Unmet needs in the treatment of autoimmunity: From aspirin to stem cells

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ABSTRACT

As rheumatologic diseases became understood to be autoimmune in nature, the drugs used to treat this group of conditions has evolved from herbal or plant derived anti-inflammatory agents, such as salicylates, quinine and colchicine to the many recently approved biological response modifiers. These new drugs, especially the anti-tumor necrosis factor agents, have shown remarkable efficacy in autoimmune diseases, and there are new agents under investigation that will provide additional treatment options. In between, the world was introduced to cortisone and all of its derivatives, as chemical synthesis led to better, more efficacious drugs with lesser side effects.

Disease modifying anti-rheumatic agents have actually been around since the first half of the 20th century, but only began to be used in the treatment of autoimmune diseases in the 1970s and 1980s. One advantage is that they have been invaluable in their ability to offer "steroid sparing" to decrease the adverse effects of steroids.

Research over the past decade has resulted in a new class of drugs that influence cytokine regulatory pathways such as the Janus associated kinase inhibitors. The promise of personalized medicine now permeates current research into new pharmacological agents for the treatment of autoimmune disease. The new appreciation for the gene–environment interaction in the pathogenesis of most diseases especially those as heterogeneous as autoimmune diseases, has led to our focus on targeted therapies. Add to that the new knowledge of epigenetics and how changes in DNA and histone structure affect expression of genes that can play a role in immune signaling, and we now have a new exciting frontier for cutting edge drug development. The history of treatment of autoimmune diseases is really only a little over a century, but so much has changed, leading to increasing lifespans and improved quality of life of those who suffer from these ailments.

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1. Introduction

Rheumatologic diseases have been described over the past hundreds of years, although the pathogenesis has remained elusive. It was only a little over 100 years ago that many of the rheumatologic diseases were shown to be associated with a defect in the immune system. Diseases such as rheumatoid arthritis and systemic lupus erythematosus were thought to be due to an autoimmune process, and the quest for the autoantibodies that drive the mechanism for the body’s targeting of its own tissues became the focus of research in this field. Early on it was the discovery of an autoantibody that cemented autoimmunity as the pathogenesis for many rheumatologic diseases, but now, a century later, there is an understanding that autoimmunity is much more complex, and that autoantibodies may or may not be the primary pathogenic mechanism for many of these diseases.

The development of drugs for the treatment of autoimmune diseases has similarly followed a course that parallels our understanding of pathogenic mechanisms. Early discoveries were fortuitous, perhaps, with herbal products and natural remedies forming the foundation of treatment prior to the 19th century. In fact, aspirin, a drug derived from the bark of the willow tree, was the mainstay of pharmacologic therapy for many years. Ensuing years brought other NSAIDs, and the class of drugs known as the anti-inflammatory agents was born. Further discoveries brought us glucocorticoids, methotrexate, and a class of drugs of unclear mechanism. While the clinical characteristics of the different rheumatic disease were only precisely defined beginning in the 1800s, early recorded history describes many cases which reflect that the disease probably has been affecting humankind for centuries. Descriptions by Egyptians, Indian and Chinese medical scholars from over 3000 years ago refer to patients with debilitating arthritis. The Ebers Papyrus dates back to 1500 BC and describes a condition that is consistent with rheumatoid arthritis. Writings from Hippocrates, Galen, Pliny the Elder and others all recommended decoctions of salicylates for the treatment of rheumatism. The Indian literature describes Ayurvedic medicine and touts the use of ginger in the treatment of rheumatoid arthritis [21,22]. The Chinese have used acupuncture for centuries [23].

In addition, Traditional Chinese Medicine (TCM) describes the use of hundreds or even thousands of herbal preparations and their combinations in treatment rheumatoid arthritis. These include Angelica sinensis or “female ginseng”, which had been used in Chinese medicine since the Han dynasty (25–200 BC) and recorded in the Chinese ancient medical textbook Shen Nong Ben Cao Jing (神農本草經) [24]. It turns out that the components of ginseng include chemicals such as phytosterols which may possess weak anti-inflammatory agents. Other herbal medications used in China include the roots of the Glycyrrhiza plant (licorice) [25–29], also since the Han dynasty and Tripterygium wilfordii, to treat a multitude of autoimmune diseases including rheumatoid arthritis and systemic lupus erythematosus [30–33].

Other medications used in various ancient cultures include Cimicifuga racemosa, Black Cohosh (the Americas) [34–36], Curcuma longa or turmeric, curcumin (China and India) [37], bromelain from pineapple stem (various cultures) [38], Centella asiatica (China and India) [39], Hashingar (India) [40], Chirayita (the Himalayas) [41], Crocus sativus or saffron (various cultures) [42], Strobalanthus callosus or karvi (India) [43], Trevisia polycarpa Bent (India) [44], Madimadi (Korea) [45], Gentiana macrophylla or Qin Jiao (China) [46], Urtica dioica (Europe) [47] and Shiraia bambusicola (China) [48]. A detailed description of these herbal products and their potential mechanism of action are beyond the scope of this paper, but suffice to say that most of these components may contain weak anti-inflammatory agents of unclear mechanism.

