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LETTERS

Conversion of staphylococcal pathogenicity islands to CRISPR-carrying antibacterial agents that cure infections in mice

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Staphylococcus aureus **and other staphylococci continue to cause life-threatening infections in both hospital and community settings[1–3]. They have become increasingly resistant to antibiotics, especially β-lactams and aminoglycosides, and their infections are now, in many cases, untreatable. Here we present a non-antibiotic, non-phage method of treating staphylococcal infections by engineering of the highly mobile staphylococcal pathogenicity islands (SaPIs). We replaced the SaPIs' toxin genes with antibacterial cargos to generate antibacterial drones (ABDs) that target the infecting bacteria in the animal host, express their cargo, kill or disarm the bacteria and thus abrogate the infection. Here we have constructed ABDs with either a CRISPR–Cas9 bactericidal or a CRISPR–dCas9 virulence-blocking module. We show that both ABDs block the development of a murine subcutaneous** ***S. aureus*** **abscess and that the bactericidal module rescues mice given a lethal dose of** ***S. aureus*** **intraperitoneally.**

SaPIs are ~15-kb genetic elements that carry *tst* and other toxic superantigen determinants and are inserted in the staphylococcal chromosomal DNA[4]. With the help of certain bacteriophages, SaPI DNA is excised from the chromosome, undergoes extensive replication, and becomes packaged in small phage-like particles. These are released upon phage-induced cellular lysis and go on to infect other cells, enabling those cells to produce the disease-causing, SaPI-encoded superantigens[5–7]. To develop ABDs, we began with a typical SaPI, SaPI2 (**Fig. 1a**), added *tetM*, to enable selection for tetracycline (Tc) resistance, and deleted the toxin genes and the two genes, *cpmA* and *cpmB*, that cause the formation of small capsids[5]. The deletion of *cpmA* and *cpmB* resulted in SaPI packaging in full-sized phage particles, thus providing an extra 30 kb of available packaging space, which could be filled with any desired cargo genes. These modifications did not diminish SaPI transfer frequency (data not shown). We then used standard allelic replacement technology to insert generic CRISPR–Cas9 and catalytically inactive CRISPR–dCas9 modules[8] with spacers targeting *agr*, a nonessential global genetic regulator of staphylococcal virulence[9].

agr is transcribed from divergent promoters P_2 and P_3; the P_2 operon includes a two-component signaling module, where AgrC is the histidine kinase receptor and AgrA the response regulator. An *agr*-encoded autoinducing peptide, AIP, is the activating ligand for the two-component signaling module, and phosphorylated AgrA upregulates both *agr* promoters. The major effector of virulence regulation is the *agr*P_3 transcript, RNAIII. The AIPs and their cognate histidine kinase receptors are highly variable and form four distinct *agr* specificity groups, with the AIPs generally cross-inhibitory between groups[10]. The *agr* response regulator and promoter region, however, are highly conserved throughout the staphylococci.

Consequently, we have used spacer guide RNAs targeting *agrA* and *agr*P_2P_3 for the ABDs with the CRISPR–Cas9 and CRISPR–dCas9 modules, respectively. As we had earlier found that SaPIs could be transferred to *Listeria monocytogenes*[11], we constructed a third ABD with a CRISPR–Cas9 spacer targeting *hly*, the gene encoding listeriolysin O. We labeled ABDs in numerical order starting with 2001, which represents the modified parental SaPI2 used for all of the constructs (**Fig. 1b**). The existing and potential future ABD constructs are illustrated in **Figure 1c**.

To develop a high-titer, phage-free source of ABD particles, we deleted the helper phage small terminase subunit (*terS)* gene, eliminating the packaging of phage DNA[12] without affecting phage or SaPI DNA replication or gene expression. This deletion enabled the production of mitomycin C–induced lysates with ABD particle titers in the 10^{10}/ml range. We have tested ABD2003 for toxicity and observed no ill effects (mice were weighed daily for 7 d and observed for signs of toxicity—weight loss, ruffled fur, ataxic gait, sluggishness etc.) from either intraperitoneal (IP) or intravenous (IV) doses of 10^{11} particles.

To demonstrate that an ABD could directly kill an infecting staphylococcus, we used ABD2003, containing a CRISPR–Cas9 module with a spacer targeting *agrA*, which was chosen because it is universally conserved among the staphylococci yet is nonessential. ABD2003 was expected to kill staphylococci by introducing a double-strand break in the chromosomal *agrA* locus. Thus, the *agrA*-targeting ABD could be used against virtually any *Staphylococcus*, whereas strains with an *agrA* deletion could be used as controls, to test for off-target effects, and for ABD2003 production, which requires the absence of the *agrA* protospacer in the producing strain.

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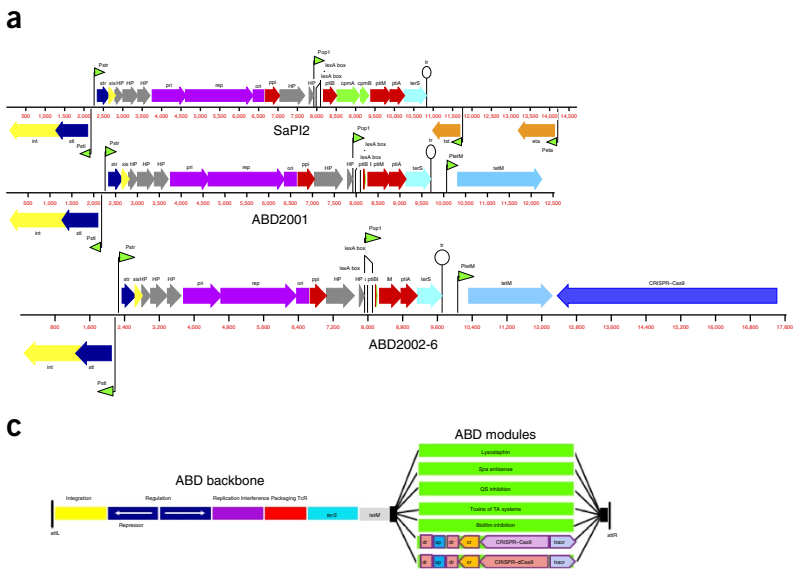

