

Biological Therapies of Immunologic Diseases

Strategies for Immunologic Interventions



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KEYWORDS

• Biotherapy • Biotherapeutic • Immunomodulatory • Monoclonal antibody

KEY POINTS

- To understand at a basic level the general classes of biological therapies.
- To understand the targets of biotherapeutics in asthma, allergies, and other immune-mediated disorders.
- To take note of up-to-date and future therapies for immune-mediated disorders.

INTRODUCTION

The immune system is a highly complex and ever-evolving network of signaling molecules and target cells, with several potential targets for therapeutic modification. Throughout the past few decades, numerous methods of immunomodulatory biological therapy have been proposed and extensively studied, yet only some have demonstrated success. The ability to concurrently alter a component of the immune system, account for several downstream effects, reduce adverse drug events, and minimize the risk of consequent autoimmunity and/or malignancy has proven extensively difficult. Nonetheless, several strategies for immunologic intervention have been successfully applied to the practice of medicine (Table 1). This article covers currently available biotherapeutic agent classes as well as potential direction for future therapy.

MONOCLONAL ANTIBODIES

Monoclonal antibodies (mAbs) have received considerable attention since their conception more than 40 years ago. By definition, mAbs are antibodies created

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Table 1 Description of biotherapeutic agent classes		
Biotherapeutic Class	Description	Examples of FDA-Licensed Products
Monoclonal antibodies	Antibodies created from a single clonal line designed to target a specific epitope	<i>Chimeric:</i> infliximab (anti-TNF) <i>Humanized:</i> omalizumab (anti-IgE), mepolizumab (anti-IL5) <i>Human:</i> adalimumab (anti-TNF), canakinumab (anti-IL1B), ustekinumab (anti-IL23/13)
Cytokines	Small secreted proteins involved in cell–cell signaling, used either in natural form or via recombinant DNA technology to serve as competitive inhibitors	<i>Natural:</i> interferons (α , β , γ) <i>Recombinant:</i> anakinra (recombinant IL-1RA)
Fc fusion proteins	Proteins consisting of the Fc domain of IgG (generally IgG1) fused to a ligand or peptide antigen, produced by recombinant genetic engineering	<i>Competitive inhibition:</i> Etanercept, rilonacept <i>Direct receptor stimulation:</i> Alefacept, romiplostin
RNA		
siRNA	Small double-stranded RNA product designed to bind, cleave, and silence a target mRNA	None
Antisense oligonucleotides	Synthesized single-stranded antisense segments designed to directly regulate mRNA expression via binding through specific Watson-Crick hybridization	Fomiversen, mipomersen, pegaptanib
Kinase inhibitors	Specific inhibitors of kinases (phosphorylating enzymes), typically acting via occupation of an active binding site	Imatinib (anti Bcr-Abl, CD117, PDGFR), nintedanib (anti-FGFR), tofacitinib (anti-JAK)
Antichemokine drugs	Small molecule inhibitors designed to target chemokine receptors	None

Abbreviations: FDA, Food and Drug Administration; FGFR, fibroblast growth factor receptor; Ig, immunoglobulin; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist; mRNA, messenger RNA; PDGFR, platelet-derived growth factor receptor; siRNA, short interfering RNA; TNF, tumor necrosis factor.

from a single B lymphocyte clone that bind a specific antigenic epitope.¹ The first mAbs were formed by Kohler and Milstein in 1975 via the hybridoma technique,² involving fusion (either chemically or virally) of immortalized myeloma cells with splenic B cells of an animal specifically inoculated with the target epitope. Cells are then grown in selective hypoxanthine-aminopterin-thymidine medium, whereby only hybridoma cells can survive. Because unfused myeloma cells lack the ability to produce hypoxanthine guanine phosphoribosyltransferase (HGPRT), they are rendered unable to replicate their own genetic material within the medium owing

to aminopterin salvage pathway inactivation³; unfused B cells, conversely, cannot survive indefinitely on account of their short life span. Fused cells, however, possess both immortality and HGPRT and can thus proliferate. Individual hybridomas are subsequently screened for antibody production and multiplied in culture.

