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Manipulating the *in vivo* immune response by targeted gene knockdown

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Aptamers, nucleic acids selected for high affinity binding to proteins, can be used to activate or antagonize immune mediators or receptors in a location and cell-type specific manner and to enhance antigen presentation. They can also be linked to other molecules (other aptamers, siRNAs or miRNAs, proteins, toxins) to produce multifunctional compounds for targeted immune modulation *in vivo*. Aptamer-siRNA chimeras (AsiCs) that induce efficient cell-specific knockdown in immune cells *in vitro* and *in vivo* can be used as an immunological research tool or potentially as an immunomodulating therapeutic.

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Introduction

The ability to manipulate immune responses *in vivo* is central to understanding the role of individual molecules and pathways in the complex networks of immune responses. Genetically modified mice, antibodies, immunoactive peptides and small molecule inhibitors are the key tools used to manipulate specific molecular pathways *in vivo* in animal models. Antibodies, peptides and inhibitors are also increasingly used therapeutically to manipulate immune responses in patients with autoimmune disease and cancer. Most of these tools modulate molecular interactions systemically in all cells with pleiotropic effects. In many research and treatment situations, however, it is desirable to augment or disrupt a molecular interaction in a particular cell type or location, especially when global modulation of the interaction is toxic. For genetic engineering, conditional knockout or expression of a transgene in a cell-specific or temporal manner can help focus on the role of a particular gene product in a specific cell or context, although perfect Cre conditional expression systems are not available for all immune cells. Production of genetically engineered mice from transfected ES cells is time-consuming and costly. Recent application of Cas/CRISPR techniques to embryos provides a shortcut to generate animals (that no longer is restricted to mice) carrying genetic mutations and deletions or reporter genes, often conditionally expressed.

