Correspondence

Geographic Variation in Human Herpesvirus 8 Seroprevalence and Possible Association with Exposure to Bites from Blood-Sucking Arthropods

To the Editor—We read with interest the recent brief report by Kazanji et al. reporting a high seroprevalence for Kaposi sarcoma (KS)–associated herpesvirus, also known as “human herpesvirus 8” (HHV-8), in Amerindians of various tribes in French Guiana as well as evidence of a reduction in HHV-8 seroprevalence related to the larvicidal campaign against *Anopheles labranchiae* in Sardinia [6] and of an uneven distribution of mosquito species among Po River valley areas with high and those with low incidences of classic KS and HHV-8 seroprevalence [7]. The promoter-arthropod hypothesis rules out a role for biological and/or mechanical vectors.

More-aggressive species—those that bite mainly outdoors and that are not primarily anthropophilic (and, therefore, are less adapted to humans)—could have the greatest potential to be promoter insects, because they elicit strong skin reactions. They include, among others, Culicinae mosquitoes (*Aedes vexans* and *Ochlerotatus caspius*), sand flies (*Phlebotomus* species), black flies (*Simulium* species), and biting midges (*Culicoides* and *Leptoconops* species).

In French Guiana, an uneven geographic distribution of a mosquito-borne disease (Mayaro virus fever) has been reported between internal and coastal areas [8] that is similar to the variation reported for HHV-8 seroprevalence [1]. The Haut Maroni and Haut Oyapock areas in which Kazanji et al. found the highest HHV-8 seroprevalence rates were also the places with the highest Mayaro virus seroprevalence rates. The main vectors of Mayaro virus fever are *Haemagogus* species (Diptera: Culicidae), especially *Hg. janthinomys*, a canopy mosquito of the rain forest habitat. The recent brief report by Kazanji et al.’s HHV-8 survey were from the arbovirus laboratory collection of the Institut Pasteur de Guyane [1], it would be of interest to know whether there is any association between the seroprevalence for HHV-8 and that for arboviruses. Because the geographic distribution of arbovirus seroprevalence can be considered a marker of the distribution of vectors, a putative association could support the promoter-arthropod hypothesis.

Considering that some of the samples from the arbovirus laboratory collection of the Institut Pasteur de Guyane [1], it would be of interest to know whether there is any association between the seroprevalence for HHV-8 and that for arboviruses. Because the geographic distribution of arbovirus seroprevalence can be considered a marker of the distribution of vectors, a putative association could support the promoter-arthropod hypothesis. It is possible that other human viruses could use the same mechanism of promoter-arthropod transmission; in this respect, it is interesting to note that a correlation between hepatitis B virus seroprevalence and arbovirus seroprevalence has been documented in the valley of Ribeira in Brazil [10].

Valeria Ascoli,1 Daniela Manno,2 and Mario Coluzzi2

1Dipartimento di Medicina Sperimentale e Patologia and 2Istituto Pasteur–Fondazione Cenci Bolognetti, Dipartimento di Scienze di Sanità Pubblica, Università La Sapienza, Rome, Italy

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Replay to Ascoli et al.

To the Editor—We thank Ascoli et al. for their comments [1]. They suggest that transmission of human herpesvirus 8 (HHV-8) among Amerindians in French Guiana might be associated with exposure to bites from blood-sucking arthropods.

In an attempt to resolve this issue, we investigated the possibility of an association between HHV-8 seroprevalence and that for 2 arboviruses (dengue and Mayaro) in 100 Amerindians living in Camopi village, which is located in Haut-Oyapock, an area of high HHV-8 seroprevalence [2]. Fifty subjects were HHV-8 seropositive, and 50 were seronegative. The 2 groups were very similar in terms of age, sex, and ethnic origin (Emerillon and Wayanpi). All serum samples were first screened by ELISA for the presence of IgM antibodies against dengue or Mayaro virus [3], and all of the samples were negative, suggesting the absence of recent infection with dengue or Mayaro virus in the studied population during the field survey. The samples were then screened by a hemagglutination-inhibition assay for IgG antibodies against dengue or Mayaro virus [4]. A sample was considered to be positive when it reacted at a dilution of 1:20 or higher. Thirty-one (63%) of the 50 HHV-8-seropositive subjects had IgG antibodies against Mayaro virus, compared with 24 (48%) of the 50 HHV-8-seronegative subjects; this difference was not significant (P = .16). For dengue virus, 6 subjects were found to be seropositive in each of the 2 groups. Although limited, these data argue against an association between HHV-8 infection and such arbovirus infections. Further studies should be performed in a larger population and should include a wide battery of different serological tests that are used to detect various arboviruses.

