COMMENTARY

Shocking superantigens promote establishment of bacterial infection

Nikolai Siemens[a,b] and Anna Norrby-Teglund[a,1]

Streptococcus pyogenes, also referred to as group A *Streptococcus* (GAS), is an exclusive human pathogen causing diseases ranging from uncomplicated infections of the throat and skin to severe invasive infections, such as necrotizing fasciitis and streptococcal toxic shock syndrome (STSS). Asymptomatic carriage, particularly in the naso- and oropharyngeal mucosa, is common. GAS is equipped with an arsenal of virulence factors (e.g., surface-associated and secreted factors that contribute to disease pathogenesis) (1). Among the secreted virulence factors, superantigens (SAgs) have been recognized as key factors mediating the systemic excessive inflammatory response associated with STSS (2). SAgs belong to a family of proteins that activate T cells in an unconventional manner bypassing the normal rules for antigen processing and presentation. SAgs bind without prior cellular processing to the major histocompatibility complex (MHC) class II molecules on antigen-presenting cells and to the variable β-chains of the T-cell receptor on $CD4^+$ and $CD8^+$ T cells. In this way, the fine MHC-peptide specificity of T cells is bypassed, allowing for activation of numerous Vβ-specific T cells and an overzealous inflammatory response. This cytokine storm is harmful to the host and underlies conditions such as STSS (2). While the role of SAgs in STSS is well established, a lingering question has been why the bacteria secrete such powerful immunostimulatory factors. To date, 14 distinct SAgs have been identified and most clinical strains express several, thus showing a redundancy of these toxins (3). In PNAS, Zeppa et al. (4) provide evidence that SAgs promote GAS nasopharyngeal colonization and infection in a Vβ-specific T-cell–dependent manner. Passive immunization with antibodies against the SAg streptococcal pyrogenic exotoxin A (SpeA) or vaccination with a SpeA toxoid devoid of superantigenic activity provided antibody-dependent protection against nasopharyngeal infection. In addition, the authors discovered an antibody-independent protective mechanism involving SAg-triggered T-cell responses that enhanced GAS infection, thereby offering an explanation as to the bacterial benefit of a SAg-triggered T-cell response (ref. 4; Fig. 1).

The concept that SAgs are critical in the establishment of GAS infection was first proposed by Kasper et al. (5), who showed that mice expressing human MHC class II molecules (e.g., mice susceptible to the action of SAgs) were easily colonized by GAS, whereas the wild-type mice readily cleared the infection. The enhanced colonization and infection was attributed to the SAg SpeA. In PNAS, the study by Zeppa et al. (4) provides additional mechanistic insight into the role of SAg as a colonizing factor. First, the authors show that passive immunization of humanized mice with anti-SpeA serum resulted in a reduced bacterial burden in the nasopharynx. Similarly, direct vaccination with inactive SpeA toxoid lacking SAg activity resulted in a strong anti-SpeA humoral response and protection against nasopharyngeal infection. However, vaccination with either SpeA or the staphylococcal SAg staphylococcal enterotoxin B (SEB) also afforded protection against GAS infection despite the absence of anti-SpeA antibodies, hence also suggesting an antibody-independent protective mechanism. The authors then explored whether T-cell responses contributed to the protective effect, and they provide evidence of the involvement of the SpeA-targeted T-cell subset, namely, $CD3^+V\beta8^+$ lymphocytes, which were reduced and less responsive in SpeA- or SEB-vaccinated mice. Thus, the data indicate that the T-cell response elicited by the SAg contributed to the noted enhanced bacterial infection. This was further supported by the finding that depletion of either $CD8^+$ T cells alone or in combination with $CD4^+$ T cells resulted in a reduced nasopharyngeal GAS burden, although an exaggerated bacterial burden for *Streptococcus pneumoniae*, a non-SAg–producing bacteria. As an additional potential explanation, the authors link the observed effects to the inflammatory state during bacterial infection. Mice lacking $CD4^+$ T cells, $CD8^+$ T cells, or both showed reduced levels of inflammation during bacterial infection, which was linked to bacterial clearance. Similarly, SpeA- and SEB-vaccinated mice that were protected against GAS, infection showed a reduced inflammatory response (4). The authors propose that SAgs target and activate Vβ-specific T cells, resulting in an inflammatory milieu and

