In: Bayer Symposium VII: The Pathogenesis of Bacterial Infections
pp 422-424 (G.G. Jackson and J. Thomas eds.) Springer-Verlag Berlin
1985

Monoclonal Antibody Protection Against *Pseudomonas aeruginosa*

R. E. W. Hancock

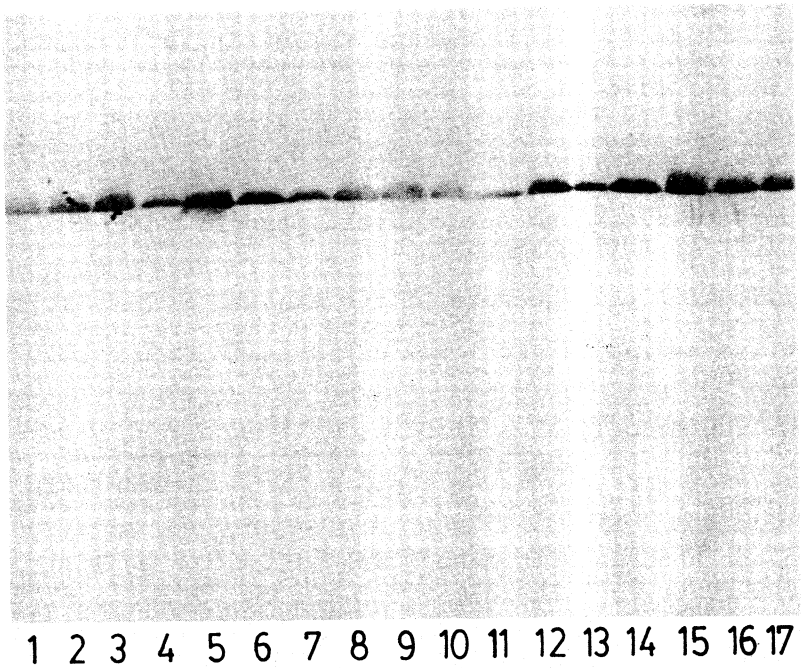
Biotechnology companies are isolating monoclonal antibodies against various bacterial products with the aim of utilizing them in prevention and therapy of infection. One that has received attention is the anti-lipid A monoclones for all gram-negative bacteria. Unfortunately, the early results have been quite disappointing. Our investigation was to see if some outer membrane proteins which are functionally conserved were also antigenically conserved and would provide a candidate antigen for a vaccine.

We have isolated monoclonal antibodies against two distinct surface epitopes of protein F of *P. aeruginosa*. Figure 1 is a Western blot that shows that all of the strains representing the serotypes of *P. aeruginosa*, which differ on the basis of different O-antigenic composition of their LPS have a single protein, protein F, of the same molecular weight, which interacts with a single monoclonal antibody against protein F. This result differs from other gram-negative organisms like *Neisseria* and *Hemophilus*. Both classes of antibodies we have studied interact with this protein. An indirect immunofluorescence assay using the monoclonal antibodies suggests that it is surface epitopes with which the monoclonal antibodies are reactive.

To test whether the antibody gave protection the monoclonal antibody was given in the tail vein of mice, either two hours or 24 hours prior to an intraperitoneal challenge. The challenge strains were PA103 or M2. Both produce large amounts of extracellular products that kill mice within 2-12 hours after the intraperitoneal challenge. The results obtained with one of the two challenge strains is shown in Fig. 2 as the per cent survival after 24 hours. The test group (upper line) received 0.1 mg of purified monoclonal antibody. The control group were given a monoclonal antibody that does not react with surface proteins. The number of surviving mice are shown at each inoculum and the differences between the groups are statistically significant by the Fisher exact test.

The results suggest that there is the potential for a vaccine component for passive protection against severe infections with *P. aeruginosa*. However, there are about 200,000 postulated vaccines in the literature and it is arguable whether or not monoclonal antibodies will become the commercial vaccines of the

Fig. 1



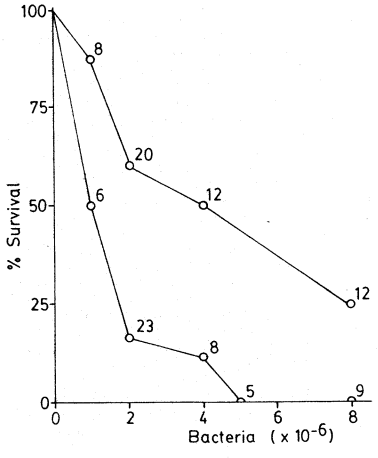


Fig. 2

future. Millions of dollars are going into their development and discussion is necessary about whether physicians and scientists believe it is a proper and practical direction for therapy.

Discussion

Q: Have the mice in which the anti-protein F antibody was produced been challenged?

A: Yes, active immunization is protective. The reason to study monoclonal antibody is to define the antigen giving protection. It is impossible to purify an outer membrane protein to homogeneity. It can be purified to only one band on the gel, but that is not antigenic purity. Therefore, there is always doubt as to the basis of the protection. We presented the antigen as protein incorporated into phosphotidyl choline vesicles (these vesicles serve as an adjuvant that could be licensed for use in humans). Our results suggest, but do not prove, that protein F was the protective antigen in active immunization.

Q: Is your implication that because of the exquisite specificity of monoclonal antibodies for a single epitope they are less likely to be effective against a virulence determinant than a polyclonal antibody would be?

A: No. Antigens for monoclonal antibodies make excellent vaccine candidates, but a battery of antibodies against a variety of porin proteins, exotoxins and maybe lipid A for anti-endotoxin may be required for full protection.

Comment: There are studies with monoclonal antibodies against pneumococcal phosphocholine and different LPS epitopes of *Salmonella* for use in passive immunization by monoclonal antibodies rather than as a basis for active immunity.

A: The major issue is whether enough of a monoclonal antibody preparation can be made to allow it to be used therapeutically or prophylactically for all of the patients who are at risk of severe infections with *Pseudomonas*. Also, antibody has a limited half-life in vivo. Such use is perhaps logistically impossible for infections of long duration. As therapy for bacteremias of short duration, monoclonal antibodies could be effective. However, few researchers have succeeded in making monoclonal antibodies of human origin in any significant amount and the dangers of using mouse immunoglobulins in man, especially if used repeatedly, must be remembered. In contrast, the use of the specific antigen that has elicited the protective monoclonal antibody could be a highly efficient form of prophylaxis.