In another study, we conducted a large seroepidemiological investigation of Mayaro virus among various ethnic groups in different parts of French Guiana. The highest seroprevalence rates were found in populations living in close contact with the forest, especially in the Haut Oyapock and Haut Maroni areas [4]. In the latter region, we had the opportunity to study 2 different populations, Wayana Amerindians and Noir-Marrons (who are of African ancestry). The respective seroprevalence rates for Mayaro virus were 19.4% and 23.7%. After adjustment for age, HHV-8 seroprevalence was found to be higher in Amerindians (20%–25%) than in Noir-Marrons (10%–15%) living near Amerindian settlements [2, 5]. These findings support the view that HHV-8 transmission is linked to socioeconomic status and cultural specificities in isolated populations (as is Epstein-Barr virus transmission) rather than the promoter-arthropod hypothesis.

Only careful epidemiological studies specifically aimed at investigating HHV-8 transmission between mother and child via bites from blood-sucking arthropods will clarify the role played by this mechanism—and its importance among other risk factors—in intrafamilial transmission of HHV-8.

Mirdad Kazanjian,1,2 Philippe Dussart,2 Patricia Torteyse,3 Renan Duszart,2 and Antoine Gessain2

1Unité de Rétrovirologie, Centre International de Recherche Médicales de Franceville, Franceville, Gabon; 2Laboratoire de Virologie, Institut Pasteur de la Guyane, Cayenne, French Guiana; 3Unité d’Epidemiologie et Physiopathologie des Virus Oncogènes, Institut Pasteur, Paris, France

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that there are at least 77 serotypes of included strains from only 4 serotypes and claim to be weak, given that the screening consider the evidence that supports that could be used for rapid molecular typing the above-described ORFs exist in the cps there is the possibility that pneumoniae; harboring region [1]. We have performed a similar inserted that these 6 ORFs, because they are of the Journal, Chuang et al. described the complete sequence of the mAgA flanking region [1]. We have performed a similar study [2]. By serotyping the original strain harboring mAgA and comparing it with cps (capsular polysaccharide synthesis) loci in both Klebsiella pneumoniae serotype K2 and K52, Chuang et al. confirmed that mAgA, which is located within the cps gene cluster, is responsible for the synthesis of serotype K1 capsule. After screening 74 strains, of which only 40 were typeable and were restricted to 4 serotypes, they found that all serotype K1 strains were mAgA positive and that all strains of the other 3 serotypes were mAgA negative. In addition, 35 of 42 strains from patients with primary pyogenic liver abscess (PLA) were serotype K1/mAgA positive. Furthermore, Chuang et al. identified 5 more open reading frames (ORFs) in the mAgA flanking region that shared the same feature. Thus, they asserted that these 6 ORFs, because they are unique to K. pneumoniae serotype K1, could be used for rapid molecular typing and early detection of PLA. However, we consider the evidence that supports that claim to be weak, given that the screening included strains from only 4 serotypes and that there are at least 77 serotypes of K. pneumoniae; there is the possibility that the above-described ORFs exist in the cps gene clusters of serotypes that were not tested.

In Fang et al.’s study of mAgA, this same group of investigators searched for virulence genes in K. pneumoniae strains that cause PLA by mini-Tn5 transposon mutagenesis [3]. They obtained 2500 mutants from a PLA strain and selected 20 “less virulent” mutants. However, they chose less mucoid mutants either on the basis of gross appearance or by the string test but did not assay directly by any virulence test, such as the serum-resistance assay or the phagocytosis assay. In light of this selection bias, we think that they have identified the gene that influences capsule synthesis rather than the gene that determines virulence.

Among the mutant genes, many of which were identified as part of the cps gene cluster, Fang et al. found a 1.2-kb ORF that fulfilled the molecular version of Koch’s postulates and that was more prevalent in PLA strains. Because this ORF did not exist in the cps gene cluster of serotype K2 and the genome of MGH-78578 (K52), they considered it to be a novel virulence gene and named it mAgA (mucoviscosity-associated gene A). The sequence of the mAgA flanking region in Chuang et al.’s study was previously reported by the same group [3].