Early mAbs were limited by the immunogenicity of murine proteins, genetic instability, and suboptimal efficacy.⁴ The first mAb approved for human use was muromonab-CD3 (OKT3, Janssen, Beerse, Belgium), an IgG2a murine antibody targeting the T-cell CD3 receptor to treat solid organ rejection⁵; owing to adverse effects including formation of human antimouse antibodies, its use has largely been superseded by newer biotherapeutics. By the late 1980s, techniques to humanize mAbs were developed,⁶ and in the late 1990s, entirely human mAbs generated by phage display⁷ and transgenic mice⁸ were developed (Fig. 1). With phage display, mRNA is isolated from human B lymphocytes and reverse transcribed into complementary DNA (cDNA) using polymerase chain reaction amplification at heavy and light chain variable regions. The products are ligated and introduced into a phage vector and inserted into *Escherichia coli* to generate an antibody library.⁹ Although this process immortalizes cDNA clones, which can be used repeatedly, it typically generates antibody fragments (Fab or scFv) that must still be incorporated into a full antibody. More than 400 mAbs have been developed to date, with usefulness in multiple immune diseases. A standardized nomenclature system exists, with murine mAbs given the suffix “-omab,” chimeric mAbs (which contain only animal variable regions) designated “-ximab,” humanized mAbs (mouse CDR only) “-zumab,” and fully human mAbs “-umab.”

In addition to the use of phage display, the efficacy of mAbs is enhanced by genetically modifying the Fc region. Termed “Fc optimization,” this process is accomplished either by point mutations or by defucosylation of the *N*-linked oligosaccharides,^{10,11} with the intent to improve mAb affinity for the FcγRIIIA receptor, thereby improving natural killer cell-mediated target destruction via antibody-dependent cellular cytotoxicity. The half-life of mAbs is prolonged by modifying interactions with the salvage pathway neonatal Fc receptor, FcRn, accomplished by point mutations in the CH2-CH3 domain in cetuximab and bevacizumab,¹² and by the addition of polyethylene glycol.

Biologic targets of mAbs are extensive; most are directed versus cell surface receptors or ligands, serving to inhibit a downstream signaling pathway. Many emerging mAbs target inflammatory cytokine families; for treatment of rheumatoid and juvenile idiopathic arthritis, for example, anti-tumor necrosis factor mAbs have been used since the late 1990s,¹³ and within the past 10 years, additional mAbs directed against IL-1β (canakinumab)¹⁴ and IL-6 (tocilizumab)¹⁵ have proven effective. Psoriasis therapy now includes anti-IL-17a (secukinumab, ixekinumab)^{16,17} and anti-IL-12/IL-23 (ustekinumab)¹⁸ mAbs. mAbs for treatment of atopic disorders target Th2 cytokines, with 2 anti-IL-5 (mepolizumab, reslizumab) mAbs^{19,20} approved for use in eosinophilic asthma and another (benralizumab) coming down the pipeline²¹; the anti-IL-4/IL-13 mAb dupilumab has also proven quite effective in phase III trials for atopic dermatitis.²² In addition to targeting T-cell cytokines, a highly successful mAb, rituximab, targets B-cell CD20; interestingly, lymphocyte phenotyping in treated patients showed that CD4⁺ T cells could potentially be used as a biomarker for response to therapy.²³ Although the clinical indications for mAbs are expanding continually, commercial success overall is often and unfortunately limited by high cost²⁴ and adverse effects, including cytokine release syndrome and acute hypersensitivity reactions.²⁵

