triggering of signaling and recruitment of numerous innate immune cells such as mast cells, neutrophils, and macrophages to psoriatic plaques.<sup>10</sup> Tocilizumab, an anti-IL-6 receptor (IL-6R) recombinant mAB, blocks IL-6 signal transduction by binding to IL-6R embedded in the cell membrane and floating in soluble form in the blood. It also can dissociate already formed IL-6/IL-6R complexes, thereby effectively halting downstream signal transduction pathways that lead to joint inflammation and destruction in RA.<sup>11</sup>

### SELECTIVE ADHESION MOLECULE INHIBITORS/INTEGRIN RECEPTOR ANTAGONISTS

Integrin molecules are conducive to lymphocyte trafficking by facilitating adhesion and migration from the vasculature into inflamed tissue. Integrin molecules are expressed on the surface of activated lymphocytes. Integrins interact with their receptors, which are the cell-adhesion molecules (CAMs) present on vascular endothelium. This interaction enables lymphocytes to migrate across the endothelium into tissues such as the brain and gut.<sup>12</sup> Integrins  $\alpha 4\beta 1$  and  $\alpha 4\beta 7$  are implicated in multiple sclerosis and IBD, respectively.

Integrin receptor antagonist therapies are mABs that serve as decoys for the CAM receptors. They bind to integrin molecules to prevent interaction with CAMs, ultimately blocking migration of the activated T-lymphocytes into inflamed tissues. Selectivity of adhesion-molecule inhibitors can vary; for instance, natalizumab modulates lymphocyte trafficking in both the central nervous system and gut, while vedolizumab is specific to the gut. Increased specificity is advantageous for targeting desired tissues and limiting adverse effects, such as progressive multifocal leukoencephalopathy, which is a Black Box Warning for natalizumab. The currently available integrin receptor antagonist therapies are humanized mABs. Overall, the goal of integrin receptor antagonist therapy is to reduce migration of activated T-cells and limit progression of chronic inflammation.

### B-CELL DEPLETING THERAPIES

B-cells play an important role in numerous autoimmune diseases. In healthy individuals, auto-reactive B-cells are removed from both the bone marrow and peripheral circulation prior to causing significant harm. In autoimmune diseases, a defect causes these auto-reactive B-cells to escape

notice and produce antibodies, present “self” antigens, and produce various cytokines implicated in the disease process.

Monoclonal antibodies directed at B-cells are generally focused at B-cell depletion. The primary agent used for B-cell depletion in autoimmune diseases is rituximab, a mAB directed against the CD-20 antigen on B-lymphocytes. CD-20 is present on more than 95% of B-cells and plays a part in B-cell activation and cell-cycle progression. When rituximab binds to CD-20, it activates complement-dependent and antibody-dependent B-cell cytotoxicity, and B-cell apoptosis.<sup>13</sup>

### OTHER BIOLOGIC RESPONSE MODIFIERS (BRMS)

#### Co-stimulation Modulators

T-cells require two signals from antigen-presenting cells (APCs) to undergo activation: antigen presentation by histocompatibility molecules and a co-stimulatory signal provided by molecules on the APCs. In the CD80/86-CD28 co-stimulatory pathway, CD80 or CD86 on APCs binds with CD28 on the surface of T-cells and causes T-cell activation, proliferation, and cytokine production. Co-stimulatory pathways may also be inhibitory, as seen with cytotoxic T-lymphocyte antigen-4 (CTLA-4). CTLA-4 binds to CD80 or CD86, resulting in inhibition of T-cell responses by preventing release of interleukins and blocking cell-cycle progression.<sup>14</sup> This pathway is important in the pathogenesis of RA, where activated T-cells are present in inflamed synovium.

Co-stimulation modulator antibodies target the signals required for co-stimulation of the T-cells to occur. Abatacept, a fusion protein used in the treatment of RA, consists of the extracellular domain of human CTLA-4 protein and the modified Fc region of human IgG1. The CTLA-4 portion of abatacept binds CD80/86 on APCs, thereby blocking the interaction between CD28 and APCs required for T-cell activation. The result of this blockade is a long-lasting attenuation of T-cell response.<sup>15</sup> Belimumab, a human IgG1 $\lambda$  recombinant mAB used in the treatment of systemic lupus erythematosus, targets B-lymphocyte stimulator (BLyS), the co-stimulator for B-cell survival and function. BLyS binds to BLyS receptors and promotes the survival of autoantibody-producing B-cells by preventing their selection and apoptosis. Belimumab binds to soluble BLyS, preventing interaction with BLyS receptors and thereby decreasing B-cell survival and production of autoantibodies.<sup>16</sup>

#### Interleukin BRMs

IL-1 is a system consisting of two pro-inflammatory ligands and the naturally occurring antagonist IL-1Ra. In RA, levels of IL-1 are elevated in plasma and synovial fluid.<sup>17</sup> Anakinra, a recombinant human IL-1Ra, binds to IL-1 receptors to prevent intracellular signaling leading to cell activation and biological responses.<sup>18</sup>

**Table 1.** Currently Approved Monoclonal Antibodies and Biologic Response Modifiers

Generic Name	Brand Name	Class	Clinical Uses
Adalimumab	Humira®	TNF inhibitor	Rheumatoid arthritis, Psoriatic arthritis, Plaque psoriasis, Crohn's disease, Ulcerative colitis, Ankylosing spondylitis, Juvenile idiopathic arthritis
Etanercept	Enbrel®		
Certolizumab pegol	Cimzia®		
Golimumab	Simponi®		
Infliximab	Remicade®		
Anakinra	Kineret®	IL-1 antagonist	Rheumatoid arthritis
Canakinumab	Ilaris®	IL-1 inhibitor	Juvenile idiopathic arthritis
Tocilizumab	Actemra®	IL-6 antagonist	Rheumatoid arthritis, Juvenile idiopathic arthritis
Ustekinumab	Stelara®	IL-12 and IL-23 inhibitor	Psoriatic arthritis, Plaque psoriasis
Secukinumab	Cosentyx®	IL-17A receptor antagonist	Plaque psoriasis, Ankylosing spondylitis, Psoriatic arthritis
Ixekizumab	Taltz®	IL-17A receptor antagonist	Plaque psoriasis
Abatacept	Orencia®	Co-stimulation blocker of CD-28	Rheumatoid arthritis, Juvenile idiopathic arthritis
Belimumab	Benlysta®	BlyS inhibitor	Systemic lupus erythematosus
Vedolizumab	Entyvio®	Integrin receptor antagonist	Crohn's disease, Ulcerative colitis
Natalizumab	Tysabri®	Anti-integrin antibody	Crohn's disease, Ulcerative colitis, Multiple sclerosis
Rituximab	Rituxan®	Anti-CD20 antibody	Rheumatoid arthritis, Lupus nephritis, Idiopathic thrombocytopenic purpura, Graft-versus-host disease

**Table 2.** Monoclonal Antibodies and Biologic Response Modifiers in the Pipeline

Generic Name	Stage in Development	Trial Names	Class	Clinical Uses
Brodalumab	Phase III clinical trials	AMAGINE and AMVISION	IL-17A receptor monoclonal antibody	Plaque psoriasis, Psoriatic arthritis, Ankylosing spondylitis
Guselkumab	Phase II clinical trials	X-PLORE	IL-23 monoclonal antibody	Plaque psoriasis
Tildrakizumab	Phase III clinical trials	-	IL-23 p19 subunit monoclonal antibody	Plaque psoriasis
Anifrolumab	Phase III clinical trials	-	IFN Type I receptor monoclonal antibody	Systemic lupus erythematosus
Ozoralizumab	Phase II clinical trials	-	Anti-TNFα Nanobody	Rheumatoid arthritis
Etrolizumab	Phase III clinical trials	-	Integrin inhibitor	Crohn's disease

**CONCLUSION**

Monoclonal antibodies and other BRMs have allowed for targeted drug therapy in managing various autoimmune diseases. A number of immune pathways have been exploited in the development of mAbs by targeting cytokines, cell-adhesion molecules, co-stimulation signals, and B-cells (Table 1). Promising new agents are in the pipeline (Table 2), providing additional options for managing autoimmune conditions.

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# Systemic Lupus Erythematosus: A Review of the Clinical Approach to Diagnosis and Update on Current Targeted Therapies

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## ABSTRACT

Systemic lupus erythematosus (SLE) is a chronic, complicated and challenging disease to diagnose and treat. The etiology of SLE is unknown, but certain risk factors have been identified that lead to immune system dysfunction with antibody formation and immune complex deposition. This immune system dysregulation causes organ injury, contributing to the variable manifestations and relapsing-remitting course of the disease. Criteria were created to aide in the diagnosis, focusing on clinical manifestations and antibody profiles specific to SLE. Treatment options are limited to a few medications to control the inflammation and decrease organ damage. Continuing investigations into the pathogenesis of SLE has led to new discoveries, making more medications available to treat this difficult disease.