[a]Center for Infectious Medicine, Karolinska Institutet, Karolinska University Hospital, S-141 86 Stockholm, Sweden; and [b]Department Genetics of Microorganisms, Interfaculty Institute for Genetics and Functional Genomics, University of Greifswald, Greifswald D-17489, Germany

Author contributions: N.S. and A.N.-T. wrote the paper.

The authors declare no conflict of interest.

See companion article on page 10226.

[1]To whom correspondence should be addressed. Email: anna.norrby-teglund@ki.se.

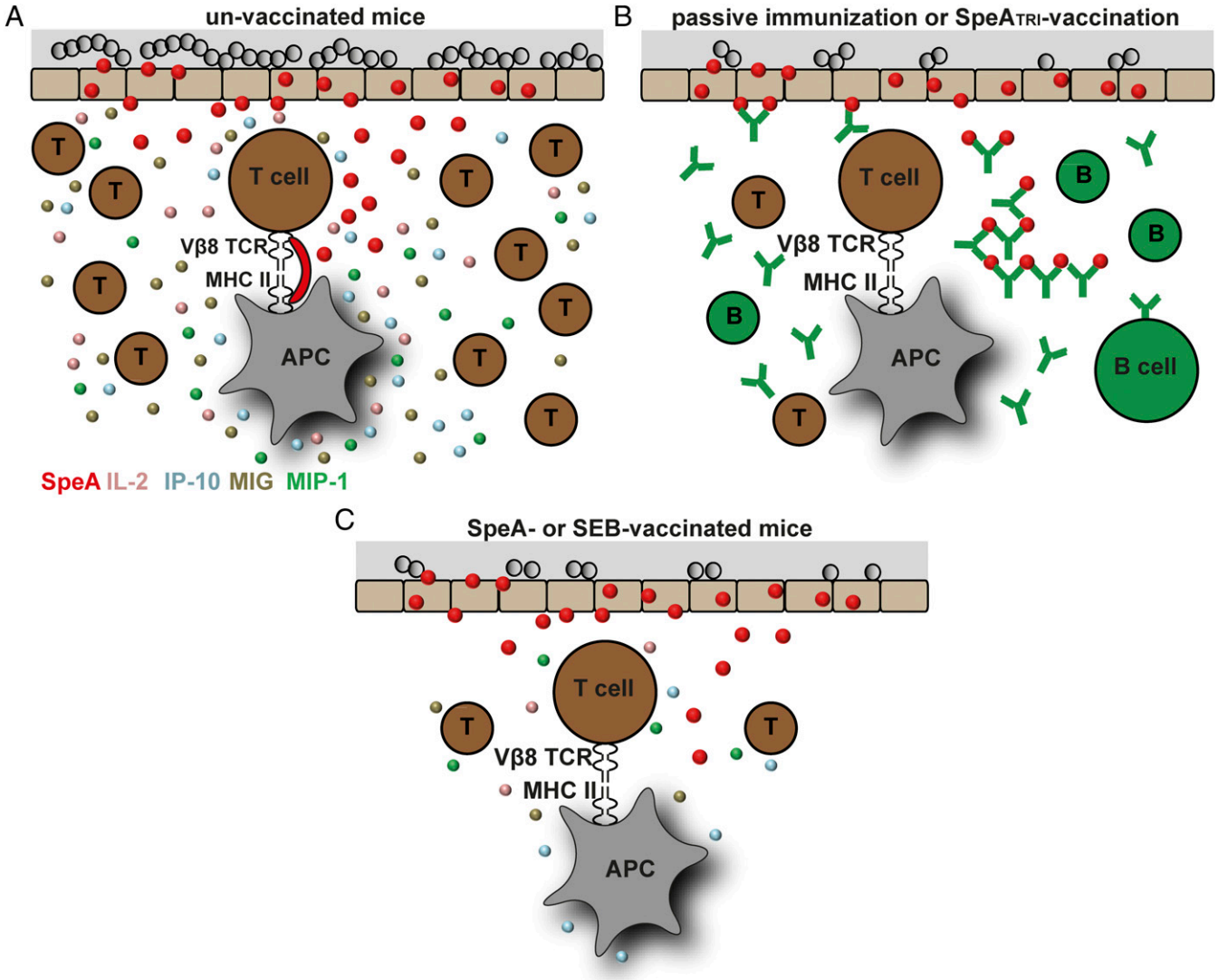

Fig. 1. Impact of SpeA vaccination on nasopharyngeal infection by GAS. (*A*) GAS uses SAgs (e.g., SpeA) to establish nasopharyngeal infection by manipulating Vβ8-specific T cells and inducing an inflammatory response. (*B*) Antibody-mediated protection from GAS infection due to passive anti-SpeA serum immunization or $SpeA_{TRI}$ vaccination [i.e., vaccination with a triple mutant containing alanine substitutions at all three positions (SpeAL41A/L42A/Y100A)]. (*C*) In the absence of SAg-driven T-cell activation, as seen in SpeA- or SEB-vaccinated mice, the inflammatory response is diminished, rendering the mice more resistant to infection. APC, antigen-presenting cell; TCR, T-cell receptor.

remodeling of the nasopharyngeal environment, which promotes bacterial colonization. The exact mechanisms involved, including if remodeling occurs and the specifics of the remodeled nasopharyngeal environment, have yet to be shown. On this note, a recent paper by Shaler et al. (6) demonstrated that SAgs can activate mucosa-associated invariant T (MAIT) cells and that MAIT cells represent a substantial source of the SAg-elicited cytokine response. Hence, the contribution of the $CD8^+$ MAIT cells might also be of interest to assess in this setting.

The work by Zeppa et al. (4) highlights the potential of targeting SAgs as vaccine candidates for GAS infections. The work shows striking protection against GAS infection by both passive and active immunization with SAgs. The benefit of blocking SAgs has been demonstrated in STSS, where i.v. polyspecific immunoglobulins (IVIGs) have been used as adjunctive therapy. IVIGs contain a broad spectrum of SAg-specific antibodies that efficiently neutralize the SAg-mediated T-cell activation and cytokine release. Clinical trials, albeit small, have reported a survival benefit of IVIG in patients with STSS (7–11). However, the data provided by Zeppa et