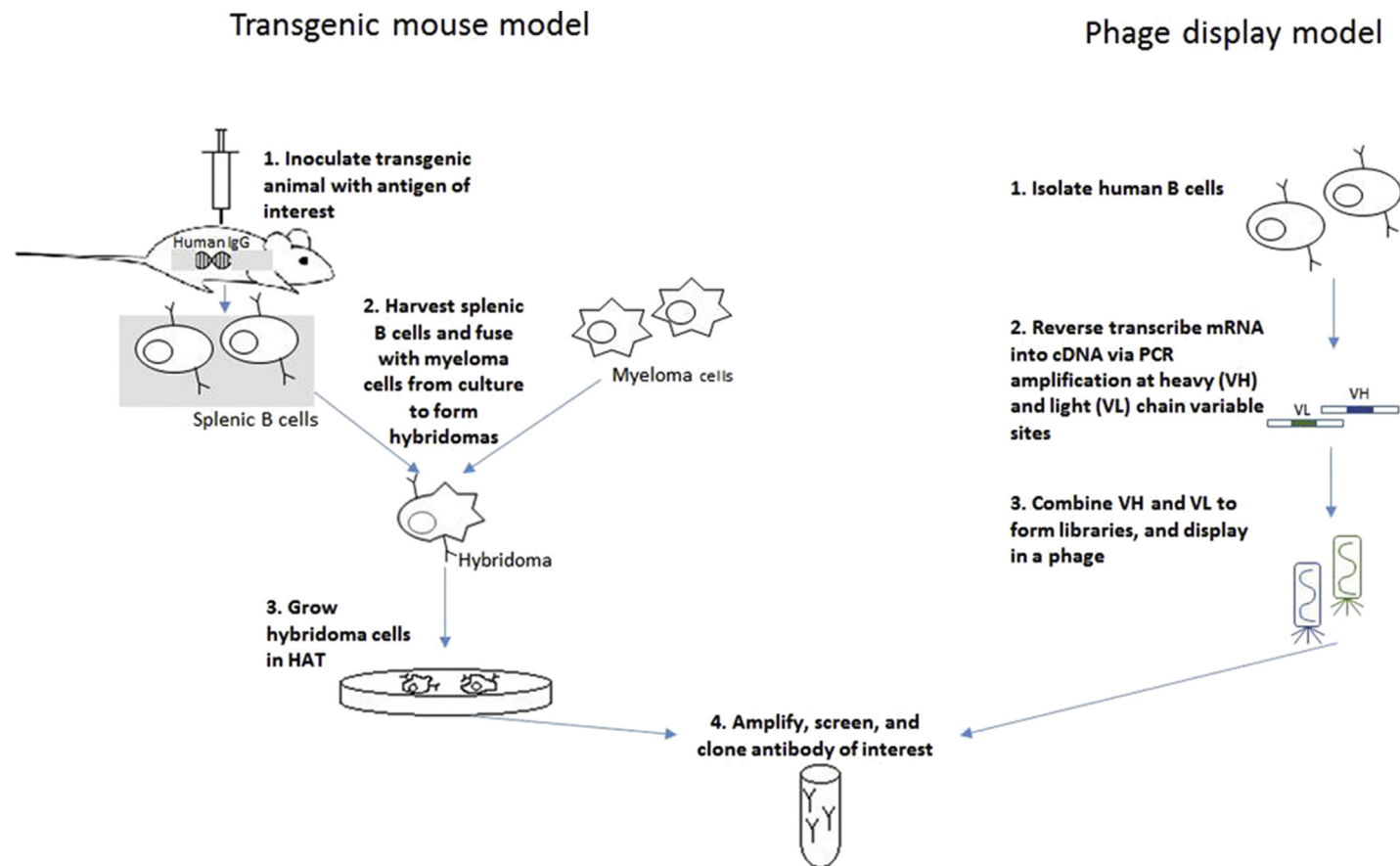


Fig. 1. Formation of monoclonal antibodies. HAT, hypoxanthine-aminopterin-thymidine; mRNA, Messenger RNA; PCR, polymerase chain reaction.

CYTOKINES

Cytokines are small secreted proteins involved in cell–cell signaling, with key roles in immune response regulation, homeostasis, and autoimmunity.^{26,27} Whereas cytokine families are targeted by many emerging biotherapeutics, cytokines themselves are used as therapy in multiple immune-mediated diseases, both in unaltered form to serve natural roles and in genetically modified form to competitively inhibit *in vivo* cytokines. There is current clinical usefulness for multiple classes of cytokines, including interferons (IFNs) and interleukins.