**KEYWORDS:** systemic lupus erythematosus, antibodies, autoimmunity, treat to target, B-cell depletion and modulation, interferon blocking agents

## SLE EPIDEMIOLOGY

SLE is seen worldwide, with incidence and prevalence rates differing geographically. Studies have shown that the incidence rate of SLE around the world is about 1 to 10 per 100,000 person-years, while the prevalence rates range from 20–70 per 100,000 person-years.<sup>1</sup> In the United States (US), the all race incidence was found to be 5.1 per 100,000 person-years<sup>2</sup> and the prevalence was estimated to be over 300,000 persons.<sup>3</sup> SLE predominantly affects women, with a reported peak female-to-male ratio of 12:1 during the childbearing years.<sup>2</sup> The disease can also be seen in children and the elderly with a narrower gender distribution. Studies have shown racial/ethnic variations, with SLE being more common in non-Caucasian persons, occurring three to four times more often in African-Americans.<sup>2</sup> In addition to African-Americans, Hispanics and Asians develop SLE more frequently than Caucasians.<sup>2</sup> In these populations, SLE tends to be more active and severe, with a higher risk of relapses and organ system involvement or damage.<sup>4</sup> Even with advances in diagnosis and treatment of the disease, the mortality risk in patients with SLE is higher than that of the

general population. For newly diagnosed patients, the 5-year survival rate is over 90% and the 15 to 20 year survival rate is about 80%.<sup>1</sup> Worse outcomes and higher mortality risk correlated with this ethnic disparity, which may be influenced by a lower socioeconomic status as well.<sup>4</sup>

## SLE PATHOGENESIS

The etiology of SLE is unknown. Certain risk factors have been identified and shown to contribute to disease susceptibility or activate the immune system causing an inflammatory response, ultimately leading to the development of the disease. Predisposition to SLE is influenced by genetic factors. The female predominance in SLE, may be explained, in part, by the contribution of certain hormones.<sup>5</sup> Environmental factors, such as smoking, exposure to ultraviolet light, viral infections, and specific medications (e.g. sulfonamide antibiotics) are known to trigger SLE.<sup>5,6</sup> The pathogenesis of SLE is complex with contribution from many components of the immune system. With the underlying genetic predisposition and in response to various triggers, the balance of the immune system shifts towards reacting against itself, rather than self-tolerance. T and B cells become activated, leading to antibody production and eventual immune complex formation. These complexes circulate and deposit in critical tissues causing organ injury.

## SLE DIAGNOSIS

Classification criteria have been derived for SLE, mainly for research purposes, to achieve population homogeneity among research studies. The American College of Rheumatology (ACR) published criteria in 1982, which were revised in 1997 (**Table 1**). The Systemic Lupus Collaborating Clinics (SLICC) international group undertook the evaluation and further revision of the above criteria resulting in a new classification system that is based on clinical and immunologic manifestations (**Table 1**). In an actual clinical practice setting, both criteria were analyzed; it was determined that the SLICC 2012 criteria were more sensitive and may allow patients to be classified with SLE earlier in the disease course.<sup>7</sup> In the clinical setting, these criteria can be used as an aid in diagnosis, but formal diagnostic criteria for SLE are lacking.

**Table 1.** Classification Criteria for Systemic Lupus Erythematosus<sup>a</sup>

	ACR 1997 <sup>b</sup>	SLICC 2012 <sup>c</sup>
<b>Cutaneous</b>	1. Malar Rash 2. Discoid Rash 3. Photosensitivity 4. Oral or Nasopharyngeal ulceration	1. Acute cutaneous lupus (including malar rash, photosensitive lupus rash) OR Subacute cutaneous lupus 2. Chronic cutaneous lupus (including discoid rash) 3. Oral or nasal ulcers 4. Nonscarring alopecia
<b>Joints</b>	5. Nonerosive arthritis - involving ≥ 2 peripheral joints characterized by pain, swelling or effusion	5. Synovitis - involving ≥ 2 peripheral joints characterized by swelling or effusion or tenderness and ≥ 30 minutes of morning stiffness
<b>Serositis</b>	6A. Pleuritis (pleuritic pain/rub or pleural effusion) OR 6B. Pericarditis (by EKG, rub, or pericardial effusion)	6. Serositis (any of the following) - pleurisy - pleural effusions - pleural rub - pericardial pain - pericardial rub - pericardial effusion - pericarditis by EKG
<b>Renal</b>	7A. Persistent proteinuria (> 0.5g/day or > 3+ dipstick) OR 7B. Cellular casts	7. Renal (any of the following) - urine protein/creatinine (or 24 hour urine protein) > 0.5g/24hr - red blood cell casts
<b>Neurologic</b>	8A. Seizures OR 8B. Psychosis	8. Neurologic (any of the following) - seizures - psychosis - mononeuritis multiplex - myelitis - peripheral or cranial neuropathy - acute confusional state
<b>Hematologic</b>	9A. Hemolytic anemia OR 9B. Leukopenia (<4,000/mm <sup>3</sup> on ≥ 2 occasions) OR 9C. Lymphopenia (<1,500/mm <sup>3</sup> on 2 occasions) OR 9D. Thrombocytopenia (<100,000/mm <sup>3</sup> )	9. Hemolytic anemia 10. Leukopenia (<4,000/mm <sup>3</sup> at least once) OR Lymphopenia (<1,000/mm <sup>3</sup> at least once) 11. Thrombocytopenia (<100,000/mm <sup>3</sup> at least once)
<b>Immunologic</b>	10A. Anti-dsDNA OR 10B. Anti-Sm OR 10C. Antiphospholipid Antibody (any of the following) - anticardiolipin antibodies (IgG or IgM) - lupus anticoagulant - false positive syphilis test or > 6 months (confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test) 11. Positive antinuclear antibody	1. Antinuclear antibody 2. Anti-dsDNA 3. Anti-Sm 4. Antiphospholipid antibody (any of the following) - lupus anticoagulant - false-positive RPR - medium or high titer anticardiolipin (IgA, IgG, or IgM) - anti-β <sub>2</sub> glycoprotein I (IgA, IgG, or IgM) 5. Low complement - low C3, C4, CH50 6. Direct Coombs test
<b>Classification of SLE</b>	- Satisfy four out of the 11 criteria	- Satisfy four of the criteria, including one clinical criterion and one immunologic criterion OR - biopsy-proven nephritis compatible with SLE and with ANA or anti-dsDNA antibodies
<b>Sensitivity<sup>12</sup></b>	83%	97%
<b>Specificity<sup>12</sup></b>	96%	84%

Abbreviations: SLICC = Systemic Lupus International Collaborating Clinics; ACR = American College of Rheumatology; SLE = systemic lupus erythematosus; Anti-dsDNA = anti-double-stranded DNA; Anti-Sm = anti-smith antibody; EKG = electrocardiogram; RPR = rapid plasma regain; CH50 = 50% hemolyzing dose of complement

a. Adapted from Epidemiology and Classification of Systemic Lupus Erythematosus. In: Hochberg MC, Silman AJ, Smolen JS, Weinblatt ME, Weisman MH, eds. *Rheumatology*. 6th ed. Philadelphia, PA: Mosby/Elsevier; 2015:1021-1025

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## SLE CLINICAL AND LABORATORY MANIFESTATIONS

SLE has a variable, relapsing-remitting course and clinical symptoms vary between patients, depending on which organ systems are affected. The above criteria incorporate the major and common organ systems that can be affected in SLE including skin, mucus membranes, joints, kidneys, brain, lungs, heart and hematologic system (Table 1). Clinical and laboratory surveillance is also important to assess and monitor for the development of any new symptoms or findings. A serious manifestation of SLE, with resultant increased morbidity and mortality, is lupus nephritis (LN). Treatment is based on the findings on a kidney biopsy. Neuropsychiatric involvement is rare but difficult to diagnose. It may not correspond to overall SLE activity. SLE patients may also have comorbidities, further complicating their disease. Atherosclerosis is common, presenting as coronary artery disease (CAD), or cerebral or peripheral vascular diseases. CAD is linked to increased morbidity and mortality, with SLE women aged 35-44 years old being more than 50 times more likely to have a myocardial infarction than women of a similar age without SLE.<sup>8</sup> Even though traditional cardiovascular risk factors do not fully explain the accelerated rate of atherosclerosis in SLE patients, they should be addressed routinely and modified to prevent further morbidity or mortality.