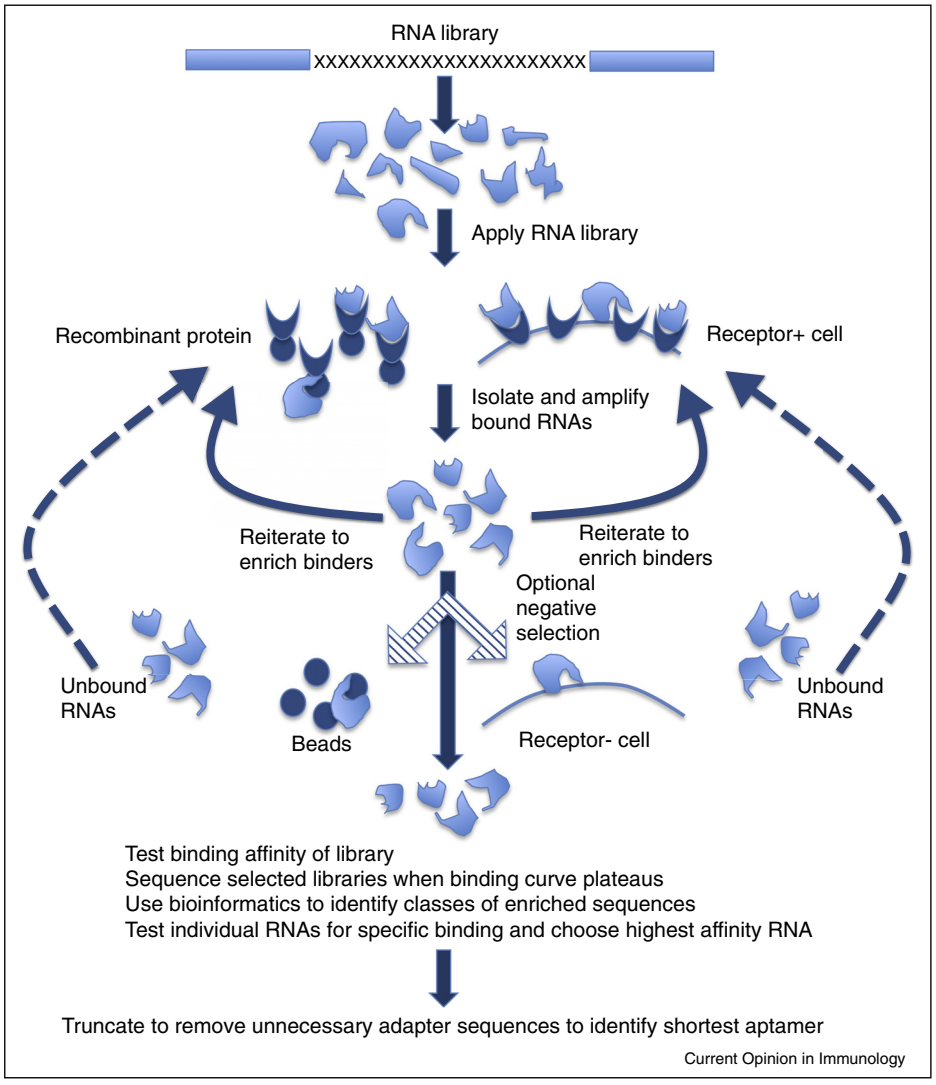
The discovery of RNA interference provides an alternate strategy for manipulating gene expression *in vivo* using small interfering RNAs (siRNAs). Recent clinical studies have shown dramatic gene knockdown in the liver (as much as 98%), which is durable (lasting a month or more) and does not induce innate immune recognition by RNA sensors [1]. However, neither of the methods used to deliver siRNAs to hepatocytes (RNA encapsulated into lipid nanoparticles or conjugated to GalNAc, which binds to the hepatocyte asialoglycoprotein receptor) nor most of the strategies used to knockdown gene expression in other cells work for immune cells, even *in vitro*. In fact lymphocytes and other hematopoietic cells are probably the most challenging cell to transfect [2]. However, aptamers-structured RNAs or DNAs selected for high affinity binding to a protein, such as a cell receptor, or other molecule — provide a workable solution for targeted gene knockdown. Chemical conjugation of an aptamer to one strand of an siRNA to generate an aptamer-siRNA chimera (AsiC) [3••,4] provides a robust and flexible strategy for targeted gene knockdown in immune cells that works *in vivo* [5,6,7•,8,9•,10]. In one study, *in vivo* knockdown of 80% was achieved in CD4T cells [7•], while in another vaccine-activated CD8+ T cells were knocked down by 50%. Moreover, aptamers, which usually have nanomolar affinity, can be selected for agonistic or antagonistic activity against their target [11,12] and can be covalently linked or conjugated to other aptamers, peptides, small molecules including toxins, and RNAs, providing a flexible platform for targeted manipulation of cells recognized by the aptamer. Here we describe the use of aptamers and aptamer conjugates for immune modulation.

Aptamer selection

Aptamers are identified from large libraries of DNA or RNA that are composed of invariant linker regions joined to a variable region of 20–40 nucleotides using a procedure called SELEX (systematic evolution of ligands for exponential enrichment) that was devised 25 years ago