A classic example of cytokine therapy is the use of IFNs. IFN- α , a type I IFN produced by leukocytes involved in innate response to viral infections, has been used as medical therapy since the 1970s, when purified topical preparations of IFN- α were deemed helpful in the prevention of viral respiratory infections²⁸ and in the treatment of herpesviridae skin infections.²⁹ A subsequent study by the same group showed success in the treatment of chronic hepatitis B infection with parenteral IFN- α .³⁰ Recombinant forms of IFN- α are currently used to treat hepatitis B and C, various cancers, and subsets of patients with hypereosinophilic syndromes.^{31,32} IFN- β was initially used in the 1980s, when human fibroblast IFN- β administered via lumbar puncture to 10 multiple sclerosis patients resulted in improvement,³³ and this cytokine, in various forms, is still used in multiple sclerosis therapy.³¹ After discovering the augmentation of macrophage oxidative metabolism by IFN- γ , its use in chronic granulomatous disease was initiated in the early 1990s³⁴; it remains in use in recombinant form today.

Interleukins are also used in recombinant form as competitive endogenous inhibitors. Anakinra is a recombinant nonglycosylated interleukin-1 receptor antagonist (IL-1RA), a natural member of the IL-1 cytokine family that serves as an inhibitor of IL-1 β . Prepared from cultures of genetically modified *E coli* using recombinant DNA technology, anakinra possesses an additional methionine at the amino terminus and competitively binds the IL-1 receptor.³⁵ Although initially marketed for rheumatoid arthritis, it has also shown recent success in cryopyrin-associated periodic syndromes (CAPS), because these patients have mutations in the cryopyrin gene leading to unregulated IL-1 β production,³⁶ and in the treatment of systemic onset juvenile idiopathic arthritis³⁷ and adult-onset Still's disease.³⁸ Pitrakinra is a similarly modified human IL-4 that competitively binds the alpha subunit of the IL-4 receptor (IL-4R α), acting as a competitive antagonist of both IL-4 and IL-13.³⁹ Pitrakinra reduced asthma exacerbations in a small subset of trial subjects, and a significant association was found between response and polymorphisms in the IL4RA gene.⁴⁰ Unfortunately, possibly owing to study design, further research on this biologic seems to have ceased. Other medical uses for interleukins include recombinant IL-11 (oprelvekin), a hematopoietic cytokine responsible for megakaryocyte maturation, for the prevention of thrombocytopenia in patient undergoing myelosuppressive chemotherapy,⁴¹ and IL-2 has a role in treatment of cancers.

FC FUSION PROTEINS

Fusion proteins used in biological therapy are created by cDNA coding for 2 separate proteins through the use of recombinant genetic engineering. The most well-used fusion proteins are the Fc fusion proteins, which involve linking the Fc domain of human IgG (generally IgG1) to another peptide, typically a ligand or peptide antigen.¹¹ Fusion with the Fc domain confers the ability to interact with high affinity Fc receptors and prolongs half-life via interaction with neonatal salvage FcRn receptors, as mentioned.⁴² Most Fc fusion proteins serve to competitively inhibit receptor binding

(abatacept, aflibercept, belatacept, etanercept, riloncept), although 2 Food and Drug Administration (FDA)-approved Fc fusion proteins function as direct receptor stimulators (alefacept and romiplostin). Whereas the Fc fusion proteins are modified by Fc optimization as described for mAbs,¹⁰ recent focus has shifted to developing new fusion platforms including the potential use of IgG3, IgG4, IgA, and IgM constructs.^{11,43}

Two unique Fc fusion proteins are riloncept and dulaglutide. Riloncept is distinctive in that it is a dimeric fusion protein consisting of extracellular portions of both the IL-1 receptor component IL-1R1 and its accessory protein IL-1RAcP, enabling it to bind and neutralize IL-1 β but also act on IL-1 α and IL-1RA with reduced affinity; it was the first drug licensed for use in CAPS.⁴⁴ Dulaglutide, although used to treat type 2 diabetes, is worth noting given its distinction as the first licensed Fc fusion protein containing a non-IgG1 subclass Fc region (IgG4).⁴⁵ The 10 currently FDA-licensed Fc fusion proteins have found clinical efficacy in organ rejection, macular degeneration, CAPs, thrombocytopenia, rheumatoid arthritis, diabetes, and psoriasis. One potentially promising Fc fusion initially excited the asthma community yet failed to show efficacy in phase III trials was altrakincept, a soluble IL4R Fc fusion protein⁴⁶; although an mAb targeting IL-4 was also ineffective in asthma treatment,⁴⁷ perhaps the ability to additionally target IL-13, as with dupilumab, could provide success.

