CORRESPONDENCE

Mastitis and Human Immunodeficiency Virus Transmission: Chemokines and Maternal Monocytes

To the Editor—Semba et al. [1] linked inflammatory processes in the lactating breast with the risk of transmission of human immunodeficiency virus (HIV) [1]. Although this is a compelling set of data, we should like to draw attention to three difficulties with the study.

First, the study would have been considerably stronger had a milk cell count or bacterial count—the reference standards for mastitis—been done. A significant component of the HIV load is located in the cellular fraction of milk, particularly in monocyte/macrophage cells, although this proportion changes with phases of lactation and probably with mastitis too [2]. This virus burden does not appear to have been estimated by use of the techniques described. Milk contains chemokines and cytokines, which also increase in mastitis and alter cell activation status in the mother's breast and the neonatal intestine. To describe infectivity in mastitis, it therefore becomes critical to estimate the contribution of increased cell-bound intracellular virus and free virus together with the effects of increased cytokines and chemokines that may alter the neonatal defenses [3, 4]. There may be protective effects of milk to prevent neonatal infection by HIV; these will not be detected if only some of the data relating to infection or inflammation are collected.

Second, the use of milk sodium levels makes it difficult to determine with precision cases of mastitis, because this measure increases nonspecifically in hand-expressed milk samples, as used in this study. Sodium concentration also changes in individual mothers, depending on their stages of lactation [5]. The use of 12 mmol/L as a cutoff appears to have been made on a statistical basis without prior studies of the Malawian population under review or the sample collection system used in the study. Other clinical data have shown that many healthy mothers have sodium concentrations >12 mmol/L with normal potassium levels, normal cell counts, and no evidence of mastitis [5]. Thus, although there appears to be an association of raised sodium levels with HIV transmission, it is not clear whether this is due to mastitis.

Finally, Semba et al. [1] described the use of antibiotics to treat mastitis, suggesting that the data do not relate to breast engorgement but to a truly infective bacterial condition in one-third of Malawian mothers. This rate is considerably higher than that observed in the United Kingdom or The Gambia, where <3% of lactating mothers receive antibiotics for mastitis. Is this a feature of malnutrition in the Malawian population, such as lower vitamin A levels, for instance? Published mastitis rates vary extensively, and none is derived from a large prospective study relevant to a developing country. Mastitis is recognized as being most common at the beginning and end of lactation and is relatively rare in midlactation, yet the mothers were reviewed at this point in lactation. It is also unclear whether all mothers with mastitis transmitted their infection to their infants. If so, the rate of ~30% transmission by breast-feeding is higher than those cited elsewhere, particularly in recent studies from South Africa [6].

The association proposed by Semba et al. [1] is important to investigate further, as it will probably be relevant to the vertical transmission of human T cell lymphotropic virus types 1 and 2—and perhaps also to cytomegalovirus, Epstein-Barr virus, and human herpesvirus 7—and therefore will be important to significant numbers of mothers worldwide. A genetic predisposition to mastitis, observed in all mammals, needs to be defined, as do associations between chemokine genotypes and this route of infection. The role of inflammation in promoting transplacental retroviral infection has been investigated more thoroughly, and clinical data are available. By contrast, mastitis or breast inflammation in nursing mothers merits considerably more attention.

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References

1. Semba RD, Kumwenda N, Hoover DR, et al. Human immunodeficiency virus load in breast milk, mastitis, and mother-to-child transmission of human immunodeficiency virus type 1. J Infect Dis **1999**; 180:93–8.
2. Southern SO. Milk-borne transmission of HIV: characterization of productively infected cells in breast milk and interactions between milk and saliva. J Hum Virol **1998**; 1:328–37.
3. Michie CA, Tantschler E, Schall T, Rot A. Physiological secretion of chemokines in human breast milk. Eur Cytokine Netw **1998**; 9:123–9.
4. Lewis P, Nduati R, Kreiss JK, et al. Cell-free human immunodeficiency virus type 1 in breast milk. J Infect Dis **1998**; 177:34–9.
5. Michie CA. The immunological composition of milk changes in mothers having problems with lactation [abstract]. Pediatr Res **1999**; 45:913.
6. Coutsoudis A, Pillay K, Spooner E, Kuhn L, Coovadia HM. Influence of infant-feeding patterns on early mother-to-child transmission of HIV-a in Durban, South Africa: a prospective cohort study. Lancet **1999**; 354:471–6.

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Reply

To the Editor—We agree with Michie and Lynn [1] that there are further unanswered questions regarding the nature of mastitis and vertical transmission of human immunodeficiency virus (HIV) in developing countries. Knowledge in this area is advancing rapidly. Mastitis is a common and neglected problem of breast-feeding women [2]. Our study in Malawi provides

some early insight into mastitis, HIV load in breast milk, and vertical transmission. Because we used archived breast milk samples to assess mastitis in our study, we did not perform leukocyte counts or cultures [3]. We noted the need for further investigation of the microbiology and treatment of mastitis in developing countries. Cell-free HIV load was measured in breast milk, and the measurement of cell-associated HIV in human milk remains to be elucidated in the laboratory. Elevated breast milk sodium : potassium ratios and elevated sodium concentrations are associated with elevations of inflammatory and immunologic mediators in breast milk, including lysozyme, lactoferrin, secretory leukocyte protease inhibitor, transforming growth factor β-2, interleukin-8, and RANTES [4–7], all suggesting the presence of inflammation.

Breast milk sodium concentrations are high in colostrum, during mastitis, and at the termination of weaning. Milk sodium concentrations can also be elevated with breast engorgement and during lactation failure with a poorly feeding infant. Careful, nontraumatic collection of hand-expressed milk samples does not result in nonspecific elevation of breast milk sodium, and the attribution of elevated breast milk sodium to milk collection methods practiced in recent studies is inconsistent with the available chemokine and cytokine data [4–7]. The use of 12 mmol/L as a cutoff for sodium concentrations in breast milk was 3 SD above the mean for human milk sodium as measured by ion-selective electrodes [3]. It should be noted that, in the older literature, higher breast milk sodium concentrations have been reported when atomic absorption spectroscopy and related methods were used, and caution is needed when comparing studies and milk sodium values that have been obtained by use of different laboratory techniques.

The incidence rates of mastitis from the older literature are underestimated, because earlier studies tended to estimate the incidence of mastitis on the basis of clinic visits [2]. The prevalence of mastitis among women in Malawi at 6 weeks postpartum, as assessed by elevated breast milk sodium concentrations, is consistent with recent studies of mastitis from developing countries such as Bangladesh [4], Tanzania [5], and South Africa [8]. In addition, recent longitudinal studies in the United States, Finland, and Australia suggest that clinically apparent mastitis occurs in 20%–33% of breast-feeding women [2]. The highest incidence of mastitis is observed in the first several weeks postpartum [2, 4, 5, 8]. Our study in Malawi assessed mastitis at 6 weeks postpartum during the period of the highest known incidence of mastitis. The proportion of mothers with mastitis who transmitted HIV to their infants was clearly shown in table 2 [3]. Between ages 6 weeks and 12 months, as shown in table 2, the overall rate of HIV transmission through breast-feeding in this study was ~5%, not 30%, and this rate is consistent with other studies conducted in the same study population [9].

As suggested by Michie and Lynn [1], the association between mastitis and transmission of HIV, as shown in Malawi [3], has relevance for vertical transmission of other viruses and merits attention. Other studies have also demonstrated that mastitis is a risk factor for vertical transmission of HIV in Kenya and South Africa [8, 10]. Mastitis, as indicated by elevated sodium : potassium ratio, is associated with elevated HIV load in breast milk and is primarily a unilateral process [8]. Evidence is accumulating that mastitis may be a key risk factor for HIV transmission through breast milk, and it is potentially amenable to treatment and prevention [2, 5].