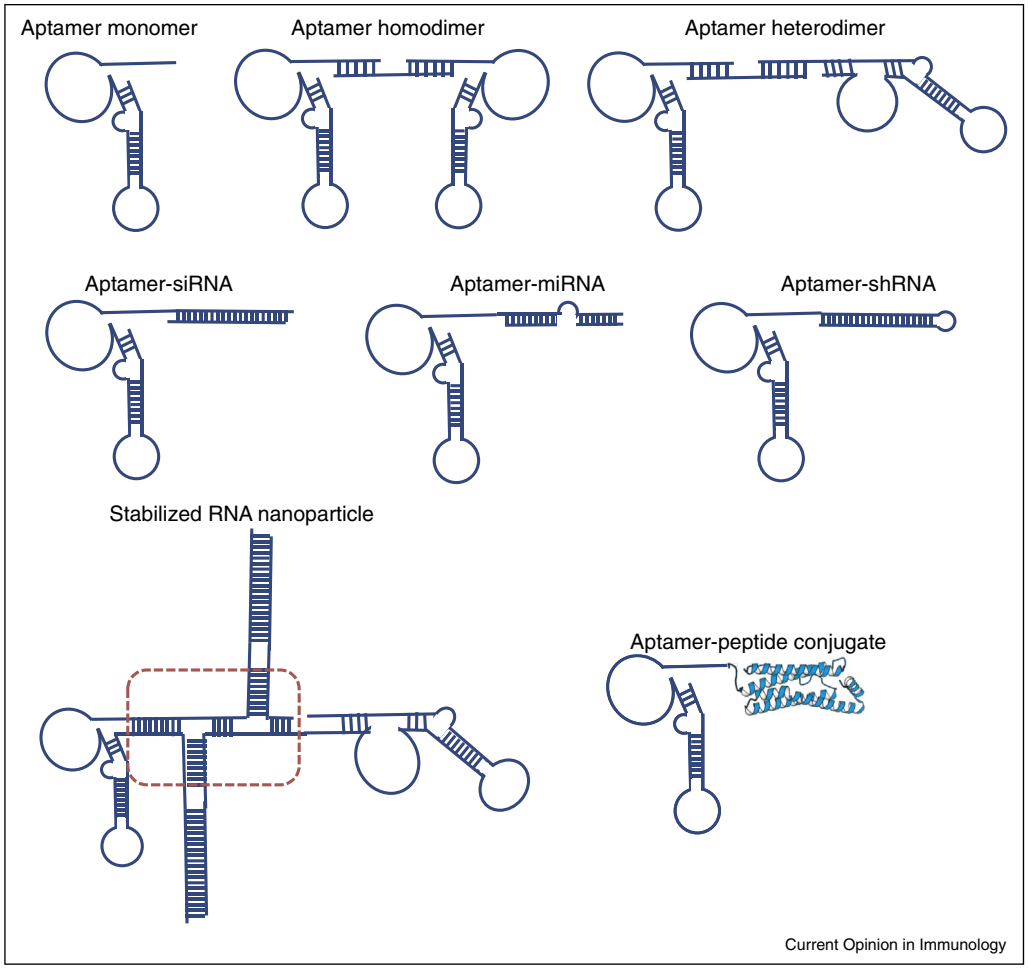
[13,14] (Figure 1). In the original method, oligonucleotide sequences are applied iteratively to immobilized proteins to select for sequences that bind with high affinity (in the pM–nM range) [15–17]. The sequences that bind are isolated, amplified and reapplied in multiple rounds of selection. The enriched library after multiple rounds is tested for binding K_D and selection is continued until a binding plateau is reached. The sequences that persist are then cloned and sequenced to obtain families of candidate aptamer sequences that bind with high affinity to the target protein. In the original version, aptamer selection often required 15–20 rounds of selection, a laborious process that sometimes failed to identify a suitable aptamer. Once selected, aptamers (and aptamer conjugates) can be *in vitro* transcribed and purified or chemically synthesized. Synthesized RNAs are easier to use for most laboratories especially for quantities need for *in vivo* experiments. RNAs <60 nt in length can be commercially synthesized, although cost increases and efficiency declines with length. Thus the shorter the aptamer, the better, especially when used as multimers or conjugates to other functional RNA moieties. Selection with shorter libraries leads to shorter sequences, without sacrificing the ability to identify high affinity hits. The

Figure 1



Aptamer selection using SELEX. An RNA library with a fixed number of varying residues sandwiched between invariant linkers is incubated with immobilized protein (left) or cells bearing the target receptor (right). Bound RNAs are isolated, amplified using RT-PCR and reapplied to the target protein or cell iteratively until binding of the enriched RNAs plateaus. Positive selection for one target could be alternated with selection for a cross-reacting target (such as mouse vs human or for related isoforms or homologues). Selection on cells has the advantage of selecting for receptors in their native conformation or can be used to optimize internalization. Once binding has plateaued, enriched RNAs are sequenced and the sequences analyzed by bioinformatics to identify groups of related sequences that bind to different epitopes.

Figure 2



Examples of useful aptamer designs. Aptamers can be used on their own, as multimers that are homotypic or heterotypic, or as conjugates (or ligated to) other RNAs (siRNAs, miRNAs, shRNAs and potentially mRNAs), proteins, toxins, fluorophores or small molecule drugs. A highly stable nanoparticle designed based on a phage motor RNA core region (boxed in red) can be derivatized with aptamer(s) or other active RNAs [68].

selected sequence can often be truncated from either or both ends without loss of affinity, since the invariant adapter sequences used for cloning often do not contribute to binding (Figure 2).

