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Letters to the Editor

Poliovirus-Specific Immunoglobulin M Antibodies during Diagnosis of Acute Poliomyelitis or Postpoliovirus Syndrome or Monitoring of Vaccine Responsiveness

The μ-capture assay to measure poliovirus-specific immunoglobulin M (IgM) antibodies for three poliovirus serotypes in serum and cerebrospinal fluid (CSF) in 114 patients with clinically determined acute poliomyelitis at Karachi, Pakistan, employed ^{35}S-methionine-radiolabelled polioviruses. In the microwell format, the appearance of poliovirus-specific IgM antibodies appeared a sensitive and specific test procedure for laboratory confirmation of poliomyelitis. During the first 15 days of patient illness, more poliomyelitis cases were confirmed by an intrathecal immune IgM response than by isolation of virus in the stool specimens (3). The microwell poliovirus IgM format (3) might also be ideal for monitoring the intrathecal IgM response in patients with postpoliomyelitis syndrome who manifest progressive muscular dystrophy decades after the initial episode of acute poliomyelitis (1). Presently, intrathecal immune reactivity is measured in CSF during electrophoresis of undiluted CSF specimens in agarose gel followed by a passive transfer to polyvinyl difluoride membrane coated with the poliovirus antigen. Following glutaraldehyde-induced cross-linkage of immunoglobulin with the membrane, it is possible to stain the poliovirus-specific IgM oligoclonal bands. Investigations with 36 patients, 16 men and 20 women, of postpoliomyelitis syndrome for intrathecal immune reactivity enabled detection of IgM oligoclonal bands in 21 patients, with no bands in any of the control group with a childhood poliomyelitis or a neuromuscular disease (4). The μ-capture assay would enable extended monitoring of intrathecal IgM response in patients with postpoliomyelitis syndrome in industrialized countries.

The use of radiolabelled materials in the μ-capture assay for poliomyelitis (3) is an obstacle against its extended diagnostic use in developing countries, where the routine diagnostic laboratories lack facilities for handling radioactive materials. Appropriate modifications would be needed in order to eliminate the use of radioactive materials and toward adaptation of the assay format for quantification of poliovirus IgM in saliva. The immune response in serum and CSF pairs in 114 patients in Pakistan (3) has been distinct, with no correlation in antibody titers in CSF-serum pairs. The salivary IgM quantum might well be an equally useful marker for a specific diagnosis of poliomyelitis. The sensitivity and specificity for hepatitis A virus-specific IgM in saliva samples obtained with a treated absorbent pad have been 100% (51 of 51 samples) and 98% (46 of 47 samples) in relation to serum antibody titers. Moreover, the decline of hepatitis A IgM in oral samples was parallel to, although somewhat more rapid than, that of hepatitis A IgM in serum samples (5). The utility of the μ-capture immunoassay for saliva rather than blood or CSF would be obvious in remote locations with poor facilities for health care and the absence of trained personnel to obtain CSF by lumbar puncture from patients labelled clinically as patients with acute poliomyelitis.

Conventionally, the immune response to live attenuated or enhanced potency inactivated poliovirus vaccine has been monitored through the quantification of IgG-class poliovirus antibodies in serum (2). Ready availability of simplified saliva-based techniques for poliovirus-specific IgM and IgA would enable one to ascertain whether there was a "window" phase between IgM and Ig response or some vaccinees responded by a selective IgA response. Even the patients with live poliovirus vaccine-induced or associated paralytic poliomyelitis might demonstrate an abnormal immune response.

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Author's Reply

We acknowledge with pleasure Dr. Arya's interest in our μ-capture assay for poliovirus-specific IgM-class antibodies. We also share Dr. Arya's vision on the implications of this technique in the control of poliomyelitis, and we are happy to say that the new applications he proposed are in progress in our laboratory. Some time ago we analyzed a set of CSF and serum specimens from patients suffering from postpoliomyelitis syndrome by using the published μ-capture technique with radiolabeled antigen (1). The results have been submitted for publication elsewhere (2). Another interesting point is the virus-specific IgM response in saliva. Studies aimed at the use of salivary IgM response in laboratory diagnosis of poliomyelitis and in monitoring vaccine responses are in progress. It is true that especially in developing countries the routine diagnostic laboratories lack facilities for handling radiolabeled

antigen. So far, our attempts to use the μ-capture technique with enzyme-conjugated antigen have resulted in lessened sensitivity of the assay, and further work is necessary to establish an enzyme immunoassay method for measuring poliovirus-specific IgM antibodies.

