

# Effect of a Putative B Cell Superantigen on Complement

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Staphylococcal protein A (SPA), a cell wall component of *S. aureus*, binds not only to the Fc fragment of IgG, but also via an alternative site to the Fab fragments of immunoglobulins (Igs) independent of heavy chain isotype.<sup>1,2</sup> In man, this binding is restricted to Igs with V<sub>H</sub>3 heavy chains and occurs at a site(s) outside the conventional antigen-binding region.<sup>2,3</sup>

An increasing body of evidence has indicated that SPA shares many analogous properties with T cell superantigens, and it has thus been characterized as a B cell superantigen.<sup>3,4</sup> Unlike a T cell superantigen, however, a B cell superantigen has the ability to react with potentially large amounts of soluble B cell antigen receptors in the serum. Encountering such a large reservoir of reactive Igs could have deleterious effects on the host, particularly if this interaction leads to activation of the complement cascade with resultant tissue inflammation. The addition of SPA to serum has been shown to cause activation of the complement cascade. This activation has been attributed to the binding of the classical site on SPA to the Fc region of IgG.<sup>5</sup> It is also possible, however, that complement activation is caused by the interaction of SPA with Fab regions of V<sub>H</sub>3<sup>+</sup> Igs. The generation of such complement-activating "immune complexes" by SPA may represent a novel and important biologic activity of a B cell superantigen.

Therefore, we sought to determine *in vitro* if the interaction of the alternative site on SPA with the Fab region of Ig molecules causes complement activation. Using a total hemolytic complement assay, we demonstrated that SPA, abrogated of its IgG Fc-binding activity by hyperiodination (mod-SPA), causes complement consumption when incubated with human serum (TABLE 1). To further test this hypothesis, we determined whether the interaction of SPA with polyclonal IgM or a panel of monoclonal IgM proteins (representative of human V<sub>H</sub> gene families) led to C1q binding in an ELISA. These proteins were first analyzed in an SPA-binding ELISA to determine which bind to SPA. Our results (data not shown) demonstrated that polyclonal IgM bound SPA as well as four out of six V<sub>H</sub>3<sup>+</sup> IgM proteins. No proteins from other V<sub>H</sub> gene families had binding activity. Because only a subset of V<sub>H</sub>3<sup>+</sup> Ig molecules is known to bind SPA, it was anticipated that some of our V<sub>H</sub>3<sup>+</sup> IgM monoclonals would also not bind SPA.<sup>3</sup> To determine if the interaction of the IgM proteins with SPA led to binding of C1q, biotinylated SPA was incubated with either human polyclonal IgM or the monoclonal IgM

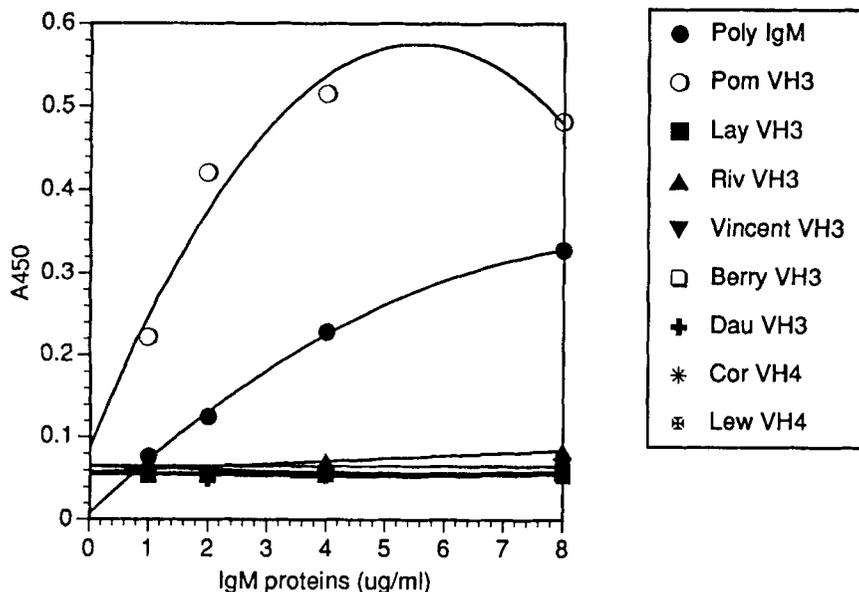
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**TABLE 1.** The Effect of mod-SPA on the Total Hemolytic Complement Activity of Human Serum