RNA

Significant recent advances in RNA therapeutics have made it possible to target the specific nucleic acids involved in protein synthesis of particular genes of interest. Currently used RNA therapeutic techniques include double-stranded RNA-mediated interference (RNAi) and single-stranded antisense oligonucleotides (ASOs). RNAi, a posttranscriptional gene silencing mechanism, occurs naturally in human cells; in this process, a long, double-stranded RNA induces the cleavage and silencing of host mRNA, thereby preventing translation of mRNA to protein via integration with the RNA-induced silencing complex (RISC) and the endonuclease Argonaute 2.⁴⁸ This can be accomplished through the use of short interfering RNA (siRNA) or microRNA (miRNA), 2 types of double stranded noncoding RNA that possess subtle but important differences. The most widely examined RNAi molecules, siRNAs consist of a “passenger” sense strand that is degraded and a “guide” antisense strand that leads the RISC to the target mRNA and binds with full complementarity, inducing 5' cleavage and silencing.⁴⁹ The antisense strand of miRNA, however, is only partially complementary to the target mRNA, and gene suppression is induced via translational repression; although this often leads to imperfect binding in therapeutic studies, a conceivable advantage of this process is the potential for miRNA to bind multiple genes.⁵⁰

RNAi molecules face numerous challenges, including instability against extracellular and intracellular degradation (the half-life of naked siRNA is approximately 15 minutes),^{50,51} weak cellular uptake, low potency, and the potential for “off-target” effects via binding to other mRNA products; improvements include the addition of viral, small molecule, and nanoparticle carriers.⁵² Moreover, the human innate immune system is inherently trained to recognize double stranded RNA as foreign, thereby inducing type 1 IFNs. This process is prevented by the careful introduction of chemical modifications. Although no RNAi product is yet approved by the FDA, some siRNA-based therapies have shown promise in the treatment of “undruggable” cancer mutations,⁵³ although the best clinical response seems to be tumor stabilization rather than complete response. In respiratory inflammatory diseases, *in vivo* studies have used miRNA

and siRNA to target IL-4, STAT-6, GATA3, c-kit, and other cytokines with variable success, although no drug has progressed past phase II trials.⁵⁰ Ongoing phase II trials for ALN-CC5, an siRNA targeting complement component C5, have shown promise in treatment of paroxysmal nocturnal hemoglobinuria,⁵⁴ and its creator Alnylam is pre-clinically evaluating its value in the treatment of atypical hemolytic uremic syndrome and myasthenia gravis.

Unlike siRNA and miRNA, ASOs are synthesized single-stranded antisense segments designed to directly regulate mRNA expression via binding through specific Watson-Crick hybridization.⁵¹ ASOs are the longest studied RNA-based drugs, having been discovered more than 2 decades ago. ASOs are subject to similar limitations as siRNAs, including recognition by Toll-like receptors and other innate pattern recognition receptors, and unmodified ASOs are unable to cross plasma membranes given their negative charge⁴⁸; to bypass these impediments, ASOs have undergone considerable modifications; reflective of sequential changes, ASOs are typically referred to as first, second, and third generation.⁴⁹ First-generation modifications include alterations to the phosphodiester backbone, primarily via phosphorothioate (PS) bonds, to increase affinity and prevent degradation.^{49,54} Second-generation modifications include improving cellular uptake and target affinity via sugar moiety alterations such as 2'-O-methyl, 2'-O-methoxymethyl (2'-OMOE), and locked nucleic acid alterations,⁵¹ and recent third-generation developments center around chimeric "gapmer" ASOs, in which a central "gap" segment that activates RNase H is flanked by 2 to 5 chemically modified nucleotides on the 2' ends that improve stability.⁴⁸ As of today, 3 oligonucleotide products are licensed. The first oligonucleotide to receive FDA approval in 1998, fomivirsen, is a first-generation PS-ASO used in the treatment of cytomegalovirus infection in immunocompromised individuals⁴⁹; the other approved ASOs are mipomersen, a second-generation ASO used in familial hypercholesterolemia,⁵⁵ and pegaptanib, a pegylated antivascular endothelial growth factor aptamer used in wet macular degeneration.⁵⁶

