Understanding autoimmune disease: new targets for drug discovery

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A more complete understanding of the mechanisms that drive autoimmune diseases has begun to be translated into therapeutic options with significant clinical consequences. A clear example of this is the introduction of biological therapies, which have provided new therapeutic avenues, as well as validated the mediators (TNFα, IL-6), mechanisms (T cell costimulation, leukocyte migration), and cellular players (T and B lymphocytes) of the disease process itself. New discoveries into the role of Th17 and regulatory T cells and the epigenetic regulation of cytokine expression may offer novel intervention strategies to satisfy the unmet medical needs that still exist in these diseases.

Introduction

Autoimmune diseases (see text Box 1) affect approximately 5% of the human population [1] and are associated with high morbidity and mortality. They are chronic debilitating diseases that often attack young adults, especially women, and their social and economic impact is enormous [2].

For many years, the treatment of autoimmune diseases has been based on non-selective immunosuppressant or cytotoxic drugs that in general offer a limited clinical efficacy. The introduction of biological therapies in the late nineties opened a new era in the treatment of autoimmunity, and exerted a significant impact on the disease course and the quality of life of patients. There are currently 13 biologicals either approved or in advanced clinical trials, the vast majority in rheumatoid arthritis (RA), multiple sclerosis (MS) and/or Crohn's disease. An example of the current therapeutic armamentarium of these three diseases, depicting both small molecules and biologicals, is shown in Figure 1.

Biologicals have faster onset of action and higher efficacy than the existing therapies, but they are expensive, some patients do not respond adequately, and they can cause severe side effects. Treatment with Tysabri (natalizumab) has been associated with the development of progressive multifocal leucoencephalopathy, an opportunistic infection of the brain caused by the JC-virus [3]. Other biologicals have been reported to induce autoimmune disorders, like idiopathic thrombocytopenic purpura (ITP) [4], lupus-like syndrome or vasculitis [5]. All these drugs offer significant improvement in the quality of life of patients, but their risk/benefit has to be carefully analyzed and frequently revisited.

Clinical trials with biologicals have significantly contributed to the changes observed in the treatment paradigm of autoimmune diseases in recent years. On the one hand, there is an increasing use of add-on or combination therapies in established disease. On the other hand, clinical studies in RA and MS have proven that treating the disease as soon as the first clinical symptoms appear may prevent tissue destruction [6,7]. Both drug combinations and early disease intervention place an emphasis on safety, and this is an unmet need that novel therapies should address.

Recent scientific discoveries have shed light on basic mechanisms operating in autoimmunity and may offer novel strategies for therapeutic intervention. For example, the field has recently seen the incorporation of new T cell lineage members [8–10], like the Th17 cells, that have been shown to play a prominent role in tissue injury in autoimmunity [8], or a new subset of regulatory cells, the inducible regulatory T cells (iTreg) [9]. Studies performed by different laboratories strongly suggest that T cell differentiation into the different subsets (Figure 2) is a highly plastic process that results from the combination of signals provided by antigen-presenting cells through the T cell receptor, the local cytokine environment and the interactions with resident tissue cells [11,12]. Thus, modulation of certain cytokines in specific compartments

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BOX 1

Autoimmune diseases are chronic inflammatory diseases of unknown etiology that results from the combination of a genetic susceptibility, environmental factors and immune dysregulation. The breakdown of mechanisms controlling immune tolerance is believed to be responsible for the attack of self-antigens by autoreactive lymphocytes, leading to tissue injury. Defects in lymphocyte activation and innate immune mechanisms, sustained leukocyte and lymphocyte infiltration, massive production of cytokines, and secretion of autoantibodies, are common features of the different diseases.

Autoimmune diseases may affect a particular organ or tissue, or have a systemic manifestation. Some examples of diseases belonging to each category are indicated below:

Nervous system: multiple sclerosis, myasthenia gravis

Skin: psoriasis, pemphigus vulgaris, vitiligo

Gastrointestinal system: Crohn's disease, ulcerative colitis, primary biliary cyrrhosis

Blood: autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura

Endocrine glands: type I diabetes mellitus, Grave's disease, Hashimoto's thyroiditis

Blood vessels: anti-phospholipid syndrome, Wegener's granulomatosis

Kidneys and lungs: Goodpasture syndrome

Systemic: rheumatoid arthritis, systemic lupus eryhtematosus, scleroderma, Sjogren's syndrome, ankylosing spondylitis, dermatomyositis.

There are approximately 70 human diseases currently classified as autoimmune. They differ in terms of incidence (number of new patients diagnosed each year), and prevalence (number of people with the disease at a specific time). The most common diseases are psoriasis, Grave's disease, rheumatoid arthritis (RA) and thyroiditis. Others, like Goodpasture syndrome, are very rare. Women are more affected than men and, in diseases like lupus, 85% of patients are females.

The classical therapies of most autoimmune diseases consist of disease-modifying agents like methotrexate, leflunomide, azathioprine, sulphasalazine or cyclosposphamide. Corticosteroids are usually administered for a limited period of time, during the relapses or exacerbations of the disease. In some conditions, intravenous immunoglobulin therapy is used. Drugs like methotrexate are frequently used in more than one disease, but other treatments are disease-specific, like the interferon beta formulations or glatiramer acetate of multiple sclerosis. Diseases like type I diabetes are very difficult to treat owing to the fact that, at the time of diagnostic, most of the pancreatic islets have already been destroyed.