ABD	Size (kb)	Insertions	Function
ABD2001	12.5	tetM	ABD backbone
ABD2002	17.6	tetM CRISPR–Cas9 nontargeting	Generic CRISPR–Cas9
ABD2003	17.6	tetM CRISPR–Cas9 agrA	Causes lethal double-strand break in agrA
ABD2004	17.6	tetM CRISPR–Cas9 hly	Causes lethal double-strand break in hly (listeriolysin O)
ABD2005	17.6	tetM CRISPR–dCas9 nontargeting	Generic CRISPR–dCas9
ABD2006	17.6	tetM CRISPR–dCas9 agr	Blocks virulence by inhibiting expression of agr locus

Figure 1 SaPI and ABD structures. (**a**) Genetic maps of SaPI2 and its ABD derivatives. Yellow, *int* and *xis*; dark blue, regulation; gray, hypothetical genes; purple, replication; red, phage interference; green, capsid morphogenesis; cyan, terminase small subunit (*terS*); orange, toxin genes; light blue, tetracycline resistance (*tetM*); royal blue, CRISPR module. (**b**) ABD constructs. ABD2001 was derived from the prototypical SaPI2 by deleting toxin genes *tst* and *eta* and the capsid morphogenesis genes *cpmA* and *cpmB*. ABDs 2002–2006 were derived from ABD2001 by the insertion of the listed genes. (**c**) ABD scheme. From *attL* to *tetM* is the ABD backbone. Black boxes represent the cloning site. Green rectangles represent different modules that have been or will be added to the backbone. Targeting spacers are listed after a slash. dr, direct repeat; sp, spacer; cr, crRNA leader; tracr, tracr RNA.

To test for the killing activity of ABDs, we used several *S. aureus* strains, including RN1, USA300 LAC and other *agr*+ clinical isolates, with ABD2003. We also tested ABD2004 with *L. monocytogenes*. We present Petri dish photographs (**Fig. 2**) to show the killing results: platings were done with 10^6 particles/ml and 10^9 cells/ml, and 0.1-ml aliquots were plated on Trypticase soy broth (TSB) with Tc 5 µg/ml (Tc5). Colonies seen with the nontargeting spacer (ABD2002) (~10^4–10^5 per plate) represent ABD-receiving cells; those few seen with the *agrA*-targeting spacer (ABD2003) were formed by ABD-receiving cells that were not killed by the ABD and represent CRISPR-resistant mutants. Results for these strains are illustrated in **Figure 2a**.

For *L. monocytogenes*, strain SK1442, we used ABD2004, which carries *CRISPR–Cas9* and *hly*. We observed approximately equivalent cell killing by this ABD, but no killing by ABD2002, which contains the nontargeting CRISPR–Cas9 (**Fig. 2a**).

For a more quantitative analysis, cell suspensions were sonicated to disrupt clumps, and equal aliquots were mixed with varying numbers of ABD2003 particles and plated on nonselective plates for survivors (which represent cells that were not infected by an ABD particle) and on Tc5 plates for CRISPR-resistant transductants. **Supplementary Table 1** shows the results for ABD2003 (targeting *agrA*) with RN1. Essentially identical results were obtained with the other strains. These results confirmed the expected behavior of the three CRISPR modules *in vitro* and led to preclinical testing of ABD2003 in mice. For the *in vivo* tests of ABD2003, we used two different murine infection models with a luminescent *agr*P3-*lux*-carrying RN1 derivative[13]: a subcutaneous (SC) abscess model[14] and an intraperitoneal (IP) lethality model[15].

For the SC model, we used hairless mice and a dose of 4×10^8 organisms (the number of staphylococci in a culture is actually about triple the number of colony-forming units because of the natural clumpiness of the organism, and we refer to the actual number of organisms rather than the number of colony-forming units throughout), which uniformly caused a ~2 cm abscess that opened and drained within 72 h. Since there is poor circulation in the subcutaneous space, we needed to deliver the ABD particles to the same site as the bacteria. For this, we injected the bacteria via a cannula into the subcutaneous tissue space, followed immediately by the ABD particles. With an ABD particle:bacteria ratio of 10, abscess formation was completely prevented by ABD2003 and was slightly diminished by ABD2002, which contains CRISPR–Cas9 with a nontargeting spacer (we have observed that incoming SaPI or ABD particles cause a slight inhibition of growth; data not shown) and was unaffected by PBS alone (**Fig. 3a**,**b**). Essentially the same result was obtained with an ABD particle:bacteria ratio of 3, while a ratio of 1 reduced the size of the abscess but did not fully block it. Although these experiments showed good efficacy, we recognize that the injection of ABD particles at precisely the same location as the infecting bacteria is a somewhat artificial test. As a more realistic test of therapeutic efficacy, we needed to demonstrate that particles could be administered at one site and reach the infecting bacteria at a second site.

As it has been reported that phage can cure a subcutaneous staphylococcus infection if administered IP[16], we performed a second test of ABD efficacy in the SC model by injecting the ABD particles IP. These results demonstrated that ABD2003 particles can reach and eradicate bacteria in one compartment, tissue or site when administered at a distant site (**Fig. 3c**,**d**).

The final test of ABD2003 was for intraperitoneal lethality. For this test, the mean lethal dose of bacteria was ~5×10^9, and we used ABD2003 at three different ABD particle:cell ratios: 40, 4 and 0.4, with 5 groups of 15 mice each (**Fig. 3e**,**f**). Particles were injected at a nearby site immediately following the bacteria. In this experiment, all 15 untreated mice died within 12 h, as did all of 15 treated with CRISPR–Cas9 with a nontargeting spacer (ABD2002). For mice treated with graded doses of ABD2003 particles, 14 of 15 survived at ABD particle:cell ratio 40, 8 of 15 at ratio 4, and 2 of 15 at ratio 0.4.

