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Antibody-Recruiting Molecules: An Emerging Paradigm for Engaging Immune Function in Treating Human Disease

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Abstract

Synthetic Immunology, the development of synthetic systems capable of modulating and/or manipulating immunological functions, represents an emerging field of research with manifold possibilities. One focus of this area has been to create low molecular-weight synthetic species, called antibody-recruiting molecules (ARMs), which are capable of enhancing antibody binding to disease-relevant cells or viruses, thus leading to their immune-mediated clearance. This article provides a thorough discussion of contributions in this area, beginning with the history of small-molecule-based technologies for modulating antibody recognition, followed by a systematic review of the various applications of ARM-based strategies. Thus, we describe ARMs capable of targeting cancer, bacteria, and viral pathogens, along with some of the scientific discoveries that have resulted from their development. Research in this area underscores the many exciting possibilities at the interface of organic chemistry and immunobiology, and is positioned to advance both basic and clinical science in the years to come.

Keywords

Antibody-Recruiting Molecules (ARMs); synthetic; bifunctional molecules capable of inducing antibodies to bind disease-relevant proteins; cells, or organisms; Synthetic immunology; the creation of synthetic systems that perform complex immunological functions; Antibody; protein that is produced by B-cells which identifies and neutralizes disease associated objects; Immunomodulators; rationally-designed molecules that can affect components of the immune system; Immunotherapy; disease treatment that utilizes; induces; suppresses, or enhances components of the immune system; bifunctional molecule; a molecule that possesses two distinct binding targets; ternary complex; an assembly containing three distinct species; held together either through covalent or non-covalent bonds

Introduction

The introduction of cowpox (*vaccinia*) virus immunization by Edward Jenner in 1796 was a landmark moment in the history of medicine.(1) Not only did Jenner's vaccination strategy ultimately lead to the eradication of smallpox, it made clear the extraordinary power of a person's own immune system for warding off deadly illness. Subsequent advances, including the implementation of passive antibody therapy or "serum therapy" in the late 1800s,(2) and Milstein and Kohler's report of the first engineered monoclonal antibody in the 1970s,(3) have paved the way for a new revolution in immunotherapeutics focused on

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monoclonal antibody-based drugs. The first member of this class, Muromonab-CD3 (anti-CD3 or OKT3),(3) was cleared by the FDA in 1986 for treating transplant rejection, and since that time, the number of antibody drugs has increased dramatically; 31 agents are currently approved for clinical use, and more than 300 are undergoing clinical trials.(4–7) Furthermore, in 2011 alone, antibody-based therapies grossed $44.6 billion worldwide and this number has been predicted to increase in the upcoming years.(6, 8, 9)

The surge in popularity of monoclonal antibodies can be easily understood in light of their many extraordinary properties. Antibody molecules are readily generated against a variety of disease-relevant targets, some of which have been conventionally considered "undruggable".(10) Additionally, because antibodies often interact with their targets with excellent affinity and specificity, undesirable side-effects related to off-target binding are thought to be low relative to traditional small-molecule-based therapeutics. Finally, antibodies may elicit therapeutic responses by a variety of mechanisms including inhibition of protein function,(11, 12) targeting the delivery of cytotoxic drugs,(13–15) and triggering immune effector responses.(16–18)

There are two mechanisms by which antibodies can trigger immune-mediated cytotoxicity: engagement of plasma complement proteins and/or direct activation of immune effector cells. The former process, termed complement-dependent cytotoxicity (CDC), begins upon cell-surface immobilization of certain members of the complement protein family (e.g., C1q) by opsonizing antibodies. This event initiates a downstream proteolytic cascade culminating in direct cell lysis or recruitment of complement-receptor-expressing effector cells, ultimately leading to target cell clearance.(19) Alternatively, binding of the antibody's crystallizable fragment (F_c) to Fc-receptors expressed on the surface of various immune cells can lead to receptor crosslinking, followed by target cell phagocytosis or the release of potent oxidizing agents and protein toxins (e.g., granzyme and perforin).(20) These processes are termed antibody-dependent cellular phagocytosis (ADCP), and/or antibody-dependent cellular cytotoxicity (ADCC), respectively.(19) Importantly, because F_c receptor-mediated cytotoxicity can enhance the processing and presentation of disease-relevant antigens, these cellular mechanisms have the capacity to give rise to long-lasting adaptive immunity.(21–24)

These advantages notwithstanding, antibody-based therapeutics suffer from certain limitations that arise primarily from their high molecular weights and peptide structures. For example, antibody administration can result in systemic inflammatory response syndrome, IgE-mediated acute anaphylactic reactions, serum sickness, and cytokine release syndrome, all of which have the potential to be life-threatening.(25) In addition, because antibody therapeutics contain "non-self" protein sequences, they can elicit host immune reactions, thus counteracting their efficacy.(25) Although the widespread "humanization" of antibody protein sequences has drastically reduced the potential for undesired immune responses, such reactions remain problematic clinically.(26, 27) Another limiting factor is that individuals can express different allelic variants of F_c receptors, which can directly impact the efficacy of "naked" monoclonal antibodies intended to act through interaction with these receptors.(28) Antibody-toxin conjugates, developed to address such difficulties, are associated with other drawbacks. These include premature drug release arising from antibody–drug linker instability, which can lead to toxic effects in normal tissues.(29) In addition, identifying optimal levels of drug conjugation can be difficult; low loadings may render treatment ineffective, while high loadings may lead to excess toxicity or a loss of antigen specificity.(30) Other limitations of antibody-based therapeutics include a lack of oral bioavailability, difficulties in standardization and characterization, and high costs.(25, 30–32)

Researchers have begun to investigate the development of low molecular weight species ("small molecules") that possess the complex functional properties of antibodies and other biologics. Because small molecules are generally inexpensive to produce, optimizable for oral bioavailability,(29) and unlikely to cause unwanted allergic or immunogenic responses, (31) these agents may be able to address many of the limitations of biologics without compromising their advantages. These studies have motivated efforts in Synthetic Immunology, a field focused on developing synthetic systems and strategies for controlling and/or creating human immunity.(33, 34) Despite still being in its infancy, this research area has already given rise to a number of exciting strategies, with potential applications in both basic and biomedical science.