2. Treatment of rheumatic diseases in ancient times

While the clinical characteristics of the different rheumatic disease were only precisely defined beginning in the 1800s, early recorded history describes many cases which reflect that the disease probably has been affecting humankind for centuries. Descriptions by Egyptians, Indian and Chinese medical scholars from over 3000 years ago refer to patients with debilitating arthritis. The Ebers Papyrus dates back to 1500 BC and describes a condition that is consistent with rheumatoid arthritis. Writings from Hippocrates, Galen, Pliny the Elder and others all recommended decoctions of salicylates for the treatment of rheumatism. The Indian literature describes Ayurvedic medicine and touts the use of ginger in the treatment of rheumatoid arthritis [21,22]. The Chinese have used acupuncture for centuries [23].
Fig. 1. Timeline of discovery of drugs for autoimmune diseases. 1. Canakinumab was approved for its use in CAPS in 2009. 2. Methotrexate has been known since the 1940s, but only approved for treatment of autoimmune diseases in 1998. 3. The initial use of cyclosporin was in transplant medicine; its immunosuppressive effects led to its being used in autoimmune diseases.

Synthesis of aspirin

1. Synthesis of salicylic acid from phenol

\[
\begin{align*}
\text{C}_6\text{H}_5\text{OH} & \rightarrow \text{C}_6\text{H}_4\text{OH} \\
\text{C}_6\text{H}_4\text{OH} & \rightarrow \text{C}_6\text{H}_4\text{COOH} \\
\text{C}_6\text{H}_4\text{COOH} & \rightarrow \text{C}_6\text{H}_4\text{COOH} + \text{H}_2\text{O}
\end{align*}
\]

2. Synthesis of aspirin from salicylic acid

\[
\begin{align*}
\text{C}_6\text{H}_4\text{COOH} & \rightarrow \text{C}_6\text{H}_4\text{COOH} \\
\text{C}_6\text{H}_4\text{COOH} & \rightarrow \text{C}_6\text{H}_4\text{COOH} + \text{H}_2\text{O}
\end{align*}
\]

The mechanism of action of aspirin is through its effect on the cyclooxygenase pathway. The mechanism was discovered in 1971 by John Robert Vane, who demonstrated that aspirin inhibited production of prostaglandins and thromboxanes. There are two cyclooxygenases, COX-1 and COX-2. Aspirin inhibits COX-1 irreversibly, which distinguishes it from other COX-1 inhibitors. Other NSAIDs, such as ibuprofen and diclofenac, inhibit COX-1 reversibly. Besides its action on the cyclooxygenase pathway, aspirin also has recently been found to act on other pathways, including modification of the cellular signaling pathway via NF-κB. In addition, aspirin has been found to be able to activate AMP-activated protein kinase. Finally, it has been proposed that the acetyl group of aspirin also can have an effect on acetylating cellular proteins, and thereby exerting an effect on posttranslational regulation of protein function.

Several NSAIDs have since become popular substitutes for asthma, including ibuprofen, naproxen, ketoprofen, tolmetin, ketorolac, diclofenac, piroxicam, and many others. These drugs are classified based on their chemical structure. Table 1 shows a breakdown of the available NSAIDs.

Merck and Pfizer were the two companies who were working on producing a COX-2 inhibitor, which would provide the anti-inflammatory effects of the earlier drugs and would have less gastrointestinal side effects. In 2004, Merck voluntarily withdrew their product rofecoxib from the market because of concerns of a risk of heart attacks and strokes. While it will perhaps never be known whether or not this was a true finding, this left Pfizer with the only COX-2 inhibitor available, named celecoxib (after removal of valdecoxib, Pfizer’s other COX-2 inhibitor, in 2005). Celecoxib is now widely used for the treatment of rheumatoid arthritis, osteoarthritis, pain, ankylosing spondylitis and painful menstruation. A meta-analysis performed in 2006 failed to find an increased risk of cardiovascular events in COX-2 patients when compared to patients receiving non-specific NSAIDs or placebo.

Quinine is derived from the bark of the cinchona tree. It possesses multiple effects, including anti-inflammatory, analgesic, antipyretic and anti-malarial effects. Its stereoisomer, quinidine, has anti-arrhythmic effects. Quinine has a bitter taste and is a crystallized alkaloid that is present in some beverages such as tonic water or bitter lemon. The use of quinine traces back to the 18th century as an anti-malarial drug, but its use extends far earlier than that and is a natural remedy used by the Quechua of Peru as a remedy for shivering. Although its primary use was as an anti-malarial, it has also been used for other ailments, including arthritis. Adverse effects include cinchonism and idioopathic thrombocytopenic purpura (ITP).

3.2. Colchicine

Colchicine is derived from the plant, *Colchicum autumnale* (the autumn crocus). It has a long history of use in ancient times, described in the Ebers papyrus as a treatment for swelling and rheumatism. Further evidence of its use appeared in the *De Materia Medica* by Pedanios Dioscorides during the first century AD and in later publications from Persia, England and France. The pure compound was extracted from the native plant in 1920 by Pelletier and Caventou [49,50]. Colchicine is now used in the treatment of gout, familial Mediterranean fever, Behcets disease and pericarditis, though it is only approved for the treatment of the two former diseases. Its mechanism of action is through inhibition of microtubule polymerization by its binding to tubulin [51]. Colchicine thereby inhibits mitosis, as mitosis cannot proceed in the absence of tubulin. Side effects of colchicine include hypovolemic shock, renal failure and death. It has a very low therapeutic index. There is no antidote, though there are means to reduce toxicity, including glutamate or aspartate [52,53].