The limitations of current disease-modifiers are a slow onset of action, a moderate efficacy that declines after several years of treatment, and side effects, the most common being hepatotoxicity, myelosuppression and/or general immunosupression.

may have a significant impact on the predominance of one or another cell phenotype.

Epigenetics, the study of heritable changes in gene function that occur without a change in the DNA sequence, is a rapidly evolving field that is likely to lead to the discovery of future therapies. It not only provides the mechanisms to explain T cell lineage differentiation and reprogramming [12–14], but it can also explain how environmental factors may trigger autoimmunity in diseases like systemic lupus erythematosus [15]. This area is still in its infancy, but a better knowledge of the role of epigenetic mechanisms operating in the different autoimmune diseases may help to tailor specific therapies.

This article will review some of the current and future potential targets for the treatment of autoimmune diseases in the light of these discoveries. For this purpose, targets have been grouped according to their putative role in blocking cytokines, cell migration, or T cell and B cell function. For each group, a table with drugs that are in active clinical development according to www.clinicaltrials.gov or the company websites are included. Finally, possible strategies aimed at modulating epigenetic mechanisms will also be discussed.

Inhibition of cytokine synthesis and/or signalling

Cytokines are direct mediators of inflammation and participate in the regulation of the immune response. They have been reported to play a fundamental role in the regulation of the phenotype of effector T cells (Figure 2). An imbalance between proinflammatory (TNFα, IL-12) and antiinflammatory (IL-10, TGFβ) cytokines may favor the induction of autoimmunity, chronic inflammation and tissue damage. The pleiotropic functions of cytokines make these mediators very attractive targets.

Biologicals have demonstrated that the blockade of proinflammatory cytokines like TNFα or IL-6 can prevent disease progression in RA. Among the novel cytokines, the most promising targets are IL-23 and IL-17 (Table 1). Genetic studies indicate an association between certain IL-23R alleles and susceptibility to psoriasis [16] and intestinal bowel diseases [17] and this has been translated successfully into the clinic. Ustekinumab, an antibody directed against p40, the common chain of IL-12 and IL-23, has recently been approved in psoriasis. Regarding IL-17, the neutralization of this cytokine has been reported to ameliorate the disease in animal models of RA and MS, confirming the important effector function of this cytokine [18,19]. An anti-IL-17 antibody (AIN457, Novartis) is in clinical trials for various autoimmune indications. Genomic association studies may be fundamental to establish which cytokines are particularly relevant in the different autoimmune diseases [16,17,20].

Attempts to block the biological effects of cytokines with oral small molecules inhibitors of kinases have been actively pursued. The best examples are p38 mitogen-activated protein kinase (MAPK) inhibitors, mainly of the α and β isoforms. Inhibitors of p38MAPK block the synthesis of TNFα and other proinflammatory cytokines (IL-1, IL-8) induced by several stimuli both *in vitro* and *in vivo*. They have been postulated to be the oral alternative to the anti-TNF biologicals. At least five different compounds (BIRB-796, Scio-469, VX-475, VX-702, pamapimod) have been tested in clinical trials of RA [21] or Crohn's disease [22], and eventually failed owing to either toxicity or poor clinical efficacy. It has often been argued that toxicity may have precluded using high enough doses in the clinic, which would explain the modest efficacy observed with all compounds. Recent discoveries, however, point to a different reason for their failure as they suggest that the p38 pathway may be more complex than anticipated. Clinical trials with Scio-469 have shown that the levels of C-reactive protein (CRP) in patients diminish at the beginning of the treatment (−48% at 2 weeks) but then rebound (−1% at 12 weeks), suggesting