Autoantibody production is fundamental to the pathogenesis of SLE. These autoantibodies are directed against nuclear or cytoplasmic antigens and are known as antinuclear antibodies (ANA). ANA's are included in the diagnostic criteria (Table 1) and are seen in more than 95% of SLE patients. Other antibodies have been identified that are recognized based on their targeted autoantigens and are collectively known as anti-extractable nuclear antigens (ENA). Anti-double stranded DNA antibody (anti-dsDNA) is highly specific (95% specific) for SLE, especially with renal disease. Anti-Sm antibodies (antibodies against Sm core particles) are unique and highly specific for SLE with renal disease, although seen in only about 20-30% of SLE patients overall. Other antibodies may be seen in SLE, but are not specific for the disease and can be seen in other autoimmune conditions. For example, anti-ribonucleoprotein (anti-RNP) is seen in 30-40% of SLE patients, but is highly associated with mixed connective tissue disease. Anti-Ro (anti-SSA) and anti-La (anti-SSB) are seen in 40% of SLE patients, but have a stronger association with Sjogren's syndrome. Anti-ENA antibodies are used as serologic markers for SLE. Anti-dsDNA antibodies and complement components (C3 and C4) may be used to monitor SLE activity, especially in the setting of lupus nephritis.<sup>9</sup> Another set of antibodies seen in 30-40% of SLE patients are the antiphospholipid antibodies, which are lupus anticoagulant, anticardiolipin antibodies, and anti- $\beta_2$ -glycoprotein 1 antibodies. About 10-15% SLE patients can have antiphospholipid syndrome, manifested by recurrent venous or arterial thrombosis or pregnancy morbidity.<sup>8</sup>

## TREAT TO TARGET

Utilization of corticosteroids (CS) in SLE management began in the 1950s and was a major important therapeutic milestone. However, challenges in SLE treatment remain to this day. Retrospective studies have provided evidence that increased disease activity in rheumatologic or autoimmune disorders is related to future organ damage and death.<sup>10</sup> In response to this finding, the "treat to target" strategy to achieve disease remission was established and attainable in rheumatoid arthritis. This concept is gaining momentum in the care of SLE patients with new treatment options available and/or emerging medications in the research pipeline.<sup>11</sup> Currently there are only three agents, in addition to CS, that are FDA approved for SLE treatment. The challenge has been to create guidelines for the management and treatment of SLE due to the lack of quality evidence for almost all aspects of SLE, except lupus nephritis.

## BASIC THERAPY

Management of SLE patients begins with basic recommendations including avoidance of sunlight and use of high-SPF sunscreen (> 35), with screening and counseling for modifiable cardiovascular risk factors such as cigarette smoking and uncontrolled HTN. Family planning discussions should be considered with SLE patients of reproductive age. Supplementation of calcium and vitamin D is recommended. The general approach to the use of pharmacological agents depends on specific organ involvement and is tailored to other SLE patient characteristics, such as ethnicity and comorbid conditions.

## ANTIMALARIALS

Antimalarial medications, such as hydroxychloroquine (HCQ), have proved useful in treating milder manifestations of lupus including dermatitis, arthritis and constitutional symptoms. The exact mechanism of action of antimalarials is unknown. Support for the use of HCQ as background therapy in patients with SLE emerged after a pivotal Canadian study found that HCQ reduced flares in SLE patients compared to subjects in whom the medicine was withdrawn.<sup>12</sup> Subsequent analysis linked HCQ to the reduction of organ damage, thrombosis and improvement in survival. HCQ has been shown to favorably modulate lipid profiles in patients receiving CS.<sup>13</sup>

## CORTICOSTEROID TREATMENT IN SLE

Considered the cornerstone of SLE treatment, CS have the major advantage of rapid control of SLE activity, from controlling skin or joint disease, to severe and life threatening complications such as vasculitis and nephritis. CS are often given orally, but for severe life-threatening complications, intravenous forms of CS are usually administered.<sup>14</sup> Short

term use of CS may be necessary and often convenient to control SLE flares, but long-term use is related to significant side effects. Doses of prednisone greater than 10–19 mg a day increase the risk of cardiovascular events 2.4 times compared to daily doses below 9 mg.<sup>15</sup> The risk of long term CS use on skeletal health is well established and will not be addressed here.

### FROM OTCS TO CHEMOTHERAPY

In addition to antimalarials, non-steroidal anti-inflammatory drugs (NSAIDs) and traditional agents used for rheumatoid arthritis, such as methotrexate, have been used to control mild to severe arthralgias and arthritis. Immunosuppressive drugs such as azathioprine (AZA) in doses of 2-2.5mg/kg/d may be used as steroid sparing agents to treat the various manifestations of SLE. For the most life threatening SLE organ manifestations such as neuropsychiatric, LN, pulmonary hemorrhage and systemic vasculitis, high-dose or pulse steroids as the initial treatment are being used, usually followed by high potency steroid sparing agents. Cyclophosphamide (CYC), an alkylating agent, has emerged as the gold standard medication for the management of lupus nephritis after an NIH study which showed that SLE patients on CYC had better renal survival than patients on CS alone.<sup>16</sup> The dose and regimen for CYC was standardized since the NIH experience, but based on the Euro-lupus trial, the lower dose of CYC can be safely used in selected populations, without compromising its efficacy.<sup>17</sup> CYC is used as induction therapy to further decrease inflammation and decrease disease activity. Due to its significant toxicity, most SLE patients are managed with maintenance medications that include AZA or mycophenolate mofetil (MMF) to reduce the frequencies of flares.<sup>14</sup> Nearly 25 years since the publication of the pivotal NIH study of the use of CYC for the management of LN, MMF proved to be non-inferior and for some populations, non-Caucasians, even superior to CYC as an agent for induction of remission of LN.<sup>18</sup>

### B-CELL DEPLETION OR MODULATION

B cells play a central role in the pathogenesis of active lupus through cytokine production, presentation of self antigens, activation of T cells and antibody production. Better understanding of B cell function in SLE pathology directed investigators to conduct trials of rituximab (RTX) for the treatment of severe SLE. RTX is a chimeric mouse/human monoclonal antibody (mAb) against the CD20 antigen on B cells, rapidly decreasing B cells, hence reducing inflammation. The important study of the utilization of RTX in lupus nephritis did not meet the primary end point of reduction in proteinuria; despite reducing the level of complements and dsDNA.<sup>19</sup> Critics of the study point to possible faulty study design (small number of patients, use of high dose of steroids, short study time) as a reason for not reaching

statistical significance. Further, the suboptimal response to RTX may be related to immune complex-mediated advanced kidney injury rather than antibody production related damage. Still, “off label” RTX is used as a second-line agent in lupus complications like neuropsychiatric SLE and vasculitis in addition to its proven efficacy in idiopathic thrombocytopenic purpura (ITP) and autoimmune hemolytic anemia (AHA). The most common side effects of RTX are related to post infusion complete blood count (CBC) abnormalities. Epratuzumab is another B cell therapy targeting the CD22 molecule on B cells. CD22 is responsible for B cell activation and function. This anti-CD 22 agent, that modulates B cell response, has entered late phases of clinical trials with promising preliminary data.<sup>20</sup> Epratuzumab was first studied in trials which ended prematurely due to shortage of the drug. Data analysis showed that epratuzumab may decrease disease activity in SLE, but the authors were unable to draw definitive conclusions. Another trial, studying the same molecule, showed improvement in most areas of SLE activity, from mucocutaneous to renal and neuropsychiatric manifestations. Headache and nausea were the most common side effects.<sup>21</sup>

### TARGETED THERAPIES

BLyS (a B lymphocyte stimulator) is responsible for B cell survival in some SLE patients and is targeted by belimumab, a new FDA-approved drug. This fully human mAb binds to BAFF (B-cell activating factor) receptor on mature B cells decreasing their activation, antibody secretion and possibly preventing T cell activation as well. Belimumab was found to be beneficial in patients with SLE-related dermatitis, mucositis and arthritis, but was not specifically studied in LN. In one clinical trial, a subgroup of patients with elevated dsDNA and low C3 and C4, benefited from this medication the most.<sup>22</sup> Belimumab may constitute a viable, but expensive, option to treat SLE patients who are not responding or intolerant to first line therapies. It has an acceptable safety profile.

### INTERFERON BLOCKING AGENTS

Interferon  $\alpha$  (INF) has been linked to accelerated disease activity and is the main target of antimalarial therapy in SLE.<sup>23</sup> INF $\alpha$  blocking therapies entered phase II clinical trials and show promising results in moderate to severe SLE.<sup>24</sup> Preliminary data presented in abstract form in 2014 showed promising results with sifalimumab, mAb against INF $\alpha$ . This INF inhibitor reduced baseline moderate to severe SLE mucocutaneous involvement, as well as decreased arthritis and fatigue scores. It did not improve serological markers of active disease, such as dsDNA and complement levels. For this medication, the overall safety data was acceptable, with infections and headache as the most commonly reported adverse effects. Novel studies capitalize on INF $\gamma$  with INF $\gamma$  gene expression seen in peripheral blood of subjects with

autoimmune disorders, such as SLE. A recent randomized controlled trial of a mAb against INF $\gamma$  (molecule AMG 811) used in subjects with mild to moderate SLE showed dose dependent modulation of INF gene expression and reduction of the inflammatory protein linked to the prediction of future flares and level of disease activity.<sup>25</sup>

## CONCLUSION

SLE remains a challenging disorder that requires an interdisciplinary approach with a team of health-care providers to diagnose, manage and tailor treatment to individual patient needs. Continued dedication and research into the pathogenesis of SLE to identify specific immunologic targets for potential therapies, will bring more exciting new medications and hope to SLE patients to better control this difficult and unique disease.

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# Pemphigus: Pathogenesis to Treatment

CHRISTOPHER DIMARCO, MD

## ABSTRACT

Pemphigus vulgaris (PV), pemphigus foliaceus (PF), and paraneoplastic pemphigus (PNP) are a group of rare and fatal blistering diseases involving autoantibodies that target desmosomal proteins. The pathogenesis of pemphigus involves the production of activated B-cells and IgG with stimulation by IL-4 by T-helper 2 cells. Clinically these diseases present most often with epidermal erosions of the mucosae and skin caused by rapid rupturing of flaccid bullae. These lesions correlate histologically with splits forming in the epidermis, leaving a blister roof composed of a few cell layers. Standard treatment of pemphigus involves oral corticosteroids, often with the addition of adjuvant therapies, to improve disease control, minimize corticosteroids side-effects, and increase the odds of remission.