We focus here on *a*ntibody-*r*ecruiting small *m*olecules (ARMs), which we define as synthetic, bifunctional molecules capable of inducing antibodies to bind disease-relevant proteins, cells, or organisms (Figure 1). Simultaneous association of ARMs with antibodies and surface-exposed receptors results in the formation of ternary complexes, which can elicit antibody-dependent immune effector responses. By convention, we define the two regions of ARMs as the target-binding terminus (TBT), which recognizes the disease-associated protein target (either present on cell/viral surfaces or free in solution), and the antibody-binding terminus (ABT), which associates with anti-hapten antibodies. Notably, we have chosen to limit the scope of this Review to technologies in which synthetic (i.e., non-recombinant), organic ligands are employed to control the immunological functions of antibody proteins; therefore, a variety of interesting and important systems, including antibody-drug conjugates,(35) antibody-based recombinant constructs (e.g., bispecific antibodies, diabodies, and others), (36–38) synthetic vaccines, (39) and ligand-templated supramolecular assemblies, (40–44) are not covered in detail herein.

As early as the 1970's, artificially engineered systems were shown to redirect antibody responses to the surfaces of cells that are ordinarily non-immunogenic. For example, liver, spleen, and red blood cells covalently modified with small molecule haptens, such as trinitrophenyl (TNP) groups, were shown to induce antibody-dependent cell-mediated immune responses.(45, 46) Furthermore, anti-DNP antibodies of IgG and IgM isotypes were shown to target liposomes labeled with dinitrophenyl (DNP) groups, leading to the induction of a complement-mediated cytotoxic response.(47) In all of these cases, antibody targeting and cytotoxicity were shown to be hapten-dependent; that is, antibodies were directed as a result of covalent DNP or TNP labeling. Although hapten labeling in these cases was non-specific, these early studies were critical in demonstrating that *antibody-mediated immune responses could be templated by "non-native," synthetic materials.*

Subsequently, strategies utilizing rationally-designed bifunctional systems to redirect the immune response against disease-relevant targets began to emerge. For example, chimeric proteins consisting of IgG or IgM Fc domains fused to human CD4, the cell-surface receptor target of HIV gp120, were shown to bind both to the complement protein C1q and to Fc-receptors.(48–50) These "immunoadhesins" were further shown to enhance immune effector response selectively against HIV infected cells in the presence of peripheral blood mononuclear cells (PBMCs), while inflicting minimal background cytotoxicity on uninfected cells. These pioneering studies represent the first evidence that rationally-designed, bifunctional molecules could specifically target immune-mediated functions to pathogenic proteins.

Subsequent research efforts expanded on these findings by demonstrating that proteins derivatized with small-molecule haptens could also possess immunomodulatory properties. For example, soluble CD4 covalently modified with the dinitrophenyl (DNP) motif was shown to mediate the formation of a quaternary complex between gp120, anti-DNP

antibodies, and soluble complement protein C1q.(51) Similarly, a dimeric Fab fragment ($F(ab')_2$) directed against the T-cell marker anti-thymocyte globulin (ATG), covalently labeled with fluorescein, was shown to induce selective cytotoxicity against T-cells in the presence anti-fluorescein antibodies and complement proteins.(52) Additionally, treatment of fluorescein-immunized mice with this molecule resulted in the clearance of peripheral ATG-expressing T-cells. Together these studies were critical in setting the stage for developing bifunctional ARMs.

Anti-hapten Antibodies Used in ARM Strategies

To date, ARMs have incorporated two types of functionality at the ABT: (1) small molecule ligands for "endogenous" antibodies, or (2). rationally-designed functional handles, which require delivery of pre-formed antibody-small molecule conjugates or pre-immunization for induction of selective antibody responses. Perhaps the most common targets in the first category include the galactosyl-(1–3)-galactose (α-Gal) carbohydrate epitope and the 2,4-dinitrophenyl (DNP) motif. Intriguingly, 2–8% of circulating antibodies in the bloodstream are believed to recognize the α-Gal trisaccharide,(80, 81) and these are believed to arise following exposure to this carbohydrate on the surfaces of cells derived from prokaryotes and non-primate eukaryotes.(82) Although DNP and other nitroarenes are not likely the products of biosynthesis,(83) unlike α-Gal, approximately 1% of circulating antibodies in the human bloodstream have been shown to recognize this epitope.(83, 84) Although the origin of anti-DNP antibodies is not known, one potential route of human inoculation involves exposure to DNP-containing dyes, preservatives, and/or pesticides,(85) which have been detected as environmental contaminants throughout the United States.(86, 87) An alternative hypothesis is that dietary ingestion of proteins and/or peptides containing nitroaromatic amino acids, formed in foods during the cooking process,(88) leads to adaptive immune responses against hapten-containing neo-epitopes,(89–91) perhaps in a TLR-independent manner.(92)