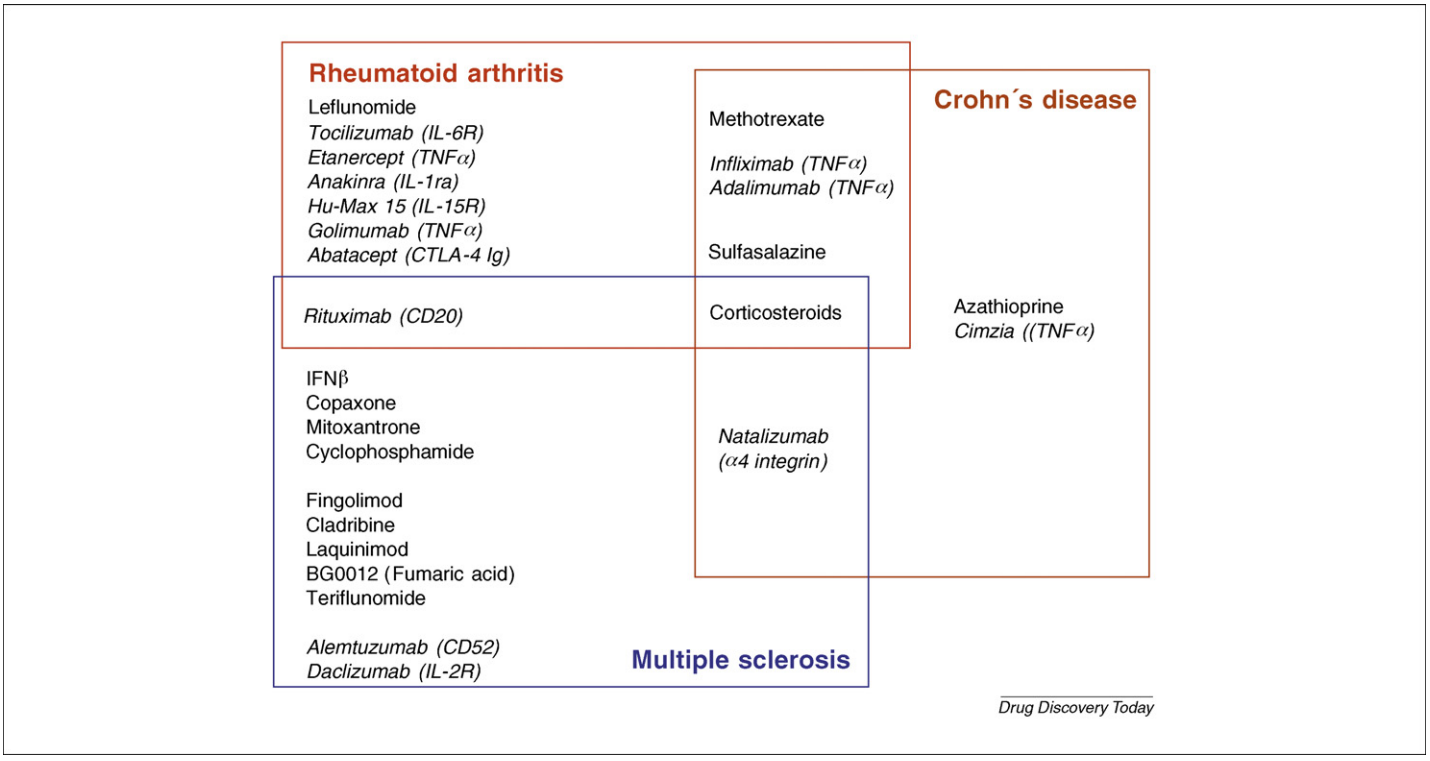

FIGURE 1

Current drugs approved or in advanced clinical trials in RA, MS and Crohn's disease. Biologicals appear in italics with their targets in parenthesis. Therapies at the intersection of the disease boxes indicate they are efficacious in more than one disease.