The rapidity and success of aptamer selection has dramatically improved in the last few years. With high throughput sequencing and bioinformatics, enriched sequences can often be identified after only 5–6 rounds of selection with a much improved success rate [18–20]. Sequences that might have been lost because of bias in PCR amplification are retained. The nucleotides, most often RNAs, are often chemically modified (for example with 2′-F-pyrimidines) to enhance stability, increase the ability to select aptamers against hydrophobic or negatively charged molecules, and minimize binding and activation of innate immune nucleic acid sensors. The SELEX procedure can also be used to identify aptamers that bind and/or are internalized by specific cell types [21,22]. By toggling between positive and negative selection [23] with cells that express or do not express a desired receptor (e.g. using receptor-negative cells transfected or not to express a targeted receptor), aptamer selection can focus on aptamers that recognize a receptor in its native context or that are efficiently internalized into cells. One company, SomaLogic, has identified proprietary aptamers against over a thousand human proteins found in the blood, often with pM affinity, including many with immune function, as a proteomic diagnostic. Table 1 provides a list of published aptamers that recognize immune receptors and mediators. Aptamers have been selected to recognize either human or mouse proteins, but occasional aptamers have been found fortuitously to recognize cross-reactive epitopes (Table 1). Cross-reactive aptamers could be very advantageous for drug development. This is particularly true of cancer immunotherapy applications,

Table 1

Aptamers that bind to immune mediators and receptors

Target	Mouse	Human	RNA or DNA	Reference	Note
Receptor/coreceptor					
CD4		X	RNA	[69]	
CD8		X	DNA	[62]	
IgM		X	RNA	[70]	
Costimulatory/inhibitory receptor					
CD28	X		RNA	[52]	
CTLA-4	X	X	RNA	[55]	
Activation marker					
OX40	X	X	RNA	[51,71]	
4-1BB	X		RNA	[16]	
IL-10 receptor	X		RNA	[56]	Antagonist
CD30, RANK	X	X	RNA	[72,79]	Cross-reactive, recognizes several TNF receptors
IL-17RA	X		DNA	[53]	Antagonist
CCR5		X	RNA	[10]	Blocks HIV-1 infection
BAFF		X	RNA	[63]	
Adhesion molecule					
L-selectin		X	RNA and DNA	[73]	
DEC205	X		RNA	[66]	
Innate immune receptor					
RIG-I		X	RNA	[67]	Agonistic, antiviral effect
Immune mediator					
IL-17A	X	X	RNA	[11]	Cross-reactive, antagonistic
MCP-1	X	X	RNA	[75]	Cross-reactive, antagonistic
IgE		X	RNA	[76]	
TGF-β1		X	DNA	[77]	
IgG	X	X	RNA	[74,78]	Binds Fc domain
IFN-γ		X	DNA	[32]	

where preclinical immune manipulation research needs to be done in immunocompetent mice bearing mouse tumors with aptamers that recognize mouse antigens. However, preclinical safety and efficacy in humans cannot be easily tested with mouse-restricted aptamers. Cross-reactive aptamers can in principle be generated by toggling selection between mouse and human proteins or cells [23].

Aptamers versus antibodies

Aptamers can be thought of as nucleic acid antibodies. Both are able to discriminate between highly related molecules with pM to low nM affinities for their targets. In principle, aptamers could substitute for antibodies in many research and therapeutic applications. Recent studies have shown how aptamers can be used instead of antibodies for multicolor flow cytometry [24], intracellular flow cytometry [25], cell selection [26–28], ELISA assays [29], immunohistochemistry [30], fluorescence *in situ* hybridization [31], surface plasmon resonance [32] and *in vivo* imaging [33]. Aptamers can also be used to quantify protein and RNA levels, including in multiplexed assays [34–39]. Nonetheless, *in vivo* applications of aptamers for research or therapeutics are just beginning to be explored. One aptamer against VEGF applied intraocularly is an FDA-approved drug (Macugen) for macular degeneration and about a dozen others have been or are being tested in clinical trials [40–42].