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References

1. Michie CA, Lynn W. Mastitis and human immunodeficiency virus transmission: chemokines and maternal monocytes [letter]. J Infect Dis **2000**; 181: 800.
2. Semba RD, Neville MC. Breast-feeding, mastitis, and HIV transmission: nutritional implications. Nutr Rev **1999**; 57:146–53.
3. Semba RD, Kumwenda N, Hoover DR, et al. Human immunodeficiency virus load in breast milk, mastitis, and mother-to-child transmission of human immunodeficiency virus type 1. J Infect Dis **1999**; 180:93–8.
4. Filteau SM, Rice AL, Ball JJ, et al. Breast milk immune factors in Bangladeshi women supplemented postpartum with retinol or β-carotene. Am J Clin Nutr **1999**; 69:953–8.
5. Filteau SM, Lietz G, Mulokozi G, Bilotta S, Henry CJK, Tomkins AM. Milk cytokines and subclinical breast inflammation in Tanzanian women: effects of dietary red palm oil or sunflower oil supplementation. Immunology **1999**; 97:595–600.
6. Semba RD, Kumwenda N, Taha TE, et al. Mastitis and immunological factors in breast milk of lactating women in Malawi. Clin Diagn Lab Immunol **1999**; 6:671–4.
7. Semba RD, Kumwenda N, Taha TE, et al. Mastitis and immunological factors in breast milk of human immunodeficiency virus–infected women. J Hum Lact **1999**; 15:301–6.
8. Willumsen J, Filteau SM, Coutsoudis A, et al. Subclinical mastitis and breast milk viral load among lactating women infected with HIV-1 in South Africa [abstract]. In: Program and abstracts of the Second Conference on Global Strategies for the Prevention of HIV Transmission from Mothers to Infants, September 1999, Montreal, **1999**:67.
9. Miotti PG, Taha TET, Kumwenda NI, et al. HIV transmission through breastfeeding: a study in Malawi. JAMA **1999**; 282:744–9.
10. Embree JE, Njenga S, Datta P, et al. Risk factors for postnatal mother-to-child transmission of HIV-1 [abstract]. In: Program and abstracts of the Second Conference on Global Strategies for the Prevention of HIV Transmission from Mothers to Infants, September 1999, Montreal, **1999**:62.

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Preliminary Falsification of EIA Screening Is Cost-Effective in the Two-Step Serodiagnosis of Lyme Disease

To the Editor—We read with great interest the article by Trevejo et al. [1] that described a simplified procedure for the laboratory diagnosis of Lyme disease (LD). The authors assumed as "certain" the clinical diagnosis of LD and on this basis calculated the sensitivity of 3 different diagnostic approaches. We suggest that there is some bias in the assumption that 25 EIA-positive serum samples plus 2 more Western immunoblot (WB)–confirmed samples, among equivocal results, sum up to a sensitivity of 41%, by the authors' simplified method, in the early phase of LD. Similarly, the assumption was made that 39 EIA-positive sera, with no WB confirmation of equivocal results, give a sensitivity of 71% in the convalescent phase. The authors ignore that 6 (22%) of 27 and 23 (59%) of 39 EIA-positive results were disproved by WB. We cannot understand what scientific reasoning allows the omission of the WB-unconfirmed, EIA-equivocal results and the inclusion of WB-unconfirmed but EIA-positive cases.

As far as we know, the "etiologic certainty" of a given infection can be determined only by the isolation of a causative organism, which is difficult to do for LD. At present, the best available parameter of relative certainty seems to be WB confirmation, on the basis of which the sensitivity of other diagnostic approaches should be calculated. It is possible, of course, for the scientific community to decide that a physician-diagnosed erythema migrans rash ⩾5 cm is the most sensitive determination of early LD. If so, a further simplified and cost-effective approach would be not to do any EIA screening.

The discrepancies between the screening tests and WB confirmation indicate that in the serodiagnosis of LD a major problem is specificity, since some unrelated disease conditions other than spirochetes and flagellated bacterial infections interfere with the results [2]. This is of great concern for the diagnosis of patients with symptoms compatible with late LD who live in geographic areas in which LD is not known to be endemic. We were very surprised that Trevejo et al. [1] reported an EIA-screening specificity of 97%, which we believe was likely due to use of a small control group.

In a setting of unknown endemicity for LD, we carried out a large case-control study with subgroups including healthy and sick subjects. Tests with the same commercial polyvalent EIA (Vidas; BioMérieux Vitek, Hazelwood, MO) used by Trevejo et al. [1] resulted in negative findings for 167 (88%) of 189 subjects. Only 2 (9%) of 22 positive or equivocal EIAs were confirmed by Western blot. Major sources of interference included acute phase and asymptomatic infectious mononucleosis (specificity, 78%) and polyclonal hypergammaglobulinemia (specificity, 87.5%) [3]. Recently, in various acute infections of viral or bacterial etiology, we found a specificity of 78%. When we included the results for our case patients, 21 serum samples were unequivocally WB negative whereas 40 were either EIA positive (19 samples) or EIA equivocal (21 samples) (EIA vs. WB specificity, 47.5%). In the series presented by Trevejo et al. [1], the specificity of EIA versus WB was 75% in the early LD serum specimens but only 37% in the convalescent samples. In our experience, 22.5% of positive/equivocal EIA results were due to Epstein-Barr virus (EBV) viral capsid antigen (VCA) IgM interference. Thus, in view of the low (or perhaps absent) endemicity in our setting, we decided to routinely falsify positive/equivocal EIA tests with EBV VCA IgM, to reduce the waste of resources. Immunoblot confirmation was done only on specimens for which falsification failed. If the suspicion of LD is high, we suggest that WB should be postponed until the interfering factor is cleared, if this is possible; if it is not, an immunoblot, however performed, can be interpreted with the knowledge that an interfering factor is present. When possible, polymerase chain reaction using biologic fluids could provide a further interpretative key [4].

In our country the cost per specimen of the approach recommended by the Centers for Disease Control and Prevention, calculated for reagents only, is $84.70; the cost per specimen of the falsification approach is $9.12. Until better-standardized and less cross-reactive reagents are marketed [5, 6], the falsification approach (by EBV IgM in our study but perhaps by other approaches in different settings) allows cost savings and, most importantly, avoids laborious immunoblots—the results of which often cannot be interpreted—and, at least in part, misdiagnosis of LD.

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References

1. Trevejo RT, Krause PI, Sikand VK, et al. Evaluation of two-test serodiagnostic method for early Lyme disease in clinical practice. J Infect Dis **1999**;179: 931–8.
2. Golightly MG. Laboratory considerations in the diagnosis and management of Lyme borreliosis. Am J Clin Pathol **1993**;99:168–73.
3. Piras MA, Porqueddu EM, Porcu PL, Aceti A. Diagnosis, treatment and prevention of Lyme Disease. JAMA **1998**;280:1050–1.
4. Priem S, Ritting MG, Kamradt T, Burmester GR, Krause A. An optimized PCR leads to rapid and highly sensitive detection of *Borrelia burgdorferi* in patients with Lyme borreliosis. J Clin Microbiol **1997**;35:685–90.
5. Bakken LL, Case KL, Callister SM, Bourdeau NJ, Schell RF. Performance of 45 laboratories participating in a proficiency testing program for Lyme disease serology. JAMA **1992**;268:891–5.
6. Bakken LL, Callister SM, Wand PJ, Schell RF. Interlaboratory comparison of test results for detection of Lyme disease by 516 participants in the Wisconsin State Laboratory of Hygiene College of American Pathologists proficiency testing program. J Clin Microbiol **1997**;35:537–43.

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Reply

To the Editor—Piras and Aceti [1] point out some current limitations of Lyme disease (LD) diagnosis and associated laboratory serology—specifically, the uncertainty of serology in the absence of culture confirmation [2]. Although culture confirmation remains the reference standard for the diagnosis of LD, it is not typically used in a clinical setting.

Our study evaluated several serologic testing approaches among a patient population with a high pretest likelihood of infection with LD, based on residence in an area highly endemic for LD and the presence of a physician-diagnosed erythema migrans rash ⩾5 cm in diameter [3]. We agree that the simplest and least costly approach for a patient with a physician-diagnosed erythema migrans rash and endemic exposure is "treat, don't test." However, in primary care practice, many persons with this presentation are tested.

Comparative evaluation of serologic testing approaches among our study population demonstrated that the use of Western immunoblotting only as an arbiter of equivocal results from EIA tests of serum samples was more sensitive and less costly than the CDC-recommended approach of applying Western blot (WB) to EIA-positive as well as EIA-equivocal samples [4]. All approaches were highly specific and were based on testing of geographic controls (selected from an area highly endemic for LD, as were the patients) who did not have a history of LD or recent symptoms suggestive of LD. The question of cross-reactivity has been studied by others [5] and was not an objective of our study.

We continue to support the use of the CDC-recommended 2-test approach for the serodiagnosis of LD [4], because of the all-too-common practice of performing serologic testing for LD on patients with a low pretest probability of LD. For those patients with a low likelihood of LD, the utility of following EIA-positive results with WB has been demonstrated [5, 6].