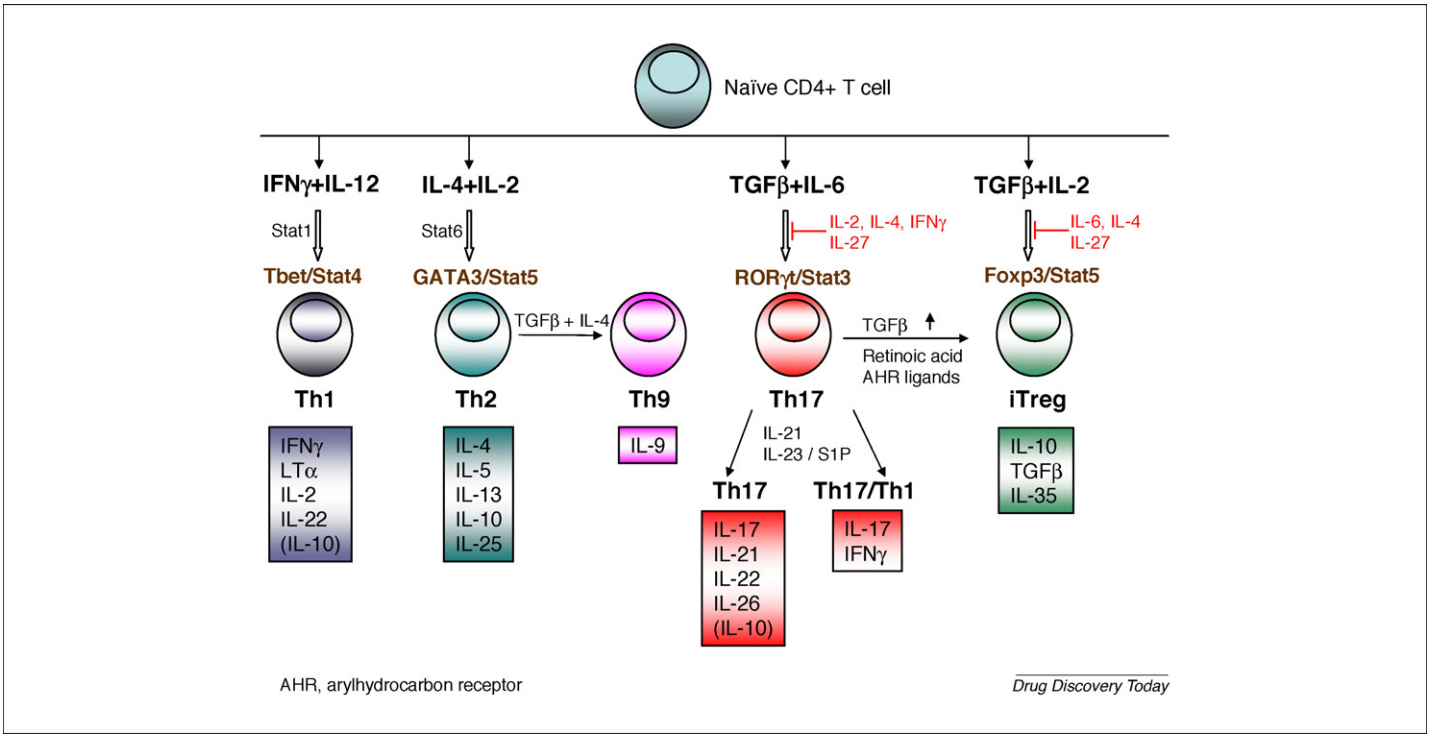

FIGURE 2

$CD4^+$ T cell lineage differentiation. The driving cytokines, their characteristic transcription factors, their regulators, and the cytokines produced by each subset are depicted (modified from [11]).

TABLE 1

Inhibition of cytokine synthesis and/or biological effects

Target mechanism	Rationale	Drug, company	Current status
JAK kinase *Inhibitors*	Blockade of multiple cytokine receptor signalling	CP-690,550, *Pfizer* INCB018424 and INCB28050, *Incyte* R348, *Rigel*	Phase 3 in RA, Crohn's and psoriasis Phase 2 in RA Phase 1
MEK kinase *Inhibitors*	This kinases mediate signals from various growth factors and proinflammatory cytokines	ARRY-438162, *Array BioPharma*	Phase 2 in RA
IL-12/23 *Antibody*	Blockade of p40 chain, common to IL-12 and IL-23, master regulators of the immune response	Ustekinumab, *Centocor*	Pre-register in psoriasis Phase 2/3 in Crohn's
IL-2 *Antibody*	Prevent clonal expansion of autoreactive T cells	Zenapax, *Biogen and PDL BioPharma*	Phase 2 in MS, type I diabetes and uveitis
IL-17 *Antibody*	Sustains chronic inflammation. Stimulates the production of inflammatory cytokines and chemokines that promote the recruitment of neutrophils and macrophages.	AIN457, *Novartis*	Phase 2 in Crohn's, psoriasis, and uveitis Phase I/II in RA and ankylosing spondylitis
IFNγ *Antibody*	Inhibition of NK cell development. Inhibition of macrophage activation. Induction of chemokines.	MEDI-545, *MedImmune*	Phase 2 in SLE

the existence of a compensatory mechanism to p38 inhibition [23]. Other kinases explored as alternative targets to p38 have been MK2, one of the p38 substrates [24], the MEK1/2 kinases (See Table 1) and Cot/Tpl2 [25]. No compounds against MK2 or Cot/Tpl2 seem to be in clinical development.

Another strategy to inhibit cytokine signalling is the inhibition of the JAK tyrosine kinases associated with cytokine receptors. Receptor stimulation leads sequentially to JAK activation by phosphorylation, receptor phosphorylation, STAT protein recruitment and STAT activation and dimerization [26]. The STAT dimer then functions as a transcription factor, translocating to the nucleus and activating the transcription of multiple response genes. The JAK family of kinases comprises four different members, namely JAK1, JAK2, JAK3 and TYK2, which control the signal transduction of a number of cytokine receptors and hematopoietic factors [26,27]. JAK-deficient cell lines and mice have validated the essential role of each protein in receptor signalling: JAK1 in class II cytokine receptors (IFN and IL-10 family), those sharing the gp130 chain (IL-6 family) and the common gamma chain (IL-2 family); JAK2 in hematopoietic factors (Epo, Tpo, GM–CSF, IL-3, IL-5) and type II IFNs; JAK3 in receptors sharing the common gamma chain (IL-2 family); and Tyk2 in the receptors of IL-12, IL-23 and type I IFNs [27].

The restricted expression pattern of JAK3 in the lymphoid system along with its role in the signalling of the IL-2 family of receptors, which includes potential targets like IL-15 and IL-21, makes JAK3 an attractive target for arthritis. From an efficacy standpoint, however, a non-JAK3 inhibitor could theoretically be efficacious as well since IL-6, a clinically validated target through the use of tocilizumab, an anti-IL6R antibody [28], requires essentially the gp130-associated JAK1. A similar rationale could apply to IFNγ (JAK1/JAK2) or GM–CSF (JAK2/JAK2). The disadvantage of non-JAK3 inhibitors could be potential adverse effects associated with inhibition of all cytokines that signal through JAK1 or of hematopoietic factors like Epo signalling through JAK2. In addition, the high sequence homology of the JAK's kinase domains makes the generation of JAK-specific inhibitors difficult.

At present, two JAK inhibitors, CP-690,550 (Pfizer) and INCB018424 (Incyte), with distinct JAK enzymatic profiles have reached phase II clinical trials for RA. The available data indicate that both compounds have a rapid onset of action and demonstrate efficacy in a high percentage of patients [29,30]. Dose-dependent side effects, such as reductions in hemoglobin and neutrophil counts and dyslipidemia [31] have also been reported for CP-690,550. Advanced clinical phases for these compounds will help to clarify the efficacy/safety ratio of JAK inhibitors in arthritis.