**KEYWORDS:** pemphigus vulgaris, pemphigus foliaceus, paraneoplastic pemphigus, desmoglein 1, desmoglein 3, corticosteroids

## INTRODUCTION

Pemphigus includes a group of blistering diseases involving autoantibodies that target proteins found in the desmosome, intercellular adhesion protein complexes. Most forms of pemphigus are classified as being a subtype of pemphigus vulgaris (PV), pemphigus foliaceus (PF), or paraneoplastic pemphigus (PNP). They are a rare group of disorders that have an incidence of 2-10 cases per one million inhabitants in some areas of the world and a prevalence of 0.1-0.7 per one hundred thousand inhabitants.<sup>1,2</sup> Pemphigus was a highly fatal disease until the introduction of corticosteroids (CS) which have reduced its mortality rate from 75% to less than 10% ,with most morbidity and mortality today due to iatrogenic causes rather than the disease itself.<sup>3,4</sup> The one exception is paraneoplastic pemphigus, which has a mortality rate around 50% ,most often due to pneumonia, the associated malignancy, or pulmonary involvement, resulting in bronchiolitis obliterans, despite treatment.<sup>5</sup>

## PATHOGENESIS

The pathogenesis underlying all forms of pemphigus involves the development of autoantibodies to the desmosomal

proteins, which can be found in many areas of the body, but which play a major role in the epidermal layers of the integumentary system. PV and PF are caused primarily by antibodies to desmoglein 1 (Dsg 1) in PF, desmoglein 3 (Dsg 3) in mucosal dominant PV, or both in mucocutaneous PV.<sup>6</sup> Dsg 1 and 3 are found in varying amounts in the epidermis of the skin and mucosa. Dsg 1 is found in higher amounts in the upper layers of the epidermis, especially on the skin, while Dsg 3 is found in the lower layers of the epidermis with higher concentrations in the mucosa and skin.<sup>2,6</sup> It is this variability in distribution which explains the 3 distinct clinical diseases.

The disease usually occurs in patients with certain HLA genotypes who generate B-cells responsible for the specific autoantibodies. The activation of these B-cells requires a complex interaction with CD4+ T helper 2 (Th2) cells and it is this Th2 cell over-activation that leads to the autoantibody production that is necessary for PV and PF.<sup>1,2,6</sup> Th2 cells are known for secreting multiple interleukins (IL), of which IL-4 plays a major role in pemphigus and the humoral immune response.<sup>2</sup> IL-4 promotes antibody production by primed B cells and an isotype switching from IgG1 to IgG4 antibodies which have been shown to be important in the active form of PF and PV.<sup>2,6</sup> IL-4 also perpetuates the disease by causing naïve CD4+ T cells to differentiate into Th2 cells.<sup>6</sup> The production of autoantibodies and epitope binding is sufficient to cause loss of adhesions between desmosomes leading to separation of keratinocytes which is directly related to disease activity.<sup>1</sup> Therefore the disease does not require other components of the immune system for activity, such as complement or cytotoxic T cells. Based on this pathogenesis, treatment for pemphigus focuses primarily on the prevention of antibody production and prevention of isotype switching from an IgG1 to IgG4. When pemphigus enters remission there is a known upregulation of IL-10 and a T helper 1 response that induces antibody isotype switching from IgG4 back to IgG1.<sup>1,2,6</sup> Tumor necrosis factor  $\alpha$ , IL-1, and other cytokines also play a smaller role in the pathogenesis of pemphigus.<sup>2</sup>

PNP is unique from PV and PF in that it may contain autoimmune antibodies to Desmoglein 1 and 3, but has more specific antibodies to envoplakin and periplakin.<sup>7</sup> While envoplakin and periplakin are the most specific for PNP, patients with this disease can develop multiple autoantibodies primarily to desmosomal proteins, including the

plakin family of proteins (plectin, BP230, and desmoplakin), desmocollins, and alpha-2-macroglobulin-like antigen-1.<sup>5,8</sup>

**CLINICAL**

While pemphigus is classified as an auto-immune blistering disease, usually the most prominent findings are epidermal erosions from rapid rupturing of blisters with thin roofs. PV often begins with oral erosions primarily involving the buccal and gingiva mucosae. If patients have developed antibodies to both Dsg 1 and 3, they will likely manifest erosions and flaccid bullae on the skin over weeks to months. Generally the chest, face, scalp, upper back, and areas of trauma are common sites for cutaneous involvement.<sup>1,6,9,10</sup> PF often can present very similarly to the cutaneous involvement of PV. Clinical differences include the lack of mucosal involvement and an exfoliative presentation due to the shallow depth that the erosions occur in the epidermis.<sup>9</sup> (Table 1)

PNP, due to the presence of multiple different autoantibodies, may have a more variable clinical presentation. All patients present with severe involvement of at least a single mucosal surface, with the majority reporting oral involvement. However, there is a high percentage of patients who have involvement of the ocular, genital, and nasal mucosa.<sup>5</sup> Up to two thirds of patients will have cutaneous involvement presenting with classic erosions of pemphigus. But as many as 50% of patients will present with cutaneous lesions similar to erythema multiforme, bullous pemphigoid, and lichen planus. The most commonly reported malignancies with PNP are lymphoid malignancies, most often non-Hodgkin lymphoma and chronic lymphocytic leukemia, followed by Castleman disease, thymoma, and a mix of other solid organ tumors.<sup>5,7</sup> Of note, only two thirds of patients will have been diagnosed with a malignancy when presenting with PNP.<sup>9</sup>

The diagnosis of any patient with a clinical suspicion for

pemphigus is best confirmed with a combination of histopathology and laboratory testing. Most commonly a biopsy of a fresh vesicle or the edge of a blister, with adjacent non-blistered skin, should be performed for histopathology. A biopsy of normal skin at least 1cm away from any blistered or inflamed skin should also be obtained and sent for direct immunofluorescence (DIF).<sup>9</sup> The key histological feature of pemphigus is an intra-epidermal split with the loss of adhesion and separation of normal appearing keratinocytes referred to as acantholysis. In PV, the histology shows suprabasilar split with acantholysis of keratinocytes and DIF will be positive for intercellular IgG involving the entire epidermis. PF will have a subcorneal split with acantholysis of keratinocytes and a DIF showing positive intercellular staining in the upper epidermal layers.<sup>9</sup> PNP can have a histology and DIF with variable amounts of suprabasal acantholysis, lymphocytic infiltrate, and necrotic keratinocytes.<sup>7</sup> (Table 1) Histopathology and DIF can have overlapping features between the various forms of pemphigus. But the histologic picture may be non-diagnostic and serologic studies are recommended. Enzyme-linked immunosorbent assay (ELISA) to quantitate Dsg antibody titers can be done or, if unavailable, serum should be sent for indirect immunofluorescence (IIF) on monkey esophagus for a qualitative measurement of serum Dsg antibodies.<sup>8-10</sup> Specific to PV, ELISA can be used to monitor Dsg 3 antibodies which can correlate with disease severity.<sup>10</sup> Specific to PNP, if suspected, IIF can be performed on monkey or rat bladder urothelium which lacks Dsg 1 and 3 but still contains plakins making it a specific test for PNP.<sup>5,8</sup>

**TREATMENT**

Due in part to its rarity and the lack of standard definitions for tracking disease activity, studies on the treatment of pemphigus are few and limited by small sample sizes.<sup>3</sup> First-line

**Table 1.** Summary of disease classification, clinical features, autoantibody targets, histological, and immunofluorescence findings.