4. Gold salts

“Gold salts” have been used in the treatment of rheumatoid arthritis since 1935 [54,55]. The use of gold salts is known as aurotherapy or...
chrysotherapy. In rheumatic arthritis, gold salts are usually administered intramuscularly. It takes a long time to take effect and is associated with multiple side effects, including discoloration of the skin when exposed to sunlight. The mechanism of gold salts is unknown, although there have been studies that suggest they interfere with the uptake and binding of molecules to MHC Class II proteins [56]. Examples of gold salts include auranofin [57] and aurothiogluucose. Auranofin has been shown to inhibit IL-1β and TNFα in macrophages [57]. A Cochrane Database review in 2000 showed that the use of gold salts lead to improvements in joint inflammation and reduced disease activity [58]. However, the side effects including chrysisis, which is an accumulation of gold compounds in skin and other tissues, acute renal failure, heart disease, and hematologic disease has led to a reduction in the use of gold salts in the treatment of rheumatoid arthritis [59–66].

5. Steroids

The discovery of theelin, theelol and estradiol by Edward A Doisy in the early 1930s opened the door to extensive research into a class of anti-inflammatory drugs that would have a remarkable impact on the treatment of autoimmune diseases. In 1932, Otto Rosenheim and Harold King identified the perhydrocyclo-pentanophenanthrene structure that forms the basis of cholesterol and related compounds. In 1935, Edward Kendall was studying compounds from bovine adrenal glands and called them compounds A, B, C, D, E and F. In 1936, he crystallized compound E from bovine adrenal glands, while at the same time Tadeus Reichstein discovered a similar substance and called it substance Fa. During the Second World War, it was found that pilots of the Luftwaffe were able to tolerate higher altitude flying with lower ambient oxygen after being administered adrenal extracts. A significant amount of research funding was funneled into the study of this group of compounds, at the expense of the newly discovered antimicrobial agents. This funding subsequent disappeared after the war, but in the 1940s, Phillip Hench of the Mayo clinic was studying the use of these drugs in a patient with rheumatoid arthritis, and found that compound E had remarkable clinical efficacy, reversing the crippling effects of her disease.

Subsequently, further clinical trials demonstrated that the initial dose could be reduced with similar efficacy, and the efficacy was shown to be reproducible. Compound E was eventually renamed Cortisone, in order to avoid confusion with Vitamin E. Hench, Kendall and Reichstein won the Nobel prize for their work on cholesterol metabolism. And an entire industry of development of synthetic corticosteroids was born. Fig. 3 illustrates the molecular backbone of the corticosteroid molecules.

The result of subsequent research in new steroid drugs was targeted to find drugs that would have greater efficacy and fewer side effects. It became obvious once corticosteroid were widely used in the treatment of autoimmune diseases that they were accompanied by significant adverse effects when used chronically. It was discovered that introduction of a 1,2 double bond in the A ring of cortisone yields prednisone, which was associated with increased anti-inflammatory effects and decreased side effects. Performing the same action on cortisol yielded prednisolone. It was also discovered that 16α hydroxylation retains glucocorticoid activity with no increase in salt and fluid retention. 16α methylation further increases anti-inflammatory activity. Fluorination at 9α increased anti-inflammatory potency, but also increased protein loss, potassium loss, sodium retention and edema. Combination of several manipulations on the perhydrocyclo-pentanophenanthrene nucleus by 9α fluorination, 1–dehydrogenation and 16α methylation yields the drug dexamethasone. Dexamethasone and betamethasone are molecules of similar structure and are considered to be epimers of each other. (Fig. 4).

These manipulations of the basic steroid or cholesterol ring has provided clinicians with a group of drugs that has been life changing for patients suffering from autoimmune diseases for the last 60 years [67]. They are still some of the more commonly used drugs, but new drugs have been constantly investigated because of the significant side effects of steroids, and the use of these newer drugs has been targeted for their “steroid-sparing” effects. Table 2 summarizes the currently available DMARDS.

6. Disease modifying anti-rheumatic drugs (DMARD)

DMARDS are a group of medications used to treat rheumatoid arthritis. These drugs are called disease modifying agents because not only do they provide reduction in symptoms of pain and inflammation over time, but they also reduce or prevent joint damage. In doing so, they also preserve joint structure and function [68]. However, their symptom relieving properties are such that improvements are not seen immediately, but over a period of 1–3 months. The benefit of their use is that they act as “steroid sparing” agents on one hand, and also over the long term can reduce the need for non-steroidal anti-inflammatory agents (NSAIDS). The DMARD do not act through a common mechanism, but may affect different pathways and cellular processes to exert their beneficial effect [69].

6.1. Azathioprine

Azathioprine was originally synthesized in 1957 as a prodrug for mercaptopurine. It acts as a purine synthesis inhibitor and inhibits the proliferation of T and B cells, and is thus useful as a cancer drug. It has been widely used since the 1950s in transplantation immunology, cancer treatment and autoimmune diseases [70–73]. The main severe side effect of azathioprine is bone marrow suppression. It is used in multiple autoimmune diseases including systemic lupus erythematosus [74], pemphigus [75], inflammatory bowel disease [76–79], autoimmune hepatitis, myasthenia gravis [80] and various vasculitidis, but its only FDA approved uses are for kidney transplantation and for rheumatoid arthritis [81,82].

6.2. Chloroquine and hydroxychloroquine

Chloroquine is an antimalarial drug. It has been used for the treatment of rheumatoid arthritis. It was developed in 1934, although it was probably used in some form well before that, and is a lysosomotropic agent. It has been associated with retinal toxicity, and while its mechanism of action in treating malaria is probably related to its characteristic of accumulating in lysosomes, its activity in autoimmune disease is more likely related to its weak anti-inflammatory activity, or possibly through its effects on apoptosis. Hydroxychloroquine, a derivative of chloroquine, is much more widely used in the treatment of rheumatoid arthritis and systemic lupus erythematosus. Like its related drug, it affects lysosomes. Hydroxychloroquine has several anti-inflammatory effects, including its ability to block toll-like receptors, and to increase the lysosomal pH in antigen presenting cells [83]. Increasing the pH of lysosomes leads to reduced cellular processing and this appears to affect immune function including phagoctysis, chemotaxis and oxidative burst activity [84].