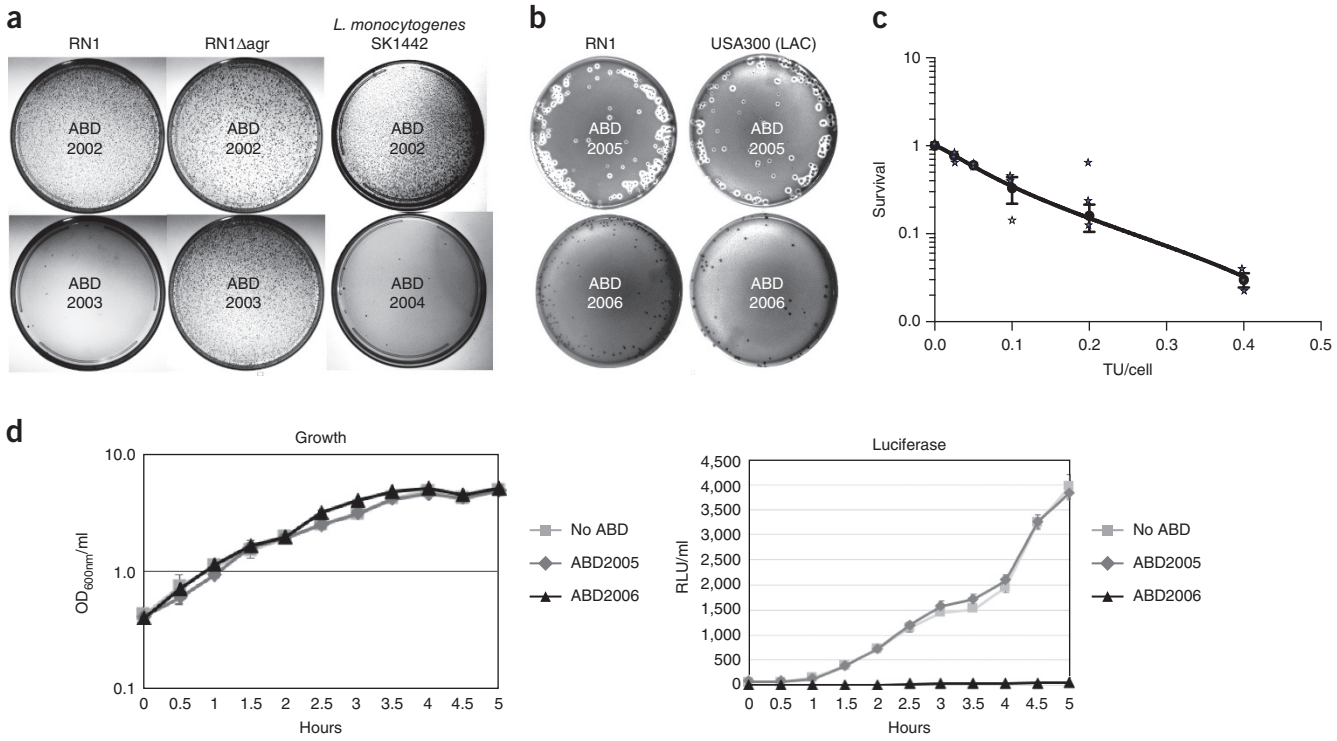

Figure 2 ABD activities *in vitro*. (**a**,**b**) Plating tests to demonstrate ABD antibacterial activities *in vitro*. (**a**) Killing of *S. aureus* by ABD2003 and of *L. monocytogenes* by ABD2004. Suitable dilutions of ABD2002, ABD2003 or ABD2004 particle preparations were mixed with RN1, RN1Δ*agr* or *L. monocytogenes* SK1442, plated on Tc5, and incubated at 37 °C for 48 h. (**b**) Inhibition of hemolytic activity by ABD2006. Suitable dilutions of ABD2005 or ABD2006 particle preparations were mixed with RN1 or USA300 LAC, plated on sheep blood agar plates supplemented with Tc5, and incubated at 37 °C for 48 h. These experiments have each been done more than 5 times with similar results. (**c**) Dose–response curve for ABD killing. Data from **Supplementary Table 1** are presented as a semi-log plot of surviving cells vs. ABD2003 particle dose; TU, transfer units. Results are averages of the 3 experiments presented in **Supplementary Table 1**. Error bars represent s.e.m., where $n = 3$ experiments. (**d**) Inhibition of *agr* expression by ABD2006. Strains with integrated ABD2001, ABD2005 and ABD2006 and a control strain without any ABD were incubated with shaking at 37 °C. Two 100-µl samples were withdrawn at each time point and assayed for growth (optical density (OD) at 600 nM) and relative luciferase units (RLU) in a Molecular Devices luminometer. Graphs show averages of experiments performed in triplicate with error bars representing s.e.m., where $n = 3$ experiments.

As it was important to determine whether the system would be widely applicable, we tested ABDs and phages on a series of strains in our collection. Here 100 µl of equivalent dilutions providing about 10^5 transfer units of ABD2002 (which carries *CRISPR–Cas9* with a nontargeting spacer) and ABD2003 (which carries *CRISPR–Cas9* with an *agrA*-targeting spacer) were mixed with about 10^8 cells of each of the 14 strains listed in **Table 1** and plated on TSB-Tc5 plates. ABD2002 was transduced at very high frequency (~10^4 colonies/plate) to 11 of the 14 strains and at a somewhat lower frequency for 1, and for these 12 strains, there were extremely few (usually <10) ABD2003 tetracycline-resistant transductants, which is the usual result for killing by ABD2003 (**Table 1**). The few survivors are probably CRISPR-resistant mutants. The two strains (4850 and 17855) that were transduced at very low frequency by ABD2002 were essentially insensitive to the ABDs. The occurrence of such strains is obviously problematic for a therapeutic agent. Staphylococcal phages (and, therefore, ABDs) adsorb nonspecifically to wall teichoic acid (WTA), resulting in very broad host specificity; however, there is a minor WTA variant that results in resistance to most phages[17], which may be responsible for the observed resistance.