al. (4) suggest a different approach by considering SAgs as potential vaccine candidates to halt colonization and establishment of infection. Until now, the key candidate for vaccines against GAS infections has been the classical virulence factor M-protein, and there have been several efforts made to develop M-protein–based vaccines (12). M-protein is encoded by the *emm* gene and, to date, over 200 *emm* types are reported (13). The heterogeneity of the M-proteins is defined by the hypervariable N terminus, which makes the development of a potential multivalent vaccine against GAS a challenging task. Also, there are data to suggest that the hypervariable N terminus region is only weakly immunogenic (14, 15). The work by Zeppa et al. (4) suggests consideration of streptococcal SAgs for inclusion within a multivalent vaccine. One potential advantage is that although streptococcal SAgs are immunologically distinct (5), they are still limited to only 14 types (3). In addition, the results presented by Zeppa et al. (4) suggest that there might be a cross-reactive protective effect, at

least for SAgs sharing Vβ specificity, such as the Vβ8-targeting SEB. Zeppa et al. (4) demonstrate that vaccination with SEB rendered the mouse splenocytes poorly responsive to any of the Vβ8-targeting SAgs, which correlated with protection from nasopharyngeal infection by GAS. An efficient vaccine providing protection against the many different serotypes of GAS would be a significant achievement in the field, not the least considering poststreptococcal sequelae, such as rheumatic fever and rheumatic heart disease, which represent a major health burden in developing low-income countries. However, several questions remain, such as whether this observation is limited to a nasopharyngeal GAS infection or whether the knowledge generated in the study by Zeppa et al. (4) can be extended to other types/sites of GAS infections as well as to other SAg-producing strains, such as the prominent human colonizer and pathogen *Staphyloccocus aureus*. Overall, the study highlights a new role for SAgs as critical colonizing factors, thereby providing a plausible explanation for the redundancy of these toxins in GAS strains. The study also underscores the importance of using experimental models with SAg-susceptible cells (i.e., cells expressing human MHC class II and SAg-reactive Vβ-specific T cells) in studies of GAS colonization and establishment of infection.