ASO delivery is generally accomplished via parenteral injection, although inhaled ASOs have been trialed. AIR645 was a 2'-OMOE inhaled antisense oligonucleotide targeting mRNA encoding the alpha subunit of the IL-4 receptor (IL-4R α). Although groundbreaking in that it was the first inhaled antisense oligonucleotide of its kind, it unfortunately failed to show efficacy after phase I trials.⁵⁷ In regard to immune-mediated disease, a few novel products have recently been created. TPI ASM-8 (Pharmaxis, New South Wales, Australia) is a drug containing 2 ASOs targeting the CC chemokine receptor 3 (CCR3) and the IL-3, IL-5, and granulocyte-monocyte colony stimulating factor common beta chain, designed to block atopic inflammation. This medication showed promise and safety in the treatment of mild atopic asthma in phase I and II trials,⁵⁸ but unfortunately further progress has not been made in the past 4 years. Additionally, a fascinating antisense molecule targeting GATA3, the transcription factor essential for Th2 development, is showing potential in phase II trials for eosinophilic allergic asthma⁵⁹; termed SB010, this DNA enzyme (DNAzyme) contains antisense base pairs targeting GATA3 mRNA on its ends and a builtin enzyme hgd40 in its center, serving as the catalytic domain to cleave and silence GATA3 mRNA.

SMALL MOLECULE KINASE INHIBITORS

Within the past 2 decades, the paradigm of chemotherapy biotherapeutics has shifted dramatically from cytotoxicity to the development of specific targeted therapies.⁶⁰ Once a potential target is identified and validated, a chemical library is screened to identify a lead compound, which is then optimized and used as a foundation for

chemical modification. This modified small molecule drug is subsequently clinically validated by biomarker-led trials.⁶⁰ After discovering the involvement of protein kinases in cancer pathogenesis, significant efforts to create small molecule kinase inhibitors resulted in the identification of numerous lead compounds via these methods. Kinases activate substrates via phosphorylation by transferring phosphate from ATP to an hydroxyl group; aberrant phosphorylation has implications in numerous autoimmune and inflammatory conditions.⁶¹ The first successfully licensed small molecule kinase inhibitor was imatinib, a modified 2-phenylaminopyrimidine designed to suppress Abelson (Abl) kinase in Bcr-Abl expressing leukemia cells via occupation of the active site of the Abl protooncogene.⁶² Interestingly, imatinib also selectively inhibits tyrosine kinases c-kit (CD117) and platelet-derived growth factor receptor (PDGFR), and it is now used in a subset of systemic mastocytosis patients who are negative for the c-kit D816V gain-of-function mutation⁶³ and for hypereosinophilic syndrome patients possessing the FIP1L1–PDGFRA fusion kinase mutation.⁶⁴ The success of imatinib combined with the sequencing of the human kinome in 2002⁶⁵ and advances in high-throughput biochemical profiling led to influx of protein kinase inhibitors, with 33 FDA approved as of 2015.⁶¹