Sensitivity to helper phage 80α and lytic phage K[18] also varied among these strains, with 12 of the 14 being totally resistant to one or the other or both (**Table 1**). Certain strains (such as RN9130, RN408, RN4282, RN5006, RN5007 and RN5934) that were resistant to the two phages were sensitive to transduction and killing by the ABD. This indicates that ABDs will have considerably broader host ranges than individual phages. Aside from WTA variation, most differences in staphylococcal phage sensitivity are well known to be due mostly to postadsorption intracellular blockage[19], which can affect any step in the complex phage life cycle. These blockage mechanisms would not affect ABD activity, since the expression of only one ABD gene is sufficient, and presumably would not affect other types of ABD-mediated cell killing, such as that mediated by bacteriocins or lytic enzymes, nor would they affect ABD-mediated cell inhibition, inhibition of biofilm formation, or virulence inhibition, because all of these effects depend solely on the expression of the ABD cargo.

A second goal was to develop ABD particles that could block bacterial functionality without directly killing the organism. These would be expected to diminish host-mediated resistance, to avoid the massive release of toxic bacterial contents, to block virulence, or to interfere with biofilms. Here, we used a *CRISPR–dCas9*- and *agr*P_2P_3-containing ABD, ABD2006—designed to block staphylococcal virulence by inhibiting *agr* expression—in comparison with ABD2005, which contains *CRISPR–dCas9* with a nontargeting spacer. These tests used hemolytic activity, which is upregulated by *agr*, as a test for *agr* function. **Figure 2b** shows sheep blood agar plates with colonies of RN1 and USA300 containing either ABD2005 or ABD2006, both integrated at the SaPI2 *att* site. The ABD2005 colonies of either strain showed strong hemolytic activity whereas those containing ABD2006 showed none, indicating that ABD2006 blocks *agr* activity. We also tested for

