Letters to the Editor

Streptococcus pneumoniae Flow-Cytometric Phagocytosis Assay

In a recent article, Jansen and coworkers described the use of highly encapsulated Streptococcus pneumoniae strains in a flow-cytometric assay for the assessment of the phagocytic capacity of serotype-specific antibodies (1). This assay is an elegant and easy-to-perform flow-cytometric method, which allows the measuring of the phagocytic capacity of large numbers of pneumococcal vaccine antisera. The results look promising, as the interassay variation is low and the correlation with postvaccination immunoglobulin G concentrations is good.

In order to strengthen the potential of this method to exclusively identify anticapsular antibodies in vaccinated individuals, the authors have used hyperimmune rabbit antisera raised in our laboratory against a hydrophobic protein fraction of S. pneumoniae. These antisera did not recognize protein epitopes at the surface of heat-inactivated pneumococci that were grown to log phase on three consecutive days.

We regret that our antisera are poorly described in the article. This might lead to confusion. As a matter of clarification, we have raised hyperimmune rabbit sera against a fraction of surface-associated hydrophobic proteins that were extracted from different pneumococcal strains. Indirect immunocytometric analysis has confirmed that the sera recognize components exposed by encapsulated pneumococci. In addition, the in vitro phagocytic capacity of the sera was high with non-heat-inactivated pneumococci that were grown to log phase. Importantly, both antigenic surface exposure and phagocytic capacity of the sera were independent of the capsular type and the genotype of the pneumococcus (2).

The experiments described by Jansen and coworkers using the hyperimmune sera raised against the fraction of surface-associated hydrophobic pneumococcal proteins have been carried out independently in our laboratory. Heat inactivation was omitted for obvious reasons. In the experiments using non-heat-inactivated pneumococci that were grown to stationary-phase or to log phase on three consecutive days, the hyperimmune sera demonstrated phagocytic capacity (Fig. 1). Based on these observations, we conclude that the flow-cytometric phagocytosis assay described by Jansen and coworkers is also suitable for measuring antibodies that recognize protein epitopes exposed at the surface of encapsulated pneumococci.

REFERENCES

1. Jansen, W. T. M., J. Gootjes, M. Zelle, D. V. Madore, J. Verhoef, H. Snippe, and A. F. M. Verheul. 1998. Use of highly encapsulated Streptococcus pneumoniae strains in a flow-cytometric assay for assessment of the phagocytic capacity of serotype-specific antibodies. Clin. Diagn. Lab. Immunol. 5:703–710.

2. Overweg, K., R. de Groot, A. F. M. Verheul, A. Kerr, T. J. Mitchell, and P. W. M. Hermans. Opaque antibodies directed against hydrophobic surface proteins of Streptococcus pneumoniae confer passive protection in mice. Unpublished data.

Author’s Reply

The purpose of the described phagocytosis assay is to measure the phagocytic capacity of serotype-specific antibodies. Using highly encapsulated, heat-inactivated pneumococci, only anticapsular polysaccharide antibodies were able to promote phagocytosis. As we stated in Discussion, a potential disadvantage of the use of heat treatment is the potential denaturation of protein epitopes on the pneumococcus. Therefore, when the purpose is to evaluate the opsonic capacity of antipneumococcal protein antibodies, live and heat-inactivated strains should initially be compared.

We never intended to suggest that the described protein antisera of Overweg and coworkers were not able to promote phagocytosis of non-heat-inactivated strains. We agree that their protein antisera do promote phagocytosis of non-heat-inactivated bacteria and thereby may have protective capacities.

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