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References

1. Piras MA, Aceti A. Preliminary falsification of EIA screening is cost-effective in the two-step serodiagnosis of Lyme disease [letter]. J Infect Dis **2000**; 181:802.
2. Trevejo RT, Krause PJ, Sikand VK, et al. Evaluation of two-test serodiagnostic method for early Lyme disease in clinical practice. J Infect Dis **1999**; 179: 931–8.
3. American College of Physicians. Guidelines for the laboratory evaluation in the diagnosis of Lyme disease. Ann Intern Med **1997**; 127:1106–8.
4. Centers for Disease Control and Prevention. Recommendations for test performance and interpretation from the Second National Conference on the Serologic Diagnosis of Lyme Disease. MMWR Morb Mortal Wkly Rep **1995**; 44:590–1.
5. Johnson BJ, Robbins KE, Bailey RE, et al. Serodiagnosis of Lyme disease: accuracy of a two-step approach using a flagella-based ELISA and immunoblotting. J Infect Dis **1996**; 174:346–53.
6. Dressler F, Whalen JA, Reinhardt BN, Steere AC. Western blotting in the serodiagnosis of Lyme disease. J Infect Dis **1993**; 167:392–400.

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TT Virus: Evidence for Transplacental Transmission

To the Editor—Epidemiology and pathogenic properties of the recently described TT virus (TTV) are under intensive scrutiny. There is little doubt that TTV is transmitted through blood and blood products, as shown by the high prevalence rates we and others have observed in polytransfused persons [1–3]. For example, in 660 persons tested by nested polymerase chain reaction (PCR), we found no specific pathologies that correlated with enhanced infection rates except in polytransfused hemophiliacs, who were 73% TTV positive versus 50% for the overall group [1]. Furthermore, by use of a real time PCR assay, which permits an accurate quantitative evaluation of viremia and a very efficient detection of TTV DNA, hemophiliacs exhibited the highest TTV loads when compared with healthy subjects and other patient groups (authors' unpublished data). On the other hand, there is also no doubt that the spread of TTV infection in the general population is much too great to be explained solely on the basis of transmission through blood. Indeed, prevalence rates of up to 90% have been observed, although extreme variation has been reported depending on geographic origin and, possibly of more importance, on the sensitivity of the detection method used [1, 3–5]. This has led to a search for additional routes of transmission. Thus, the demonstration that TTV is shed in stool suggests an important role for the orofecal route [6].

Evidence has also been sought for mother-to-child transmission. In a recent letter, Simmonds et al. [7] reported that all tested newborns aged 0–3 months were TTV-DNA negative [7]. Because 75% of children aged 3 months to 1 year were TTV positive, Simmonds et al. concluded that the infection is not acquired in utero but occurs early in postnatal life, possibly from environmental sources. However, our evidence differs. By using real time PCR as the detection method, we found TTV in the serum of 15 of 18 Italian mothers tested and in 12 of paired cord sera. Of importance, the virus loads in the cord were in the same range as those in the mothers (table 1), thus excluding the possibility that cord blood had been contaminated with maternal blood during collection and suggesting that TTV had entered the fetus well before delivery. The reasons for

Table 1. Presence and loads of TTV DNA in paired sets of maternal and cord sera, as determined by a sensitive real time polymerase chain reaction (PCR) assay.

	Maternal serum[a]		Cord serum	
Serum	TTV detection[b]	DNA copies/mL	TTV detection[b]	DNA copies/mL
1	−	$< 5 \times 10^2$	−	$< 5 \times 10^2$
2	−	$< 5 \times 10^2$	−	$< 5 \times 10^2$
3	−	$< 5 \times 10^2$	−	$< 5 \times 10^2$
4	+	4.8×10^6	−	$< 5 \times 10^2$
5	+	1.9×10^4	−	$< 5 \times 10^2$
6	+	1.0×10^4	−	$< 5 \times 10^2$
7	+	9.5×10^5	+	9.6×10^3
8	+	5.5×10^4	+	4.3×10^3
9	+	1.4×10^4	+	2.5×10^4
10	+	1.1×10^4	+	3.0×10^3
11	+	1.1×10^4	+	1.5×10^4
12	+	7.0×10^3	+	1.1×10^4
13	+	1.4×10^4	+	1.9×10^4
14	+	9.8×10^3	+	1.3×10^4
15	+	2.6×10^4	+	8.6×10^3
16	+	1.2×10^4	+	6.0×10^3
17	+	1.7×10^4	+	1.3×10^4
18	+	8.0×10^4	+	2.0×10^4

NOTE. Lower limit of sensitivity of PCR, 5.0×10^2 DNA copies/mL; dynamic range, $\geqslant$5 logs; interassay reproducibility, within 0.6 log. All maternal and cord sera were tested simultaneously in triplicate at least twice.
[a] Informed consent was obtained from all mothers.
[b] Positive samples were confirmed by amplification and sequencing of 175-bp fragment in open reading frame 1 of the TTV genome (nt 2281–2455).

the difference between our results and those of Simmonds et al. are unclear. It is possible that the exquisite sensitivity of our real time PCR permitted the demonstration of TTV levels that would have escaped detection by other methods. Alternatively, it is possible that, similar to observations for the human papillomaviruses and other viruses, variants or subspecies of TTV exist that present important biologic and epidemiologic differences. In any case, our data support the concept that TTV can cross the placenta and that transmission in utero is an important means of TTV diffusion.

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References

1. Maggi F, Fornai C, Morrica A, et al. High prevalence of TT virus viremia in Italian patients, regardless of age, clinical diagnosis, and previous interferon treatment. J Infect Dis **1999**;180:838–42.
2. Simmonds P, Davidson F, Lycett C, et al. Detection of a novel DNA virus (TTV) in blood donors and blood products. Lancet **1998**;352:191–5.
3. Leary TP, Erker JC, Chalmers ML, Desai SM, Mushahwar IK. Improved detection systems for TT virus reveal high prevalence in humans, non-human primates and farm animals. J Gen Virol **1999**;80:2115–20.
4. Takahashi K, Hoshino H, Ohta Y, Yoshida N, Mishiro S. Very high prevalence of TT virus (TTV) infection in general population of Japan revealed by a new set of PCR primers. Hepatol Res **1998**;12:233–9.
5. Okamoto H, Takahashi M, Nishizawa T, et al. Marked genomic heterogeneity and frequent mixed infection of TT virus demonstrated by PCR with primers from coding and noncoding regions. Virology **1999**;259:428–36.
6. Okamoto H, Akahane Y, Ukita M, et al. Fecal excretion of a nonenveloped DNA virus (TTV) associated with posttransfusion non–A-G hepatitis. J Med Virol **1998**;56:128–32.
7. Simmonds P, Prescott LE, Logue C, Davidson F, Thomas AE, Ludlam CA. TT virus—part of the normal human flora? [letter]. J Infect Dis **1999**;180:1748–9.

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Neglected Opportunities

To the Editor—How would the press, politicians, and funding agencies react to evidence that a new vaccination strategy reduced human immunodeficiency virus (HIV) transmission by 75%, required only a single priming dose and no boosters, was expected to be active against diverse HIV strains, and could be made available immediately at minimal cost? We would expect a flurry of press attention and editorial comment and calls for an emergency program to implement the strategy in the shortest possible time.

Such a report was recently published by Lavreys et al. [1], but the "vaccination strategy" was not an injection but male circumcision. In this prospective cohort study, uncircumcised status was an independent risk factor for HIV transmission (hazard rate ratio, 4.0) in a multivariate analysis that controlled for confounding demographic and behavioral factors. However, this important and well-designed study, which overcame major limitations of previous studies, has thus far sparked little notice. In this it is similar to previous studies of circumcision and transmission of HIV/sexually transmitted disease, most of which, although less rigorously controlled and mostly retrospective, reached similar conclusions.

Lavreys et al. [1] convincingly argue that the accumulated epidemiologic evidence and the biologic plausibility are strong enough to justify implementation of the strategy now, pending more data on acceptability. For those who remain unconvinced, a randomized controlled trial of adult circumcision is entirely feasible and could be rapidly undertaken by skeptics.

It is worth asking, however, why this promising strategy has been investigated at such a leisurely pace (as pointed out by Lavreys et al., 150 years and counting) and why, even now, it has failed to capture the imagination or enthusiasm of more than a handful of investigators. Understanding the factors that

have obstructed attention to this potential opportunity may allow us to develop this and other inadequately explored preventive options more effectively in the future.

Possible explanations include the low-tech and decidedly nonnovel nature of the intervention; distaste for the procedure; the momentum of a decades-long swing of pediatrician and parent opinion away from circumcision as a painful procedure with insufficient documented medical benefits; a perceived inconsistency in the promotion or even the study of male circumcision, while decrying female "circumcision" (actually clitorectomy); the false assumption that implementation would necessarily require neonatal circumcision and, thus, would take decades to accrue a benefit; and the unproven assumption that attitudes toward circumcision are immutable consequences of religion and culture. Neglecting pursuit of this opportunity on the basis of any of these reasons is unscientific and regrettable.