Other kinase inhibitors with nononcologic indications include nintedanib, a small molecule tyrosine kinase inhibitor specific for fibroblast growth factor receptor, vascular endothelial growth factor receptor, and PDGFR, approved in 2014 for treatment of idiopathic pulmonary fibrosis,⁶⁶ and agents dubbed “jakinibs” that target the cytoplasmic janus kinase (JAK) tyrosine kinase family.⁶⁷ There are 4 JAKs—JAK 1, 2, 3 and TYK2 (tyrosine kinase 2), each associated with a multitude of cytokines. Genome-wide association studies have linked JAKs to the development of autoimmune diseases, including lupus, Crohn’s disease, and Behcet’s,²⁷ and mutations in downstream signal transducer and activator of transcription (STAT) proteins cause immunodeficiencies. Tofacitinib, a JAK1, 3, and 2 inhibitor, the first jakinib developed for treatment of autoimmune disease, is used in rheumatoid arthritis⁶⁸; although noninferior to etanercept for psoriasis and psoriatic arthritis,⁶⁹ it was not approved for this use. The first FDA-approved jakinib was ruxolitinib, which targets JAK1 and JAK2, initially approved for the treatment of myeloproliferative disease, but successful in a phase II trial for treatment of rheumatoid arthritis.²⁷ Another JAK1/2 inhibitor, baricitinib, has recently shown efficacy in the treatment of refractory rheumatoid arthritis as well, with phase III results published last month.⁷⁰ Jakinib immunomodulators allow the inhibition of multiple cytokine signals and show promise for future therapy, especially in patients who have failed traditional disease-modifying antirheumatic drugs. Adverse effects to consider include increased rates of infections as seen with other immunomodulatory medications, notably an increased risk of varicella zoster infections in tofacitinib,²⁷ and increases in total cholesterol likely owing to effects of IL-6, which promotes insulin resistance, and altered lipoprotein kinetics.⁷¹

ANTICHEMOKINE SMALL MOLECULE INHIBITORS

In the field of asthma and allergic disease, attention has been recently directed toward the development of small molecule antichemokine drugs.⁷² Chemokines, by name, are chemoattractive cytokines that facilitate leukocyte recruitment and trafficking via G-protein-coupled receptors; unchecked chemokine stimulation can lead to unwanted immune responses and autoimmunity. Although antichemokine small molecule drugs have generally proven unsuccessful,⁷³ likely owing to redundancy in the chemokine system, there are some potentially positive recent developments in the field. CCR3 is the primary eosinophil chemokine (eotaxin) receptor, an enticing target

for atopic disease. Although several small molecule CCR3 antagonists have been developed; unfortunately, none have yet progressed past phase II trials despite initial successes, primarily owing to failure to achieve efficacy.⁷⁴ One compound, BMS-629623, a novel CCR3 antagonist created by Bristol-Myers Squibb (BMS) via extensive structural modification of a prior urea-based Dupont-Merck that unfortunately antagonized CYP2D6, is reportedly undergoing phase I trials.⁷⁵ Another essential component of asthma pathogenesis involves Th2 lymphocyte recruitment. Potential chemokine receptor targets on the Th2 surface include CCR4, CCR8, and CXCR4. Thus far, blockade of CCR4 and CCR8 has regrettably shown no effect on Th2 recruitment in animal models,^{76,77} although treatment of mice with the small molecule AMD3100, a CXCR4 antagonist, significantly reduced numerous inflammatory mediators in a mouse model of asthma.⁷⁸ Despite offering seemingly lucrative targets, chemokine receptor antagonists as a class have unfortunately produced scant success thus far.

SUMMARY AND FUTURE CONSIDERATIONS

Although the complete inventory of every available biotherapeutic approach to immune mediated disease is quite exhaustive, this review provides a thorough summary of general past and current methods under investigation. Potential immune system targets are vast, and the means by which to alter them even more so. The field of biotherapeutics is rapidly expanding with recent advances in biomolecular and genetic engineering, allowing for the creation of new molecules with targeted specificity and effective in vivo delivery. In addition to the methods described, fascinating new discoveries include the ability to create synthetic antibody mimetics derived from nonimmunoglobulin scaffold proteins,⁷⁹ the conjugation of oligonucleotides to siRNA products,⁸⁰ and the increasing knowledge of genomic and epigenomic engineering products. Notable advances in epigenomics include the use of transcription activator-like effectors, which are often fused to functional domains to create custom DNA-binding products to target specific genes for degradation by transcription activatorlike effector nucleases,⁸¹ and prokaryotic clustered regularly interspaced short palindromic repeat proteins and associated Cas nucleases to target specific DNA sequences using programmable RNAs^{82,83}; these interventions allow the possibility of correcting genetic abnormalities. As we move into the era of personalized precision medicine via discoveries in effective biomarkers, all of these methods carry the potential for worthwhile and efficacious clinical applications.

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