Blockade of cell trafficking

Cell infiltration is a crucial event in chronic inflammation and one of the main mechanisms responsible for tissue damage. The main players of this process, selectins, integrins and chemokines are obvious points for intervention and strategies directed against all of them have been explored. Two anti-integrin antibodies, Tysabri (against α4β1 and α4β7) and Raptiva (anti-CD11a), have shown that the inhibition of lymphocyte trafficking is a valid approach for the treatment of MS, Crohn's and psoriasis.

The chemokine family provides multiple targets [32] with the potential of blocking the migration of specific cell subpopulations [33]. Several small molecule antagonists, as well as anti-receptor and anti-chemokine antibodies progressed to clinical trials [34] but all of them failed owing to lack of efficacy. To our knowledge, the only agent with reported positive results in an autoimmune disorder is Traffic$^{®}$ (Chemocentryx), an antagonist of CCR9, a receptor expressed in gut-homing T cells (Table 2). The drug is currently in clinical trials for Crohn's disease, ulcerative colitis and celiac disease. A new target in the family emerged with the discovery that Th17 cells express CCR6 [35], although this receptor is also expressed in regulatory T cells [36]. Receptors like CCR7 or CXCR5, which exhibit a more restricted pattern of expression and play a constitutive role, have not been exploited therapeutically [37].

A way of overcoming the redundancy and promiscuity of chemokines is the inhibition of PI3Kγ. This kinase is a key downstream signalling component of a wide range of chemokine

TABLE 2

Blockade of cell trafficking

Target mechanism	Rationale	Drug, company	Current status
CCR9 *Antagonism*	Prevents migration of gut-homing T cells.	Trafficet®, *ChemoCentryx*	Phase II/III in Crohn's Phase II in ulcerative colitis and celiac disease
S1P receptors *Agonism*	Blood lymphopenia	Fingolimod, *Novartis*	Phase 3 in MS
		S1P1 agonist *Actelion*	Phase 2 in MS, psoriasis
S1P phosphate lyase *Inhibitor*	Blood lymphopenia	LX2931, *Lexicon*	Phase 1
PI3k γ/δ *Inhibitors*	Integrate signalling pathways from both GPCR and cytokine receptors	AS605240, *Merck-Serono*	Preclinical

TABLE 3

B cell and T cell targeted therapies

Target approach	Rationale	Drug, company	Current status
CD20 *Antibody*	Depletion of B cells expressing CD20 antigen	Rituximab *Roche* Ocrelizumab, *Roche*	Market for RA Phase 2 in SLE, MS and Sjögren's syndrome Phase 3 in RA
CD19 *Antibody*	Depletion of B cells expressing CD19 antigen	Ofatumumab, *GSK*	
CD22 *Antibody*	Depletion of IgM + IgD + mature B cells	Epratuzumab *Medimmune*	Phase 2 in SLE
BLys/APRIL *Fusion protein*	Inhibition of BLyS and APRIL, two potent stimulators of B cell maturation, proliferation, and survival	Atacicept (TACI-Ig) *Zymogenetics/Merck-Serono*	Phase 2 RA and SLE
BLyS *Antibody*	Promotes B cell differentiation, proliferation and survival	Belimumab *HGS/GSK*	Phase 3 in SLE
CD52 *Antibody*	Depletion of T and B cells, NK cells and monocytes	Alemtuzumab, *Genzyme*	Phase 3 in MS
CD80/86 *Fusion protein*	Blockade of T cell costimulation	Abatacept (CTLA-4-Ig), *Bristol Myers Squibb*	Market for RA Phase 2 in lupus nephritis and diabetes Phase 3 in Crohn's disease
Syk kinase *Inhibitors*	Blockade of signalling from B cell receptor	Fosfamatinib *Rigel*	Phase 2 in RA and SLE

receptors and could thus be a potential pan-chemokine blocker [38]. Neutrophils and macrophages from PI3Kγ knockout mice show impaired activation through chemoattractants and poor chemotactic responses [39]. Several small molecule inhibitors of PI3Kγ have been developed [40] and one of them, AS605240, has been shown to reduce glomerulonephritis and prolong lifespan in a mouse model of lupus [41]. Dual gamma/delta inhibitors may offer a higher therapeutic potential than selective gamma inhibitors because PI3Kδ has important roles in T cell and B cell signalling. There are currently no PI3K inhibitors in clinical trials in autoimmune diseases.

The trafficking of lymphocytes can also be blocked by interfering with the sphingosine pathway. This novel strategy emerged with the discovery of fingolimod, an oral drug that has shown efficacy in patients with relapsing–remitting MS and is currently in several phase 3 trials. After being phosphorylated, fingolimod is converted into an agonist of 4 of the 5 receptors of the endogenous lipid sphingosine-1 phosphate (S1P), namely S1P1, S1P3, S1P4 and S1P5. Fingolimod causes blood lymphopenia, mainly due to the inhibition of the exit of naïve lymphocytes from secondary lymphoid organs, during the immunosurveillance process these cells carry out. This is believed to prevent autoreactive cells to infiltrate the central nervous system and destroy the myelin sheath. At therapeutic doses, the mean total lymphocyte count in peripheral blood of fingolimod-treated patients is reduced by 61%. Both CD4+ and CD8+ naïve and central memory T cells are selectively depleted, but not effector memory T cells [42]. If the safety profile proves to be acceptable [43], fingolimod could be one of the first oral treatments of MS.