6.3. Cyclophosphamide

Cyclophosphamide was first introduced in the 1950s to treat multiple cancers including brain tumors, lymphoma, leukemia and other solid tumors [85–90]. Cyclophosphamide is a nitrogen mustard alkylating agent. It acts by inhibiting DNA replication by attaching an alkyl group to the guanine resides. It has significant side effects so its use is generally limited to advanced disease states such as lupus nephritis [91,92]. Side effects include acute myeloid leukemia, hemorrhagic cystitis and bladder cancer, and infertility. Cyclophosphamide is also indicated in severe rheumatoid arthritis and granulomatosis with polyangiitis (GPA, Wegener’s granulomatosis). Cyclophosphamide and its related compound, ifosfamide are often administered in conjunction with 2-mercaptopoanol sulfonate sodium (mesna), which is an organosulfur compound which decreases the side effects of cyclophosphamide by
reacting its sulfhydryl group with the vinyl group of acrolein, a toxic metabolite of cyclophosphamide [93]. Aggressive hydration is also used to increase clearance of cyclophosphamide to reduce the incidence of hemorrhagic cystitis [94].

6.4. Cyclosporin

Cyclosporin is another plant derived medication that has been found to have immunosuppressive properties. Cyclosporin is a calcineurin inhibitor, and thereby blocks the signaling pathway of the T cell receptor. It inhibits calcineurin by binding to the cytosolic protein, cyclophilin [95]. Cyclosporin has broad clinical uses. Its immunosuppressive effects were discovered in 1972, and it was studied in transplant patients in the

![Fig. 3. The basic building block of steroids — the perhydrocyclo-pentanophenanthrene ring structure.](image)

![Fig. 4. The evolution of corticosteroids used in the treatment of autoimmune diseases.](image)
Table 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>Date of introduction</th>
<th>Mechanism of action</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azathioprine</td>
<td>1966</td>
<td>Purine synthesis inhibitor</td>
<td>A prodrug for mercaptopurine, bone marrow suppression is major adverse effect</td>
</tr>
<tr>
<td>Chloroquine and hydroxychloroquine</td>
<td>1934, 1955</td>
<td>Increases lysosomal pH</td>
<td>Inhibits association with class II MHC proteins</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>1957</td>
<td>Nitrogen mustard alkylating agent</td>
<td>Significant side effects</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>1972</td>
<td>Calcineurin inhibitor</td>
<td>Used in many other conditions including urticaria and atopic dermatitis</td>
</tr>
<tr>
<td>D-penicillamine</td>
<td>1956</td>
<td>Unknown</td>
<td>Not recommended to be used concomitantly with other DMARDs</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>1947</td>
<td>Inhibits purine metabolism and activation of T cells</td>
<td>Not approved for use in autoimmune diseases until much later</td>
</tr>
<tr>
<td>Minocycline</td>
<td>1972</td>
<td>Inhibits NF-κB nuclear translocation</td>
<td>Can induce autoimmune disease itself</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>1950</td>
<td>Unknown</td>
<td>Originally used in rheumatoid arthritis because of antimicrobial properties</td>
</tr>
<tr>
<td>Leflunomide</td>
<td>1990</td>
<td>Pyrimidine synthesis inhibitor</td>
<td>Not recommended for use in autoimmune disease until much later</td>
</tr>
<tr>
<td>Mycophenolic acid</td>
<td>1998</td>
<td>Inhibitor of purine metabolism</td>
<td>More expensive than azathioprine, also used in long term treatment of GPA</td>
</tr>
</tbody>
</table>

DMARDS = diseases treated with disease-modifying antirheumatic drugs. GPA = granulomatosis with polyangiitis.

6.5. D-penicillamine

D-penicillamine is a chelating agent. After its identification as a chelating agent, its first use was in the treatment of Wilson’s disease in 1956 [100]. Subsequently, D-penicillamine has been used in the treatment of rheumatoid arthritis [101] and scleroderma [102]. Like minocycline, D-penicillamine, while it has a role in the treatment of autoimmune diseases, paradoxically, has also been associated with the appearance of autoimmune diseases [103–107]. Other side effects of D-penicillamine are skin rashes in up to 15% of patients, mouth ulcers, nausea and loss of appetite, and impaired kidney function. A less common but serious side effect is bone marrow suppression, specifically aplastic anemia [108].

6.6. Leflunomide

Leflunomide is a pyrimidine synthesis inhibitor that had been used in various diseases prior to the 1990s. It acts as a general cytostatic agent and affects many cell types. It has been shown to inhibit T cell proliferation and to block progression of rat lymphocytes beyond the S phase of the cell cycle, thus inhibiting entry into the G2 or M-phases [109]. In the 1990s, the Food and Drug Administration approved leflunomide for use in systemic lupus erythematosus and rheumatoid arthritis. A study of 43 patients with juvenile idiopathic arthritis who were nonresponsive to methotrexate were treated with leflunomide. Of the 43 patients, 19 interrupted leflunomide. Overall, the baseline 28-joint Disease Activity Score (DAS28) dropped from 5.57 to 3.74 with a p value of < 0.001 [110].

