Antibodies to Block Staph Virulence

Michael Otto[1,*]
[1]Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, The National Institutes of Health, 903 South 4th Street, Hamilton, MT 59840, USA
*Correspondence: motto@niaid.nih.gov
DOI 10.1016/j.chembiol.2007.10.001

Antibodies rationally designed against staphylococcal virulence signals inhibit the development of experimental disease by passive immunization [1], indicating great potential for therapeutic approaches against staphylococcal and other bacterial infections.

Infections with *Staphylococcus aureus* are on a dangerous rise. While these bacteria have threatened hospitalized patients and individuals with predisposed risk factors for a long time, and more than any other bacterial pathogen, we are now facing hypervirulent strains of Staph that are attacking healthy people outside health care settings [2]. Similar to the majority of hospital-associated strains, these strains carry methicillin resistance (methicillin-resitant *S. aureus* or "MRSA"), which makes treatment with methicillin-type antibiotics impossible. Already, the majority of all skin and soft tissue infections reporting to the emergency departments of U.S. hospitals are due to this so-called community-associated MRSA (CA-MRSA) [3] and there is a great danger that the acquisition of even more resistance factors by CA-MRSA strains will result in a public health catastrophe.

Therefore, there is an urgent need for the development of novel anti-staphylococcal therapeutics. Due to the multiple antibiotic resistance found in Staph and many other bacteria, some researchers have proposed targeting bacterial virulence instead of essential genes, thus only attacking the expression of harmful molecules while leaving the bacteria alive [4]. This approach is believed to minimize the development of resistance. However, both drug and vaccine development for staphylococcal infections is extremely difficult owing to a pronounced functional redundancy of virulence determinants. Thus, finding a way to eliminate several virulence determinants at the same time has become a major goal in these endeavors. For example, inhibition of the enzyme that catalyzes the anchoring of surface enzymes to the staphylococcal cell wall—proteins of extreme importance for the establishment of an infection—would eliminate a series of virulence determinants of similar function [5].

Specifically, global regulators of virulence have been in the focus of virulence-targeted approaches to find novel drugs, not only in staphylococci, but in many bacterial pathogens. The best studied and probably most important virulence regulator in staphylococci is the accessory gene regulator *agr*, which has been investigated in detail in the laboratory of Richard Novick at New York University. This system is responsible for adapting bacterial physiology and virulence factor expression to changing environmental conditions, dependent on the density of the bacterial population [6]. Except for a certain eclipse phase, the activity of *agr* increases with the size of the bacterial population during infection, which has been shown using in vivo expression studies [7]. Notably, Dr. Novick and coworkers, together with other groups including ours, have found that *agr* signals through an exported autoinducing peptide (AIP), whose overall structure is conserved, but whose amino acid sequence differs between subgroups [8, 9]. Importantly, AIPs from different origins have the common feature of inhibiting *agr* expression in nonrelated groups, thereby preventing expression of a series of important virulence factors [8, 10]. In fact, it has been shown that the administration of an inhibiting AIP together with an infectious strain leads to a significant reduction in *S. aureus* infectivity in a mouse subcutaneous infection model [8].

However, a major drawback of the crossinhibiting AIP-based strategy for the prevention of staphylococcal diseases is the relative instability of the unusual thiolactone ring structure of staphylococcal AIPs. Therefore, systemic application of crossinhibiting AIPs has so far not been successful and the development of quorum-sensing inhibitors for therapeutic use has not been pursued with the same intensity as in Gram-negative bacteria (which use chemically different signals such as the homoserine lactones) [11]. Therapeutic use of AIPs would only be possible if one were to find a molecule that combines *agr* inhibiting features with significantly improved stability.

Now, as an elegant alternative to overcome the AIP instability problem, Park et al. [1] report on the use of anti-AIP antibodies to inhibit *agr* function (Figure 1). In this issue of *Chemistry & Biology*, they show that antibodies rationally designed against the AIP of one *S. aureus agr* subgroup specifically prevent *agr* expression and *S. aureus* disease in an animal model of abscess formation. Notably, the antibodies also provided protection when used for passive immunization, i.e., when given before infection. These findings take the research on anti-*agr* drugs to a new level and increase our hope that we may one day successfully target this key staphylococcal virulence regulator to control infection. While the general applicability of the presented approach will need to be shown for all *S. aureus agr* subtypes, especially for type 1 (the most prevalent among MRSA), it appears that the anti-AIP antibodies have very good chances to be of general use. Likely, a broader spectrum *S. aureus*

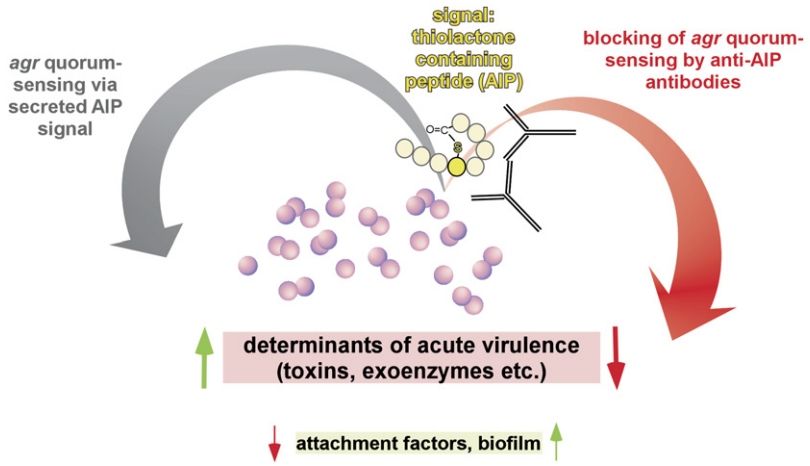

Figure 1. Inhibition of Quorum-Sensing Controlled Virulence Determinants by Antibodies against the *Staphylococcus aureus agr* Signal

