Glanders is a debilitating disease with no vaccine available. Murine monoclonal antibodies were produced against Burkholderia mallei, the etiologic agent of glanders, and were shown to be effective in passively protecting mice against a lethal aerosol challenge. The antibodies appeared to target lipopolysaccharide. Humoral antibodies may be important for immune protection against B. mallei infection.

In an attempt to generate specific antibodies that could be useful in the specific diagnosis of glanders and in prevention of disease, monoclonal antibodies (MAbs) were generated in BALB/c mice. In determinations of antibody specificity or efficacy against infection, results were evaluated for statistical significance by linear regression analysis or by analysis of variance. Survival distributions were compared by Kaplan-Meier methods. All tests were at the 95% confidence level (two tailed) (14).

To generate monoclonal antibodies, mice were injected intraperitoneally (i.p.) with 100 μg of irradiation-killed mid-log-phase B. mallei China 7 strain (ATCC 23344) cells and given a second injection 14 days later. Three days after the booster injection, a splenocyte suspension was prepared from the mouse with the highest enzyme-linked immunosorbent assay titer against B. mallei and fused with the murine myeloma cell line P3X63-Ag8.653 (7). Primary hybridoma culture supernatants were screened for antibody activity by enzyme-linked immunosorbent assay with irradiated B. mallei- or B. pseudomallei-coated microtiter wells, and B. mallei-positive hybridomas were subcloned by limiting dilution. Thirty-two clones reacted with B. mallei antigens and were selected for further specificity screening (data not shown). Of these, four anti-B. mallei clones were finally selected, based on their strong reactivity with B. mallei and absence of reactivity with the closely related B. pseudomallei (8). Antibodies were purified by protein A chromatography; purity was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Culture supernatants from the four selected hybridomas, designated 1G2-ID3, 1G3-1, 9C1-2, and 8G3-1B11, reacted with B. mallei even at high dilutions of culture supernatants tested (Table 1). At each dilution tested, absorbencies of microtiter wells coated with B. mallei antigens were significantly greater (P < 0.003) than absorbencies of microtiter wells coated with B. pseudomallei antigens.

To determine the ability of the anti-B. mallei MAbs to specifically capture B. mallei antigens in solution, B. mallei or B. pseudomallei cell lysates were added to microtiter wells precoated with individual anti-B. mallei MAb. Antigen capture was detected by adding a heterologous anti-B. mallei MAb conjugated to biotin (Table 2). All four anti-B. mallei MAbs

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Burkholderia mallei, pseudomallei, glanders, monoclonal antibodies, passive protection
were capable of capturing *B. mallei*, but not *B. pseudomallei*, antigens in solution (*P* < 0.0001). The negative control MAb termed F1-04-A-G1, specific for the F1 capsular antigen of *Yersinia pestis* (2), did not bind to either of the two *Burkholderia* cell lysates (*P* > 0.05). The ability of the MAbs to specifically recognize *B. mallei* and not the closely related *B. pseudomallei* antigens in solid phase, as well as in solution, suggests their potential value as specific diagnostic tools in differentiating glanders and melioidosis in clinical conditions.

We then determined the antigenic specificity of the four anti-*B. mallei* MAbs by immunoblot analysis of *B. mallei* separated by SDS-PAGE with 10 to 20% precast Tricine gels. The ability of the MAbs to specifically recognize *B. mallei* and not the closely related *B. pseudomallei* antigens in solid phase, as well as in solution, suggests their potential value as specific diagnostic tools in differentiating glanders and melioidosis in clinical conditions.

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least at the dose of antibodies (1.0 mg/mouse) used in the experiment. However, our results using an in vitro system of *B. mallei* infection of J774 murine macrophages suggest that the number of intracellular pathogens decreased in infected cells cultured in the presence of anti-*B. mallei* MAbs and complement, compared to that in infected cells cultured in the absence of antibodies and complement (unpublished observations). Therefore, it is conceivable that circulating antibodies at sufficient concentrations could have a bacteriostatic or bactericidal effect on intracellular pathogens in vivo. Similar passive protection against i.p. *B. pseudomallei* infection was previously reported. Polyclonal antisera specific for the polysaccharide of *P. pseudomallei* LPS were shown to passively protect against an i.p. *P. pseudomallei* infection in a diabetic rat model of melioidosis (3). Likewise, murine MAbs specific for *B. pseudomallei* polysaccharide passively protected mice challenged i.p. with *B. pseudomallei* (9). Thus, the presence of circulating antibodies at the initial stages of infection may contribute to protective immunity against glanders and melioidosis, two clinical conditions caused by the phylogenetically closely related *B. mallei* and *B. pseudomallei*, respectively (8). However, as in most diseases caused by intracellular pathogens, in addition to specific humoral immunity, a
robust and specific cell-mediated immunity would in all likelihood be necessary for eliminating infected cells and controlling pathogenesis.

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The research described in the manuscript was conducted in compliance with the Animal Welfare Act and other federal statues and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

We dedicate this paper to the memory of Tran C. Chanh—gifted researcher, caring mentor, and friend.

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