Although antibodies sometimes have higher affinity than aptamers, aptamers have important advantages. Aptamers can be chemically synthesized, which is cheaper and avoids the batch-to-batch variation of producing recombinant antibodies in biological systems. Moreover, aptamers are stable even at high temperatures (self-refolding if denatured) and have a long shelf-life. Antibodies (even humanized antibodies) typically generate anti-idiotypic responses that can lead to reduced effectiveness when given repeatedly. When chemically modified, aptamers and aptamer conjugates do not stimulate innate immunity, and nucleic acids are not known to elicit antibodies. This means that in principle they can be used repeatedly without attenuation or immune side effects. However, antibodies to nucleic acid therapeutics may not have been looked for carefully. Patients with autoimmune diseases often develop antibodies to nucleic acid–protein complexes; it is possible that aptamer-based therapeutics could generate such immunogenic complexes.

Aptamers can be linked in a modular fashion to other aptamers or functional groups (siRNAs, fluorophores, peptides, toxins, drugs) either covalently using straightforward chemistry or via short adapter sequences added to an end of an aptamer sequence that base-pair to a complementary nucleotide sequence on other molecules [43,44]. Aptamers linked to other RNAs covalently or by base-pairing can be easily designed by any laboratory,

while linkage to peptides or small molecules requires chemical expertise. Some examples of such multifunctional designs that have been used to manipulate immune cells are described below.

Aptamers are smaller than antibodies (a 40 nt long aptamer is ~12 kDa) and are usually below the cutoff for renal filtration. Thus, although chemically modified aptamers and aptamer conjugates are nuclease resistant and often stable in serum and body fluids for days [7•], they are rapidly excreted when injected intravenously, which can limit the effectiveness and durability of systemically injected aptamers/aptamer conjugates. Antibodies on the other hand circulate for a month or more. Although a long half-life means that the effect of antibodies is sustained, the extremely long half-life of antibodies can be problematic in the clinic if they cause toxicity, which is not easily reversed. However, for many uses, aptamer conjugates, unlike antibodies, function inside cells, where the chemically modified RNA can last for weeks. Thus, the half-life in the circulation does not correlate with the durability of the therapeutic response, and need only be long enough (a few hours or a day) to be efficiently internalized into the intended target cell. The pharmacokinetic properties of aptamers for intravenous use can be improved by avoiding renal filtration by conjugation with polymers, such as polyethylene glycol (PEG), or by multimerization to produce a molecule above the renal cutoff [42,45]. However, rare anaphylactic reactions to PEG that caused a death recently necessitated terminating a Phase III clinical trial of a thrombolytic aptamer being developed for cardiac surgery (C. Rusconi, personal communication), suggesting that PEGylated nucleic acids should be avoided for future therapeutics. Aptamers and aptamer conjugates can also be incorporated into small nanoparticles or LNPs, but at the risk of entrapping a large proportion of the reagent in the filtering organs, especially the liver. One ingenious nanoparticle design uses the core sequence of a self-assembling phage RNA motor to produce self-assembling highly stable RNAs that can be derivatized with aptamers, fluorophores, siRNAs, or toxins to produce multifunctional cell-targeted RNAs [46–48]. It is unclear how much derivatization of particles with high affinity aptamers alters the distribution of particles from the liver to the desired tissue.

We recently found that subcutaneous administration of naked aptamer-siRNAs (AsiCs) targeting CD4 or an epithelial cancer receptor concentrate in aptamer-targeted cells, leading to systemic gene knockdown (i.e., the CD4-AsiC causes efficient knockdown in the spleen and distal lymph nodes) [49] (and JL, unpublished). Subcutaneous administration of aptamers and aptamer conjugates should provide a good solution to the poor pharmacological properties of intravenously injected small RNAs, enabling aptamers to substitute for antibodies in many *in vivo* applications.

