Letters to the Editor

Characterization of Mycobacterium bovis BCG Vaccines by DNA Fingerprinting by a Standardized Methodology

The well-coordinated efforts by van Embden et al. (5), in nine laboratories located in six different countries on three continents, toward a standardization of the methodology for DNA fingerprinting of Mycobacterium tuberculosis are indeed meritorious. By generation of strain-specific patterns, the standardized method is targeted toward detection of variable numbers and genomic positions of IS6110. The method should support the comparison of data obtained from different laboratories. Moreover, use of the method may provide straightforward information on the dynamics of international tuberculosis transmission, relative infectivity, virulence, and drug resistance of M. tuberculosis isolates. Furthermore, the technique, following appropriate modifications, would be very valuable in the characterization of innumerable Mycobacterium bovis BCG vaccine strains (3) in global use.

Administration of BCG vaccines during early infancy is associated with regional lymphadenitis and abscess formation in approximately 1% of vaccinated children (2, 4). BCG vaccines may induce lupus vulgaris: at least 62 reports of BCG vaccine-associated lupus vulgaris have been documented. Two such children who received BCG vaccination at 6 years of age required treatment with rifampin, ethambutol, and isoniazid (6). The severity and/or the occurrence of lymphadenitis and suppuration caused by different vaccine strains will vary. These strains have been differentiated through their MPB70 and mycobse B content. MPB70 is a unique BCG-specific antigen that elicits a delayed-type hypersensitivity in guinea pigs sensitized with viable BCG cells, while mycobse B is related to colonial morphology (3). Characterization of the MPB70 and mycobse B contents of the three parent strains, Glaxo 1077, Tokyo 172, and Pasteur 1173Pz, has been of little assistance in defining heterogeneity in vaccine strains. While most of the BCG strains have a single copy of IS986 at the same chromosomal site, the Brazilian, Japanese, and (former) USSR strains have an additional copy at a different location (1).

The standardized methodology (5) applied to BCG vaccine strains would assist in an in-depth characterization of the chromosomal DNAs of Swedish, Beijing, Prague, Dutch, Indonesian, and Dakar strains. The clones, if any, responsible for regional lymphadenitis or lupus vulgaris could possibly be recognized and eventually eliminated from prospective BCG vaccine batches. Any enhanced reactivity of a vaccine lot could also be investigated by a genetic amplification of M. bovis DNA fragments in biopsy or aspiration fluid from enlarged lymph nodes.

REFERENCES

Author’s Reply

Dr. Arya raises the interesting issue of the recognition of differences in postvaccination reactogenicities of the various M. bovis BCG strains by polymorphic DNA markers. We have indeed tried to find such genetic differences, using most of the polymorphic genetic markers known for M. tuberculosis complex strains (3). Except for the two M. bovis BCG groups that are distinguished by the presence of either one or two copies of IS6110, we have found a small rearrangement in the chromosomal region that borders one of the copies of this insertion sequence (IS) element. This region contains multiple direct repeats (DRs) of 36 bp, separated by spacer sequences of about the same size (2). This remarkable chromosomal locus is polymorphic among M. tuberculosis complex strains, most likely because of homologous recombination between DRs and because of IS-mediated rearrangements (1).

We have investigated 45 BCG strains, vaccine strains as well as patient isolates, and we encountered only one strain with a small rearrangement in the DR region of the chromosome. This strain had been isolated from the urine of a patient who had undergone a bladder instillation with BCG to prevent tumor relapse after resection of a superficial bladder cancer. It should be noted that the parental vaccine strain did not show this new polymorphism. Therefore, the BCG variant carrying the rearrangement likely was present in the vaccine prior to administration to this patient. It is presently unknown whether this variant was selected from the urine because it had properties different from those of the parental strain, such as increased adhesion to bladder epithelial cells. As far as we know, this is the only example of an additional DNA polymorphism among M. bovis BCG strains.