The second class of ABT that has been used in ARM strategies is composed of non-native antigens. Although such motifs by definition are not expected to bind pre-existing antibody proteins, humoral immune responses against these epitopes are readily induced by immunization with the hapten of interest conjugated to a carrier protein. One advantage of this approach is that haptens with useful chemical and/or physical properties can be chosen. For example, anti-fluorescein antibodies can easily be induced through immunization with protein conjugates of fluorescein isothiocyanate (FITC),(62, 93) and the haptens' photophysical properties provide a convenient handle for binding and imaging studies. Another useful immunization-dependent strategy exploits the unique properties of catalytic aldolase antibodies, which can form covalent adducts with 1,3-diketones (Figure 2, 1 → **3; 2** → **4**) or β-lactam (Figure 2, 5 → **6**) functionalities.(65, 94–97) Antibodies specific for the 1,3-diketone functionality can either be administered passively or generated through reactive immunization with a 1,3-diketone-KLH conjugate.(98)

Applications of Antibody Recruiting Small Molecules in Disease Targeting

Advances in synthetic and biophysical chemistry have enabled researchers to construct bifunctional small molecules against a broad variety of structurally unrelated disease-relevant targets. Due to their modular nature, ARMs are able to form immuno-modulatory ternary complexes with various macromolecular species, simply as a function of the structure and recognition properties of the TBT. Thus, applications of the ARM strategy have included both cancer and infectious disease (Table 1), and suggest a number of additional possibilities for future therapeutic development.

Cancer

Cancer is one of the leading causes of death worldwide, and is believed to be responsible for one in every four deaths in the United States.(99) Traditional treatment options for patients suffering with malignancies include surgical resection, direct irradiation, and cytotoxic chemotherapy, all of which are frequently associated with severe side-effects.(100–103) Recently, biologic agents and cellular immunotherapies have emerged as popular alternatives for cancer treatment, and due to their high specificity, these modalities have the potential to address many of the problems associated with traditional chemotherapies (e.g., off-target effects, etc.).(104) Indeed, with the inclusion of monoclonal antibodies into the repertoire, cancer therapeutics grossed $18.5 billion in sales in 2009 alone. Despite these successes, available anticancer agents remain inadequate for most patients suffering with cancer, and the demand for novel, targeted therapies is growing.(105) To this end, ARM technologies may represent promising alternatives for these patients, and have the potential both to complement, and improve upon, available anti-cancer modalities.

Barbas and colleagues were among the first investigators to demonstrate the benefits of combining small molecules with antibody proteins for cancer-relevant applications. These researchers have primarily exploited a “catalytic monoclonal antibody,” mAb 38C2, capable of reacting with the 1,3-diketone moiety to form an enaminone (Figure 2, 1 → **3; 2** → **4**). mAb 38C2 has been conjugated to various β-diketone-containing small-molecule TBTs to generate “chemically programmed antibodies” capable of recognizing various cell-surface targets. For example, the conjugate cp38C2 (Figure 2, 3),(65–67, 73) targets the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins,(106, 107) cell-surface proteins that are highly overexpressed in a wide variety of cancers, including ovarian, cervical, breast, and melanoma.(108, 109) This construct has been shown to mediate CDC and ADCC against M21 cells, and it demonstrated remarkable efficacy in human M21 melanoma cell xenograft models (81% average reduction in tumor growth after 42 days).(67) Compound cp38C2 also can inhibit metastasis of M21 tumor cells in female SCID mice, more than doubling their median survival versus untreated controls.

Subsequent studies from the Barbas group eliminated the requirement to use exogenous antibody proteins in their chemically-programmed antibody strategy. Using syngeneic murine colon cancer (CT26) and melanoma (B16) models, it was demonstrated that wild-type BALB/c mice, pre-immunized with a diketone hapten (JW-KLH), produced aldolase antibodies capable of forming covalent adducts with synthetic diketone groups. Once “programmed,” these animals were treated with either **1** or cRGD-dk, a cyclic-peptide-based integrin-targeting conjugate, and tumor-specific ADCC responses were observed, resulting in approximately 75% tumor reduction compared with vehicle control.(73)

Another useful function of small molecule conjugates with aldolase antibodies, termed “CovX-bodies,” is to improve the pharmacokinetic properties of therapeutic compounds. For example, mAb 38C2 conjugate **4** has been employed as a delivery vehicle for a metabolically-labile small-molecule inhibitor of endothelin A (ET_A),(68) a receptor involved in neovascularization and implicated in cancer, renal failure, heart failure, and hypertension.(110–112) This conjugate exhibited efficacy in a murine xenograft model of human prostate cancer (PC-3) and demonstrated up to a 45% inhibition of tumor growth versus controls. This antibody-attachment strategy has also been employed to minimize toxic side-effects of the HIV entry inhibitor Aplaviroc,(61) and to stabilize peptide-based targeting agents such as VEGF(77) and angiogenesis inhibitors of thrombospondin-1.(113) Indeed, a CovX-body containing a peptide-based angiopoietin-2 inhibitor (CVX-060) is currently being evaluated in a Phase II clinical trial in patients with advanced renal cell carcinoma.(2, 114)

Recent work from the Barbas lab has taken the ARM concept into a variety of novel directions. For example, these authors have demonstrated that the catalytic antibody mAb 38C2 can be conjugated with bifunctional small molecules, enabling them to target two different surface macromolecules: the integrin receptors ($\alpha_v\beta_3$ and $\alpha_v\beta_5$) and the luteinizing hormone releasing hormone receptors (LHRH-R).(74) More recent investigations have shown that conjugating mAb 38C2 to ARC245 (**5**), an RNA aptamer capable of binding VEGF, can increase the ARC245 serum half-life from minutes to 21 hours.(76) Given the flexibility of available screening methods for identifying selective, high-affinity aptamers, (115, 116) this strategy has the potential to greatly accelerate the process of TBT discovery against numerous disease-relevant targets.

