JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1992, p. 3298–3301
0095-1137/92/123298-04$02.00/0

Vol. 30, No. 12

Letters to the Editor

Autoagglutination and Latex Particle Agglutination Assays

Paulsson et al. (3) recently reported on the use of a latex particle agglutination assay to rapidly identify strains of coagulase-negative staphylococci (CoNS) which produce surface receptors that recognize fibronectin, vitronectin, laminin, and collagen. To perform the assay, latex beads were coated nonspecifically with one of the aforementioned proteins and mixed with a suspension of the bacterial strain to be evaluated. Those strains which express surface receptors cause agglutination of the latex particles coated with the corresponding protein. As a negative control, the authors examined for autoagglutination of each bacterial strain in buffer alone. After reading their original study (22) regarding the use of this method to evaluate the binding of *Staphylococcus aureus* to surface-active proteins, we also attempted to adapt the assay to evaluate the binding of CoNS to fibronectin and chymotryptic fragments of fibronectin in 1990. At first, we thought that the procedure worked well, noting a marked difference in the ability of different strains to agglutinate fibronectin-coated latex particles, until we examined the agglutination of uncoated latex particles a control. Much to our dismay, nearly one-fourth (12) of the 51 strains examined caused a 4+ agglutination of uncoated latex particles in the absence of autoagglutination in buffer alone. The remaining strains produced a variety of agglutination reactions in the presence of uncoated latex ranging from none to 3+. We originally thought that the agglutination of uncoated latex particles was directly proportional to cell hydrophobicity, but studies performed by Dr. Michael Pfaller (then at the University of Iowa Medical Center) on a number of our strains showed no correlation between hydrophobicity and the observed agglutination reaction. As a result of these findings, we abandoned efforts to use this assay. The authors did not indicate whether the agglutination of uncoated latex particles was examined during the course of their study. Is it possible that the agglutination of latex particles by strains of CoNS is nonspecific in certain instances and is actually diminished when the particles are coated with proteins? An example of this hypothesis would be as follows. Assume that the strain *Staphylococci capitis* LK 499 (ATCC 27840) shown in Table 1 in their study strongly agglutinates uncoated latex beads. The coating of the beads with fibronectin, vitronectin, laminin, and gelatin but not collagen I abolishes this activity, which would then give the appearance of binding to collagen-coated beads. We have previously observed that the coating of polystyrene tissue culture wells with either fibronectin or chymotrypic fragments of fibronectin significantly reduced the adhesion of five slime-positive strains of *Staphylococcus epidermidis* to plastic (1). We would be most interested to learn whether Dr. Paulsson and colleagues observed the same phenomenon and what was done to overcome this obstacle.

REFERENCES

1. **Dunne, W. M., P. L. Havens, and F. T. Counter.** 1989. Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 640, p. 208.
2. **Naidu, A. S., M. Paulsson, and T. Wadström.** 1988. Particle agglutination assays for rapid detection of fibronectin, fibrinogen, and collagen receptors on *Staphylococcus aureus*. J. Clin. Microbiol. **26**:1549–1554.
3. **Paulsson, M., Å. Ljungh, and T. Wadström.** 1992. Rapid identification of fibronectin, vitronectin, laminin, and collagen cell surface binding proteins on coagulase-negative staphylococci by particle agglutination assays. J. Clin. Microbiol. **30**:2006–2012.