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2. **Roivainen, M., E. Kinnunen, and T. Hovi.** Twenty-one patients with strictly defined postpoliomyelitis syndrome: no poliovirus-specific IgM antibodies in the cerebrospinal fluid, submitted for publication.

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Detection of *Salmonella typhi* by PCR

In their paper, Song et al. describe a PCR-based diagnostic test for the detection of *Salmonella typhi* (3). The authors employed a nested PCR approach using as their first pair of primers an *S. typhi*-specific forward primer (ST 1) and a common flagellin gene-derived reverse primer and nested to them a second set of primers. In this second set, the forward primer (ST 3) was originally also thought to be *S. typhi* specific (1) but was later found to share the same DNA sequence with other type *d* flagellin genes (2). Therefore, it is not surprising that in the nested PCR, applied to cultured salmonella, an amplified product was obtained when *S. typhi* as well as *S. muenchen* DNA was used as template. In the Discussion section, the authors doubt (quite rightly) the significance and actual probability of finding *S. muenchen* in blood samples. However, in order to avoid this problem altogether, the authors could have used, as we are in our laboratory, a different forward primer for the first PCR which is derived from the DNA sequence located just upstream to primer ST 1 (1, 3), i.e., 5′ TATGCCGCTACATATGATGAG 3′ (1), together with primer ST 2 (1, 3). The use of these primers in the first round of DNA amplification should result in an amplified product using either *S. typhi* or *S. muenchen* DNA template (and also DNA from other *Salmonella* species expressing flagellar antigen *d*). Primer ST 1, which is the *S. typhi*-specific primer, should then be used together with primer ST 4 (3) for the nested reaction. As blood PCR was positive only for the nested reactions, false-positive amplification due to sample contamination with other *Salmonella* species (like *S. muenchen*) will be avoided.

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Ed. Note: *The author of the published article declined to respond.*

Treatment of Late Lyme Disease: a Challenge to Accept

We have read with great interest Liegner's guest commentary (1). We present a case which supports Liegner's opinions concerning the diagnosis and treatment of Lyme disease.

A 41-year-old male physician suffered a tick bite on his back. Forty-four hours later, the tick was traumatically removed by a dermatologist. Prophylactic oral doxycycline (200 mg/12 h) was administered for 5 consecutive days. Three months later, he complained of fatigue, febricula, and asthenia. The patient ignored these symptoms until he noted generalized and progressive muscle hypotrophy with fasciculations, accompanied by nonspecific neurologic, gastrointestinal, genitourinary, and cardiorespiratory symptomatology 27 months after the tick bite. He was initially diagnosed as having a psychiatric disorder by several specialists. Ancillary studies included complete blood count, erythrocyte sedimentation rate, biochemical profiling, serum immunoglobulins (Igs), thyroid hormones, human immunodeficiency virus, *Treponema pallidum*, *Brucella mellitensis*, *Salmonella typhi*, Epstein Barr virus serum antibody titers, tuberculin testing, chest X ray, electrocardiogram, electroencephalogram, echocardiography, abdominal ultrasound, and central nervous system (CNS) magnetic resonance imaging. Results were within the normal range except for a high serum IgM titer and partial IgA deficiency. Forty-one months after the tick bite, the patient was diagnosed by a neurologist as having probable encephalomyelitis due to *Borrelia burgdorferi*. Serum and cerebrospinal fluid (CSF) antibody titers to *B. burgdorferi*, as well as CSF cytology and biochemistry, were negative. Thus, no antibiotic therapy was initiated until further clinical deterioration was very evident. He received 2 g of ceftriaxone daily for 4 weeks. Marked early clinical improve-