Disease	Clinical Features	Autoantibodies	Histology	Direct Immunofluorescence	Indirect Immunofluorescence
Pemphigus Vulgaris – Mucosal Dominant	Mucosal erosions and flaccid bullae	Desmoglein 3	Suprabasilar split with acantholysis	Intercellular IgG on the entire epidermis	Intercellular IgG on Monkey Esophagus
Pemphigus Vulgaris – Mucocutaneous	Cutaneous and mucosal erosions and flaccid bullae	Desmoglein 1 and 3	Suprabasilar split with acantholysis	Intercellular IgG on the entire epidermis	Intercellular IgG on Monkey Esophagus
Pemphigus Foliaceus	Cutaneous erosions and exfoliative dermatitis	Desmoglein 1	Subcorneal split with acantholysis	Intercellular IgG on the upper epidermis	Intercellular IgG on Monkey Esophagus
Paraneoplastic Pemphigus	Severe mucosal involvement, pemphigus-like, erythema multiform-like, or lichen planus-like cutaneous lesions	Envoplakin Periplakin Plectin BP230 Desmoplakin Desmocollin Desmoglein 1 and 3 Alpha-2-macroglobulin-like antigen-1	Suprabasilar split with acantholysis, lymphocytic infiltrate, and necrotic keratinocytes	Intercellular IgG on the entire epidermis	Intercellular IgG on Monkey Esophagus, Rat Bladder, and Monkey Bladder

therapy for all forms of pemphigus should be CS. Initial daily doses equivalent to 0.5 to 1.0 mg/kg of prednisone are recommended. However, smaller studies have shown that there may be no difference in outcomes for either initial dose.<sup>3</sup> IV methylprednisolone has been shown in pemphigus patients to decrease tumor necrosis factor  $\alpha$  and interleukin 6.<sup>2</sup>

With the initiation of a CS it is common practice to also start an adjuvant therapy for disease control. The exact mechanism of immunosuppressive medications in pemphigus is unknown but it is believed that these therapies act by inhibiting B cell and autoantibody production which contribute to disease activity.<sup>2</sup> Adding an adjuvant agent is proven to lower the risk of relapse. However this effect is lost when comparing specific adjuvant medications. Also, adjuvant therapy does not improve remission rates, time to disease control, time to relapse, or the incidence of death in pemphigus.<sup>3,4</sup>

In addition to traditional immunosuppressive medications, another recently utilized adjuvant is intravenous immunoglobulin (IVIG). IVIG has been shown to decrease IL-1 levels in patients with PV and also provide immune modulation and reconstitution.<sup>2</sup> IVIG also causes a decrease in IgG4 and IgG1 antibodies to Dsg 1 and 3 within 2 weeks of therapy.<sup>11</sup> A recent meta-analysis demonstrated that IVIG was the only adjuvant that improved disease control compared to more traditional immunosuppressive medications.<sup>4</sup> In combination with CS, IVIG has been shown to induce clinical improvement in over half of treated patients.<sup>11</sup>

B-cell depleting therapies have also been studied as standard adjuvant therapy for the treatment of pemphigus and have increased remission rates up to 65%.<sup>10</sup> Rituximab is a monoclonal antibody against the CD20 surface glycoprotein on mature B cells while sparing plasma cells. In pemphigus there is a decrease in autoantibodies to Dsg and peripheral blood B cells that lasts several months. Those levels may rise with the return of peripheral B cells and this may signal a relapse. However, not every patient with this reconstitution relapses, suggesting a restoration of immune tolerance.<sup>10</sup>

Tumor necrosis factor  $\alpha$  inhibitors have also been studied as adjuvant therapy as well in pemphigus. However, these agents may not be as successful in inducing remissions and the role of TNF- $\alpha$  in pemphigus is still not well understood.<sup>2</sup> While IL-4 has been shown to play a major role in the pathogenesis of pemphigus and currently there are medications that block IL-4, such as dupilumab, no studies evaluating its role in the treatment of pemphigus have been published.<sup>6</sup>

Expert consensus panels have convened to define goals for treating patients with pemphigus as well as the doses required before considering a treatment to be a failure.<sup>9,12</sup> Per such consensus, "disease control" was defined as no new lesions forming and established lesions improving over several weeks. CS doses should be maintained until no new lesions have developed for at least 2 weeks and most erosions have healed.<sup>9,12</sup> Doses of 1.5 mg/kg of prednisone or an alternative CS equivalent should be used daily for 3 weeks with or without an adjuvant before a patient has been deemed to

have failed treatment. Failed adjuvant doses are defined as 12 weeks of daily oral regimens of 2mg/kg of cyclophosphamide, 2.5 mg/kg of azathioprine, 3 grams of mycophenolate mofetil, or a weekly dose of methotrexate at 20 mgs.<sup>12</sup>

European guidelines have since recommended that all patients with pemphigus be treated with prednisone initially. Second-line therapy involves the addition of azathioprine, mycophenolate mofetil, or mycophenolic acid as an adjuvant. Third-line therapy is the replacement of the failed adjuvant with an anti-CD20 antibody, IVIG, immunoadsorption, cyclophosphamide, dapsone, or methotrexate.<sup>9</sup> An alternative proposed algorithm included starting all pemphigus patients with CS and an adjuvant initially. If the treatment fails after 3 months of therapy the adjuvant therapy should be replaced with rituximab at 4 weekly doses of 375 mg/m<sup>2</sup>. For patients with PNP, CS with rituximab as an adjuvant are recommended as first line therapy, often due to the concurrent Non-Hodgkin lymphoma.<sup>13</sup>

## CONCLUSION

Despite the rarity of pemphigus in the general population, research continues to better elucidate the mechanisms underlying this group of diseases. Treatment regimens with long-term remissions and new medications are being evaluated as potential treatment options.

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# Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP): Clinical Features, Diagnosis, and Current Treatment Strategies

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## ABSTRACT

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is an acquired immune-mediated disorder characterized by weakness and sensory deficits that can lead to significant neurological disability. The diagnosis is based on a combination of clinical examination findings, electrodiagnostic studies, and other supportive evidence. Recognizing CIDP and distinguishing it from other chronic polyneuropathies is important because many patients with CIDP are highly responsive to treatment with immunosuppressive or immunomodulatory therapies. This review summarizes the clinical features, diagnosis, and current treatment strategies for CIDP.

**KEYWORDS:** chronic inflammatory demyelinating polyradiculoneuropathy, CIDP, polyneuropathy, immune-mediated neuropathy

## INTRODUCTION

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is an acquired, immune-mediated disorder characterized by progressive symptoms of proximal and distal muscle weakness, often accompanied by sensory deficits. CIDP is a common, albeit frequently underdiagnosed condition with an estimated prevalence of 1 to 2 per 100,000 adults.<sup>1</sup> Distinguishing CIDP from other chronic sensorimotor polyneuropathies is imperative as numerous therapeutic options are now available.

## CLINICAL FEATURES

In adults, peak incidence occurs at 40-60 years of age with a slight male predominance.<sup>2</sup> Classic presentation of CIDP is slow progression of both proximal and distal muscle weakness. Predominantly distal weakness may occur but this finding should prompt further investigation to exclude other types of neuropathy (as discussed below). Although weakness predominates in CIDP, the majority of patients also have sensory symptoms such as numbness or paresthesias, classically in a stocking-glove pattern. On examination, there may be diminished sensation to multiple modalities. Deep tendon reflexes are absent or reduced. Gait may be wide based and unsteady. Cranial nerve and bulbar involvement

is rare, occurring in only 10-20% of patients.<sup>3</sup> Autonomic involvement is also rare and typically mild.<sup>4</sup> Symptoms follow either a progressive or relapsing course, with a relapsing course being more likely in younger individuals.<sup>5</sup>

## CIDP VERSUS GULLAIN-BARRE SYNDROME

CIDP and acute inflammatory demyelinating polyradiculoneuropathy (AIDP or Guillain-Barre Syndrome) may share many clinical features but can be distinguished primarily based on the time from onset to peak of clinical symptoms. AIDP is a monophasic illness that typically occurs with acute onset and progresses to a clinical nadir over a period of less than four weeks.<sup>6</sup> It is often associated with an antecedent event such as vaccination or diarrheal illness. By comparison, CIDP symptoms typically progress for a period greater than 8 weeks. Unlike in AIDP, patients with CIDP may experience a relapsing course of symptoms and onset is only rarely preceded by vaccination or illness.<sup>7</sup> Additionally, in CIDP involvement of the cranial nerves, respiratory muscles, and autonomic nervous system is more rare than in AIDP. In some cases the temporal delineation outlined above may be difficult and only observation over time can clarify whether the clinical course is that of AIDP or CIDP. Another consideration is that of treatment-related fluctuation of symptoms. Approximately 8-16% of AIDP patients can show a clinical deterioration within 8-9 weeks after their initial improvement or stabilization following immunotherapy.<sup>8</sup>

## PATHOGENESIS

CIDP is an immune-mediated disorder generated from both cellular and humoral immune responses that are directed against peripheral nerve antigens, leading to demyelination and often secondary axonal loss.<sup>9</sup> Studies of the pathogenesis of CIDP suggest that activated T lymphocytes invade the peripheral nervous system through derangement of the blood-nerve barrier. Once within the peripheral nervous system these activated T cells generate pro-inflammatory cytokines and produce cytotoxic activity against myelin.<sup>9</sup> The myelin sheath is composed of numerous proteins, many of which are being investigated as possible targets for antibody responses in CIDP. Potential auto-antigens include myelin protein zero, myelin basic protein, connexin 32, and



gangliosides.<sup>9</sup> Overall, the mechanisms for these immune responses and the precise peripheral nerve antigens that are targeted have not been fully elucidated. Further research may assist in defining subtypes of disease and how they respond to particular treatments. For example, recent research has demonstrated that patients with antibodies against paranodal proteins contactin-1 (CNTN1) and neurofascin-155 (NF155) comprise a specific phenotype of CIDP that is refractory to first line therapies.<sup>10</sup>

## DIAGNOSTIC WORK-UP

### Diagnostic criteria

As CIDP has become better recognized, researchers and professional societies have proposed various diagnostic criteria based on clinical features, specific electrodiagnostic criteria, and ancillary studies including nerve biopsy or lumbar puncture. Unfortunately, consensus is lacking. Review of the details of the various diagnostic criteria and their differences is outside the scope of this review. In general, the diagnosis of CIDP is primarily based on clinical presentation and electrodiagnostic studies, whereas CSF analysis and histologic studies provide additional supportive data in selected cases.