Reports from the Paulson and Bundle groups have disclosed a class of ARMs for targeting B-cell lymphomas through multivalent interactions with CD22,(71, 72) a cell-surface regulator of B-cell signaling overexpressed in malignant cells.(117) To this end, a bifunctional molecule (**7**) was constructed containing an *o*-nitro phenol element at the ABT to bind endogenous anti-nitrophenol (anti-NP) antibodies and a glycan sequence at the TBT for binding CD22. By complexing this construct with decavalent anti-NP IgM, these authors demonstrated an increase in binding avidity for CD22-expressing cells of approximately two orders of magnitude versus control conditions lacking anti-NP IgM. Follow-up studies demonstrated that further increases in TBT valency, through conjugation to a polymer support, led to 100-fold higher levels of anti-NP IgM recruitment to target B-cells versus unconjugated compounds. (72)

The Low group has developed ARMs targeting the folate receptor (FR), a cell-surface protein overexpressed in many cancers, by utilizing folic acid at the TBT and either fluorescein (**8**) or DNP at the ABT.(62–64) For example, when co-administered with IL-2, construct **8** enhanced median survival by 250% in wild-type female BALB/c mice grafted with FR positive M109 lung tumors and pre-immunized with BSA-FITC or KLH-FITC.(62) Co-administration of this fluorescein-folate construct with both IL-2 and IFN-γ enhanced survival by 300%. Follow-up studies suggested that anti-tumor effects resulted from ADCP and ADCC mechanisms,(63) and substitution of the fluorescein group with DNP did not diminish efficacy in these models.(64) Notably, FR-targeted ARMs were found to induce long-lasting immunity against FR-expressing tumors; re-challenge of mice previously treated with M109 tumor cells led to rejection of tumor without introduction of any additional ARM.(118) Although details relevant to the mechanism of this memory effect were not reported, depletion of both CD4+ and CD8+ T cells resulted in no protective immunity upon tumor re-challenge. These results suggest the intriguing possibility that ARMs can be utilized as therapeutic vaccines,(119) consistent with data reported for monoclonal antibody therapies.(120–123)

Seeking to exploit the widespread prevalence of endogenous anti-α-Gal antibodies in the human bloodstream,(69) Kiessling and coworkers synthesized bifunctional constructs containing known integrin-binding functionality at the TBT and the α-Gal trisaccharide motif at the ABT (**11**). These ARMs proved effective at inhibiting integrin-mediated cell adhesion, recruiting anti-α-Gal antibodies, and mediating complement-dependent cytotoxicity against various cancer cells using normal human serum as the sole source of anti-α-Gal antibodies and complement proteins.(70) Cytotoxicity studies comparing this ARM with a toxin-conjugate in which the α-Gal trisaccharide was replaced by doxorubicin, revealed the antibody-recruiting agent to be more selective; construct **11** only exhibited activity against cells expressing high levels of integrin, while the doxorubicin conjugate proved cytotoxic to cells expressing both high and low levels of integrin. Based on these findings, the authors concluded that non-linear increases in antibody binding avidities due to

multivalent interactions enable the ARM-based agents to select for cells expressing more than a "threshold" level of target receptor.

Anticancer efforts in the Spiegel laboratory have focused on the development of antibody-recruiting small molecules directed against prostate cancer cells, called ARM-Ps.(75, 124) These bifunctional molecules contain a glutamate urea moiety at the TBT for targeting the prostate specific membrane antigen (PSMA). This surface-bound protein is overexpressed in most subtypes of prostate cancer cells,(125) as well as in the neovasculature of many solid tumors (e.g., glioblastoma multiforme,(126) bladder cancer,(127) gastric and colorectal cancer(128)). An ARM-P derivative containing 8 oxyethylene units in the linker, called ARM-P8 (**9**), was found to possess an optimal compromise between affinity to PSMA and ability to form ternary complex. Follow-up studies demonstrated that ARM-P8 could mediate ADCC against PSMA-expressing prostate cancer cells in the presence of anti-DNP antibodies and peripheral blood mononuclear cells (PBMCs). Interestingly, high concentrations of ARM-P8 were found to serve as auto-inhibitors of ternary complex formation, both in biophysical and cell viability assays. Analogous observations have been made previously in systems involving bifunctional molecules that template ternary linkages, and these observations support ternary complex formation as a necessary pre-condition for ARM-P8-mediated ADCC.(63, 71) Furthermore, the amount of ternary complex was shown to be directly dependent on the concentration of antibody, indicating that levels of anti-DNP antibody in serum can directly affect the efficacy of ARM-P8.(75)

DUPA, an ARM-P8 homolog, was recently evaluated in a humanized NOD/SCID mouse model. Thus, NOD/SCID animals transfused with human PBMCs and xenografted with either LNCaP (PSMA-positive) or PSMA-negative (PSMA-negative) tumors were immunized with DNP-KLH.(78) DUPA administration was found both to inhibit tumor growth and prolong survival in an antibody- and PSMA-dependent fashion. Animals that were not pre-immunized against DNP, or that were xenografted with PSMA-negative DU145 tumors, were unaffected by DUPA treatment. Taken together, these studies provide further support the potential utility of ARMs in clinical applications.