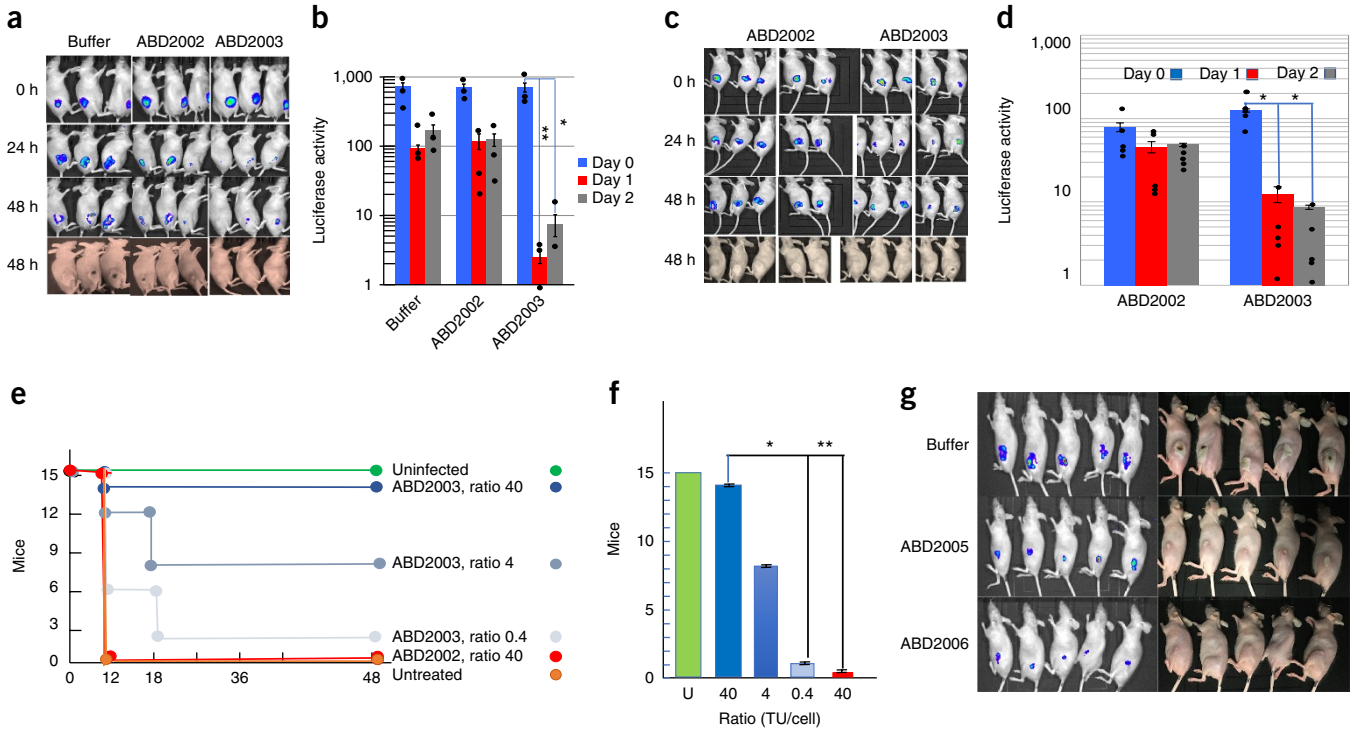

Figure 3 ABD blockade or cure of murine infections. (**a**,**b**) Blockade of SC murine infections by ABDs. Bacteria, 4×10^8, were injected into the SC space of hairless mice through a cannula, followed by 1.2×10^9 ABD2002 or ABD2003 particles through the same cannula. Bacteria were imaged in the *in vivo* imaging system (IVIS) immediately and after 24 and 48 h. (**a**) The IVIS images for luciferase activity (top three rows) and photographs of the abscesses in the same mice (bottom row). (**b**) A quantitative analysis of the luciferase signals. Error bars represent s.e.m., $n = 3$ animals. $^{**}P = 0.01$ and $^{*}P = 0.05$, determined by the one-tailed Fisher's exact test. Luciferase signals were quantified using Living Image software (Perkin-Elmer, Inc.). Experiment done once. (**c**,**d**) Treatment of an SC murine abscess by IP administration of ABD particles. Mice were injected SC with 4×10^8 organisms, strain RN1 containing an *agr*P_3::*lux* fusion, followed immediately by 8.8×10^9 ABD particles IP. IP treatment with ABD particles at the same dose was repeated 1 h later. Mice were imaged in the IVIS immediately after the first ABD injection, and again after 24 and 48 h. (**c**) IVIS images and abscess photographs. (**d**) Quantitative analysis of luciferase signals. Each bar represents the average of the luciferase signals for the five mice in each group at the three time points shown. Error bars represent s.e.m., $n = 5$ animals. Significance was evaluated by the one-tailed Fisher's exact test; $^{*}P = 0.05$. Abscesses at 48 h are the prominent white areas in the mouse flanks, present in all five of the ABD2002 mice but in only one of the ABD2003 mice. Difference in abscess formation is significant at the 5% level ($P = 0.0476$), one-tailed Fisher's exact test, $n = 5$ animals. Experiment done once. (**e**,**f**) Rescue by ABD2003 of mice given a lethal IP dose of staphylococci. Groups of 5 mice were infected IP with 5×10^9 RN1 cells. This was followed immediately by IP injection of different numbers of ABD2003 particles. Mice were monitored for 48 h. (**e**) Time course of mouse deaths. (**f**) A graphical representation of the final results, in which the groups of mice were pooled and statistics calculated on the pooled groups; U, uninfected; TU, transfer units. Error bars represent s.d. for each set of 3 pools; $^{*}P = 0.01$; $^{**}P = 0.004$, calculated by the one-tailed Fisher's exact test, $n = 5$. (**g**) Blockage of abscess formation by an *agr*-inhibiting ABD. Hairless mice were infected with 4×10^8 RN1 cells through a Teflon cannula, followed immediately by 4×10^9 ABD particles, either ABD2005 (nontargeting) or ABD2006 (targeting the *agr*P_2P_3 promoter region), as indicated. Mice were imaged in the IVIS immediately and after 18 and 42 h. The 42-h images are shown. Fisher's exact test, on the basis of visualization of abscesses, gave a $P = 0.0079$ for ABD2006 vs. either ABD2005 or untreated; $n = 5$ animals. Experiment done once.

inhibition of *agr* during growth, using these two RN1 derivatives plus two others: an ABD-negative one and one carrying ABD2001, each containing an *agr*P_3-*lux* (luciferase) reporter. ABD2006 completely inhibited *agr* expression, as shown by the absence of expression of the *agr*P_3-*lux* reporter during growth, whereas strains carrying no ABD, carrying ABD2001 or carrying ABD2005 showed strong and equivalent luciferase induction (**Fig. 2c**). All four strains grew indistinguishably. These results indicate that ABD2006 completely inhibited *agr* expression, whereas ABD2001 and ABD2005 had no effect.

We have previously shown that inhibition of *agr* expression by a heterologous AIP can attenuate (but not totally block) a subcutaneous abscess in hairless mice[20]. Accordingly, we tested the effects of ABD2006 (CRISPR–dCas9 *agr*P_2P_3) on subcutaneous abscess formation in the mouse. Using the cannula design as above, we found that ABD2006 at an ABD:bacteria ratio of 3 completely blocked abscess formation, whereas ABD2005 did not (**Fig. 3g**), though the abscesses were smaller than those seen in untreated animals. This effect remains unexplained. Although we did not do a contemporaneous comparison of AIP vs. ABD in this model, we suggest that blocking *agr* expression by the ABD may be more effective than blocking it by the AIP. Note that in the treated mice, which did not develop any abscess, bacteria were still seen at the injection site, as revealed by a persistent and weak luciferase signal, consistent with inhibition of virulence but not loss of viability. The persistent signal is assumed to represent weak residual *agr* expression.

These data demonstrate excellent efficacy for CRISPR-carrying ABDs in two mouse infection models. However, the ABD system is not without potential problems, including concomitant packaging of unwanted host genes, induction of resident SaPIs, recombination with resident SaPIs, or recombination with plasmids carrying virulence or resistance genes. Other possible problems include the occurrence of

Table 1 ABD and phage host ranges

S. aureus strains	Genotype	SaPI	*agr* group	Transduction frequency[a]		Phage sensitivity	
				ABD2002 (control)	ABD2003 (killer)	80α	Phage K
RN1	NCTC8325 wt	SaPIΔ6	I	H	F	S	R
RN450	RN1 Δ all 3 phages[b]	SaPIΔ6	I	H	F	S	R
RN12134	RN1 Δ*agr*	SaPIΔ6	I	H	H	S	R
Newman	wt	SaPIΔ6	I	I	F	S	S
Newman7B4	Newman Δ all 4 phages[b]	SaPIΔ6	I	H	F	S	S
RN4282	wt	SaPI1	I	H	F	R	S
N315	wt	SaPI2	II	H	F	R	S
RN4850	wt		IV	L	F	R	S
RN9130	502A ΔpT502A		II	H	F	R	R
17855	wt			L	<1[c]	R	R
RN408	PS29 wt			H	F	R	R
RN5006	wt			H	F	R	R
RN5007	wt			H	F	R	R
RN5934	wt			H	F	R	R

[a]H, high frequency; I, intermediate frequency; L, low frequency; F, a very few colonies, probably CRISPR-resistant mutants; wt, wild type. [b]Strain NCTC8325 has three prophages and strain Newman has four. Δ indicates deletion of all three or all four, respectively. [c]<1 indicates that there were no transductants.

resistance to the ABDs, and stable establishment of only a fraction of incoming ABDs carrying nonlethal cargo.