### Nerve conduction studies and Electromyography (EMG)

Electrodiagnostic studies are key for determining if the underlying pathology is demyelinating or axonal. Hallmark findings of a demyelinating disorder in a nerve conduction study may include evidence of conduction block, prolonged distal latencies, slowing of conduction velocity, or absent/delayed F responses.<sup>2</sup> The pattern of demyelination seen on these studies may be patchy or multifocal, in contrast to hereditary demyelinating polyneuropathies such as Charcot-Marie-Tooth disease, where demyelination is more uniform and conduction block is not seen. The needle EMG may reveal signs of secondary axonal loss.

### Lumbar Puncture

Similar to AIDP, in CIDP there may be elevation of CSF protein with a normal cell count (albuminocytologic dissociation). Sampling of the CSF is not necessary in every patient suspected to have CIDP but may help further support the diagnosis in certain cases. Finding a pleocytosis in the CSF should prompt consideration of alternative diagnoses.

### Nerve biopsy

A nerve biopsy may be considered in the workup of CIDP; however the diagnostic value is controversial. In patients with classic CIDP, the hallmark pathology includes demyelination and re-myelination changes, however this is only seen in about one-half to two-thirds of biopsies.<sup>11</sup> Other findings that may be seen include nerve edema, nerve fibrosis, and inflammatory infiltrates.<sup>11</sup> Unfortunately, the most prominent abnormalities in CIDP may lie in the proximal nerve segments or roots, which are not amenable to biopsy,

and secondary axonal changes may obscure the underlying demyelinating process.<sup>9</sup> However, nerve biopsies can be useful to identify or exclude other etiologies including amyloid or vasculitic, toxic, or hereditary neuropathies.

### Imaging findings

MRI studies of CIDP patients may show gadolinium enhancement or enlargement of the nerve roots or the lumbosacral/brachial plexi, thought to reflect chronic inflammation and demyelination/re-myelination. In addition, advanced neuromuscular ultrasound techniques are now being investigated for utility in the diagnosis of CIDP,<sup>12</sup> though ultrasound is still experimental in its applications for polyneuropathy.

### Other laboratory workup

The differential diagnosis of CIDP is broad. Depending on the clinical scenario, a variety of laboratory studies may be considered to rule out neuropathy from other causes, including (but not limited to) toxicology screen, hemoglobin A1c, thyroid function studies, hepatitis profile, HIV antibody, serum immunofixation, Lyme titers, vasculitic markers, and angiotensin converting enzyme. Hereditary neuropathies, in particular the demyelinating forms of Charcot-Marie-Tooth disease, must also be considered in the differential diagnosis, especially in cases where there is a family history of neuropathy.

## TREATMENT

Treatment is aimed at stopping the inflammatory response to prevent further demyelination and secondary axonal injury. The mainstays of treatment for CIDP include corticosteroids (CS), intravenous immunoglobulin (IVIg), and plasma exchange.

CS have been used in the treatment of CIDP for many years. While there is no strong evidence from controlled trials for oral CS, they are used commonly in practice and with good effect. Initial treatment with oral prednisone is typically high dose at 60-100 mg per day.<sup>13</sup> Once the patient is stabilized clinically the dose is slowly tapered. Unfortunately, CS cause many undesirable systemic side effects so alternative dosing regimens have been considered. Trials comparing pulsed dexamethasone to standard daily prednisone therapy show no significant difference in efficacy.<sup>14</sup> Another small study comparing IV methylprednisolone to oral prednisone and IVIG demonstrated no difference in efficacy and fewer side effects as compared to prednisone.<sup>15</sup> Alternate day dosing of oral prednisone may also be considered. There is no clearly preferred regimen for CS administration in CIDP.

IVIg has proven to be an effective alternative to CS<sup>16</sup> with generally fewer side effects.<sup>17</sup> There are no strong guidelines regarding dosing and frequency of IVIG. Typically a loading dose of 2 g/kg is given over 2-5 days but subsequent maintenance therapy is variable and dependent upon how rapidly

the patient relapses. Maintenance doses may range from 0.4-2 g/kg given as frequently as every 3-4 weeks.<sup>13</sup> Patients can be maintained on IVIg long-term but weaning or discontinuing IVIg may be considered after a period of clinical stability of about six months or more. As with the dosing, there are no universal guidelines for tapering or discontinuing the medication and it is done on an individual basis. Side effects of IVIg include increased risk of thromboembolic events, renal dysfunction, and aseptic meningitis. Subcutaneous immunoglobulin, administered weekly, is more cost-effective and may be a consideration for patients who do not tolerate IVIg well but more data is needed to establish whether it provides the same efficacy as the IV formulation.<sup>18</sup>

Plasmapheresis is another treatment modality that has demonstrated efficacy in small trials.<sup>19, 20</sup> However, it is more time consuming and invasive than IVIg, requiring the placement of a central venous catheter rather than a peripheral intravenous line. It can be used as initial therapy in a patient with prominent weakness followed by other, less invasive immunotherapy, or in some cases may be used for long-term treatment.

## REFRACTORY CASES

First line therapy for CIDP typically consists of IVIG, CS, plasmapheresis, or some combination of these agents. Other treatments may be considered in patients with refractory disease but strong supportive data for their efficacy is generally lacking. Additionally, many of these second- and third-line agents pose the risk of rare but serious side effects and should be considered with caution.

Cyclophosphamide and cyclosporine A have both shown positive results in small case series.<sup>21, 22</sup> Unfortunately they also pose the risk of significant side effects and use should be considered with caution. A small study of azathioprine showed no benefit in patients on oral prednisone therapy<sup>23</sup> though there may be anecdotal support for its use. Methotrexate has been reported to yield some benefit in case reports, but a randomized, placebo-controlled trial of oral methotrexate (adjuvant to IVIg or corticosteroid maintenance) demonstrated no significant clinical benefit.<sup>24</sup>

Rituximab is another consideration in patients not responsive to traditional therapies but more research is needed to establish its potential benefit; so far a significant treatment effect has not been proven in CIDP. However, as described above, recent data may suggest that rituximab is beneficial in a subset of treatment-resistant patients with antibodies against node of Ranvier proteins CNTN1 and NF155.<sup>10</sup> Limited data suggests that alemtuzumab may also offer an alternative to traditional therapies for patients with refractory illness<sup>25</sup> but further studies are needed and its use is experimental at this time. There have been several trials of interferons (interferon-alfa 2a and interferon beta 1a) that did not demonstrate efficacy.<sup>26,27</sup>

Experimental treatments such as peripheral blood stem cell transplantation, have not demonstrated safety or efficacy to date.<sup>28</sup> There is little data regarding non-pharmacological interventions such as regular exercise but physical therapy referral should be considered for patients with CIDP for gait training and fall prevention when clinically indicated.

## CONCLUSIONS

Recognition of CIDP in a patient presenting with chronic neuropathy is crucial because treatments such as CS, IVIg, plasmapheresis, and other alternative agents may yield significant benefit with increased quality of life and reduction in disability. Future directions include advancing our understanding of the underlying pathogenesis of CIDP and honing the diagnostic criteria. Further research is needed to establish the optimum treatment doses and durations for established therapies and to further investigate the utility of the alternative, less well-studied agents.

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# Autoimmune Cytopenias: Diagnosis & Management

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## ABSTRACT

The autoimmune cytopenias are a related group of disorders in which differentiated hematopoietic cells are destroyed by the immune system. Single lineage disease is characterized by the production of autoantibodies against red cells (autoimmune hemolytic anemia [AIHA]), platelets (autoimmune thrombocytopenia [ITP]) and neutrophils (autoimmune neutropenia [AIN]) whereas multilineage disease may include various combinations of these conditions. Central to the genesis of this disease is the breakdown of central and/or peripheral tolerance, and the subsequent production of autoantibodies by both tissue and circulating self-reactive B lymphocytes with support from T helper lymphocytes. These disorders are classified as primary (idiopathic) or secondary, the latter associated with an underlying malignancy, systemic autoimmune disease, infectious disease or a specific drug. Non-specific immunosuppression with corticosteroids remains the first-line therapy for many of these disorders, and although associated with high response rates, is compromised by significant toxicity and high relapse rates. Management of patients with chronic refractory autoimmune cytopenias who have failed first-line and second-line (cytotoxic immunosuppressant therapy and or splenectomy) is particularly complex, with definitive treatment in select patients requiring hematopoietic stem cell transplantation. Given the toxicity concerns of non-selective immunosuppressants, development of therapeutic regimens that avoid steroids has progressed rapidly in recent decades.