Immunological applications of aptamers for *in vivo* immune cell inhibition and activation

Immune cell activation to block checkpoint inhibition has recently taken center stage for cancer immunotherapy with promising results in a few tumors using blocking antibodies against CTLA-4, PD-1 and PDL1. Mouse studies have shown that aptamer-based strategies may be able to accomplish the same goal and may provide opportunities for more targeted checkpoint inhibition in tumors to minimize side effects caused by systemic inhibition of T cell inhibitors. Aptamer-mediated interventions could be useful in other settings where the immune response has a prominent role in pathogenesis, including transplant, autoimmune disease, inflammatory disease and infection. Aptamers already generated against CD4 and CD8, costimulatory and inhibitory receptors, integrins, and activation markers on T cells, RIG-I and soluble immune mediators (Table 1) provide a potential toolbox for immunotherapy research and potentially for treatment.

Monomeric or multivalent aptamers can replace antibodies to block or activate immune receptors or mediators. *In vivo* activity has been shown using a DNA L-selectin aptamer to block lymphocyte trafficking [50], multimeric OX40 or CD28 aptamers to enhance the potency of a DC-based vaccine and tumor immunity [51,52], and an IL17RA aptamer injected into inflamed joints to reduce arthritis [53,54]. The first *in vivo* example of using aptamers to overcome checkpoint blockade showed that a multivalent CTLA-4 aptamer (tetramer of a 35 nt aptamer linked to an adapter) could inhibit CTLA-4 and enhance tumor immunity in mice comparably to CTLA-4 antibody [55]. An alternate approach to overcoming checkpoint blockade used aptamers that recognize a TNF family receptor 4-1BB (CD137) on activated T cells, linked by an adaptor to form a dimer, to costimulate CD8 T cells [16]. This study showed that intratumoral injection of the dimer into subcutaneous P815 mouse tumors was as effective as anti-4-1BB at stimulating intratumoral CD8 T cells and inhibiting tumor growth and promoting survival. The dimer used in this study was probably not suitable for intravenous use because it was small enough to be rapidly excreted. Yet another study showed that an IL-10R blocking aptamer injected intravenously could inhibit tumor growth as potently as an IL-10R antibody [56].

One of the problems of using antibodies for checkpoint blockade or T cell activation is that they cause T cell activation systemically, not just in the tumor, leading to serious toxicity due to autoimmunity and cytokine storm from global T cell activation. Two clever strategies were designed with aptamers to activate CD8 T cells selectively in the tumor. In one approach [57•], the 4-1BB aptamer dimer was linked by an adaptor to an aptamer that recognizes the tumor, in this case PSMA, a prostate

cancer surface membrane protein. These bifunctional aptamer constructs, but not mixtures of the individual components, protected mice from subcutaneous and metastatic tumors that expressed PSMA. Moreover the bifunctional tumor-targeting, T cell-activating RNA increased infiltration of tumor-specific CD8 T cells into the tumor, but did not cause nonspecific immune activation in the spleen and lymph nodes, as was seen in mice treated with the same concentration of 4-1BB antibody. Another approach [58] linked the 4-1BB aptamer to aptamers targeting two molecules that are secreted by many tumors and captured by the tumor stroma, VEGF and osteopontin. After intravenous injection the VEGF aptamer-containing construct selectively concentrated in a VEGF-producing tumor and potently suppressed a variety of tumors, including metastatic lung lesions and spontaneously arising gliomas, with much less systemic immune activiation and toxicity than the 4-1BB antibody or aptamer on its own or mixtures of the dimeric 4-1BB aptamer and the VEGF aptamer. Both of these applications of aptamer technology take advantage of the flexibility of this platform for easily designing and constructing multifunctional RNAs. Unlike more complex antibody-based multifunctional proteins, these multifunctional RNAs are not expected to induce antibodies that would limit repeated use.