Wm. Michael Dunne, Jr.
Departments of Pathology and Pediatrics
Baylor College of Medicine
Houston, Texas 77030

Eileen Burd
Department of Pathology
Medical College of Wisconsin
Milwaukee, Wisconsin 53201

Author's Reply

Dunne and Burd discussed the problem of using uncoated latex beads as a control in the absence of autoagglutination in diagnostic particle agglutination assays with reference to our publication (6). Such latex beads, as well as polystyrene beads, usually express amphiphatic properties and have been used by Lachica and Zink (2) and others to develop sensitive assays to discriminate hydrophobic from hydrophilic cells of *Yersinia enterocolitica*. These tests have been compared with hydrophobic interaction chromatography, the salt aggregation test, and other tests in a recent review (7). Already in early studies in our laboratory, we carefully investigated the influence of culture media, ionic strength, and pH of the buffer on the latex assay (4). We have observed that *S. aureus* and also many coagulase-negative staphylococci express high surface hydrophobicity, as determined by various tests, especially when grown on blood agar (3). For this reason, we proposed that latex particles should be coated with proteins to block hydrophobic and surface charge properties of these beads, analogous to how microtiter enzyme-linked immunosorbent assay plates are coated with albumin, skim milk, or gelatin to prevent nonspecific protein binding. However, since cell surface lipoteichoic acid and specific high-affinity albumin-binding proteins of group A, C, and G streptococci bind albumin and since collagen-binding surface proteins of these organisms bind denatured collagen (gelatin), neither albumin nor gelatin should be used as a control. Since several fibrinogen and immunoglobulin commercial kits are used today in many laboratories to rapidly identify clumping factor and protein A-producing staphylococci we believe that the observation by Dunne and Burd and our own experience strongly suggest that uncoated latex and other polymer beads should be avoided as controls. The uncoated beads express a strong hydrophobicity and a highly negatively charged surface. These properties change when the beads are coated with proteins (Table 5 in reference 6). Beads coated with proteins which are known to not interact with the pathogens are the most relevant control. In our study we used ovalbumin in the blocking buffer to reduce nonspecific binding. Latex beads coated with the 150-kDa fragment of fibronectin (lacking the 29-kDa staphylococcal binding N terminus of fibronectin), interacting with eucaryotic cells, are an appropriate negative control for staphylococci. We are now evaluating covalent

coupling of these proteins to commercially available activated latex beads (Seradyn Inc., Indianapolis, Ind.). This will probably allow us to add detergents to protein-coated beads mixed with bacterial suspensions to avoid nonspecific reactions.

We have also found that preadsorption of fibronectin, vitronectin, fibrinogen, albumin, and plasma to various polymer surfaces influences subsequent adherence of staphylococci to the surface (5). It is well known that these proteins adsorb differently to different surfaces and adsorb differently if there is more than one protein present (1). Hence, a domain of, e.g., fibronectin to which staphylococcal strains bind can be exposed when the protein has been adsorbed to one polymer surface in the presence of a buffer but may not be when the protein has been adsorbed to the surface in the presence of serum, plasma, or other protein solutions.

REFERENCES

1. **Fabrizius-Homan, D. J., and S. L. Cooper.** 1991. A comparison of the adsorption of three adhesive proteins to biomaterial surfaces. J. Biomater. Sci. Polymer Edn. **3**:27–47.
2. **Lachica, R. V., and D. L. Zink.** 1984. Determination of plasmid-associated hydrophobicity of *Yersinia enterocolitica* by a latex particle agglutination test. J. Clin. Microbiol. **19**:660–663.
3. **Ljungh, Å., S. Hjertén, and T. Wadström.** 1985. High surface hydrophobicity of autoaggregating *Staphylococcus aureus* strains isolated from human infections studied with the salt aggregation test. Infect. Immun. **47**:522–526.
4. **Naidu, A. S., M. Paulsson, and T. Wadström.** 1988. Particle agglutination assays for rapid detection of fibronectin, fibrinogen, and collagen receptors on *Staphylococcus aureus*. J. Clin. Microbiol. **26**:1549–1554.
5. **Paulsson, M., and Å. Ljungh.** Unpublished data.
6. **Paulsson, M., Å. Ljungh, and T. Wadström.** 1992. Rapid identification of fibronectin, vitronectin, laminin, and collagen cell surface binding proteins on coagulase-negative staphylococci by particle agglutination assays. J. Clin. Microbiol. **30**:2006–2012.
7. **Wadström, T.** 1990. Hydrophobic characteristics of staphylococci: role of surface structures and role in adhesion and host colonization, p. 315–333. *In* R. J. Doyle and M. Rosenberg (ed.), Microbial cell surface hydrophobicity. American Society for Microbiology, Washington, D.C.