HIV-prevention efforts have been largely directed toward promotion of condoms and the search for a vaccine. After nearly 2 decades, a vaccine still appears to be far off, and, although condoms are an important tool for HIV prevention, the epidemic has nonetheless continued its steady expansion. The enormous benefits of a vaccine, should one prove feasible, and the proven efficacy of condoms, when people are willing to use them, should not blind us to opportunities to explore and implement alternative means of HIV prevention.

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References

1. Lavreys L, Rakwar JP, Thompson ML, et al. Effect of circumcision on incidence of human immunodeficiency virus type 1 and other sexually transmitted diseases: a prospective cohort study of trucking company employees in Kenya. J Infect Dis **1999**; 180:330–6.

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Helicobacter pylori, Lifestyle Risk Factors, and Adenocarcinoma of the Esophagus

To the Editor—We read with interest the article by Blaser [1], in which a compelling argument is made to link the continuing decline in *Helicobacter pylori* infection rates to increases in gastroesophageal reflux disease, Barrett's esophagus, and esophageal adenocarcinoma. The hypothesis is consistent with data indicating lower adenocarcinoma rates, despite higher squamous rates, among US blacks (table 1) [2] and the report of significantly higher *H. pylori* infection rates in blacks (70%) than in whites (34%) in an asymptomatic US population [3]. Alternative explanations for lower adenocarcinoma rates in blacks, such as lower consumption of alcohol and tobacco products or lower prevalence of obesity, a factor associated with increased risk of adenocarcinoma [4], are less plausible, given substantially higher rates for squamous cell carcinoma of the esophagus in blacks and similar levels of obesity in the two populations [5].

Table 1. Esophageal cancer incidence rates (SEER data, 1988–1992).

Race	Squamous cell		Adenocarcinoma		Ratio (male/female)
	Male	Female	Male	Female	
White	1.90	0.88	2.14	0.22	9.73
Black	12.36	3.48	0.47	0.17	2.76
Ratio	0.15	0.25	4.55	1.29	

NOTE. Incidence rates are per 100,000 person-years and are age standardized to the world population. SEER is the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute of the United States.

Although the accumulating evidence suggests that *H. pylori* is protective against esophageal adenocarcinoma, data in table 1 indicate that this is only part of the picture. The male/female adenocarcinoma rate ratios (whites, 9.73; blacks, 2.76) are higher than the white/black rate ratios (males, 4.55; females, 1.29), despite similar *H. pylori* infection rates in men and women [3]. Thus, although lower *H. pylori* infection rates may explain higher adenocarcinoma rates in whites, they cannot explain substantially higher rates in men.

The etiology of esophageal adenocarcinoma remains to be explained. The existing evidence suggests that obesity [4] and gastroesophageal reflux disease [6] may be important risk factors. The prevalence of obesity has increased in line with esophageal adenocarcinoma rates over the past few decades and now exceeds 50% in many North American and European populations [5]. In addition, alcohol and tobacco are established risk factors for squamous cell carcinoma of the esophagus and may help to explain substantially higher male adenocarcinoma rates. Although infection with *H. pylori* may significantly reduce the risk of esophageal adenocarcinoma, an excess risk is likely to persist in those with predisposing lifestyle risk factors, such as overweight persons who smoke. Due consideration of these risk factors is clearly warranted.

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References

1. Blaser MJ. Hypothesis: the changing relationships of *Helicobacter pylori* and humans: implications for health and disease. J Infect Dis **1999**; 179:1523–30.
2. Parkin DM, Whelan SL, Ferlay J, Raymond L, Young J. Cancer incidence in five continents. Vol VII. Lyon, France: International Agency for Research on Cancer, **1997** (publication no. 143).
3. Graham DY, Malaty HM, Evans DG, Evans DJ Jr, Klein PD, Adam E.

Epidemiology of *Helicobacter pylori* in an asymptomatic population in the United States. Gastroenterology **1991**; 100:1495–501.
4. Chow WH, Blot WJ, Vaughan TL, et al. Body mass index and risk of adenocarcinoma of the esophagus and gastric cardia. J Natl Cancer Inst **1998**; 90:150–5.
5. Kuczmarski RJ, Flegal KM, Campbell SM, Johnson CL. Increasing prevalence of overweight among US adults: the National Health and Nutrition Examination Surveys, 1960 to 1991. JAMA **1994**; 272:205–11.
6. Lagergren J, Bergstrom R, Lindgren A, Nyren O. Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. N Engl J Med **1999**; 340:825–31.

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Reply

To the Editor—Maric and Cheng [1] make important points about the epidemiology of adenocarcinoma of the esophagus by comparing rates among US blacks and whites and among males and females. Essentially all diseases have multifactorial causes; the challenge is to identify those characteristics that are associated with the highest risk of disease development. Over the past 25 years, adenocarcinoma of the esophagus has been rapidly increasing among persons in developing countries [2]. When disease rates change rapidly, environmental factors usually play the critical role. The fact that the increase in esophageal adenocarcinomas is disproportionate in different ethnic groups and among men, compared with that among women, implies differential exposure to the offending factor(s). As Maric and Cheng indicate, differences in body mass index are clearly one part of the puzzle.

The rates for many diseases differ substantially for men and women. Despite nearly similar rates of exposure to *Helicobacter pylori,* duodenal ulcer rates are 2–4-fold higher among men than among women, a differential that cannot be entirely explained by known risk factors such as smoking. For noncardia gastric cancer, another disease associated with *H. pylori,* rates among men also are substantially greater than those among women [3]. However, analysis of incidence data indicates that rates among both men and women rise appreciably with age; the age at which the big increase begins in women is shifted ~10 years later than that for men, and the slopes of the 2 curves after the 10-year shift are nearly identical [4]. Thus, the differential in disease rates can be largely explained by a protective factor that women have for ~10 years longer than men. A similar phenomenon has been observed for atherosclerotic heart disease (ASHD), and most evidence suggests that female hormones are involved in the protective effect. ASHD rates increase substantially among women after menopause, in essence paralleling trends among men. The rise in esophageal adenocarcinomas is relatively recent, and the numbers are not yet large; however, in the future, analysis of age-specific rates by sex may be useful.

Regardless of these considerations, it now has become clear that gastroesophageal reflux disease (GERD) is the most important risk factor for the development of esophageal adenocarcinoma [5] and that GERD is becoming more prevalent [6]. It is critically important to identify the triggers for this phenomenon; my hypothesis is that the lack of *cag*$^{+}$ *H. pylori* strains (which I term "acagia") is a major risk factor for development of Barrett's esophagus and subsequent adenocarcinoma.

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References

1. Maric RN, Cheng KK. *Helicobacter pylori,* lifestyle risk factors, and adenocarcinoma of the esophagus [letter]. J Infect Dis **2000**; 181:805–6.
2. Devesa SS, Blot WJ, Fraumeni JR. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. Cancer **1998**; 83: 2049–53.
3. International Agency for Research of Cancer. Monographs on the evaluation of carcinogenic risks to humans: infection with *Helicobacter pylori.* Lyon, France: IARC, **1994**; 60:177–240.
4. Sipponen P, Hyvarinen H, Seppala K, Blaser MJ. Review article: pathogenesis of the transformation from gastritis to malignancy. Aliment Pharmacol Ther **1998**; 12(Suppl 1):61–72.
5. Lagergren J, Bergstrom R, Lingren A, Nyren O. Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. N Engl J Med **1999**; 340:825–31.
6. El-Serag HB, Sonnenberg A. Opposing time trends of peptic ulcer and reflux disease. Gut **1998**; 43:327–33.

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Reemergence of Invasive *Haemophilus influenzae* Type b Disease in Alaska: Is It Because of Vaccination with Polyribosylribitol Phosphate Outer Membrane Protein Complex (PRP-OMPC) or Failure to Vaccinate with PRP-OMPC?

To the Editor—We read with interest the article by Galil et al. [1] on the reemergence of invasive *Haemophilus influenzae* type b (Hib) disease in a well-vaccinated population in remote Alaska. The authors describe a dramatic decline in cases of invasive Hib disease among Alaska Natives following near-exclusive vaccination with the Hib conjugate vaccine polyribosylribitol phosphate outer membrane protein complex

(PRP-OMPC; Merck, West Point, PA), from January 1991 until December 1995. Following a change in Hib vaccine regimen to DTP-HbOC (diphtheria and tetanus toxoids and pertussis vaccine adsorbed and Hib conjugate vaccine [diphtheria CRM_{197} protein conjugate]; American Home Products, Pearl River, NY), in January 1996, 10 cases of invasive disease were reported during the next 12 months, prompting a 15-day cross-sectional study of oropharyngeal Hib carriage in children aged 1–5 years during the spring of 1997. The intensity of Hib carriage (e.g., the number of colonies per plate) was not quantified. Galil et al. conclude that "cases of invasive disease increased, suggesting on-going Hib transmission despite widespread vaccination.... Widespread vaccination with PRP-OMP[C] Hib conjugate vaccine did not eliminate carriage in this population" [1, p. 101]. We have a number of concerns about the authors' interpretation of their results.