Resistance to ABDs can be caused by entry exclusion or postentry interference involving either DNA degradation or gene expression. Most *S. aureus* phages adsorb nonspecifically to the common WTA. A minority of strains (ST395) have a variant WTA that is not recognized by typical phages. To enable ABDs to target ST395 strains, we would use a helper phage with an ST395 receptor[17]. Resistance due to DNA degradation could be circumvented by modifying problematic restriction site(s) or using different SaPI backbones.

CRISPR resistance can affect either the CRISPR itself—for example, loss of spacer—or the host cell; for example, unlinked host gene(s) or loss of protospacer. In a sample of 15 mutants, we found both types: 5 had retained both the spacer and the protospacer and were therefore due to mutations in unlinked host gene(s) and the other 10 had simply lost the spacer. Resistance would be circumvented by including in the ABD a non-CRISPR module with a similar mode of action. Therefore, at least two different antibacterial modules would be included in any ABD destined for clinical use.

A different problem arises for ABDs that must be functional for an extended period of time. These would have to be equipped with a replicon or with an efficient integration mechanism, and would be subject to resistance based on interference with replication or integration, in addition to the above.

Nevertheless, the ABD system appears to have great potential as a therapeutic modality and to have decided advantages over existing antibacterial strategies. The addition of extra antibacterial modules should circumvent the resistance problem, and because of their specificity, the ABDs will have no impact on the microbiome. Though formally akin to phage therapy, they are predicted to have major advantages over phage therapy: (i) Efficiency: a therapeutic phage generally functions by killing its bacterial target—which means using the entire metabolic machinery of the bacterial cell. An entering ABD molecule needs only to express its antibacterial gene(s). Bacteria have developed a wide variety of phage resistance mechanisms, affecting virtually every step in the phage reproductive cycle. Most of these mechanisms would not affect an ABD. (ii) Versatility: phage genomes are not greatly expandable—for example, coliphage λ with more than about 3 kb of added DNA[21] becomes defective[22]—whereas ABD genomes can accommodate >30 kb of added DNA, over twice their genome size, without functional detriment. This, for example, would enable the insertion of additional killing modules to cover for CRISPR resistance. (iii) Broader host range than any individual phage. (iv) Nonkilling alternatives: spacers that block cell growth, cell division, virulence gene expression, biofilm formation, etc. can readily be added to *dCas9*, and genes with these properties can be incorporated independently of CRISPRs. (v) *Trans* activity: genetic systems can be incorporated that encode secretable inhibitors, such as quorum sensing inhibitors, lysins, toxins, etc., which can act at an infection site on bacteria that have escaped infection by the ABD.

We also suggest that the ABD system is by far the most effective delivery system for CRISPR and other explicit antibacterial functions. It is substantially more effective than the phagemids described by Bikard *et al.*[23] and Citorik *et al.*[24] which are based on rolling circle and other plasmids and phages, some of which are notoriously unstable and none of which can be transferred at high enough frequency to cure an infection. The results described here point the way to further development of the system, using other types of antibacterial modules directed against bacterial viability, growth and biofilm formation and directed against Gram-negative as well as other Gram-positive pathogens.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper

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AUTHOR CONTRIBUTIONS

R.P.N., H.F.R. and G.R. planned and discussed the experiments. H.F.R. had the initial idea; G.R. and H.F.R. did the cloning; G.R. performed the *in vitro* tests; I.R.-P. and R.P.N. did the mouse experiments; D.J. did the DNA preparations; and R.P.N. did the microbiological work and wrote the manuscript.

COMPETING INTERESTS

A patent application has been filed by New York University, with G.R., H.F.R. and R.P.N. as inventors, on the basis of results presented in this paper.

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ONLINE METHODS

Bacterial strains and culture conditions. Bacterial strains are listed in **Supplementary Table 2**. Bacteria were grown in CY[25] or LB broth with rotary shaking at 37 °C or on Trypticase soy agar (Difco) with antibiotic supplements as needed for plasmid maintenance or ABD selection. Tetracycline (Tc), erythromycin and chloramphenicol were all used at 5 μg/ml. Phage lysates were prepared with half-strength CY broth at 32 °C. Phage was added at about 10^7/ml to exponential bacterial cultures at about 10^8 cells/ml. Lysis was usually complete by about 3 h. For lysogenic strains, lysates were prepared by induction with mitomycin C (2 μg/ml), starting at about 10^9 cells/ml, with gentle shaking at 32 °C. Lysates were filter sterilized (0.22 μm). For preparation of ABD particles, mitomycin C was added to 500-ml exponential cultures in CY broth at 10^9 cells/ml. Cultures were incubated with slow shaking at 32 °C until lysis (2.5–3 h) and centrifuged at 10,000*g* in a Sorvall SLA-3000 rotor. Supernatants were treated with DNase I and RNase I, both at 25 μg/ml for 3 h at 37 °C, then filter sterilized; PEG800 (10%) and NaCl (0.5 M) were added and the suspension was held at 4 °C overnight, centrifuged at 9,500*g* in the Sorvall SLA-3000 rotor, and then gently resuspended in 10 ml phage buffer (10 mM Tris, pH 7.5, 10 mM $MgCl_2$, 68 mM NaCl, 10 mM $CaCl_2$, 0.1% gelatin) and stored at 4 °C. Transduction titers were determined by diluting and plating with an *agr*-null mutant of NCTC8325 on TSB-Tc5 plates.