6.7. Minocycline

Minocycline is a broad spectrum, synthetic, bacteriostatic tetracycline class antibiotic that has been shown to have anti-inflammatory activity in autoimmune diseases. It was first synthesized in 1972, and is considered by the American College of Rheumatology to be a DMARD for the treatment of rheumatoid arthritis. Several double blind clinical trials have demonstrated its effectiveness in the treatment of rheumatoid arthritis [111–114]. Minocycline acts by inhibiting apoptosis, by downregulating pro-inflammatory cytokines such as TNFα. It also inhibits the T cell-microglial interaction leading to a disruption of cell signaling through the inhibition of NF-κB nuclear translocation. An interesting point is that minocycline use has itself also been associated with drug-induced autoimmunity, especially autoimmune hepatitis [115], suggesting that the effects of minocycline on apoptosis and other regulatory mechanisms that impact T cell function are not clearly elucidated at the present time. Other adverse effects of minocycline include gastrointestinal adverse events, hypersensitivity reactions, hyperpigmentation and intracranial hypertension [116–118].

6.8. Sulfasalazine

Sulfasalazine is a sulfa drug that was developed in the 1950s for the specific purpose of treating rheumatoid arthritis [119]. The rationale for its use is that it is an antibiotic, and it was originally believed that rheumatoid arthritis was an infectious disease. However, its metabolite 5-aminosalicylic acid also has known anti-inflammatory properties. It is continued to be used mainly because of its anti-inflammatory properties. Its use in autoimmune disease has been extended to psoriatic arthritis, ulcerative colitis [120], Crohn’s disease, and even in idiopathic urticaria [121]. Side effects include stomach ulcers, though the incidence is less than that with typical non-steroidal anti-inflammatory
drugs (NSAIDS) such as ibuprofen or naproxen. Sulfasalazine can lead to hematologic side effects, including hemolytic anemia in patients with G6PD deficiency and immune thrombocytopenia [122]. Because it is a dihydrofolate reductase inhibitor, it can cause folate deficiency, leading to a megaloblastic anemia [123].

6.9. Mycophenolate mofetil

Mycophenolic acid was first discovered in 1893 by Bartolomeo Gosio. Like cyclosporine, mycophenolic acid is derived from a fungus, in this case, Penicillium stoloniferum or Penicillium echinulatum [124]. It acts by inhibiting inosine monophosphate dehydrogenase. By inhibiting inosine monophosphate dehydrogenase, it acts as an inhibitor of the de novo pathway of purine synthesis, thus blocking the proliferation and activation of T cells [125]. It is similar to azathioprine but has fewer side effects and is often used in combination therapy in the treatment of autoimmune diseases such as lupus nephritis [126,127], small vessel vasculitis, autoimmune hepatitis [128], psoriasis and immunoglobulin A nephropathy. It is an immunosuppressive agent and therefore also used in transplant patients. Possible adverse effects of mycophenolic acid include a risk of opportunistic infections and progressive multifocal leukoencephalopathy (PML). It has also been associated with miscarriages and congenital malformations.

6.10. Methotrexate

Methotrexate, or amethopterin, had been used since 1947 in the treatment of cancer. It was first developed by Subbarao and used in the treatment of acute lymphoblastic leukemia. In the 1950s, its use extended to the treatment of solid tumors, such as choriocarcinoma, mycosis fungoides, and chorioidenoma [129–133]. It wasn’t until 1988 that the FDA approved its use in rheumatoid arthritis.

Methotrexate is an anti-metabolite and anti-folate drug. It can be administered parenterally or orally. It has been used now in many autoimmune diseases, including SLE, psoriatic arthritis, psoriasis, juvenile dermatomyositis, and Crohn’s disease. It has also been used in the treatment of eczema and in urticarial vasculitis. It is considered a safe drug when administered orally in the correct dosages, but has significant side effects when administered intravenously, including gastrointestinal bleeding or epithelial damage, central nervous system effects such as seizures or coma and bone marrow toxicity, including myelosuppression which peaks at 7–14 days. Methotrexate also has significant teratogenic effects and is designated pregnancy class X.

The mechanism of action of methotrexate in autoimmune diseases is by inhibition of purine metabolism, inhibiting T cell activation and suppression of intracellular adhesion molecules. In cancer, the mechanism is thought to be through inhibition of dihydrofolate reductase, thereby inhibiting DNA synthesis in rapidly dividing cells.

7. Biological response modifiers (BRM)

Two molecular biology technologies or discoveries merged to allow for the evolution of biological response modifiers. The discovery of cytokines and chemokines and the significant impact that they have on immune function provided a target to which drugs could be developed to attenuate an over-reactive immune system. The second discovery which occurred in roughly the same period was the ability to fuse a myeloma cell with mouse spleen cells after immunizing the mice to produce antibodies against a specific antigen [134]. This leads to the formation of hybridomas [135]. The hybridomas are selected by incubating the cells in HAT (hypoxanthine–aminopterin–thymine) medium. Since myeloma cells are HPRT negative, they will not survive in HAT medium. B cells, which are HPRT positive, that are not fused with the myeloma cells, are not immortal and will eventually die. Only B cells fused with myeloma cells will survive in HAT medium. Once a collection of hybridoma cells are identified, then they can be screened for the desired antibody and cultured in order to generate an indefinite supply of monoclonal antibodies. It is this methodology for which Kohler, Milstein and Jerne were awarded the Nobel Prize in 1984.

The upshot of these two events was that scientists could now produce monoclonal antibodies to almost any immunogenic protein. At first these antibodies were produced in mice, and this led to the formation of human anti-mouse antibodies in patients who were treated with mouse monoclonals. Subsequent research led to the development of chimeric, and human monoclonal antibodies, using approaches of transgenic mice [136] and phage display technology [137], which had led to a significantly reduced incidence of adverse reactions.