The lymphopenia evoked by S1P agonists can be mimicked by inhibiting the enzyme responsible for the degradation of S1P, named S1P lyase. The inhibition of this enzyme has been reported to increase local levels of S1P and to cause blood lymphopenia in a manner analogous to fingolimod, without significant side effects. An inhibitor of S1P lyase from Lexicon is currently in clinical trials [44].

B cell and T cell targeted therapies

B lymphocytes play multiple and relevant functions; they are efficient antigen-presenting cells, provide T cell costimulation, synthesize cytokines, fix complement and produce autoantibodies [45–47]. The first B cell targeted therapy evaluated in an autoimmune disease was rituximab, an antibody that causes B cell depletion. Rituximab is a human–mouse chimeric antibody directed against the CD20 antigen, which is present in pre-B and B cells but not on plasma cells or stem cells in the bone marrow. Rituximab depletes $CD20^+$ B cells via complement-mediated cytotoxicity, antibody-dependent cytotoxicity and/or apoptosis [48]. It is approved for RA and is currently in clinical trials in SLE and MS.

Other B cell depleting antibodies in clinical development are depicted in Table 3.

The success of rituximab paved the way for the search of alternative options to target B lymphocytes. The most advanced ones are atacicept and belimumab, two biologicals that block the effects of BLyS (B Lymphocyte stimulator) and/or APRIL (a proliferation-induced ligand). BLyS and APRIL are members of the TNF family of cytokines that modulate B cell activation, survival and/or antibody production [49]. The levels of both cytokines are elevated in patients with RA and SLE [49]. Whether these agents offer an advantage over rituximab remains to be seen.

Strategies that deplete blood lymphocytes have proven successful in preventing relapses and improving disability in MS patients. Fingolimod and cladribine [50] achieve this effect via different mechanisms. Fingolimod causes lymphopenia by retaining circulating naïve lymphocytes inside lymph nodes, whereas cladribine, a purine nucleoside analogue, has cytotoxic effects in both resting and proliferating lymphocytes. Alemtuzumab, an anti-CD52 antibody, depletes T and B lymphocytes, NK cells and monocytes [51]. This antibody, together with Tysabri, is one of the most efficacious agents in MS but its impressive efficacy is linked to the development of severe autoimmune disorders, like ITP [4].

Non-selective lymphocyte depletion may cause potent immunosuppression that can be associated to opportunistic infections and cancer. A potential safer alternative would be targeting a selective T cell subpopulation. The most promising approach is to block the function and/or the generation of Th17 cells. Th17 is a newly discovered lineage of CD4+ T helper cells, different from Th1 and Th2, which mainly synthesize IL-17 and IL-22 (Figure 2). Th17 cells seem to be the major inducers of experimental autoimmune encephalomyelitis and arthritis in mice, a function previously attributed to Th1 cells [52]. The inhibition of Th17 cell generation could be achieved by inhibiting the binding of IL-21 to its receptor with an anti-IL-21 or IL-21R antibody. JAK3 inhibition is a possible option to block IL-21 signalling with a small molecule. Endogenous S1P has also been reported to enhance the development of Th17 cells, and this could be blocked by fingolimod-like compounds [53].

Regulatory T cells (Treg) play an important role in the suppression of autoimmune responses via inhibition of self-reactive T cells [54]. Strategies aiming at increasing the number and/or the activity of Tregs are very attractive. An option consists of the administration of low doses of IL-2, an important survival factor for these cells. This concept has proven to be effective in protecting mice to develop diabetes and has prompted the Immune Tolerance Network to conduct a trial with IL-2 and sirolimus in human subjects with new onset type I diabetes mellitus [55].

The activation of the T cell receptor in the absence of a costimulatory signal leads to T cell anergy and blocks the immune