**KEYWORDS:** autoimmune cytopenias, WAIHA, CAD, ITP, AIN

## INTRODUCTION

Failure to maintain self-tolerance is the dominant pathophysiologic mechanism binding the autoimmune cytopenias, a group of disorders characterized by the immune mediated destruction of differentiated hematopoietic cells. Central tolerance is governed by apoptosis of autoreactive cells upon binding to self-antigen (negative selection), which occurs early in B and T cell differentiation in the bone marrow and thymus, respectively. In contrast, the active process of peripheral tolerance, is driven by CD4+/CD25+ regulatory

T cells (Tregs) and CD8+ suppressor T lymphocytes which maintain anergy or suppression against self-antigens. (1) Numerous mechanisms to account for central and peripheral tolerance breakdown in the context of autoimmune cytopenias have been proposed. The emergence of “forbidden clones” as proposed by Burnett more than sixty years ago, (2) hinges on the persistence of self-reactive clones that should have been deleted via central tolerance, and may play a role in autoimmunity seen in lymphoproliferative diseases or polyclonal lymphocyte activation in viral infection. Molecular mimicry in the context of viral, bacterial and mycoplasma infections may also result in the initiation and acceleration of autoimmunity due to the presence of common antigenic epitopes in proteins and carbohydrates, particularly on the surface of red blood cells. (3) Additional mechanisms to account for the failure to maintain self tolerance include neo-antigen generation by environmental agents or drugs, as observed in drug-induced AIHA, (4) and immunoregulatory disturbances stemming from the alteration of cytokine networks. Interestingly, although autoimmunity is commonly thought to arise from the interplay between environmental factors and genetic predisposition, the HLA linkages documented for various organ and systemic autoimmune diseases such as type-1 insulin-dependent diabetes, pemphigus vulgaris, systemic lupus erythematosus, rheumatoid arthritis, etc., have not yet been clearly demonstrated for the autoimmune cytopenias.

## AUTOIMMUNE HEMOLYTIC ANEMIAS

### Pathogenesis

Autoimmune hemolytic anemia (AIHA) is defined by the destruction of mature red blood cells (RBCs) by anti-RBC autoantibodies produced by autoreactive B lymphocytes facilitated as otherwise by complement. Autoantibodies can result in erythrocyte destruction via numerous mechanisms including; a) phagocytosis of erythrocytes opsonized by autoantibodies and complement by activated macrophages, b) direct erythrocyte osmotic lysis through complement fixation and sequential activation of the membrane attack complex (MAC), and c) antibody-dependent cell-mediated cytotoxicity (ADCC) mediated by cytotoxic CD8+ T cells and natural killer (NK) cells that carry membrane receptors for the Fc portion of bound immunoglobulin G (IgG). ADCC and erythrophagocytosis preferentially occur in the

spleen and lymphoid organs, whereas complement mediated destruction is primarily intravascular or occurs in the liver. AIHAs are divided into warm and cold types according to the thermal characteristics (reactivity at 37°C or 4°C) of the predominant autoantibody formed, which in large part is predicated on the antibody class (IgG versus IgM), and the chemical characteristics of the epitope (protein or carbohydrate).

**WARM AUTOIMMUNE HEMOLYTIC ANEMIA**

**Clinical Features & Laboratory Findings**

Optimal reactivity of the autoantibody at 37°C (mainly IgG1 and IgG3 subclass), defines warm autoimmune hemolytic anemia (WAIHA) which can affect all age groups and accounts for 80-90% of adult cases of AIHA. Clinical and laboratory features are shown in **Table 1**. In vivo binding of antibody and/or complement to the red blood cell (RBC) surface can be detected in a direct Coomb's or antiglobulin test (DAT). In nearly half of all WAIHA cases, these pan-reactive autoantibodies exhibit specificity for Rh protein epitopes. (5)

**Management**

Transfusion of allogeneic red cells for rapid symptomatic improvement of hypoxic anemia along with controlled non-specific immunosuppression with pharmacologic doses of corticosteroids represents front-line therapy. Initial hemoglobin stabilization and prompt symptomatic improvement is observed in up to 70–80% of patients. However, disease relapse after steroid-induced remission is common. (6) Corticosteroid non-responders can be managed with other non-specific immunosuppressants such as cyclosporin A, azathioprine and cyclophosphamide. (7) Although splenectomy has played a dominant historical role in the management of WAIHA, with the first series of patients (n = 28) described by Chertkow and Dacie in 1955, (8) data regarding durable remission remains unclear with an approximate response rate of 38-70% in patients with WAIHA. (9) Recombinant erythropoiesis-stimulating agents (ESA) represent an alternative promising treatment modality that may be more widely employed in the future, (10,11) and high dose IVIG, although less successful than in ITP, may be efficacious in some non-responder cases. (12)

Targeted therapy with Rituximab, a potent, humanized monoclonal antibody directed against CD20 on pre-B cells, mature B lymphocytes, and immature plasma cells, has been increasingly used as second-line therapy in relapsed

**Table 1.** The Autoimmune Cytopenias

Disorder	Clinical Features	Laboratory Tests	Treatment
Warm Autoimmune Hemolytic Anemia	Fatigue, jaundice	CBC, MCV, reticulocyte count, bilirubin, haptoglobin, LDH, haptoglobin, direct Coomb's test (IgG ± C3), blood smear examination for spherocytes	Steroids Splenectomy Erythropoiesis-stimulating agents (ESA) Rituximab
Cold Autoimmune Hemolytic Anemia	Fatigue, jaundice, passage of brown urine induced by cold	CBC, direct Coomb's test (C3+/IgG-), blood smear examination for agglutination, spherocytes or erythrophagocytosis (Figure 1)	RBC transfusion Chlorambucil Cyclosporine Rituximab
Autoimmune Thrombocytopenia	Bruising, bleeding from mucosal surfaces	Platelet count, antigen-specific autoantibody assays, blood smear examination to exclude pseudothrombocytopenia and confirm large platelets	Steroids Splenectomy Rituximab IVIG Thrombopoeitin (TPO) mimetics
Autoimmune Neutropenia	Recurrent infections	Total white cell, differential and neutrophil count	G-CSF

or refractory cases. Binding of rituximab to CD20-positive cells results in B-lymphocyte depletion via a combination of apoptosis, complement activation and antibody-dependent cell cytotoxicity. (13) Small case series have supported the efficacy and safety of this drug in children and adults with WAIHA with durable responses of up to 3 years. (14,15) Response rates of 33–s77% with complete remission in 29–55% have been reported in an evidence-based focused review, (16) with the beneficial effect greatest in neonates and children compared to adult patients with WAIHA. (14,15,17) Long-term side effects remain to be explored, yet mild infusion reactions including hypotension and fever are the most common complications of rituximab with a very low incidence of serious infection. (18) A battery of additional treatment modalities in various stages of the investigational and licensure pipeline include such drugs as alemtuzumab (anti-CD52), bortezomib, kinase inhibitors and IgG-specific endoglycosidase EndoS. (19-22)

**COLD ANTIBODY AUTOIMMUNE HEMOLYTIC ANEMIAS**

**Pathogenesis**

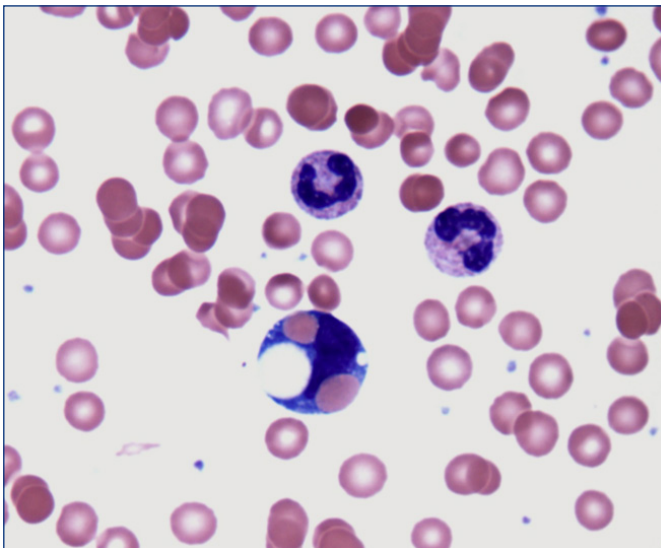
Cold antibody autoimmune hemolytic anemias are serologically characterized by autoantibodies with optimal reactivity at 4°C, with the majority of cases being either cold agglutinin syndrome (CAS) or paroxysmal cold hemoglobinuria (PCH). The autoantibodies in primary CAS are monoclonal IgM, and polyclonal IgM in secondary CAS due to infectious diseases such as Mycoplasma pneumoniae and infectious mononucleosis. The polyclonal antibodies produced in response to these infections typically demonstrate specificity to the RBC blood group antigens I and i, respectively. The antibody specificity in PCH is a polyclonal IgG immunoglobulin directed toward the P blood group antigen.

This bi-phasic hemolysin (Donath-Landsteiner antibody) reacts with RBCs in the peripheral circulation when the temperature drops below 20°C, and initiates complement fixation. Upon returning to the warmer central circulation, complement mediated erythrocyte osmotic lysis ensues. (23)

**Clinical Features & Laboratory Findings**

Clinical features are shown in **Table 1**. CAS typically manifests as moderate chronic hemolytic anemia in middle-aged or elderly patients, often with cold exacerbation of signs (acrocyanosis of the extremities), splenomegaly, anemia and mild jaundice. Prognosis is generally fair although significant mortality has been described. (24) PCH is characterized clinically by acute hemolytic anemia often with hemoglobinuria, predominantly in children with a history of a recent viral illness. Although PCH typically follows a mercurial, acute, often severe course, prognosis is excellent with the majority of cases spontaneously resolving within a few days to several weeks following onset. The direct Coomb’s test will be positive for complement (C3) and negative for IgG. Erythrophagocytosis of complement sensitized cells can be observed in the peripheral blood films of up to 80% of young children with acute transient PCH (**Figure 1**).