During the course of characterizing ARM-P8, researchers in the Spiegel laboratory observed that ARM-P derivatives with short linker regions displayed surprisingly strong PSMA-binding affinities. Follow-up biochemical, crystallographic, and computational studies, led to the serendipitous discovery of an arene-binding site on PSMA that can accommodate electron poor aromatic rings, such as DNP.(124) Although the interaction of the DNP moiety with the arene-binding site appears to involve only two amino acids, it affords a potency increase in PSMA binding of up to two orders of magnitude. Next-generation ARM-Ps that take advantage of this arene-binding site interaction are expected to show significant enhancements in PSMA binding affinity versus available derivatives.

More recently, researchers in the Spiegel laboratory synthesized an antibody-recruiting molecule called "ARM-U" (**10**), which targets the urokinase-type plasminogen activator receptor (uPAR).(79) uPAR is expressed on the surfaces of breast, colon, stomach, and bladder cancers,(129, 130) and has been used as a diagnostic marker for malignancy.(131–135) ARM-U has been shown to target uPAR at a the high affinity uPA binding site, recruit anti-DNP antibodies to uPAR-expressing A172 human glioblastoma cells, and ultimately mediate ADCP and ADCC in an antibody- and uPAR-specific manner. These studies underscore the generality of the ARM strategy for cancer treatment.

Infectious disease (Bacteria and Viruses)

The World Health Organization (WHO) has estimated that infectious agents (viruses, bacteria and parasites) are responsible for approximately 25% (15 million) of global deaths each year and are the predominant cause of mortality in developing nations.(136, 137) Vaccines are considered to be among the most successful strategies for fighting infectious disease,(138) and such strategies have been extremely successful in combating agents such as typhoid, cholera, rabies, measles, mumps, hepatitis B, rubella, tetanus and polio. Despite these achievements, current vaccination strategies are limited by difficulties in production, (139) variable levels of immunostimulation, and high costs. (140) (141, 142)

Although traditional small-molecule-based antibacterial and antiviral therapeutics have proven extremely successful, their utility has been hampered by surges of rapid resistance. (143, 144) Monoclonal antibody-based therapies have demonstrated substantial preclinical success in treating various infectious diseases;(145–148) however, despite their therapeutic promise, only a small percent of antibodies currently in development are indicated for infectious disease treatment.(148) To date, only one antibody-based antiviral agent (against respiratory syncytial virus, Palivizumab) has obtained FDA approval, and there are no clinically-approved monoclonal antibodies that target bacterial pathogens.(149, 150) Thus, there is a critical need to develop new therapeutic strategies targeting infectious agents.

Bacteria

The therapeutic arsenal against bacterial infection has largely consisted of natural products and synthetic small molecules. Conventional antibiotics act by targeting vital bacterial functions such as cell wall synthesis, protein synthesis, RNA transcription, and DNA replication. Because many bacterial species share common essential targets, these agents are often harmful to native flora as well as pathogenic microbes, and can increase host susceptibility to certain infections.(151, 152) Furthermore, the emergence of organisms that are resistant to many, if not all, available agents has proven increasingly problematic. Indeed, there has been significant recent interest in the development of monoclonal antibodies for treating drug-resistant bacteria, in part because these agents could exploit mechanisms distinct from conventional antibiotics, making them less likely to induce cross-resistance.(147) Despite this, out of 13 mAbs currently in clinical development for treating bacterial infection, none have demonstrated significant efficacy.(147) Antibacterial therapeutics with novel mechanisms of action would therefore be highly desirable.(153–155)

A variety of ARM-based antibacterial strategies have been evaluated. The first example of such an approach was reported by Bednarski and colleagues and employed a rationally designed, bifunctional molecule capable of directing anti-avidin antibodies to *E. coli* (Figure 3, 12).(53, 54) Biotin was conjugated to the *C*-glycoside of mannose, a known ligand for bacterial mannose receptors, and this construct was shown to recruit anti-avidin antibodies to the surface of *E. coli* in a manner dependent on the presence of conjugate, avidin and antibodies. These researchers further demonstrated that complexes between avidin, antibody and ARM could mediate complement- and macrophage-dependent cytotoxicity in a manner competable by α-mannopyranoside. Interestingly, the inherent multivalency of avidin significantly enhanced the millimolar binding affinity of the *C*-glycoside ligand to the mannose receptor.

Wang and coworkers expanded this concept by developing bifunctional polymers capable of redirecting endogenous anti-α-Gal antibodies to *E. coli*.(55) Using chemo-enzymatic synthesis, these researchers constructed a polymeric ARM derivative containing poly-mannose as the TBT and poly-α-Gal as the ABT (**14**). These bifunctional polymers were

shown to bind both *E. coli* mannose receptors and endogenous anti-α-Gal antibodies from human serum using competition ELISA experiments.

More recently, Whitesides and coworkers(56, 57) developed ARMs that target pathogenic bacteria by utilizing the potent antibiotic vancomycin. Polyvalent polymers containing fluorescein at the ABT and vancomycin at the TBT were synthesized (**13**) and shown to redirect anti-fluorescein antibodies to the surface of various Gram-positive bacteria (*S. epidermidis, S. pneumoniae,* and *S. aureus*). Using fluorescence microscopy and flow cytometry, the authors demonstrated that the antibody-recruiting polymer could mediate phagocytosis of opsonized bacteria in the presence of anti-fluorescein antibodies.