Pharyngeal colonization with Hib, its role in disease transmission, and the effect of vaccination on colonization remain poorly understood. Barbour [2] found Hib carriage in 8% of children 4 years after vaccination with HbOC, compared with 5% in unvaccinated children. Among Hib carriers, an inverse relationship between Hib colony count and anti-PRP concentration was found: all vaccinated Hib carriers had very low Hib colony counts. In another study, Barbour et al. [3] noted that both the mechanism and the duration of conjugate vaccine inhibition of Hib colonization are uncertain. Hall et al. [4] studied Hib carriage in Alaska Natives in 1982–1983 and found a colonization level of 6.8% despite historically very high rates of invasive Hib disease. They concluded that "mechanisms for increased exposure which would not be reflected in high carriage rates may exist for these young children" [4, p. 1190]. Furthermore, the Galil study found no differences in the prevalence of colonization between villages with and without recent cases of invasive Hib disease that would explain the outbreak cases.

A potential misinterpretation of the study would be that the elimination of Hib carriage is primarily dependent on the choice of Hib vaccine. As Galil et al. [1] suggest, other factors may have contributed to continuing Hib carriage in this population of Alaska Natives.

Never-vaccinated children, older than the population studied by Galil et al., are recognized as a source of nasopharyngeal Hib carriage [5]. Although 97% of the population surveyed had received $\geqslant 3$ doses of a Hib conjugate vaccine, no vaccine coverage information was available for children in the remaining 46 villages. Given that a convenience sample was used, it is likely that the villages chosen for the carriage survey also were more receptive to vaccination campaigns.

If age-appropriate Hib vaccination coverage were variable or if older never-vaccinated children were found to carry Hib, sources of Hib transmission could persist. In an environment of incomplete coverage, it would be impossible to definitively assess the impact of Hib vaccination on Hib carriage, because pockets of unvaccinated children could serve as reservoirs for reintroducing the organism. These populations may have served as the source of Hib transmission to young infants susceptible by virtue of vaccination with DTP-HbOC, the "unmasking effect" described by the authors.

PRP-OMPC is highly effective. In Israel, the efficacy and effectiveness of the vaccine were 98.7% and 94.9%, respectively, and cases of disease in infants <3 months old (prior to the age when one can expect a direct protective effect of vaccine) dropped sharply, consistent with vaccine-induced reduction in pharyngeal Hib carriage (herd immunity) [6]. Among the Navajo in the southwestern United States, a population similar in Hib disease incidence and risk factors to the Alaska Natives, PRP-OMPC had >95% efficacy against invasive disease in infants aged 2–18 months after 2 doses of vaccine [7].

Although the study by Barbour et al. [3] demonstrated persistent pharyngeal Hib carriage following vaccination with HbOC, several studies have documented a reduction in Hib carriage levels following vaccination with Hib conjugate vaccines [8–10]. However, he effect of Hib conjugate vaccines on carriage may vary substantially among populations with high and low rates of invasive Hib disease incidence [11]. The study by Galil et al. was conducted in a population that experienced one of the highest reported incidence rates of invasive Hib disease in the prevaccine era [12]. The only other published study on the effect of any Hib conjugate vaccine on carriage in a population with a high incidence of invasive Hib disease found that pharyngeal carriage in a Navajo population vaccinated with PRP-OMPC decreased from 4.8% to 2.3% [13].

The report by Galil et al. [1] underscores the need for further investigation of the role of pharyngeal colonization in the transmission of invasive Hib disease as well as of the effect of Hib vaccination on carriage. We agree that early seroprotection, high levels of vaccine coverage, and continued disease surveillance are crucial to attaining the goal of invasive Hib disease eradication.

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References

1. Galil K, Singleton R, Levine OS, et al. Reemergence of invasive *Haemophilus influenzae* type b disease in a well-vaccinated population in remote Alaska. J Infect Dis **1999**; 179:101–6.
2. Barbour ML. Conjugate vaccines and the carriage of *Haemophilus influenzae* type b. Emerg Infect Dis **1996**; 2:176–82.
3. Barbour ML, Body R, Crook DWM, et al. *Haemophilus influenzae* type b carriage and immunity four years after receiving the *Haemophilus influenzae* oligosaccharide-CRM_{197} (HbOC) conjugate vaccine. Pediatr Infect Dis J **1993**; 12:478–84.
4. Hall DB, Lum MKW, Knutson LR, et al. Pharyngeal carriage and acquisition

of anticapsular antibody to *Haemophilus influenzae* type b in a high-risk population in southwestern Alaska. Am J Epidemol **1987**;126:1190–7.
5. Kuroki H, Ishikawa N, Uehara S, et al. Nasopharyngeal colonization with *Haemophilus influenzae* type b among infants and children in Japan. Acta Paediatr Jpn **1997**;39:541–5.
6. Dagan R, Fraser D, Roitman M, et al. Effectiveness of a nationwide infant immunization program against *Haemophilus influenzae* b. Vaccine **1999**; 17:134–41.
7. Santosham M, Wolff M, Reid R, et al. The efficacy in Navajo infants of a conjugate vaccine consisting of *Haemophilus influenzae* type b polysaccharide and *Neisseria meningitidis* outer membrane protein complex. N Engl J Med **1991**;324:1767–72.
8. Barbour ML, Mayon-White RT, Coles C, et al. The impact of conjugate vaccine on carriage of *Haemophilus influenzae* type b. J Infect Dis **1995**; 171:93–8.
9. Mohle-Boetani JC, Ajello G, Breneman E, et al. Carriage of *Haemophilus influenzae* type b in children after widespread vaccination with conjugate *Haemophilus influenzae* type b vaccines. Pediatr Infect Dis J **1993**;12: 589–93.
10. Takala AK, Eskola J, Leinonen M, et al. Reduction of oropharyngeal carriage of *Haemophilus influenzae* type b (Hib) in children immunized with an Hib conjugate vaccine. J Infect Dis **1991**;164:982–6.
11. Ward J. Prevention of invasive *Haemophilus influenzae* type b disease: lessons from vaccine efficacy trials. Vaccine **1991**;9(Suppl):S17–25.
12. Ward JI, Lum MK, Hall DB, et al. Invasive *Haemophilus influenzae* type b disease in Alaska: background epidemiology for a vaccine efficacy trial. J Infect Dis **1986**;153:17–26.
13. Takala AK, Santosham M, Almeido-Hill J, et al. Vaccination with *Haemophilus influenzae* type b meningococcal protein conjugate vaccine reduces oropharyngeal carriage of *Haemophilus influenzae* type b among American Indian children. Pediatr Infect Dis J **1993**;12:593–9.

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Reply

To the Editor—We thank Dargan et al. [1] for their interest in our recent report of the reemergence of invasive *Haemophilus influenzae* type b (Hib) disease in rural Alaska Native infants. We appreciate their support for the key messages of our study, specifically the need for early protection in this population, high levels of vaccination coverage, continued surveillance for invasive Hib disease, and further investigation of the epidemiology of pharyngeal colonization, including the effect of Hib vaccination on carriage. The results of this study were instrumental in reestablishing polyribosylribitol phosphate *Neisseria meningitidis* outer membrane protein (PRP-OMP) vaccine as the Hib conjugate vaccine for the first dose of the Hib vaccination program in Alaska. The resumption of the use of PRP-OMP for the first dose has led to a return to lower rates of infant Hib disease in rural Alaska Natives, an indication of the unique immunologic features of this vaccine.

As Dargan et al. [1] note, our knowledge of the effect of Hib conjugate vaccination on colonization is incomplete, and the relationship between colonization and disease transmission remains unclear; however, we will clarify several points raised by their letter. The study of the effect of PRP-OMP vaccine on colonization in the Navajo population is not the only study of the effect of Hib vaccination on colonization in a high-incidence population [2]. Adegbola et al. [3], in a randomized, controlled trial of the impact of PRP–tetanus toxoid Hib conjugate vaccine on oropharyngeal carriage in The Gambia, found a 60% reduction in the prevalence of carriage (11.0% among unvaccinated children vs. 4.4% among fully vaccinated children). In addition, persistent Hib carriage has been documented in Australian Aboriginal children, despite routine vaccination with PRP-OMP vaccine; this population also experienced a high incidence of Hib disease and an epidemiologic pattern similar to that observed in rural Alaska Natives [4]. Dargan et al. note that the study by Barbour et al. [5] showed no difference in Hib colonization 4 years after immunization with *H. influenzae* oligosaccharide-CRM_{197} (HbOC) [5]. However, this study included just 60 infants who had received HbOC in an immunogenicity trial 4 years earlier and were living in a general population of unimmunized children, not a setting in which herd immunity would be expected. The more pertinent comparison is with studies of Hib colonization following widespread vaccination of the entire population. These studies convincingly document a substantial reduction in Hib colonization and provide the basis for understanding the herd immunity that occurs following widespread immunization [6–8].