Enumeration of ABD particles. Although transduction frequencies are classically determined by selective plating, it has long been known that selective plating underestimates the actual number of transduced particles by tenfold or more[26–28], and this is also the case for the ABDs (and, by implication, for the SaPIs). **Supplementary Table 1** shows the results of an experiment in which samples of 10^7–10^8 cells that had been sonicated to disrupt clumps were incubated for 20 min at room temperature in phage buffer with different numbers of particles, then diluted and plated for survivors. Adsorption was highly efficient, as filtered samples had $<10^3$ ABD transducing units/ml. **Supplementary Table 1** shows the results of survival of *agrA*-positive cells as a function of ABD multiplicity. As can be seen, the survival frequencies were much lower than expected for the listed transducing particle multiplicities, suggesting that the titer of transfer units (TU), used to determine the multiplicities, seriously underestimates the number of infective particles (IP). From the table, the KU:TU ratio is about 11. The basis of this difference is not known and is under investigation.

As also shown in **Supplementary Table 1**, ABD-resistant mutants, represented by Tc-resistant transductants in platings of the ABD2003-RN1 mixtures, occurred at a frequency of ~10^{-4} per infected cell independently of multiplicity.

ABD construction. Plasmids are listed in **Supplementary Table 3** and the sequences for ABD2001-ABD2006 can be found in **Supplementary Sequences**.

The *CRISPR–Cas9* segment was PCR-amplified from plasmid pDB114 (kind gift from Luciano Marraffini (Rockefeller University, New York) using primers CCEF and CCER and the 5-kb PCR product cloned into HincII-cut pUC18, resulting in plasmid pUC18-CRISPR-Cas9.

To insert *CRISPR–Cas9* into the SaPI2 genome, we amplified upstream and downstream sequences flanking the toxin gene region. The upstream flanking sequence was PCR-amplified using primers SCUF and SCUR. The resulting 1-kb PCR fragment was cloned into HincII-cut pUC18, resulting in pUC18-SaPI2-Up. The downstream flanking sequence was PCR-amplified using primers SCDF and SCDR. The resulting 1-kb PCR fragment was cloned into HincII-cut pUC18, resulting in pUC18-SaPI2-Dn.

Plasmid pUC18-SaPI2-Up was digested with BglII and PstI and the 1-kb SaPI2-Up fragment was gel-purified. Plasmid pUC18-CRISPR-Cas9 was digested with PstI and XhoI and the 5 kb *CRISPR–Cas9* fragment was gel-purified. Plasmid pUC18-SaPI2-Dn was digested with XhoI and XmaI and the1 kb SaPI2-Dn fragment was gel-purified. The three fragments (SaPI2-Up, CRISPR–Cas9 and SaPI2-Dn) were ligated using T4 DNA ligase. The ligated product was PCR-amplified using primers SCUF and SCDR. This PCR product was ligated to EcoRV-digested pET28b, resulting in plasmid pET28b-S2Up:CRISPR/Cas9:S2Dn. This plasmid was used for spacer incorporation. It was digested with BsaI and an annealed spacer (30 bp) was ligated to it (the spacer cloning protocol was adapted from the Marraffini spacer cloning protocol, submitted to Addgene (https://www.addgene.org/crispr/reference/). This resulted in pET28b S2Up:CRISPR/Cas9:Spacer:S2Dn. This plasmid was digested with BglII and XmaI and the 7-kb insert (S2Up:CRISPR/Cas9:Spacer:S2Dn) was gel-purified and ligated to BamHI- and XmaI-digested pMAD. This resulted in pMAD-S2Up:CRISPR/Cas9:Spacer:S2Dn, which was electroporated into RN4220 Δ*agr*::*cad*A (80α lysogen) carrying ABD2001. Allelic replacement was then performed using a standard protocol[22]. Candidate colonies were screened for allelic exchange using PCR amplification and confirmed by sequencing. This procedure was performed with three different spacers: the first one lacking any target sequence, the second targeting the *S. aureus agrA* gene and the third targeting the *L. monocytogenes hly* gene. The resulting ABDs were designated ABD2002, ABD2003 and ABD2004, respectively. Lysates were prepared from the strains carrying ABD2002, ABD2003 and ABD2004, respectively; filtered; and used to transduce the ABDs into an ABD particle-producing strain (RN450 Δ*agr*::*cadA*, Φ80α Δ*terS*).

Construction of CRISPR/dCas9-Spacer containing ABD genome. Primers and spacers are listed in **Supplementary Table 4**.

Plasmid pdCas9 was purchased from Addgene (plasmid no. 46569). pdCas9 expresses the tracrRNA, the dCas9 catalytic site mutant and a CRISPR array designed for the easy cloning of new spacers. The CRISPR–dCas9 backbone was PCR amplified from plasmid pdCas9 using primers CdCEF and CdCER. This PCR-product (5 kb) was cloned into HincII-cut pUC18, resulting in pUC18-CRISPR/dCas9.

Plasmid pUC18-SaPI2-Up was digested with BglII and PstI and the 1-kb SaPI2-Up fragment was gel-purified. Plasmid pUC18-CRISPR/dCas9 was digested with PstI and XhoI and the 5-kb CRISPR–dCas9 fragment was gel-purified. Plasmid pUC18-SaPI2-Dn was digested with XhoI and XmaI and the 1-kb SaPI2-Dn fragment was gel-purified. All three eluted fragments (SaPI2-Up, CRISPR–dCas9 and SaPI2-Dn) were ligated using T4 DNA ligase. The ligated product was PCR amplified using primers SCUF and SCDR. This PCR product was ligated to HincII-digested plasmid pACYC184, resulting in pACYC184-S2Up:CRISPR/dCas9:S2Dn.

Plasmid pACYC184-S2Up:CRISPR/dCas9:S2Dn was used for spacer incorporation. The plasmid was digested with BsaI and an annealed spacer (30 bp) was ligated to it, resulting in pACYC184-S2Up:CRISPR/dCas9:Spacer:S2Dn. This plasmid was digested with BglII and XmaI, and the 7-kb fragment (S2Up:CRISPR/dCas9:Spacer:S2Dn) was gel-purified and ligated to BamHI- and XmaI-digested pMAD plasmid. This resulted in pMAD-S2Up:CRISPR/dCas9:spacer:S2Dn.