Viruses

Although vaccine-based strategies have been extremely successful against various viral diseases, a significant number of viral pathogens that have proven refractory to such approaches (*e.g.,* HIV, herpes simplex viruses, etc) still remain.(156, 157) For the most part, available antiviral agents function by inhibiting enzymes such as reverse transcriptase, polymerase, protease, integrase, primase, and neuraminidase,(158) and their utility is limited by resistance development,(159, 160) low efficacy,(161) and the high rate of spontaneous mutation inherent to the viral lifecycle.(162) Monoclonal antibody therapies targeting viruses have experienced only modest success, and only a single such agent, which targets respiratory syncytial virus (RSV), has been approved for clinical use.(150) Novel technologies with the potential to harness the endogenous immune response in killing viral pathogens could be profoundly useful in the fight against viral diseases.

An early example of an ARM-based antiviral strategy was described by Wang and colleagues (Figure 4).(58) Using chemo-enzymatic synthesis, these researchers prepared a bifunctional molecule designed to redirect endogenous anti-α-Gal antibodies to HIV. This agent incorporated the α-Gal trisaccharide epitope at the ABT and was linked to the 36-amino acid gp41 fusion inhibitory peptide, T-20, at the TBT (**15**). The authors subsequently demonstrated that functionalization of T-20 had minimal effects on its ability to inhibit virus fusion, and that the bifunctional glycopeptide could bind anti-α-Gal IgG and IgM antibodies from human serum.

More recently, Valhne, *et al.* developed a series bifunctional glycopeptides capable of mediating immune responses against HIV-infected cells.(59) These constructs were derived by chemically linking the α-Gal disaccharide to a series of 15-mer oligopeptides derived from the gp120-binding region of CD4 (**16**). Using ELISA and immunofluorescence microscopy, the authors then showed that these bifunctional glycopeptides could redirect endogenous α-gal antibodies from human serum to both immobilized and cell-surface-expressed gp120. Additional assays then demonstrated that the presence of human antibodies enhanced the fusion inhibitory activity of the peptide by 10% versus control conditions. Interestingly, an additional 5–15% enhancement in inhibition was observed when complement-preserved human serum was used in HIV infectivity assays, which was attributed to the direct cytolytic action of complement proteins on HIV-infected cells. Glycopeptide-derived ARMs were also shown to mediate immune responses against chronically HIVIIIB/LAV-infected ACH2 cells in the presence of human serum and isolated natural killer (NK) cells via an ADCC mechanism, although some analogs proved cytotoxic even in the absence of NK cells.

The Spiegel laboratory has recently developed a non-peptidic ARM, called ARM-H (antibody recruiting molecule targeting HIV).(60) This bifunctional small molecule incorporates a derivative of the known small-molecule fusion inhibitor BMS-378806 at the

TBT,(163) along with the DNP motif at the ABT (**17**). ARM-H-mediated formation of ternary complex with anti-DNP antibodies and HIV-1 Env-expressing cells was shown to induce complement-dependent destruction of these cells. Furthermore, ARM-H can bind gp120 competitively with CD4, and also inhibit the entry of HIV-1 virus into human T-cells. Thus, ARM-H has the potential to interfere with the survival of HIV through multiple complementary mechanisms.

In general, by converting virulence factors (e.g. lectins, gp120) into recognition elements for immune-mediated destruction, ARMs have the potential to target various infectious pathogens. Although still in their infancy, such ARMs could serve as promising alternatives or adjuncts to available immunotherapies, antibiotics and antiviral agents.

Outlook

The strategies detailed herein underscore the promise of ARM technology for a range of therapeutically relevant contexts. Despite significant progress in this arena, certain obstacles remain in advancing this strategy into the clinic. For example, the different ABT types mentioned above are each likely to be associated with unique advantages and disadvantages. Although approaches that exploit endogenous antibodies are anticipated to be the most straightforward to implement in practice, their utility will likely vary between patients as a function of antibody concentrations, affinities, isotypes and sub-isotypes distributions, and other factors. Comprehensive, population-wide investigations into the prevalence and properties of known endogenous antibodies, as well as the identification of such species with entirely new binding specificities, will be critical for clinical applications. Pre-immunization strategies could afford greater control over these parameters, but would involve additional operational complexity, which may also carry increased risks of side-effects. Finally, pre-conjugated antibody-small molecule species can be constructed using a single antibody isotype (e.g., IgG1, a potent inducer of ADCC), however, such agents would likely carry many of the same limitations of available immunotherapies (e.g., dosing via injection, immunogenicity, etc.). Overall, the optimal ABT will likely depend on the specific patient and/or disease process being targeted.

While most reported TBT motifs interact with relatively well-characterized ligand–receptor systems, discovery strategies to enable unbiased targeting of disease-associated surface proteins could greatly extend the applicability of the ARM approach. To this end, modern techniques in rational ligand design,(164) and high-throughput chemistry(165) and biology(166) are likely to prove enabling, and recent strategies for the discovery of novel antibody-binding carbohydrate motifs using array technologies,(167–169) the systematic identification of antibody biomarkers for both healthy and disease states(170) and the identification of compounds capable of modulating protein-protein interactions,(171–174) provide cause for optimism along these lines. Finally, novel chemical scaffolds (e.g., for targeting multiple receptors at once),(71, 72, 74, 175) and assembly strategies (e.g., *in vivo* bioorthogonal chemistry),(176, 177) have the potential to facilitate ARM optimization. For example, improving receptor-binding profiles, decreasing molecular weight, enhancing oral bioavailability, all could serve to broaden the clinical utility of ARM agents.

By exploiting an emerging chemical understanding of complex biological systems, future efforts to rationally modulate human immunological functions have the potential to augment our ability to prevent, diagnose and treat human disease.(33) ARM-based strategies represent an important step in this direction, bridging mechanistic features of biologic agents with a detailed understanding of small molecule structure and function (Figure 5). Next-generation immunomodulators have the potential to move beyond the ARMs, enabling

precise control over immune responses, and contributing to an understanding of the molecular events underlying human disease at the resolution of atoms and molecules.