Dargan et al. [1] accurately point out that the contribution of Hib colonization in older children and adults to transmission is poorly understood. The influence of vaccination coverage rates and the regimen and vaccine used is likewise unclear. We should clarify that infants in rural Alaska are routinely vaccinated during well-child care visits and itinerant public health nurse visits, not through vaccination campaigns, as Dargan et al. suggest, and that immunization coverage levels in Alaska, including rural Alaska Native populations, are as high as, or higher than, the national average. In general, by 2 years of age >90% of Alaska Native children, including those in the villages in our study, have received ⩾3 doses of Hib conjugate vaccines. Therefore, low vaccination coverage rates are not likely to explain our observations.

Further research into the factors that contribute to the elimination of Hib colonization in various populations is urgently needed. At this time, the role of factors such as carriage in older age groups, the intensity of colonization and transmission, the vaccination schedule, and the vaccine used in Alaska is not clearly elucidated. We agree that it would be a misinterpretation of the results of this study to suggest that we would not have observed continued colonization had another Hib conjugate vaccine been used. To address the remaining questions, we have initiated several other studies that we hope will clarify the relative importance of these various factors and lead to improved strategies for elimination of Hib disease in Alaska and elsewhere.

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References

1. Dargan JM, Coplan PM, Kaplan KM, Nikas A. Reemergence of invasive *Haemophilus influenzae* type b disease in Alaska: is it because of vaccination with polyribosylribitol phosphate outer membrane protein complex (PRP-OMPC) or failure to vaccinate with PRP-OMPC? [letter]. J Infect Dis **2000**;181:806–8.
2. Takala AK, Santosham M, Almeido-Hill J, et al. Vaccination with *Haemophilus influenzae* type b meningococcal protein conjugate vaccine reduces oropharyngeal carriage of *Haemophilus influenzae* type b among American Indian children. Pediatr Infect Dis J **1993**;12:593–9.
3. Adegbola RA, Mulholland EK, Secka O, Jaffar S, Greenwood BM. Vaccination with a *Haemophilus influenzae* type b conjugate vaccine reduces oropharyngeal carriage of *H. influenzae* type b among Gambian children. J Infect Dis **1998**;177:1758–61.
4. Smith-Vaughan HC, Priprakash KS, Leach AJ, Mathews JD, Kemp DJ. Low genetic diversity of *Haemophilus influenzae* type b compared to nonencapsulated *H. influenzae* in a population in which *H. influenzae* is highly endemic. Infect Immun **1998**;66:3403–9.
5. Barbour ML, Booy R, Crook DW, et al. *Haemophilus influenzae* type b carriage and immunity four years after receiving the *Haemophilus influenzae* oligosaccharide-CRM_{197} (HbOC) conjugate vaccine. Pediatr Infect Dis J **1993**;12:478–84.
6. Takala AK, Eskola J, Leinonen M, et al. Reduction of oropharyngeal carriage of *Haemophilus influenzae* type b (Hib) in children immunized with an Hib conjugate vaccine. J Infect Dis **1991**;164:982–6.
7. Mohle-Boetani JC, Ajello G, Breneman E, et al. Carriage of *Haemophilus influenzae* type b in children after widespread vaccination with conjugate *Haemophilus influenzae* type b vaccines. Pediatr Infect Dis J **1993**;12:589–93.
8. Jonsdottir KE, Steingrimsson O, Olafsson O. Immunisation of infants in Iceland against *Haemophilus influenzae* type b. Lancet **1992**;340:252–3.

The opinions expressed in this paper are those of the authors and do not necessarily reflect the views of the Indian Health Service.

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Mortality in Serologically Unconfirmed Mediterranean Spotted Fever

To the Editor—I read with interest the article by Paddock et al. [1], who described the detection of occult mortality due to Rocky Mountain spotted fever (RMSF) by demonstrating *Rickettsia rickettsii* antigens or DNA in blood and tissues [1]. In Mediterranean countries, including Israel, spotted fever is caused by members of the *R. conorii* complex, which are antigenically related to *R. rickettsii.* The clinical course of Mediterranean spotted fever (MSF), however, is milder than that of RMSF, especially in children, and the fatality rate is considered to be <5% [2]. In 1993, Wolach and I [3] reported fatal MSF in 3 Israeli children who had presented with a febrile disease lasting 5–7 days, septic shock, mental changes, hyponatremia, and bleeding tendency. A rash, macular or purpuric, was present in 2 children and absent in the third. A presumptive diagnosis of meningococcemia or sepsis of unknown origin was entertained. The 3 children were empirically treated with β-lactam antibiotics and aggressive supportive therapy but died within 24 h of admission. The diagnosis of MSF was confirmed in 2 patients by cell culture and by animal inoculation in the third. Antibodies to spotted fever–group rickettsiae, as determined by microimmunofluorescence, were negative in 2 children, whereas a borderline titer of 1 : 80 was found in 1.

Early administration of specific therapy with tetracyclines or chloramphenicol significantly decreases mortality due to rickettsial infections [4]. Institution of such therapy, however, requires that the rickettsial etiology of the illness be suspected on clinical and/or epidemiologic grounds, because these antimicrobial drugs are not usually given empirically to febrile patients. In addition, pediatricians frequently hesitate to start therapy with tetracycline in febrile young children without a definitive proof of a rickettsial diagnosis, because of the potential risk of teeth staining. Usually the diagnosis of rickettsioses relies on serologic tests, whereas culture of the organism and polymerase chain reaction methods are not routinely used or universally available. The series of patients reported by Paddock et al. [1] and our 3 patients clearly show that serologic tests may be unreliable for diagnosing or excluding rickettsial infections, especially in persons presenting with fulminant disease. Had specific isolation techniques for rickettsiae not been attempted, the diagnosis would have been missed in our 3 patients, and their deaths would have been attributed to infections caused by unidentified pathogens. Thus, it appears that, when alternative methods for confirming the disease are not used, the diagnosis of MSF may be missed by serology, resulting in underestimation of the true case/fatality rate of the infection. Because MSF may follow an unpredictable, rapid fatal course similar to that of RMSF, it seems prudent to advise prompt administration of empirical tetracycline therapy whenever the diagnosis is suspected.

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References

1. Paddock CD, Greer PW, Farebee TL, et al. Hidden mortality attributable to Rocky Mountain spotted fever: immunohistochemical detection of fatal, serologically unconfirmed disease. J Infect Dis **1999**;179:1469–76.
2. Raoult D, Weiller PJ, Chagnon A, Claudet H, Gallais H, Casnova P. Medi-

terranean spotted fever: clinical, laboratory and epidemiological features of 199 cases. Am J Trop Med Hyg **1986**; 35:845–50.
3. Yagupsky P, Wolach B. Fatal Israeli spotted fever in children. Clin Infect Dis **1993**; 17:850–3.
4. Woodward TE. Rocky Mountain spotted fever: epidemiological and early clinical signs are keys to treatment and reduced mortality. J Infect Dis **1984**; 150:465–8.

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Reply

To the Editor—We appreciate the comments by Yagupsky [1], which underscore several of the salient features of spotted fever–group rickettsial infections that we emphasized in our report [2]. For patients, diagnosticians, and clinicians faced with the potentially tragic consequences of Rocky Mountain spotted fever or severe Mediterranean spotted fever (MSF), these points merit repeating. First, the nonspecific signs and symptoms early in the course of rickettsial infections mimic many other infectious and noninfectious syndromes. Second, the diagnostic challenges posed by these diseases are compounded by the lack of rapid and widely available confirmatory tests early in the course of the illnesses. Finally, spotted fevers have the potential to kill otherwise healthy persons in <1 week after onset of symptoms, emphasizing the need for early administration of appropriate therapy.