Immunological applications of aptamers for targeted gene knockdown

A major problem confronting tumor immunotherapy is the poor immunogenicity of tumors, which produce few tumor-specific antigens. The Gilboa laboratory used tumor-targeted PSMA AsiCs to knockdown genes responsible for mRNA quality control via nonsense-mediated decay (NMD) [59••]. This pathway surveys the pioneer mRNA transcript and degrades mRNAs that fail to pass muster. When key NMD enzymes are knocked down, the tumor expresses and presents neoantigens that T cells recognize. This approach inhibited tumor growth more potently than vaccination with GM-CSF-expressing irradiated tumor cells. A recent study of checkpoint blockade with anti-PD1 antibodies in non-small cell lung cancer showed that only tumors with many mutations respond to immunotherapy [60]. For the bulk of non-responding tumors, strategies like AsiC-mediated suppression of NMD might be needed to make these tumors visible to the immune system.

Aptamer-siRNAs have been developed to knockdown gene expression in all CD4 cells [7•,8,61] or CD8 [62] T cells. My group designed CD4-AsiCs to knockdown gene expression in all CD4+ cells susceptible to HIV infection and showed that knockdown of *CCR5*, the HIV coreceptor used in sexual transmission, or knockdown of HIV genes by vaginally applied AsiCs prevented genital transmission of HIV to humanized mice [7•,8]. Knockdown in the genital tract persisted for about 2 weeks, providing sustained protection against viral challenge. In unpublished studies, we also found that subcutaneous administration of CD4-AsiCs led to systemic knockdown (~80%) in splenic and distal lymph node CD4 T cells, but not in CD4- T cells or B cells. Thus, AsiCs can be used to accomplish cell-specific and temporal gene knockdown systemically. These AsiCs do not affect the proliferation of CD4 T cells or alter cell-surface expression of CD4 or activation markers, probably because the monomeric CD4 aptamer does not cross-link the target receptor. Moreover, sensitive qPCR assays showed no evidence of interferon or inflammatory cytokine induction, indicating that the AsiC does not provoke an innate immune response. Another approach to inhibit HIV infection relied on AsiCs incorporating an aptamer directed against HIV envelope protein to specifically target HIV-infected cells [6,43]. Another study designed CD4-AsiCs to knockdown *RORC*, the gene encoding the T_H17 master transcription factor RORγt, and then assessed CD4 T cell differentiation *in vitro* under polarizing conditions [61]. As expected knocking down RORC inhibited T_H17 cell differentiation *in vitro*. It will be interesting to examine what happens when the master transcription factors for T cell differentiation are knocked down *in vivo*. For directed gene knockdown in CD8 T cells, a DNA aptamer for CD8 was annealed via a 'sticky bridge' adapter to an siRNA and shown to induce CD8 cell-specific gene knockdown *in vitro* [62]. So far no *in vivo* experiments involving directed gene knockdown via the CD8+ receptor have been reported.

We have begun to use CD4-AsiCs to investigate the *in vivo* role of host genes in sexual transmission of HIV in humanized mice. We knocked down *TREX1*, a DNase that enables HIV to avoid activating cGAS and triggering antiviral Type I interferons (IFN) (Wheeler *et al.*, manuscript provisionally accepted, *Cell Host Microbe*). Because IFNs both have an antiviral effect and recruit and activate immune cells susceptible to HIV infection at sites of infection, it was difficult to predict what effect local IFN expression in infected cells would have on transmission. In fact *TREX1* knockdown inhibited transmission, supporting the overall antiviral effect of IFN production at the site of infection. By contrast, IFNs injected intravenously caused systemic inflammation and promoted HIV transmission. Thus aptamer-targeted gene knockdown in specific cytokine-producing cell types can provide insight into local *in vivo* cytokine function.

AsiCs bearing aptamers to cell surface receptors expressed specifically on activated lymphocytes have been used to improve production of antigen-specific memory T cells [9•], inhibit HIV infection using a CCR5 aptamer [10], and suppress proliferation of activated B cells or B cell tumors using a BAFF aptamer [63]. Inhibition of the mTORC1 complex in the mTOR pathway with rapamycin promotes differentiation of antigen-specific CD8 T cells to memory cells, which would be