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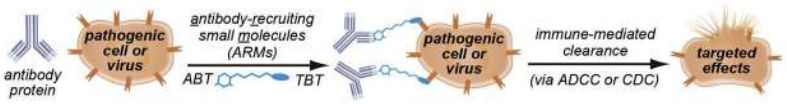

Figure 1.

Antibody Recruiting Small Molecules (ARMs). ARMs are bifunctional small molecules that function by forming ternary complexes with disease-relevant targets and endogenous antibodies. Ternary complex assembly leads to the activation of immune effector functions, followed by immune-mediated cytotoxicity and/or clearance of disease-causing species

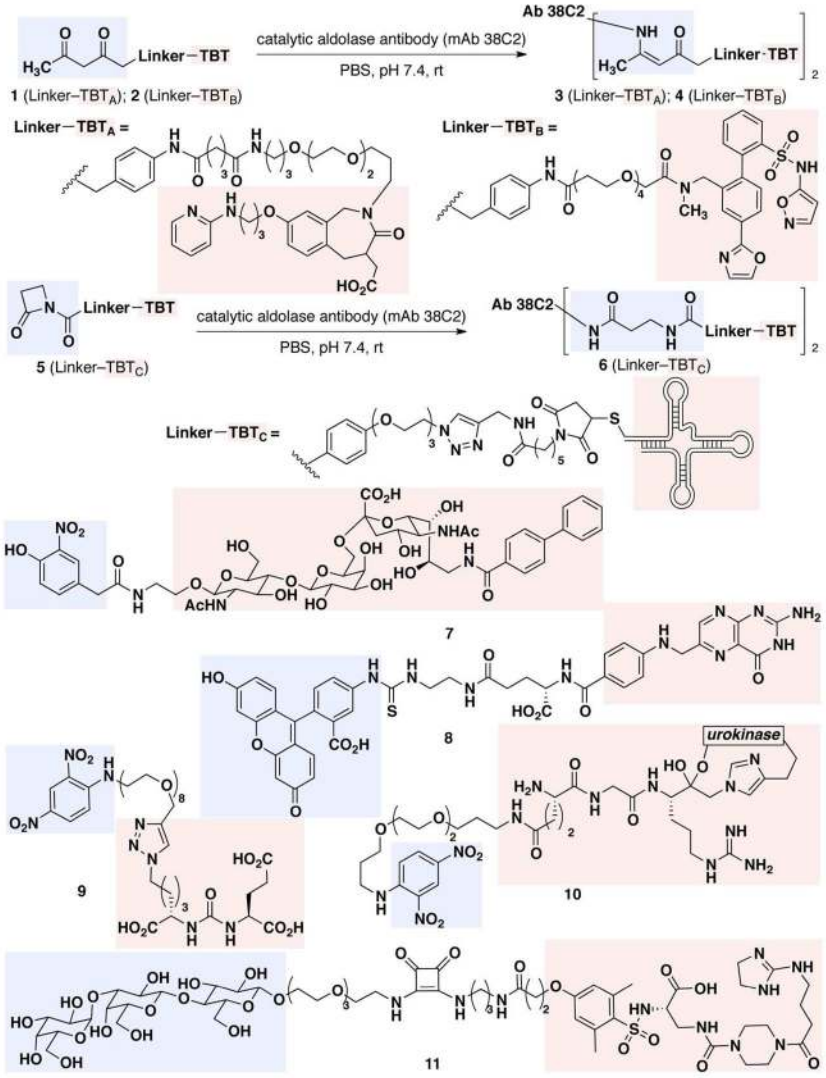

Figure 2.

Chemical structures of cancer-targeting ARMs. TBTs are highlighted in red boxes and ABTs in blue boxes