Acknowledgments

This research is supported by European Union FP7 Grant 305340 (to A.N.-T.), Swedish Research Council Grant 2014-6722 (to A.N.-T.), and Swedish Society of Medicine Grants SLS-501071 (to N.S.) and SLS-586271 (to N.S.).

1 Walker MJ, et al. (2014) Disease manifestations and pathogenic mechanisms of Group A Streptococcus. *Clin Microbiol Rev* 27:264–301.
2 Llewelyn M, Cohen J (2002) Superantigens: Microbial agents that corrupt immunity. *Lancet Infect Dis* 2:156–162.
3 Commons RJ, et al. (2014) Streptococcal superantigens: Categorization and clinical associations. *Trends Mol Med* 20:48–62.
4 Zeppa JJ, et al. (2017) Nasopharyngeal infection by *Streptococcus pyogenes* requires super antigen-responsive Vβ-specific T cells. *Proc Natl Acad Sci USA* 114:10226–10231.
5 Kasper KJ, et al. (2014) Bacterial superantigens promote acute nasopharyngeal infection by Streptococcus pyogenes in a human MHC Class II-dependent manner. *PLoS Pathog* 10:e1004155.
6 Shaler CR, et al. (2017) MAIT cells launch a rapid, robust and distinct hyperinflammatory response to bacterial superantigens and quickly acquire an anergic phenotype that impedes their cognate antimicrobial function: Defining a novel mechanism of superantigen-induced immunopathology and immunosuppression. *PLoS Biol* 15:e2001930.
7 Kaul R, et al.; The Canadian Streptococcal Study Group (1999) Intravenous immunoglobulin therapy for streptococcal toxic shock syndrome–a comparative observational study. *Clin Infect Dis* 28:800–807.
8 Linnér A, Darenberg J, Sjölin J, Henriques-Normark B, Norrby-Teglund A (2014) Clinical efficacy of polyspecific intravenous immunoglobulin therapy in patients with streptococcal toxic shock syndrome: A comparative observational study. *Clin Infect Dis* 59:851–857.
9 Norrby-Teglund A, et al. (2000) Relative neutralizing activity in polyspecific IgM, IgA, and IgG preparations against group A streptococcal superantigens. *Clin Infect Dis* 31:1175–1182.
10 Norrby-Teglund A, et al. (2005) Successful management of severe group A streptococcal soft tissue infections using an aggressive medical regimen including intravenous polyspecific immunoglobulin together with a conservative surgical approach. *Scand J Infect Dis* 37:166–172.
11 Norrby-Teglund A, et al. (1996) Plasma from patients with severe invasive group A streptococcal infections treated with normal polyspecific IgG inhibits streptococcal superantigen-induced T cell proliferation and cytokine production. *J Immunol* 156:3057–3064.
12 Dale JB, et al. (2017) Structure-based design of broadly protective group a streptococcal M protein-based vaccines. *Vaccine* 35:19–26.
13 Sanderson-Smith M, et al.; M Protein Study Group (2014) A systematic and functional classification of Streptococcus pyogenes that serves as a new tool for molecular typing and vaccine development. *J Infect Dis* 210:1325–1338.
14 Lannergård J, et al. (2011) The Hypervariable region of Streptococcus pyogenes M protein escapes antibody attack by antigenic variation and weak immunogenicity. *Cell Host Microbe* 10:147–157.
15 Lannergård J, et al. (2015) Sequence variability is correlated with weak immunogenicity in Streptococcus pyogenes M protein. *Microbiologyopen* 4:774–789.