The immunohistochemical (IHC) test described in our report uses an antibody that reacts with multiple spotted fever–group rickettsiae, including members of the *Rickettsia conorii* complex that causes MSF. In this context, we have used this assay to diagnose previously unexplained fatal illnesses caused by spotted fever–group rickettsiae other than *R. rickettsii.* Our experience reinforces the comments of Yagupsky [1] and includes confirmation of fatal spotted fever infections in 2 adult Israeli patients for whom serologic evidence of MSF was lacking. The first patient was a 31-year-old woman who died 6 days after onset of an illness characterized by fever, myalgias, headache, and respiratory insufficiency. The second patient was a 38-year-old man who died 7 days after being hospitalized for fever and vomiting. Both patients developed thrombocytopenia and petechial or purpuric rashes over the course of their illnesses. Similar to 2 of the pediatric patients described in the earlier report by Yagupsky and Wolach [3], neither of these 2 patients demonstrated diagnostic levels of antibody reactive with spotted fever–group rickettsiae when serum samples were tested by use of an indirect immunofluorescence assay. However, tissues from both patients obtained at autopsy demonstrated microscopic lesions consistent with histopathologic findings of fatal MSF [4], and IHC staining for spotted fever–group rickettsiae revealed abundant rickettsial antigens and intact rickettsiae in and around blood vessels and within reticuloendothelial cells (figure 1).

We agree with Yagupsky's recommendation to administer tetracycline antimicrobials (preferably doxycycline) to children of any age when spotted fever–group rickettsioses are considered in the differential diagnosis. The recognized propensity of tetracyclines to bind to dental enamel should not dissuade physicians from using the most effective drug for the treatment of these potentially life-threatening infections. The decision to use doxycycline in young children is never made casually, and the rationale for this choice of therapy should be discussed with the child's parents [5]. However, it should be recognized that a single short course (i.e., 5–7 days) of doxycycline should not result in cosmetically significant staining of teeth [6, 7]. For patients with Rocky Mountain spotted fever or severe MSF, there is a relatively narrow window of time during which effective antibiotic therapy dramatically reduces the risk of death [8]. Since laboratory tests available to most physicians do not assist in early diagnosis, initiation of therapy should be based on clinical, and especially epidemiologic, findings (e.g., unexplained febrile illness associated with known tick bite or tick exposure or unexplained fever with thrombocytopenia, rash, or headache occurring during spring or summer months in an area where the disease is endemic).

Isolation of *R. conorii* from patients' blood and tissues confirmed the diagnosis of spotted fever for each of the 3 pediatric patients described by Yagupsky and Wolach [3] and reflects the clinical acumen of these investigators. Although isolation is the reference standard for diagnosis, culture is seldom attempted during the acute phase of illness, even when a rickettsial infection is suspected, and cannot be performed retrospectively on autopsy specimens unless samples were appropriately collected and stored (e.g., frozen at −70°C). Biosafety level 3 practices and facilities are recommended if culture of spotted fever–group rickettsiae is attempted [9]. In contrast, IHC testing provides a method for retrospective confirmatory diagnosis on archived samples months or even years after the illness. In this context, IHC is a versatile technique that can help resolve problematic issues relating to the diagnosis of otherwise unexplained illnesses and provide new insights into the clinical course and epidemiologic features of these diseases [10].

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References

1. Yagupsky P. Mortality in serologically unconfirmed Mediterranean spotted fever [letter]. J Infect Dis **2000**; 181:809–10.
2. Paddock CD, Greer PW, Ferebee TL, et al. Hidden mortality attributable to Rocky Mountain spotted fever: immunohistochemical detection of fatal, serologically unconfirmed disease. J Infect Dis **1999**; 179:1469–76.
3. Yagupsky P, Wolach B. Fatal Israeli spotted fever in children. Clin Infect Dis **1993**; 17:850–3.

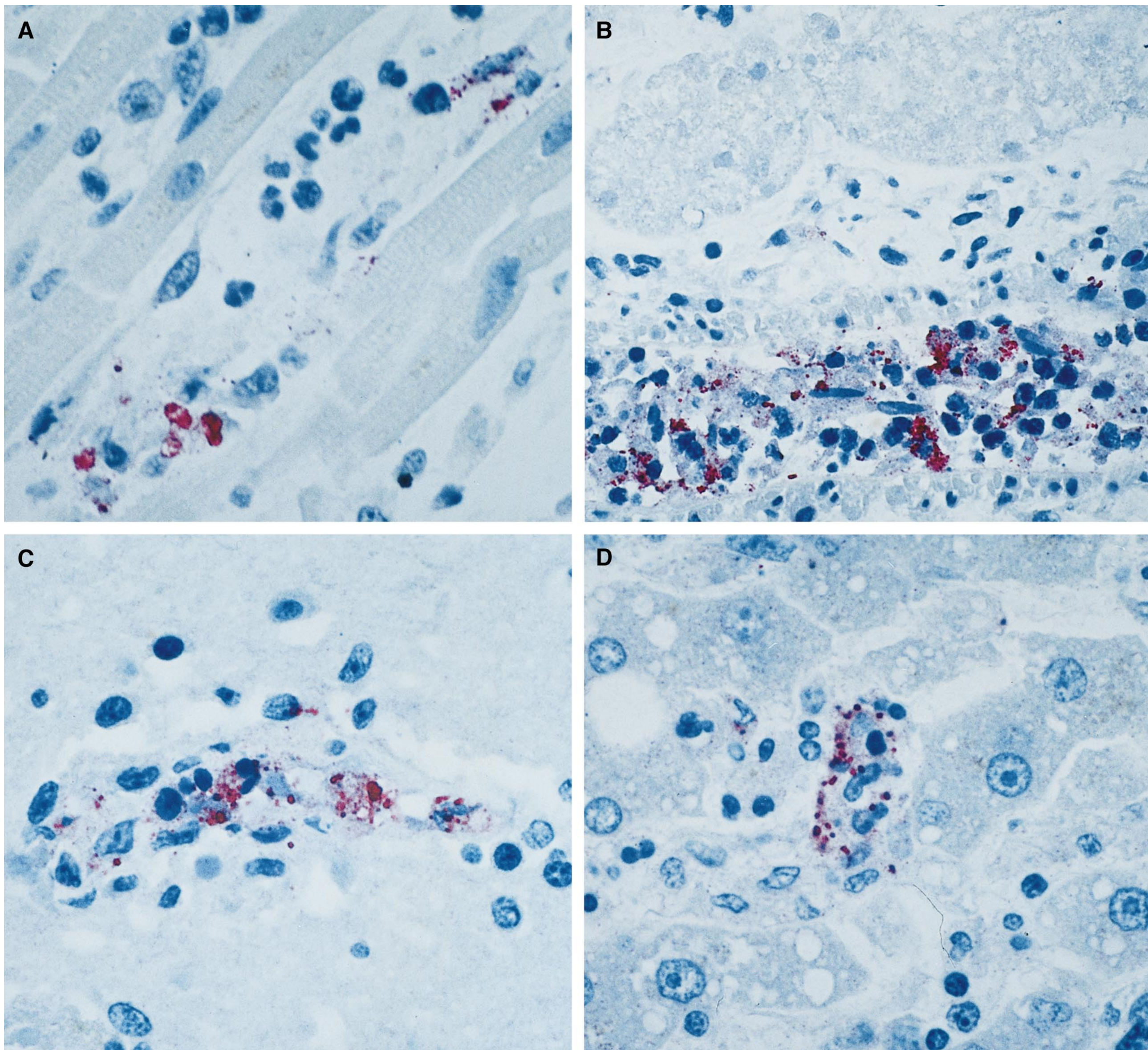

Figure 1. Immunohistochemical localization of spotted fever–group rickettsial antigens in tissues of patients with fatal spotted fever, by immunoalkaline phosphatase stain with naphthol phosphate–fast red substrate and hematoxylin counterstain. *A,* Rickettsiae and rickettsial antigens associated with damaged endothelium in myocardial interstitium (patient 1); original magnification, ×158. *B,* Abundant rickettsial antigens within denuded intravascular endothelial cells in small vessel in kidney (patient 1); original magnification, ×100. *C,* Rickettsial nodule in cerebral cortex (patient 1); original magnification, ×158. *D,* Spotted fever–group rickettsiae and rickettsial antigens in cytoplasms of Kupffer cells in liver (patient 2); original magnification, ×158.

4. Walker DH, Herrero-Herrero JI, Ruiz-Beltrán R, Bullón-Sopelana A, Ramos-Hildago A. The pathology of fatal Mediterranean spotted fever. Am J Clin Pathol **1987**; 87:669–72.
5. Abramson JS, Givner LB. Should tetracycline be contraindicated for therapy of presumed Rocky Mountain spotted fever in children less than 9 years of age? Pediatrics **1990**; 86:123–4.
6. Grossman ER, Walchek A, Freedman H, Flanagan C. Tetracyclines and permanent teeth: the relation between dose and tooth color. Pediatrics **1971**; 47:567–70.
7. Cale DF, McCarthy MW. Treatment of Rocky Mountain spotted fever in children. Ann Pharmacother **1997**; 31:492–4.
8. Kirkland KB, Wilkinson WE, Sexton DJ. Therapeutic delay and mortality in cases of Rocky Mountain spotted fever. Clin Infect Dis **1995**; 20: 1118–21.
9. Richmond JY, McKinney RW, eds. Rickettsial agents. In: Biosafety in microbiological and biomedical laboratories. 4th ed. Washington, DC: US Government Printing Office, **1999**:148–52.
10. Zaki SR, Paddock CD. The emerging role of pathology in infectious diseases. In: Scheld WM, Craig WA, Hughes JM, eds. Emerging infections 3. Washington, DC: American Society for Microbiology Press, **1999**:181–200.