T. Wadström
M. Paulsson
Å. Ljungh
Department of Medical Microbiology
University of Lund
Sölvegatan 23
S-223 62 Lund
Sweden

Zoonotic Tuberculosis in Latin America

We have read with great interest the paper written by Dr. P. del Portillo et al. (4) on a species-specific DNA fragment of *Mycobacterium tuberculosis* and would like to make some comments limited to their assertions on the bovine tuberculosis problem. The authors sustain that human tuberculosis of bovine origin is a serious health problem in Latin American countries and that, because of this, there is an urgent need to develop a specific, sensitive, and rapid diagnostic test capable of distinguishing infections caused by *M. tuberculosis* from those caused by *M. bovis*.

We fully agree with the first of those statements. Nevertheless, we cannot endorse the figures and sources presented in their support. The authors attribute to a Pan American Health Organization/World Health Organization (PAHO/WHO) publication (7) a reported rate of 70.5 new cases of human tuberculosis of bovine origin per 100,000 individuals in Latin American countries. Neither this information nor any other about human tuberculosis due to *M. bovis* can be found in the cited document. Only in the annexed Table II, 9j, on p. 336 is a global tuberculosis rate (presumably due to *M. tuberculosis* complex) of 69.3/100,000 for 1980 presented.

Two other figures–2 and 8% of *M. bovis* is isolated from pulmonary and extrapulmonary tuberculosis cases, respectively–attributed to Cetrangolo et al. (2) and Collins et al. (3) are quoted to confirm the importance of this problem. Cetrangolo et al. isolated *M. bovis* from 3.8% of 215 tuberculous patients in Buenos Aires City, and Collins et al. confirmed that 2.7% of human tuberculosis cases were due to *M. bovis* in Southeast England.

There is scarce epidemiological information on the impact of bovine tuberculosis on human health in Latin America, mainly because the bacteriological diagnosis of human tuberculosis is generally limited to the smear examination, and even when cultures are performed, the glycerol-containing Lowenstein-Jensen medium–on which *M. bovis* strains are very difficult to grow–is the only one available. In a few studies, however, cultures on a pyruvate-containing egg medium were also included. These studies originated in Argentina, where a relatively high prevalence of tuberculosis in cattle coincides with a reliable bacteriological diagnosis of human disease: percentages of human tuberculosis due to *M. bovis* ranged from 0.4 to 6.2%, and most of the patients infected with *M. bovis* were slaughterhouse or rural workers (1, 2, 6).

There are only estimations of the global importance of this problem at the regional level: according to a recent PAHO/WHO publication (5), some 7,000 new cases due to *M. bovis* may arise each year in Latin America, a rate of nearly 2/100,000 inhabitants. This figure is not negligible and shows the zoonotic relevance of the infection, even if it is far from the 70.5/100,000 stated by P. del Portillo et al.

A rapid and precise method of species identification of clinical specimens would certainly help to define the actual magnitude of zoonotic tuberculosis, and the polymerase chain reaction has emerged as the most convincing tool for this purpose. To our knowledge, however, it has not yet succeeded in discriminating *M. bovis* from the other members of the *M. tuberculosis* complex in field samples.

The tools of molecular biology are also expected to shed further light on the epidemiological chain of this infection. Nevertheless, we cannot envisage species identification through the polymerase chain reaction as an urgent solution to the problem of tuberculosis of bovine origin in Latin America. *M. bovis* is as susceptible as *M. tuberculosis* to antituberculosis drugs, and therefore, on a program basis, differentiation between the species is not relevant to curing the patient nor to controlling the transmission. The only