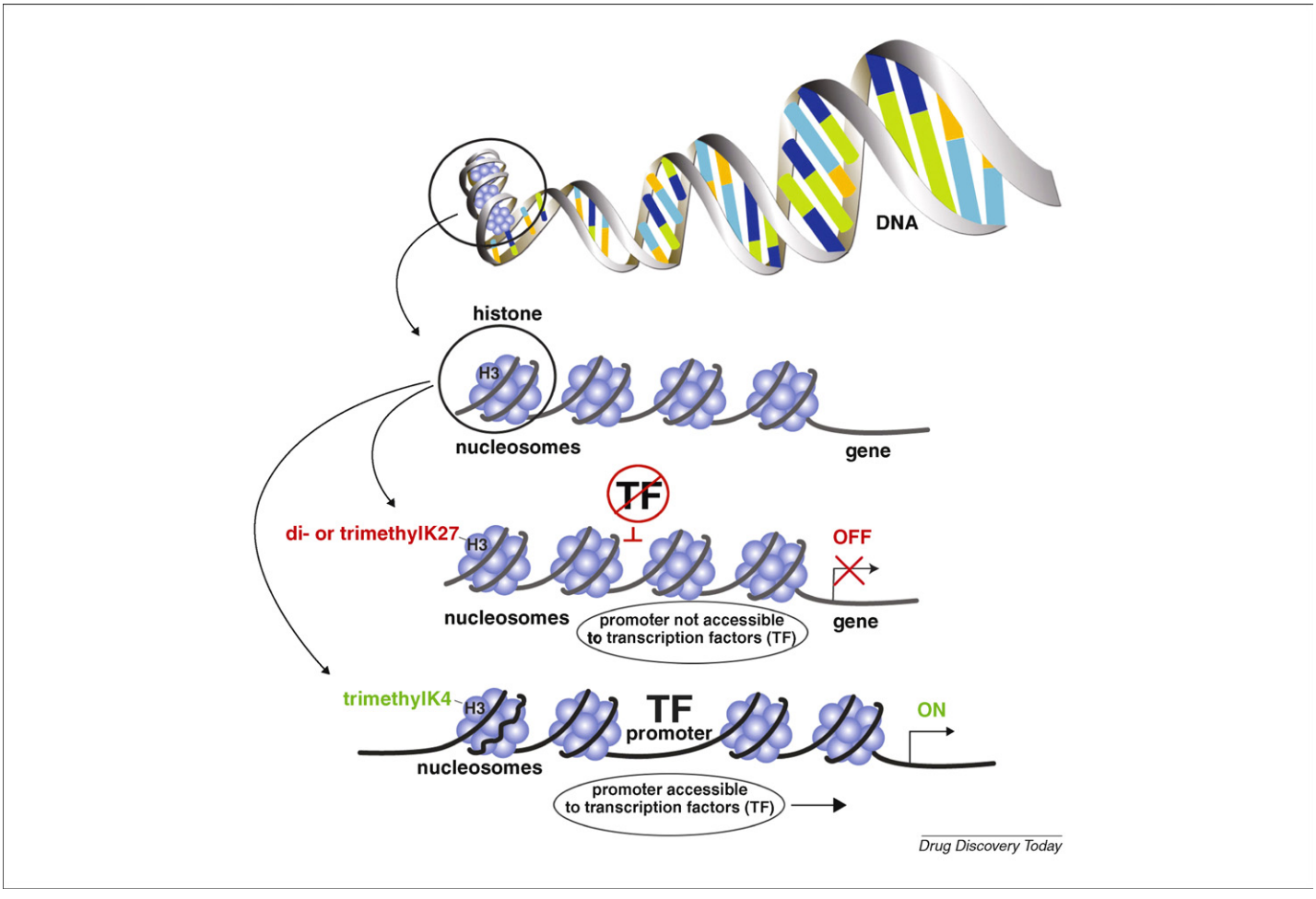

FIGURE 3

Diagram depicting the effect of histone methylation on gene transcription (see text for details).

TABLE 4

Epigenetic mechanisms

Mechanism	Rationale and additional information
DNA methylation	Failure to maintain DNA methylation levels and patterns in mature T cells may result in T cell autoreactivity and autoimmunity. Aberration of DNA methylation in T cells has been suggested to be at the basis of both idiopathic and drug-induced lupus (10). DNA hypomethylation is observed in T cells of lupus patients and is associated to LFA-1 overexpression. DNA methylation inhibitors are used in the treatment of hematological malignancies (5-azacytidine, decitabine).
Chromatin Remodeling	**Histone deacetylase (HDAC) inhibitors** Vorinostat (Merck) is an FDA approved HDAC inhibitor originally designed to treat cutaneous T cell lymphoma. Additional inhibitors are in clinical trials (51). For example, ITF2357 (Italfarmaco) is in phase 2 for active systemic onset juvenile idiopathic arthritis. Preclinical validation in rodent models of MS with Trichostatin A.
RNA silencing	**siRNA (small interfering RNA)** Produced from long strands of double-stranded RNA (DS-RNA) derived from exogenous material (virus) or endogenous transcripts. The enzyme Dicer processes the ds-RNAs into short-dsRNAs. One strand of the ds-RNA associates with the RNA-induced silencing complex (RISC), whereas the complementary strand is degraded. Total homology between the single-stranded RNA and a specific mRNA sequence results in the cleavage and degradation of the mRNA. Companies developing siRNAs are Alnylam Pharmaceuticals, Opko corporation and Sirna therapeutics (49). **miRNA (microRNA)** Short single stranded RNA (22 nucleotides) produced exclusively by the human genome that act *in trans* to regulate multiple mRNAs. They target specific RNAs by mechanisms like an incomplete base pairing and post-trancriptional gene silencing. Each individual miRNA may potentially downregulate numerous genes and pathways. miRNAs play key roles in immune modulation and in the development of regulatory T cells, and could be crucial to maintain immunological tolerance. Aberrant miRNA expression has been reported to occur in many diseases. As an example, miR-17–92 has been linked to lymphoproliferation and autoimmunity in mice (54). **Antagomirs** Synthetic oligonucleotides directed against specific miRNAs to correct increased miRNA levels that may be the result of a mutation, or reduce the normal expression of a specific miRNA to increase the expression of regulatory molecules.

response. The inhibition of T cell costimulation by using the CTLA4-Ig fusion protein, Abatacept has already been approved for RA and is in clinical trials for other autoimmune diseases, including SLE, where it has shown efficacy and an excellent safety profile.