This plasmid was electroporated into RN4220 Δ*agr*::*cad*A (Φ80α) carrying ABD2001. Allelic replacement was done as above. Candidate colonies were screened for allelic exchange using PCR-amplification and confirmed by sequencing. Two different spacers were incorporated into this basic plasmid: one with no target sequence, the other with an $agrP_2P_3$ target. These ABD2001 derivatives were designated ABD2005 and ABD2006, respectively. Both were transduced by phage 80α into an ABD particle-producing strain (RN450 Δ*agr*::*cadA*, Φ80α Δ*terS*).

Protocol for new spacer cloning in pET28b-S2Up:CRISPR/Cas9:S2Dn or pACYC184-S2Up:CRISPR/dCas9:S2Dn in *E. coli*. To clone a spacer sequence into pET28b-S2Up:CRISPR/Cas9:S2Dn or pACYC184-S2Up:CRISPR/dCas9:S2Dn, two complementary 30-nt oligonucleotides matching the target sequence (synthesized by Integrated DNA Technologies) were phosphorylated and annealed. The target sequence (protospacer) is 30 nt and is followed by the protospacer-adjacent motif (PAM), NGG.

Phosphorylation (examples for agrA*).* 1 μl SpAF (100 μM), 1 μl SpAR (100 μM), 5 μl 10× T4 ligase buffer (NEB), 1 μl T4 PNK (NEB), 42 μl ddH_2O, total 50 μl.

Annealing. 2.5 μl of 1 M NaCl was added to the phosphorylated oligonucleotide pairs. The mixture was incubated 5 min at 95 and slowly cooled to room temperature, using a thermocycler. Annealed oligonucleotides were diluted tenfold for use.

Ligation. 5 μl BsaI-digested pET28b-S2Up:CRISPR/Cas9:S2Dn or pACYC184-S2Up:CRISPR/dCas9:S2Dn (>50 ng/μl), 1 μl diluted annealed oligonucleotides, 2 μl 10× T4 ligase buffer (NEB), 1 μl T4 ligase, 11 μl ddH2O, total 20 μl, incubated at room temperature for 2 h.

Animal methods. Female C57BL6 mice 6–8 weeks old were used for intraperitoneal tests. B6.Cg-*Tyr*$^{c\text{-}2J}$*Hr*^{hr}/J hairless mice were used for subcutaneous abscess tests. Mouse usage was approved by the NYULMC IACUC. Mice were anesthetized by isoflurane or by ketamine-xylazine before injections. Mice were euthanized by CO_2 narcosis.

***In vivo* testing of ABDs.** *Subcutaneous abscess model*[14]. For treatment via cannula, hairless mice (females 6–8 weeks old) are anesthetized with ketamine/xylazine, an opening in the skin is made with an 18-gauge needle and a 3-cm length of 27 gauge Teflon tubing is inserted to a depth of ~0.5 cm. Bacteria, NCTC8325 containing pRN7141 (carrying an *agr*P$_3$-*lux* fusion), 4×10^8 cells in 50 µl phosphate-buffered saline (PBS), are injected with a 28-G needle through the cannula, followed immediately by 1.2×10^9 ABD particles, also in 50 µl PBS. Mice are imaged in an IVIS Illumina XMRS camera (Perkin-Elmer), immediately and after 4, 24, 48 and 72 h. For imaging, they are anesthetized with isoflurane. For treatment by intraperitoneal injection, hairless mice (females 6–8 weeks old) are anesthetized with isoflurane, and bacteria, NCTC8325 containing pRN7141, 4×10^8 cells in 50 µl PBS, are injected with a 28-G needle directly into the subcutaneous space, followed immediately by the injection of ABD particles, 2.5×10^{11}, in 250 µl PBS intraperitoneally. Imaging is as above.

Peritoneal lethality model. Bacteria, 4×10^9, in 100 µl PBS, are injected into the peritoneal space of 6- to 8-week-old female C57BL6 mice, followed immediately by 2.5×10^{11} ABD particles in 200 µl PBS at a nearby site. Mice are weighed daily and monitored for health status. Living Image software was used to measure the bioluminescent signals emitted by the infecting bacteria and detected with the IVIS.

Statistical methods. Analysis of mouse infection data. (i) Subcutaneous abscess induced and treated with ABD2003 via cannula (**Fig. 3a,b**). 3 groups of 3 mice each, single experiment. Luciferase signals measured using Living Image software were averaged over the 3 mice in each group, for each day, and the results analyzed by the one-tailed Fisher's exact test. The analysis is presented in the figure legend. (ii) Subcutaneous abscess treated by intraperitoneal injection of ABD particles, two groups of 5 mice each, single experiment (**Fig. 3c,d**). Luciferase signals averaged over the 5 mice in each group, for each day, and the results analyzed by the one-tailed Fisher's exact test. The analysis is presented in the figure legend. (iii) IP lethality prevented by IP injection of ABD particles at different doses. Five mice in each group, done in triplicate. Groups of mice were scored for death by 48 h and the results for the triplicate groups combined and analyzed by Fisher's one-tailed exact test, as presented in the legend to **Figure 3e,f**. (iv) Subcutaneous abscess induced and treated with ABD2006 (CRISPR–dCas9/agrP$_2$P$_3$) via cannula (**Fig. 3g**). Three groups of 5 mice each, single experiment. The IVIS crashed and luciferase signals could not be evaluated. The one-tailed Fisher's exact test, performed on the basis of visualization of the abscesses, gave a *P* value of 0.0079 for ABD2006 vs. ABD2005 and the same for ABD2006 vs. no treatment.

Life Sciences Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. All data used in this report are present in the body of the report or in the Online Methods section. Strains and sequences are available upon request.

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