Apart from monoclonal antibodies, another class of biological response modifiers are the fusion proteins. These drugs are synthesized by fusing a part of an immunoglobulin molecule with another molecule or part thereof in order to inhibit the activity of a particular cytokine. In some cases, the Fc portion of an Ig molecule is bound with a receptor against the target molecule (as in etanercept), while in other cases, the Ig molecule can be bound to a portion of the target molecule itself (as in abatacept). More complex iterations can be employed as well, and in rilonacept, we have a fusion protein that consists of the Fc portion of an IgG1 molecule bound to ligand binding domains of the extracellular part of the interleukin 1 receptor (IL1R1) and the IL-1 receptor accessory protein (L-1RαCP), thereby neutralizing IL-1. See Table 3 for indications of the various BRMs.

7.1. Blocking pro-inflammatory cytokines

7.1.1. TNFα

The first evidence of an anti-tumor factor with cytotoxic activity against lymphocytes was demonstrated by Granger et al. [138] and Ruddle et al. [139–141] in 1968. It was named lymphotoxin. In 1975, another factor was discovered to have similar cytotoxic activity, and this was named tumor necrosis factor (TNF) [142]. In 1984, it was found that the DNA sequence for these two factors was similar; tumor necrosis factor was renamed TNFα and lymphotoxin was renamed TNFβ. Activity of TNFα is through the TNFR1 and TNFR2 receptors [143]. TNFα and 2 are respectively also known as CD120a and CD120b. Activation leads to an inflammatory response which is mediated by activation of NF-κβ, the MAP kinase pathway and death signalling [144–147]. Because TNFα is a proinflammatory cytokine, its activity has been demonstrated to play a role in autoimmune diseases, including rheumatoid arthritis, psoriasis, ankylosing spondylitis, Crohn’s disease and ulcerative colitis.

There are now five anti-TNF agents currently available in the United States. The first to be approved was infliximab in 1998 for the treatment of Crohn’s disease. Infliximab is a chimeric monoclonal antibody which was developed in 1993 by Knight et al. [148]. Soon after the introduction of infliximab, Etanercept was introduced. As the name suggests, Etanercept is not a monoclonal antibody, but instead is a recombinant fusion protein, consisting of the TNF receptor 2 and the IgG1 constant region. It was developed by Beutler et al. in 1991 [149–151]. Its mechanism of action is through blockade of the TNF receptor 2 molecule. Bruce Beutler incidentally went on to share the Nobel Prize in 2011 with Jules Hoffman and Ralph Steinman for his work on innate immunity.

The third TNF inhibitor to be introduced was Adalimumab, which unlike infliximab is a human monoclonal antibody. It was discovered in 1993, but it wasn’t until 2008 that Adalimumab was approved for the use in multiple autoimmune diseases including rheumatoid arthritis, moderate to severe chronic psoriasis, ankylosing spondylitis, Crohn’s disease, psoriatic arthritis and juvenile idiopathic arthritis in children. In 2012, it was also licensed for use in ulcerative colitis.

Further development of monoclonal antibodies yielded Golimumab [152] and Certolizumab pegol. Golimumab is a humanized monoclonal antibody against TNFα, and received approval in 2009 for the treatment of adult rheumatoid arthritis, adult active psoriatic arthritis and ankylosing spondylitis. The benefit of Golimumab over infliximab is less frequent dosing of monthly injections. Certolizumab pegol is unique in that it is a...
polyethylene glycol polymer fragment bound to the Fab' fragment of a humanized monoclonal antibody against TNFα. The advantages of PEGylation of a drug are that the hydrophilic but inert polymer increases the molecular weight and improves drug solubility and stability, extends circulating life, reduces dosage frequency without a corresponding reduction in efficacy, and protection from proteolytic degradation. Certolizumab pegol was approved for the treatment of Crohn's disease and moderate to severe rheumatoid arthritis in 2008. It was approved on September 27th, 2013 for the treatment of adult patients with psoriatic arthritis, and on October 18th, 2013, UCB announced that Certolizumab pegol had been approved by the FDA for the treatment of active ankylosing spondylitis. Monoclonal antibodies to TNFα are often used in conjunction with methotrexate in the treatment of autoimmune diseases [153].

The most common side effect with all of the anti-TNF inhibitors is skin reactions. The most common significant adverse effect is a risk of increased infection [154]. These infections may include reactivation of tuberculosis and fungal infections. All patients should have a TB test before implementing anti-TNF therapy. Other side effects are rare, but include a risk of lymphoma, and neurological complications have been reported as well. Interestingly, it has also been observed that some patients who are being treated with anti-TNF agents can develop autoantibodies [155]. Anti-TNF agents have also been associated with hepatotoxicity [156].

7.1.2. IL-1

Apart from TNFα, there are other pro-inflammatory cytokines, including IL-1. IL-1 is produced by numerous cell types and is found in elevated concentrations in the serum, synovial fluid and tissues of patients with rheumatoid arthritis. Success in animal models showing that anakinra, a recombinant protein which is an antagonist of naturally occurring interleukin-1 receptor (IL-1Ra), is effective in mediating and amplifying destructive arthritis led to its introduction as an anti-inflammatory drug [157–159]. It was approved in 2001 for use in patients with moderate to severe rheumatoid arthritis who do not respond to traditional DMARDS. Unlike the monoclonal antibodies, anakinra is administered at daily subcutaneous doses of 100 mg.