The *agr* quorum-sensing regulator controls the expression of virulence determinants in a cell density-dependent fashion via secretion of an extracellular signaling peptide (autoinducing peptide, AIP). The AIP has a length of 7–9 amino acids, in which a conserved cysteine is linked to the C terminus in a thiolactone, or rarely, lactone linkage. Toxins and other determinants of acute virulence are under positive control by *agr*. Therefore, antibodies against the AIP block the development of experimental acute disease, as shown in the report by Park et al. [1]. As *agr* controls characteristic determinants of attachment and biofilm formation in an opposite fashion, the use of such antibodies for the treatment of staphylococcal infections may, however, be limited to specific types of infection.

therapeutic based on anti-AIP antibodies may easily be produced against a mixture of AIPs of all four *S. aureus agr* subgroups. Additionally, as the sequences from a variety of staphylococcal *agr*-type AIPs are known and there are reports on *agr*-type systems in other Gram-positive bacteria, the same strategy may be used for other staphylococcal and even nonstaphylococcal pathogens. Finally, to assess the chances of general applicability for bacteria that use the *agr* quorum-sensing system, it will be of central interest to investigate whether the conserved (thio)lactone structure plays a key role in antigen-antibody recognition, or whether this interaction is mainly determined by the primary amino acid sequence of the AIP hapten.

However, there are some limitations and questions that first need to be addressed before an anti-AIP antibody will be suitable for the treatment of disease. Most importantly, inhibiting the *agr* regulator in staphylococci not only leads to the downregulation, but also to the upregulation of several virulence determinants that are under opposite regulation by *agr*. For example, the already mentioned surface proteins that are involved in the establishment of an infection are for the most part downregulated by *agr* [6]. Thus, inhibition of *agr* would lead to their increased expression, the in vivo outcome of which is not known. Further, *agr* appears to regulate biofilm formation in a negative fashion [12]. Biofilm formation, the agglomeration of cells on a surface within a sticky matrix, significantly lowers the efficacy of antibiotics and innate host defense mechanisms. Indeed, *agr* negative mutants are isolated frequently from sometimes severe types of biofilm-associated staphylococcal infection and constructed *agr* mutants show increased colonization of indwelling medical devices in experimental biofilm-associated infection [12, 13].

We will have to find out whether these facts may possibly limit the applicability of *agr*-inhibiting drugs. However, the importance of *agr* in many staphylococcal infections has been demonstrated in vivo [7, 8]. Furthermore, strong expression of *agr* appears to be an important characteristic of the dangerous CA-MRSA strains [14]. Therefore, using *agr* as a target with an improved strategy as described by Park et al. [1], is an extremely promising novel attempt to find anti-staphylococcal therapeutics and control the increasing spread of *S. aureus* and especially the CA-MRSA epidemic.

REFERENCES

1. Park, J., Jagasia, R., Kaufmann, G.F., Mathison, J.C., Ruiz, D.I., Moss, J.A., Meijler, M.M., Ulevitch, R.J., and Janda, K.D. (2007). Chem. Biol. *14*, this issue, 1119–1127.
2. Chambers, H.F. (2005). N. Engl. J. Med. *352*, 1485–1487.
3. Moran, G.J., Krishnadasan, A., Gorwitz, R.J., Fosheim, G.E., McDougal, L.K., Carey, R.B., and Talan, D.A. (2006). N. Engl. J. Med. *355*, 666–674.
4. Alksne, L.E., and Projan, S.J. (2000). Curr. Opin. Biotechnol. *11*, 625–636.
5. Mazmanian, S.K., Liu, G., Ton-That, H., and Schneewind, O. (1999). Science *285*, 760–763.
6. Novick, R.P. (2003). Mol. Microbiol. *48*, 1429–1449.
7. Wright, J.S., 3rd, Jin, R., and Novick, R.P. (2005). Proc. Natl. Acad. Sci. USA *102*, 1691–1696.
8. Ji, G., Beavis, R., and Novick, R.P. (1997). Science *276*, 2027–2030.
9. Otto, M., Sussmuth, R., Jung, G., and Gotz, F. (1998). FEBS Lett. *424*, 89–94.
10. Otto, M., Sussmuth, R., Vuong, C., Jung, G., and Gotz, F. (1999). FEBS Lett. *450*, 257–262.
11. Otto, M. (2004). FEMS Microbiol. Lett. *241*, 135–141.
12. Vuong, C., Saenz, H.L., Gotz, F., and Otto, M. (2000). J. Infect. Dis. *182*, 1688–1693.
13. Vuong, C., Kocianova, S., Yao, Y., Carmody, A.B., and Otto, M. (2004). J. Infect. Dis. *190*, 1498–1505.
14. Wang, R., Braughton, K.R., Kretschmer, D., Bach, T.L., Queck, S.Y., Li, M., Kennedy, A.D., Dorward, D.W., Klebanoff, S.J., Peschel, A., et al. (2007). Nat. Med., in press.