	Protein Added (240 $\mu\text{g/ml}$ )	% Hemolysis Inhibition
Experiment #1	mod-SPA	76%
Experiment #2	mod-SPA	71%

NOTE: Normal human serum was incubated in the presence or absence of hyperiodinated SPA (mod-SPA) that was devoid of Fc IgG-binding activity. Hemolytic complement activity was then determined in a total hemolytic complement assay.

proteins (FIG. 1). Aliquots of the incubation mixtures were added to wells coated with C1q or the control protein BSA. After incubation and washing, streptavidin-peroxidase was added to the wells, which were subsequently developed with OPD substrate. In control experiments, no binding was observed when (1) biotinylated SPA alone was added to C1q-coated wells and (2) IgM was added to C1q-coated wells before incubation with biotinylated SPA. In addition, no binding to BSA-coated wells was detected under any of the above conditions. The interaction of SPA with polyclonal IgM led to binding of C1q. Because SPA will bind only  $V_{H3}^+$  IgMs, we anticipated that C1q binding would be limited to  $V_{H3}^+$  IgM/SPA



**FIGURE 1.** IgM/SPA complexes binding C1q. IgM proteins were incubated with biotinylated SPA for two hours at 37°C. Aliquots of these reaction mixtures were then added to C1q-coated wells (5  $\mu\text{g/ml}$ ) of an ELISA plate for two hours at 37°C. After washing, streptavidin-HRP was added for 1 hour at 37°C, and the wells were then washed with BSA/PBS. Wells were developed by addition of OPD substrate. Controls are as described in the text.

complexes. This hypothesis proved to be correct, although surprisingly, not all  $V_H3^+$  IgM/SPA complexes bound C1q. We are currently evaluating these  $V_H3^+$  proteins to determine the structural basis for their inability to bind C1q following interaction with SPA.

In summary, because addition of modified SPA to human serum leads to complement consumption and because the interaction of SPA with either polyclonal IgM or some  $V_H3^+$  IgM proteins leads to binding of C1q, our results suggest that the interaction of IgM with the Fab-binding site on SPA leads to complement activation. Accordingly, the *in vivo* interaction of a B cell superantigen with secreted Igs may lead to complement activation and ensuing tissue inflammation.

#### REFERENCES

1. INGANAS, M. 1981. Comparison of mechanisms of interaction between protein A from *Staphylococcus aureus* and human monoclonal IgG, IgA, and IgM in relation to the classical Fc $\gamma$  and the alternative F(ab') $_2$  $\epsilon$  protein A interactions. *Scand. J. Immunol.* **13**: 343-352.
2. ROMAGNANI, S., M. G. GUIDIZI, G. DEL PRETE, E. MAGGI, R. BIAGIOTTI, F. ALMERIGOGNA & M. RICCI. 1982. Demonstration on protein A of two distinct immunoglobulin-binding sites and their role in the mitogenic activity of *Staphylococcus aureus*. *J. Immunol.* **129**: 596-602.
3. SASSO, E. H., G. J. SILVERMAN & M. MANNIK. 1989. Human IgM molecules that bind staphylococcal protein A contain VHIII H chains. *J. Immunol.* **142**: 2778-2783.
4. SILVERMAN, G. J. 1992. Human antibody response to bacterial antigens: Studies of a model conventional antigen and a proposed model B cell superantigen. *Int. Rev. Immunol.* **9**: 57-78.
5. KRONVALL, G. & H. GEWURZ. 1970. Activation and inhibition of IgG-mediated complement fixation by staphylococcal protein A. *Clin. Exp. Immunol.* **7**: 211.