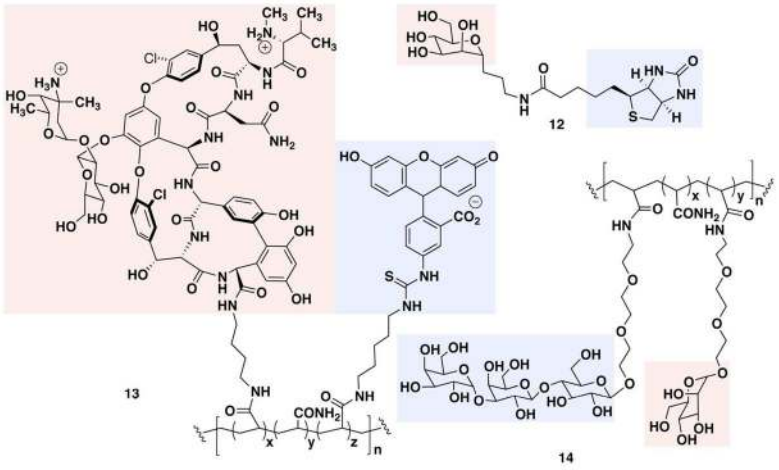

Figure 3.
Bacteria-targeting ARMs, with TBTs in red boxes and ABTs in blue boxes

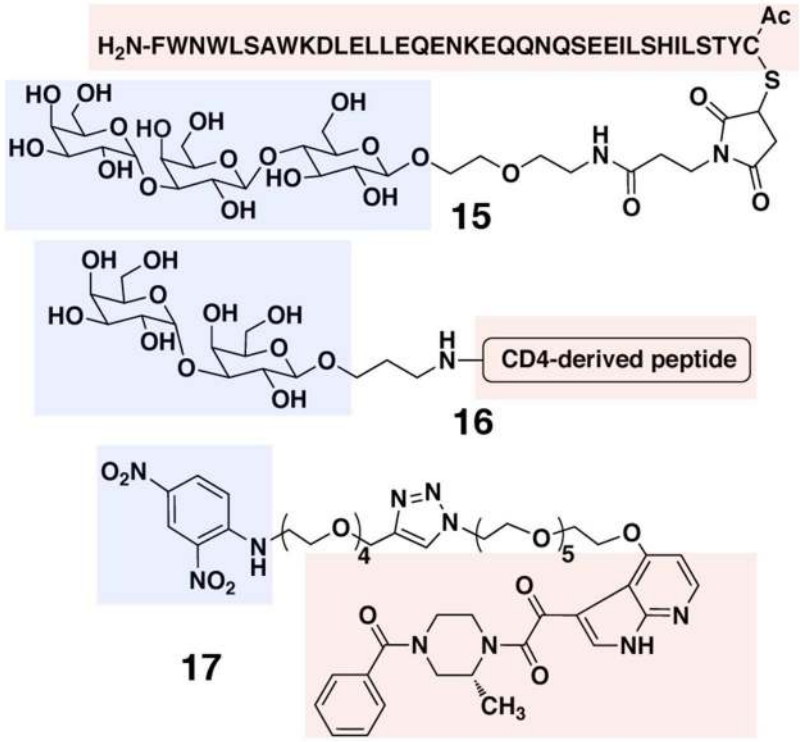

Figure 4.
Virus-targeting ARMs, with TBTs in red boxes and ABTs in blue boxes

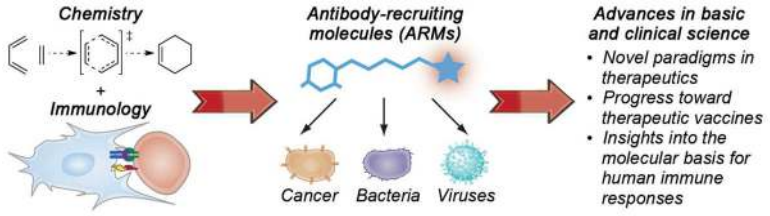

Figure 5.

The development of ARMs emerged from the confluence of many disparate fields of study, including synthetic organic chemistry and immunology. Although still in early stages of development, ARMs have the potential to contribute significantly to basic and clinical sciences.

Table 1

Summary of the applications of antibody-recruiting small-molecules to disease targets

Disease / Pathogen	Target	Antibody-binding Moiety	Molecule Type	*In vitro* response	*In vivo* model	Ab source
E. coli(53, 54)	mannose receptor	avidin	small molecule-protein conjugate	CDC, phagocytosis	-	commercial
E. coli(55)	mannose receptor	α-Gal	peptide	inhibition of agglutination	-	endogenous
Gram-positive bacteria(56, 57)	D-Ala-D-Ala	fluorescein	polymer	phagocytosis	-	commercial
HIV(58)	gp41	α-Gal	peptide	viral inhibition	-	endogenous
HIV(59)	gp120	Gal(α1–3)Gal	peptide	CDC, ADCC	-	endogenous
HIV(60)	gp120	DNP	small molecule	CDC, viral inhibition	-	commercial
HIV(61)	CCR5	β-lactam	mAb-small molecule conjugate	viral inhibition	-	monoclonal antibody
lung cancer(62–64)	folate receptor	fluorescein DNP,	small molecule	CDC, ADCC, phagocytosis	syngeneic female Balb/c	immunization
Karposi's sarcoma, colon cancer, melanoma(65–67)	integrin receptors ($\alpha_v\beta_3$ and $\alpha_v\beta_5$)	1,3-diketone	mAb-small molecule conjugate	CDC, ADCC	female nude and SCID xenografts	monoclonal antibody
prostate cancer(68)	ET_A	1,3-diketone	mAb-small molecule conjugate	opsonization	nude xenograft	monoclonal antibody
breast cancer, melanoma, osteosarcoma, Karposi's sarcoma(69, 70)	$\alpha_v\beta_5$	α-Gal	small molecule	CDC	-	endogenous
B cell lymphoma(71, 72)	CD22	nitrophenol	small molecule, polymer	opsonization	-	commercial
colon cancer, melanoma(73)	integrin receptors ($\alpha_v\beta_3$ and $\alpha_v\beta_5$)	1,3-diketone	small molecule	ADCC	syngeneic female BALB/c, and C57BL6	reactive immunization
melanoma, ovarian adenocarcinoma(74)	integrin receptors ($\alpha_v\beta_3$ and $\alpha_v\beta_5$) and LHRH receptor	β-lactam	mAb-small molecule conjugate	opsonization	-	monoclonal antibody
prostate cancer(75)	PSMA	DNP	small molecule	ADCC	-	commercial
umbilical cord endothelial cells (HUVEC)(76)	VEGF	β-lactam	RNA aptamer	inhibition of cell migration	female athymic nude mice (pharmacokinetic)	monoclonal antibody
colon adenocarcinoma breast carcinoma(77),	VEGF and Ang2	β-lactam (azetidinone)	mAb-small molecule conjugate	inhibition of receptor binding	female athymic nude mice	monoclonal antibody
prostate cancer(78)	PSMA	DNP	small molecule	ADCC	hu-PBL-NOD/SCID	immunization
colon adenocarcinoma, glioblastoma(79)	uPAR	DNP	small molecule-protein conjugate	ADCC, phagocytosis	-	commercial