**Figure 1.** Peripheral blood smear of a biphasic hemolysin positive case of paroxysmal cold hemoglobinuria (PCH) in a four-year-old child, exhibiting prominent monocytic erythrophagocytosis with occasional spherocytes.

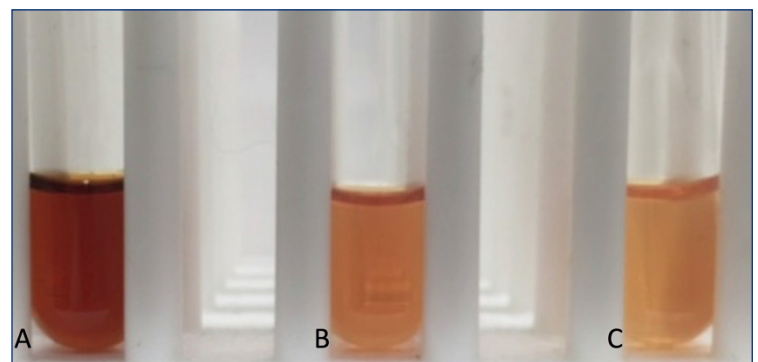


**Management**

Cold exposure avoidance, red cell transfusion for hypoxic anemia, and immunosuppression with the alkylating agents chlorambucil and/or cyclophosphamide represent front-line therapy for CAS. B lymphocyte depletion with rituximab to remove the pathologic clonal B cells, has been investigated in case reports, small retrospective series and phase 2 trials. (25,26) In these studies, rituximab monotherapy achieved partial responses in greater than 50% of patients with

CAS, complete responses in 5% and disease improvement in those who had previously received rituximab. However, median duration response of 11 months and failure rates of 40-50% remain impediments to universal implementation of this drug in the treatment of CAS. In PCH, the severe acute intravascular hemolysis (**Figure 2**), may necessitate transfusion of red blood cells along with supportive care provided in a heated room.

**Figure 2.** Three plasma samples collected from the same patient with PCH at 0 (A), 3 days (B), and 4 days (C) after admission. Hemoglobinemia resulting from intravascular hemolysis is most readily apparent in the plasma sample collected on presentation (day 0), which quickly resolved in the ensuing week. Hemoglobin concentrations (mg/dL): A = 83; B = 43; C = 28 (normal < 2 mg/dL).



**AUTOIMMUNE THROMBOCYTOPENIA**

**Pathogenesis**

Immune mediated destruction of platelets along with attenuated platelet production characterize ITP (27) which is mediated in part by anti-platelet IgG and T-cell subset abnormalities. (28,29) Using antigen-specific assays that measure autoantibodies capable of binding to platelet surface glycoproteins, anti-platelet autoantibodies can be detected in only 50-60% of ITP patients. (30) A limited number of B-cell clones produce these antiplatelet antibodies as a result of antigen-driven somatic mutation. (31) Platelets coated with autoantibody are cleared in the reticuloendothelial system by phagocytosis and possibly complement-mediated lysis. (32) Hepatic clearance of platelets by an anti-GPIb-IX mediated Fc-independent mechanism involving the Ashwell-Morell receptor may also occur. (33) Furthermore, the lack of autoantibodies in many ITP patients has led to the discovery that cytotoxic CD8+ T lymphocytes can lyse platelets in vitro and impair megakaryocyte function. (34)

**Clinical Features & Laboratory Findings**

Autoimmune thrombocytopenia (ITP) is the most common of the autoimmune cytopenias with an incidence of five out of 100,000 children per year and two out of 100,000 per year in adults. ITP may be primary, secondary to autoimmune disease, infection (CMV, HIV, Hepatitis C, Helicobacter pylori) and malignancy, drugs (35) or occur in association

with AIHA (Evan's syndrome). Common clinical features are listed in **Table 1**. Adults tend to run a chronic course, whereas shorter disease duration (approximately 6 months) and much higher spontaneous remission rates occur in children. Bone marrow biopsy indicated in patients > 60 years of age to exclude an underlying B cell malignancy may reveal normal or increased megakaryopoiesis. (36)

### Management

Corticosteroid taper and intravenous immunoglobulin represent front line therapy for ITP, with approximately 70-80% response rate in newly-diagnosed, previously untreated ITP patients. (37) However, recurrence of thrombocytopenia in the majority of patients, necessitates additional intervention. Optimal second line therapy remains uncertain, although traditionally splenectomy for steroid refractory patients has been employed at the risk of post-operative complications and 1% mortality due to septicemia. (38) High-dose dexamethasone instead of prednisone has been advocated in adults as a different strategy to avoid second-line therapy altogether. Numerous alternative strategies such as B-cell depletion with the monoclonal antibody rituximab, anti-D immunoglobulin, thrombopoiesis-stimulating agents and Fc receptor blockade have been investigated. Retrospective and prospective single-arm trials have shown a beneficial effect of rituximab therapy in adult and childhood ITP, (39) which may be boosted with combinatorial regimes involving rituximab and dexamethasone, (40) or even triple therapy with the inclusion of cyclosporine (TT4). (41) Rapid platelet responses have been observed with the thrombopoietin (TPO) receptor agonists (romiplostim and eltrombopag), although medication discontinuation is often followed by a platelet count drop to pretreatment levels. (42,43) In non-splenectomized Rhesus positive individuals with ITP, anti-D immunoglobulin therapy may be similarly efficacious as conventional treatments through the saturation of macrophage Fc receptors by opsonized red blood cells. (44) Targeted Fc receptor blockade with monovalent anti-Fc $\gamma$  receptor/albumin fusion proteins and/or neutralization of autoimmune IgG Fc by soluble Fc $\gamma$ Rs is also being pursued. (45,46) Inhibition of platelet glycoprotein desialylation with the antiviral sialidase inhibitor, oseltamivir phosphate, has resulted in significant platelet count increases in anti-GP1b autoantibody positive chronic ITP patients refractory to all other conventional therapies, representing a promising antigen specific area of future research. (47) Platelet transfusion is usually reserved only for patients with acute life-threatening bleeding (retinal or intracranial hemorrhage) due to the rapid clearance of infused platelets.

## AUTOIMMUNE NEUTROPENIA

### Pathogenesis

Autoantibodies directed against neutrophils are primarily responsible for the rare entity autoimmune neutropenia (AIN). AIN be primary or secondary to viral infections, drug-induced mechanisms, hematological malignancies

such as large granular lymphocyte leukemia, autoimmune diseases and primary immune deficiency syndromes. Antigens in the polymorphic human neutrophil antigen system (HNA), particularly HNA-1 and HNA-4, located on the Fc $\gamma$ RIIIb (CD16) and CD11b molecules respectively, are the primary targets of anti-neutrophils antibodies which can be demonstrate in up to 70% of cases. (48) Cell-mediated destruction of granulocytes may also occur due to inhibitory CD8+ cytotoxic T-cells present within the marrow space.

### Clinical Features & Laboratory Findings

As with other autoimmune cytopenias, the natural history of AIN varies between children and adults, with a relatively benign course and spontaneous remission within 6-24 months commonly occurring in children, in contrast to a more pronounced, chronic course in adults. Upper respiratory tract infections, skin sepsis, recurrent fevers, otitis media in children and chronic tiredness in adults may all be presenting signs.

### Management

Front line therapy with recombinant human granulocyte colony stimulating factor (rhG-CSF) can be used in the immediate treatment of severe infections as well as for infection prophylaxis at a decreased dosing schedule. (49) Immunosuppression, intravenous immunoglobulin and splenectomy have produced variable to disappointing results in the treatment of AIN. Rituximab likewise, has met with limited efficacy in this disorder, presumably due to the central role of the inhibitory CD8+ cytotoxic T cells. (50)

## SUMMARY

Immune mediated destruction of hematopoietic cells characterize the autoimmune cytopenias. The complexity of these cases indicate that referral to a hematologist is indicated in nearly all cases. Viral infections, autoimmune diseases, drugs, solid tumors and hematopoietic malignancies underlie many of the cases of secondary autoimmune cytopenias. Natural history variation between children and adults generally predicts higher rates of spontaneous remission and shorter disease duration in children. Non-specific immunosuppression with corticosteroids represents front-line therapy for many of these disorders yet active investigation into steroid sparing regimes has uncovered multiple new treatment modalities. Notably, autoreactive B lymphocyte depletion via targeted therapy with the humanized, chimeric monoclonal anti-CD20 antibody, rituximab, has provided durable responses in AIHA and ITP, whereas the mammalian target of rapamycin inhibitor, sirolimus, may provided safe and efficacious mono-therapy treatment for patients with refractory autoimmune multilineage cytopenias. (51) Recombinant erythropoiesis-stimulating agents may in the future become standard therapy in WAIHA, and newly vetted targets to treat ITP include monovalent Fc receptor blockade and combinatorial therapy including rituximab, dexamethasone, thrombopoietin receptor analogues and cyclosporine.

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## Conflicts of Interest

None.

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