This example shows that occasional genetic differences can be found by testing BCG strains with different polymorphic genetic markers. However, it seems unlikely that such genetic markers will indicate the presumed genetic differences that contribute to differences in postvaccination reac-
togenecities, because in general such occasional rearrangements are unlikely to affect the virulence of bacterium. Given the rarity of such rearrangements in M. bovis BCG, the presently known polymorphic genetic markers seem unsuitable to recognize BCG variants with different reactogenecities, as suggested by Arya.

REFERENCES

Clinical Isolates of Campylobacter mucosalis

Figura et al. (2) recently reported on the isolation of Campylobacter mucosalis from two children with enteritis by using a filtration technique and incubating in an H2-enriched microaerobic atmosphere. During a 30-month period, from October 1990 to March 1993, using a similar protocol, the Red Cross Hospital processed 6,111 diarrheic stool specimens from which 1,519 strains of Campylobacter, Helicobacter, or Arcobacter spp. were isolated and identified by recognized criteria (2a).

The prevalence was as follows: 645 (42.5%) Campylobacter jejuni subsp. jejuni isolates, 337 (22.2%) Campylobacter upsaliensis isolates, 187 (12.3%) Campylobacter strains dependent on an H2-enriched microaerobic atmosphere for growth (Oxoid BR 38 gaspak, no catalyst), 159 (10.5%) C. jejuni subsp. doylei isolates, 100 (6.6%) Helicobacter fennelliae isolates, 42 (2.8%) Campylobacter coli isolates, 23 (1.5%) Campylobacter hyointestinalis isolates, 11 (0.7%) Helicobacter cinaedi isolates, 8 (0.5%) Campylobacter fetus subsp. fetus isolates, 5 (0.35%) Arcobacter butzleri isolates, and 1 (0.06%) each of Campylobacter lari and Campylobacter curvus.

All 187 isolates (185 from stool samples, 1 from a blood culture, and 1 from a gastric biopsy) requiring H2 were nitrate, reductase, and oxidase positive but negative for urease, catalase, hippurate, and indoxyl acetate. They produced abundant H2S detectable with lead acetate strips and triple sugar iron agar, and they tolerated 1% glycine but not 1.5% NaCl. These characteristics are identical to those for the isolates described by Figura et al. (2) and for C. mucosalis (4, 6), but unfortunately, they are also identical to the description of Campylobacter concisus (4, 6). C. concisus has been found in the human mouth and, until recently, has only rarely been isolated from human feces. Using H2-enhanced microaerobic growth conditions, Lauwers et al. (3) isolated 94 strains of C. concisus from the stools of enteritis patients. C. mucosalis strains can be differentiated from C. concisus by growth at 25°C, susceptibility to cephalothin, and the production of “dirty yellow” colonies (4, 5).

Nine of our isolates were determined to be C. mucosalis by the above criteria. Molecular techniques were performed by established procedures. The average mol% G+C value was found to be 38.1, which is the overlap region of the two species (4, 6). DNA-DNA hybridization studies were performed, and the clinical isolates were probed twice, once with the type strain for C. mucosalis, NCTC 11000, and again with the type strain for C. concisus, NCTC 11485. None of the nine isolates reacted with the C. mucosalis probe, while six reacted strongly with the C. concisus probe and three did not react with either probe. These three may represent a new species, as preliminary studies of cellular fatty acid profiles and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) have indicated.

Differentiation of these two species on the basis of growth temperature, cephalothin sensitivity, and colony color is suspect because of strain variability (2, 6). Figura et al. (2) used the API 20 Campy system for confirmation, but we have not found this system completely satisfactory for typing. Immunotyping (7), SDS-PAGE (1), and, in particular, DNA hybridization studies (5) all allow excellent discrimination between the two species. Indeed, Roop et al. (5) reported that the DNAs from C. mucosalis and C. concisus were only 9% complementary. It is premature to base identification of C. mucosalis, which has never been isolated from humans before, solely on a very limited number of variable characteristics. Molecular studies must be done for positive identification.

REFERENCES