Epigenetic regulation of immune cell function

Epigenetic regulation is a mechanism by which genes are selectively activated or inactivated [56]. Among the proteins involved in this process are DNA methyltransferases, methyl-CpG binding proteins and histone-modifying enzymes leading to acetylation, methylation, ubiquitination or phosphorylation of histones. Gene regulation by the small noncoding RNAs [57], small interference RNA (siRNA) and microRNA (miRNA), are considered epigenetic mechanisms.

A lingering conundrum of the immune response is how do certain immune/inflammatory cells ''remember'' whether or not they should be actively transcribing specific genes, which would facilitate their participation in a given response. Recent investigations have begun to explore this question, as it relates to immune responsiveness, and have uncovered a novel mechanism for the activation or inactivation of key T lymphocyte-derived genes [58]. Both DNA methylation and chromatin remodeling have been identified as key mechanisms that dictate the T cell phenotype and categorizes the T helper (Th) cell as either a interferon-gamma producing Th1 cell or a IL-4, IL-5, IL-13 expressing Th2 cell. In the presence of lineage-specific transcription factors and appropriate polarizing cytokines, a dynamic process occurs at the amino terminus of histones H3 and H4, which either increases or decreases the susceptibility of transcription factors to specific promoters. The histone tails can be modified by acetylation, methylation, or phosphorylation, resulting in either activation or silencing of gene expression. While histone acetylation is a reversible post-transcriptional modification, other covalent additions, such as methylation, have historically been viewed as irreversible. However, with the discovery of specific histone demethylases, these chromatin modifications are now believed to be very dynamic processes.

Methylation of lysine 27 (K27) of histone 3 (H3) keeps the chromatin in a conformation such that the promoter for specific genes is not available to transcription factors and thus the gene is silenced. On the contrary, methylation of lysine 4 on histone 3 is known to open up the conformation of the chromatin and allow transcription factors access to the promoter and initiate gene expression (Figure 3). The former scenario is an important mechanism that results in the silencing of the IL-4 and IL-13 genes in Th1 cells, as H3K27 is trimethylated in these T cells, resulting in a condensed chromatin structure and no accessibility to the cytokine promoters. Additional investigations have shown that acetylation of H3 and H4 amino terminal tails can facilitate the opening of chromatin and allowing transcription factors access to specific cytokine promoters [58]. In these studies, T cells skewed to a Th2 phenotype demonstrated hyperacetylation of histone 4 at the regulatory region of the IL-4 gene. Interestingly T cells polarized to a Th1 phenotype demonstrated hyperacetylation of histone 4 at the interferon-gamma promoter site, but no histone acetylation at the regulatory region of the IL-4 gene.

Insight into the mechanism(s) of how epigenetic alterations can regulate the cytokine profile of polarized T cells offers the potential for novel drug discovery with the chromatin modifying machinery

as target. Recently the FDA approved vorinostat, a histone deacetylase (HDAC) inhibitor, as therapeutic treatment for cutaneous T cell lymphoma. Interestingly, nearly a dozen additional HDAC inhibitors are presently in clinical trials [59]. While a significant amount of drug development targeting epigenetic alterations has centered on cancer therapy, there is little doubt that these drugable targets will find an additional niche in treating immune disorders.

RNA silencing strategies (siRNA and miRNA), have recently emerged as essential regulators of gene expression [60–62]. They control transcription by changing the structure of chromatin and regulating mRNA stability and translation at the post-trancriptional level [60]. They are receiving attention as a potential approach for the treatment of cancer, but also autoimmune diseases (Table 4), as two RNA silencing mechanisms have been reported to be involved in lupus pathogenesis [57]. The complexity of these systems is such that a detailed knowledge on the interaction map of the different RNAs with the different human genes is required in order to avoid unexpectedly dramatic side effects.

Conclusions

Cytokine inhibition appears to be one of the most attractive approaches to treat autoimmune diseases. Attempts to block the effects of cytokines with oral small molecules have not been successful in the clinic to date, but preliminary results obtained with JAK inhibitors are very encouraging and point at the JAK family as one of the most attractive targets so far.

Preventing tissue damage remains an unmet medical need that could be achieved by inhibiting the migration of inflammatory cells, ideally at the early stage of the disease. Targeting Th17 cells appears to be a promising approach to prevent aggressive neutrophil and macrophage tissue infiltration. This field is novel and in the near future will provide a better understanding of the function of these cells and an avenue for drug development.

The ultimate goal to treat autoimmunity is to develop efficacious therapies that will lead to a sustained remission of the disease. This approach needs to include a strategy directed at restoring immunological self-tolerance while preserving the immune response against invading pathogens. The discovery of the microRNAs that control the genetic programs of regulatory and effector lymphocytes is an exciting field that will increase our understanding of the immune system in the near future. It is clear that the limited number of successful therapies to treat these diseases underscores the need for a better understanding of the mechanisms and players involved in autoimmunity and tolerance. However, with the continued interest in developing additional novel biologics and small molecule inhibitors there is additional hope on the horizon for patients with autoimmune disorders.

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