In addition to anakinra, canakinumab also targets the IL-1 pathway. Canakinumab is a monoclonal antibody of the IgG1κ type that is directed against the IL-1β subunit of IL-1. It does not bind to IL-1 receptor but blocks the interaction between IL-1 and its receptor. It was FDA approved for use in active juvenile idiopathic arthritis in children 2 years of age and older. Administration of canakinumab was associated with a significant response in acute phase reactants including C-reactive protein. The other indication for canakinumab is in the cryopyrin associated periodic syndromes [160] and it was also associated with an improvement in serum amyloid A levels as well. As indicated by the common usage of these drugs in autoimmune and autoimmune inflammatory conditions [161], the pathogenic mechanism may involve similarities, that may involve the inflammasome [162,163]. The third drug directed against IL-1, rilonacept, which utilizes IL-1 Trap methodology to inhibit IL-1 activity, was developed as an Orphan drug and is not FDA approved for autoimmune diseases, but is indicated for the treatment of familial cold autoimmune inflammatory syndrome (FCAS) and Muckle-Wells syndrome (MWS).

7.1.3. IL-6

The other proinflammatory cytokine, which itself has stimulatory effects on IL-1 and TNFα, is Interleukin-6 (IL-6). IL-6 acts through type 1 cytokine receptor complex consisting of IL-6Ra chain and gp 130 (CD126 and CD 130 respectively). CD130 is also known as IL-6 signal transducer. A humanized monoclonal antibody called tocilizumab is approved for the treatment of rheumatoid arthritis in combination with methotrexate or as monotherapy in those who cannot tolerate methotrexate [164]. In addition, it is one of the few monoclonal antibodies that has been FDA approved for use in children down to two years of age, as it is approved for the treatment of systemic juvenile idiopathic arthritis. As with most monoclonal antibodies, it is administered intravenously monthly.

7.2. Blocking B cells

A technique known as B cell depletion therapy has also been used in the treatment of rheumatoid arthritis [165]. The pathogenesis of autoimmune diseases and the role or autoantibodies spurred research into the inhibition of B cell activity or numbers by the use of a monoclonal antibody, and in 1997, rituximab, a monoclonal antibody directed against CD20, a protein found on the surface of B cells, was initially approved for the treatment of non-Hodgkin lymphoma. Rituximab has also been found to be effective in the treatment of rheumatoid arthritis.
in conjunction with methotrexate [166,167]. It is approved for use in refractory rheumatoid arthritis [168]. It may also be an alternative for patients who have a poor response to anti-TNF drugs [169]. Besides rituximab, more recently developed human monoclonal antibodies against CD20 include ocrelizumab, which has shown reduced immunogenicity of the molecule while providing effective treatment of rheumatoid arthritis in conjunction with methotrexate [170].

7.3. Abatacept

Abatacept is a fusion protein that consists of the Fc portion of IgG1 combined with the extracellular domain of CTLA-4. Abatacept is indicated in the treatment of rheumatoid arthritis that is not responsive to DMARDS or anti-TNFα agents [171–173]. Abatacept was introduced in 2005. The mechanism of action is through blocking of co-stimulatory signals from antigen presenting cells to T cells. Studies of abatacept in ulcerative colitis have not been promising [174], and there are ongoing clinical trials investigating its efficacy in type 1 diabetes mellitus [175], multiple sclerosis and lupus nephritis when used in combination with cyclophosphamide [176].

7.4. Ustekinumab — blocking IL-12 and IL-23

Ustekinumab is a monoclonal antibody targeting IL-12 and IL23. It was approved in 2009 for the treatment of moderate to severe plaque psoriasis, and in September of 2013 for the treatment of psoriatic arthritis. In clinical trials, it showed superior efficacy when compared with etanercept in the treatment of patients with moderate to severe plaque psoriasis [177,178]. It also showed that it was safe in clinical trials of up to 76 weeks duration of treatment [178]. It also showed 75% improvement in psoriasis area and severity index (PASI) scores in studies on patients with plaque psoriasis [179].

7.5. B cell activating factor (BAFF, BLys)

Belimumab, a new monoclonal antibody directed against B cell activating factor has been in clinical trials in the treatment of systemic lupus erythematosus. However, the trials were not done in patients with severe SLE, and the available data is not entirely convincing in that the drug was only marginally effective and there were more deaths in the treatment group compared to the placebo group [180–187]. Belimumab did allow some patients to reduce their corticosteroid dosage. Targeted therapy against BAFF has also been studied in the treatment of myasthenia gravis [188].

7.6. Janus associated kinase (JAK) inhibitors

A new class of drugs, called Janus-associated kinase (JAK) [189–210] inhibitors, is now available. Tofacitinib citrate is the first in this category of drugs and was FDA approved in 2012 for the treatment of rheumatoid arthritis. It is also undergoing trials in other autoimmune diseases including psoriasis [211–213], ulcerative colitis [214–218] and also has a role in transplantation as an immunosuppressive agent [219–221]. It has been shown to reduce joint swelling, pain and stiffness. Its mechanism of action is through disruption of the cytokines signaling pathways that utilize the JAK-STAT pathway. Unlike the BRMs, it is a small pill that is taken orally twice daily. Known adverse effects include an increased risk of infection, gastrointestinal perforation, and lymphomas and other cancers [190,193,222–224].