advantageous for T cell immunity [64]. However, rapamycin also inhibits the mTORC2 complex, leading to undesirable immunosuppressive effects (It promotes Treg development, polarizes DCs to be toleragenic, and inhibits T cell trafficking.). The Gilboa group conjugated an siRNA against raptor, a member of the mTORC1 complex, to the 4-1BB aptamer to promote development of memory CD8 T cells, avoiding the undesirable effects of broad mTOR inhibition [9•]. Intravenous injection of mice bearing transferred ovalbumin (OVA)-specific CD8 T cells with the 4-1BB-AsiC that knocks down raptor enhanced the persistence after immunization of OVA-specific T cells. The enhanced memory response was similar in rapamycin-treated mice. However, the cytolytic function of the rapamycin-treated OVA-specific CD8 T cells was impaired compared to those in the aptamer-treated mice. This functional difference translated to a strong difference in tumor immunity and survival in vaccinated mice challenged with OVA-expressing mouse melanoma cells. Thus aptamer-targeted gene knockdown can be used for cell-specific knockdown against a single gene target. This dual (cell and target gene) specificity allows for experimental designs that can pinpoint gene function in particular cell types. In therapeutic settings this fine specificity for both target cell and drug target, not achieved with drugs or antibodies, should limit toxicity and promote therapeutic benefit.

The murine CTLA-4 aptamer was used to target tumor-associated, exhausted CD8 T cells and Tregs in mice bearing several types of subcutaneous tumors or metastatic melanoma cells [65]. Intravenous injection of CTLA-4-AsiCs that knocked down *Stat3* activated tumor-associated CD8 T cells, which down-regulated PD-1 and showed increased tumor antigen-specific granzyme B expression and IFN-γ production, enhanced tumor-infiltration of tumor antigen-specific CD8 T cells and strongly reduced tumor-associated Tregs, leading to inhibition of primary tumors and metastases. The aptamer on its own had no therapeutic effect, presumably because it did not crosslink CTLA-4. Moreover, because the aptamer recognizes an evolutionarily conserved motif, the mouse aptamer cross-reacts with human CTLA-4. CTLA-4 is expressed by T cell lymphomas and some other hematological malignancies. In fact intravenous injection of the same *Stat3*-targeting CTLA-4-AsiC inhibited the growth of a human CTLA4+ T cell lymphoma in immunodeficient mice. This study did not comment on whether *Stat3*-targeting CTLA-4-AsiCs showed any indications of autoimmunity in immunocompetent mice. Head-to-head comparisons of antitumor efficacy and toxicity in tumor-bearing mice treated with CTLA-4 antibodies and CTLA-4-AsiCs should be performed to evaluate whether AsiCs have an advantage over checkpoint blockade antibodies for activating anti-tumor immunity with reduced autoimmune side effects.

Immunological applications of aptamers for antigen presentation

Aptamers have also been used to enhance antigen-presentation. One ingenious example described above [59••] used tumor-targeted AsiCs to induce tumors to express neoantigens. Another study conjugated a peptide containing the OVA T cell antigen to aptamers that recognize a dendritic cell (DC) receptor (DEC205) expressed strongly on CD8α+ DCs [66]. Immunization with the aptamer-peptide conjugate given with poly(I:C) as adjuvant induced OVA-specific CD8 T cells in mice transferred with OVA-specific CD8 T cells that strongly protected them from orthotopic tumors expressing OVA. Efficient immunization required linkage of multiple aptamers to the peptide to produce a multivalent construct. However, this construct was not as efficient an immunogen as peptide-conjugated DEC205 antibody. It is also possible to use aptamers to stimulate innate immunity to augment an immune response or inhibit viral infection. A RIG-I aptamer that lacks a 5′-triphosphate has been selected that activates IFN production and inhibits viral replication *in vitro*. This type of aptamer could be incorporated into multifunctional RNAs designed as antivirals or immunogens [67].

Conclusions

Aptamers, which can be thought of as nucleic acid antibodies, provide a flexible platform for targeted immune manipulation. Like antibodies, they can be used on their own to activate or antagonize immune mediators and receptors. They can also be readily conjugated to other aptamers, siRNAs, miRNAs, drugs, toxins, peptides, and fluorophores to produce multifunctional targeted reagents that work *in vivo* to interrogate the role of an individual molecule in the immune response and potentially could be used to design a new type of targeted immunotherapeutics. The studies described here illustrate some of the potential ways these agents could be used. However, the field is just beginning.

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