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Oropharyngeal *Candida* Colonization and Human Immunodeficiency Virus Type 1 Infection

To the Editor—Gottfredsson et al. [1], in a cross-sectional study of oropharyngeal *Candida* colonization in 83 patients with human immunodeficiency virus type 1 (HIV-1) infection, reported that only plasma HIV-1 load was predictive of *Candida* load; neither CD4 lymphocyte level nor use of antiretroviral therapy was associated with colonization [1]. They hypothesized that high levels of HIV-1 may suppress local mucosal immune responses, thereby permitting increased *Candida* colonization, and speculated that control of HIV-1 replication may restore local immune function. Their findings certainly are consistent with these possibilities. However, there are at least 2 alternative explanations that might account for the interesting findings.

Cross-sectional studies do not provide information about causality for any associations found. However, even if high levels of HIV-1 do cause increased *Candida* colonization, suppressed local mucosal immunity is not the only possible mechanism that might explain such an occurrence. It was recently shown in vitro that HIV-1 glycoproteins may promote the virulence of *Candida albicans* [2]. Thus, increased local HIV-1 replication might have a direct effect on increased *Candida* colonization, without necessarily requiring the presence of defects in local immune function. Furthermore, such a mechanism need not imply disassociation of HIV-1 effects on local mucosal immunity from those on CD4 lymphocyte counts, as would be suggested by a better correlation of *Candida* colonization with HIV-1 effects on local immune function than with effects on CD4 lymphocyte counts.

In addition, patient self-report is known to overestimate adherence to prescribed medications [3], especially for regimens requiring multiple doses daily [4]. Preliminary observations of HIV-infected current and former drug users in the Bronx, New York, showed that medication adherence assessed by electronic monitoring was significantly correlated with HIV load but that self-reported adherence was not [5]. HIV-1 load is inversely correlated with actual use of effective highly active protease inhibitor–containing antiretroviral regimens. Even though oropharyngeal *Candida* colonization was not independently associated with the reported use of protease inhibitors, its association with HIV-1 load suggests that there may well have been less use of protease inhibitors in colonized patients. Since protease inhibitors have anticandidal activity [6], it is possible that the actual use of protease inhibitors, better reflected by low HIV-1 load than by self-reported adherence, had a direct effect on reducing oropharyngeal *Candida* colonization. It would be of interest to know if the independent association of *Candida* colonization with HIV-1 load was seen in the subgroup of patients known not to have received either current or recent protease inhibitor therapy.

Until longitudinal studies are available and actual rather than reported use of chemotherapeutic agents with antifungal activity can be accurately measured and controlled for in analyses, it may be premature to conclude that HIV-1 load has a direct effect on local mucosal immunity, which promotes oropharyngeal *Candida* colonization.

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References

1. Gottfredsson M, Cox GM, Indridason ÓS, de Almeida GMD, Heald AE, Perfect JR. Association of plasma levels of human immunodeficiency virus type 1 RNA and oropharyngeal *Candida* colonization. J Infect Dis **1999**; 180:534–7.
2. Gruber A, Lukasser-Vogl E, Borg-von Zepelin M, Dierich MP, Würzner R. Human immunodeficiency virus type 1 gp160 and gp41 binding to *Candida albicans* selectively enhances candidal virulence in vitro. J Infect Dis **1998**; 177:1057–63.
3. Haynes RB, McKibbon KA, Kanani R. Systematic review of randomized trials of interventions to assist patients to follow prescriptions for medications. Lancet **1996**; 348:383–6.
4. Eisen SA, Miller DK, Woodward RS, Spitznagel E, Prybeck TR. The effect of prescribed daily dose frequency on patient medication compliance. Arch Intern Med **1990**; 150:1881–4.
5. Arnsten JH, Grant RW, Demas PA, Gourevitch MN, Schoenbaum EE. Adherence with antiretroviral therapy in HIV-infected drug users. J Gen Intern Med **1999**; 14(Suppl 2):7.
6. Cassone A, De Bernardis F, Torosantucci A, Tacconelli E, Tumbarello M, Cauda R. In vitro and in vivo anticandidal activity of human immunodeficiency virus protease inhibitors. J Infect Dis **1999**; 180:448–53.

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Reply

To the Editor—We thank Klein et al. [1] for their cogent response to our work [2] in which we reported that, among various clinical parameters in a cohort of human immunodeficiency virus (HIV)–infected patients, plasma HIV RNA levels had the most significant association with oropharyngeal *Candida* colonization. As Klein et al. point out, this association could be the result of 3 possibilities: HIV affecting mucosal

immunity, HIV affecting the *Candida* species directly, or a direct effect of protease inhibitor (PI) therapy on *Candida* species. In our report, we speculated that the first of these 3 possibilities was the most likely explanation for our observations. Although we agree with Klein et al. that the other 2 possibilities should be considered, we believe fewer data support these hypotheses.

The possibility that HIV has a direct effect on *Candida* species is provocative, and we referenced 2 studies that described the interaction of HIV glycoproteins inhibiting phagocytosis and intracellular killing of *Candida* in vitro [3, 4]. We interpreted these studies as supporting our hypothesis that HIV has an adverse effect on local mucosal immunity. To our knowledge, there is no convincing evidence in the literature to support the notion that HIV can increase the virulence of *Candida* species in vivo.

Finally, the possibility of PIs having an anticandidal effect is intriguing. In a study by Cassone et al. [5], which Klein et al. [1] reference, the PIs ritonavir and indinavir both appeared to have an anticandidal effect in a rat vaginal candidiasis model. Unfortunately, this work was unavailable to us during the preparation of our manuscript. If we assume that the anticandidal effect of PIs is significant enough to decrease oropharyngeal colonization, then it certainly seems as if the association between low levels of *Candida* colonization and low viral load measurements can be attributed in part to PI exposure. However, in our cohort of patients, we did not find that the self-reported use of PIs was associated with candidal colonization. In our report, we referenced a study showing that antiretroviral monotherapy with reverse-transcriptase inhibitors resulted in a significant reduction in oropharyngeal candidiasis [6], thus giving support to the idea that improvements in local immunity may be more important than the direct effect of PIs on yeast. Furthermore, our anecdotal experience in the clinic has shown that patients with azole-resistant thrush can have resolution of symptoms by initiation of PI-sparing highly active antiretroviral regimens. We also have seen patients known to be compliant with PI therapy, as determined by measurement of blood levels, who have high viral load measurements and thrush. All these observations cause us to be cautious in assigning any direct antifungal effect to PIs.

We agree with Klein et al. [1] that it is premature to conclude that our observations of low *Candida* colonization correlating with low viral load measurements reflect improved mucosal immunity. It was, and remains, our intention to provide a working hypothesis to explain our findings, and we hope to further support this hypothesis through ongoing prospective studies.

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References

1. Klein RS, Arnsten JH, Sobel JD. Oropharyngeal *Candida* colonization and human immunodeficiency virus type 1 infection [letter]. J Infect Dis **2000**; 181:812.
2. Gottfredsson M, Cox GM, Indridason OS, de Almeida GMD, Heald AE, Perfect JR. Association of plasma levels of human immunodeficiency virus type 1 RNA and oropharyngeal *Candida* colonization. J Infect Dis **1999**; 180:534–7.
3. Gruber A, Lukasser-Vogl E, Borg–von Zepelin M, Dierich MP, Wurzner R. Human immunodeficiency virus type 1 gp160 and gp41 binding to *Candida albicans* selectively enhances candidal virulence in vitro. J Infect Dis **1998**; 177:1057–63.
4. Pietrella D, Monari C, Retini C, Palazzetti B, Bistoni F, Vecchiarelli A. Human immunodeficiency virus 1 envelope protein gp120 impairs intracellular antifungal mechanisms in human monocytes. J Infect Dis **1998**; 177:347–54.
5. Cassone A, De Bernardis F, Torosantucci A, Tacconelli E, Tumbarello M, Cauda R. In vitro and in vivo anticandidal activity of human immunodeficiency virus protease inhibitors. J Infect Dis **1999**; 180:448–53.
6. Kinloch-De Loes S, Hirschel B, Hoen B, et al. A controlled trial of zidovudine in primary human immunodeficiency virus infection. N Engl J Med **1995**; 333:408–13.

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