7.7. Unmet needs — searching for new treatment options for autoimmune diseases

Despite the lengthy discussion on DMARDS and new biologicals, the fact is that treatment for many of the autoimmune diseases is virtually non-existent. In our previous discussion, most of the biologicals are directed towards rheumatoid arthritis and inflammatory bowel diseases. With the exception of an anti-BAFF monoclonal antibody that is being studied in the treatment of systemic lupus erythematosus, there are no biologicals approved for the treatment of primary biliary cirrhosis (PBC) [225–228], Sjogren’s syndrome, scleroderma, polymyositis and dermatomyositis, although rituximab [227] and CTLA4-Ig [229] has been studied in refractory patients with PBC. It is especially curious that it is precisely these disease in which a known autoantigen has been identified — mitochondria in PBC, Ro and La in Sjogren’s syndrome, Sc-70 in scleroderma and histidyl-tRNA-synthetase in dermatomyositis. Perhaps this illustrates the difficulty in finding efficacy through blockade of one immune pathway in the face of tremendous redundancy in immune function. Another way to look at this is that it is also quite ironic, and intriguing, that it is those autoimmune diseases in which a clear autoantibody has not been found that are most successfully treated with BRMs, for example, rheumatoid arthritis and psoriasis.

8. What’s next? — the age of genomics, proteomics, metabolomics and personalized medicine

Because of the heterogeneity of many of the autoimmune diseases, it may not be possible to define an optimal therapy that will be applicable to all patients. Genetics have been shown to play a role in disease pathogenesis and in determining whether a patient will respond to a particular treatment modality [230–234]. For example, in rheumatoid arthritis, the interleukin-6 (IL-6) -174G>C and the IL-6 receptor (IL-6R) D358A gene polymorphisms, affects a patients response to rituximab [235]. These pharmacogenetic patterns may be found in many other diseases and is the focus of many clinical trials. The ability to target optimal therapy to the right patient will have an impact in healthcare delivery, quality and costs.

Epigenetics is the reversible, hereditable change in gene activity that does not occur as a result of a change in DNA sequence. In other words, this is an additional mechanism which determines who develops a disease. Obviously, the best way to study epigenetic effects is in identical twins [10,236], but in the absence of finding sufficient pairs of identical twins, large scale population based studies must be conducted. The major mechanisms for epigenetics are through DNA methylation or histone acetylation/deacetylation [236,237]. Changing the histone structure allows for varying degrees of “openness” or accessibility of DNA, thereby impact the extent of gene expression. The role of epigenetics in the pathogenesis of autoimmune diseases is now well documented [1,232,237–242]. Although there are no current drugs that can impact epigenetics directly, knowledge of epigenetics in clinical cases has the potential for developing targeted therapy by identifying which patients respond best to which medications. The mechanism of most autoimmune diseases most likely results from a combination of factors related to genetics, epigenetics and environment [243,244].

MicroRNAs were discovered in the 1990s and their significance in autoimmune disease were appreciated in the early to mid-2000s. MicroRNAs are small non-coding RNAs that function as transcriptional or post-transcriptional regulators of gene-expression. It is thought that microRNAs act by binding to complementary sequences in messenger RNA to induce gene silencing through translation repression or target degradation [245]. There are estimated to be about 1000 microRNAs in the human genome, and each microRNA may affect multiple genes [246,247]. Some of the microRNAs that are known to impact autoimmune diseases are shown in Table 4 [246,248]. While the current thought is that microRNAs that are identified to be downregulated or upregulated in autoimmune diseases may provide additional diagnostic possibilities or better disease monitoring tools, there is also a possibility that they may also be used as therapeutic targets in the future. At the very least, microRNAs may serve as tools to predict clinical response to medications [249].

The other therapy that should not be overlooked in the treatment of autoimmune diseases is mesenchymal stem cell (MSC) transplantation.
Because the pathogenesis of autoimmune disease, by definition, is a derangement in the ability to tell self from non-self, it has been postulated that the reason for this is that the origin of the defect may be in mesenchymal stem cells. Therefore, it has been proposed that autologous hematopoietic stem cell transplantation effectively can take an existing overly self-reactive immune system that is subjected to ablative therapy, and replace it with a new more self-tolerant immune system. In the 15 years of experience with MSC in autoimmune diseases, there have been over 1500 cases of patients with autoimmune diseases including systemic sclerosis, juvenile idiopathic arthritis, rheumatoid arthritis, systemic lupus erythematosus, and idiopathic cycopenic purpura, with good results [250].

One should not forget that we are not passed the era of biological response modifiers, that there are many other cytokines and chemokines that play significant roles in the pathogenesis of autoimmune diseases. These include CXCL10 with a role in rheumatoid arthritis [251], the interleukin 7 pathway in various autoimmune diseases [252] and RANKL in the treatment of bone loss in rheumatoid arthritis [253]. Anti-CD4 has also been studied in the treatment of scurfy mice, an animal model resembling the immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) syndrome [254]. The high cost of developing biological response modifiers is inhibitory towards the production of generics that are identical to the branded drug, which has propelled the concept of using biosimilars and there efficacy in treating autoimmune diseases, providing a means for cost reduction [255].

9. Conclusions

It is important to appreciate the remarkable discoveries of the past 100 years. We now have new drugs that have better efficacy when used either in combination or alone in the treatment of autoimmune disease. Some of these drugs have more, and some have less, adverse effects than the older drugs. Most new drugs are more expensive, and this must be considered when appropriately choosing, maintaining and discontinuing the therapy for any given patient [256,257]. Another consideration is to ensure that the use of these drugs is actually clinically effective in improving symptoms in any given case, that tight control must be implemented in the use of these drugs by close monitoring of disease activity [258]. As we move through the era of biological response modifiers and into the age of personalized medicine, we begin to understand why customized management plans are important in these disease that have such great heterogeneity [259–262]. As our focus on healthcare reform and the affordable healthcare act takes center stage, it will become more and more important to provide patients with the right medications, considering the exorbitant prices of today’s new medications [263].

References


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