Fang et al. had a collection of 53 PLA strains, of which 52 were mAgA positive. They explained that the 1 PLA strain that was mAgA negative resulted from the patient’s poor medical condition (liver cirrhosis and hepatic failure), and they created the image of a 100% rate of detection of mAgA in PLA strains. Our principal objection is that, even with a similar period of strain collection done by the same group, there is a significant difference in mAgA prevalence in PLA strains between Fang et al.’s study and Chuang et al.’s study (52/53 vs. 35/42; P < .01). Whether a selection bias existed in their studies should be clarified.

In a previous surveillance study, we reported that the prevalence of serotype K1 among K. pneumoniae PLA strains was 63.4% [4]. Consequently, we screened all of our 134 PLA strains and found that all of the K1 strains were mAgA positive and that all of the non-K1 strains were mAgA negative [2]. In a more extensive survey using a collection of 495 isolates encompassing the reference strains of all 77 serotypes, Struve et al. showed that mAgA is restricted to the cps gene cluster of K. pneumoniae serotype K1 [5].

The sensitivity of the use of mAgA in the detection of PLA depends on the prevalence of serotype K1 among K. pneumoniae strains that cause this disease. A K1 prevalence rate of no more than 65% among K. pneumoniae PLA strains has been reported [5–8], except for the rates reported by Fang et al. and Chuang et al. If the actual prevalence of serotype K1 is not as high as they report, mAgA may be not a good marker for the detection of PLA.

On the basis of the evidence of the localization and serotype specificity of mAgA, we deduce that the loss of virulence and mucoviscosity of the mAgA mutant may be attributed to the disruption of the serotype K1 capsule. Thus, the serotype K1 capsule, rather than mAgA per se, is really the virulence factor in K. pneumoniae PLA strains.

Kuo-Ming Yeh,1,2 Chang-Phone Fung,3 Jung-Chung Lin,1 and L. K. Siu1

1Division of Infectious Diseases and Tropical Medicine, Department of Internal Medicine, Tri-Services General Hospital, 2Graduate Institute of Medical Sciences, National Defense Medical Center, 3Section of Infectious Diseases, Department of Medicine, Taipei Veterans General Hospital and National Yang-Ming University, and 4Division of Clinical Research, National Health Research Institutes, Taipei, Taiwan

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Reply to Yeh et al.

To the Editor—Our claim that several open reading frames (ORFs) are unique for Klebsiella pneumoniae serotype K1 is reasonable on the basis of the evidence—in our study, 36 of 36 magA-positive strains and 0 of 38 magA-negative strains were serotype K1 (P < .0001) [1]. The 38 non-K1 strains should include many serotypes other than K1–K6 (although not all 77 serotypes, of course) [1]. The studies of Yeh et al. and Struve et al. further strengthen this conclusion: magA was found to be unique for K1 after testing 134 K. pneumoniae strains [2] and after testing 495 strains that included all 77 serotypes [3].

The screening of mutants by mucoviscosity tends to identify surface-related genes; however, after confirmation, it did lead us to the first genetic locus, magA, to be associated with primary pyogenic liver abscess (PLA) [4]. Screening by serum resistance or phagocytosis assay, as suggested by Yeh et al. [5], cannot avoid the same “bias,” because resistance to serum or phagocytosis is also related to the bacterial surface. Furthermore, it is impractical to screen thousands of mutants by these 2 assays. A “nonbiased” screening method for less virulent mutants would be animal inoculation, but this is not feasible either.

Only Waal shares 20% amino acid similarity with MagA by PSI-PHI BLAST analysis; therefore, it is considered to be a novel locus [4]. We have some unpublished functional data that suggest that MagA is not a homologue of Waal. We completed sequencing the flanking regions of magA until matches with MGH78578 were found [4], but there was not enough information to annotate these 33-kb fragments precisely. After serotyping and DNA sequence comparisons with K2 and MGH78578, we recognized that many ORFs in this region are parts of the cps loci and, thus, extended DNA sequencing to the entire cps region [1].

Our first magA prevalence study did show the prevalence rates of magA in PLA and noninvasive strains to be 98% (52/53) and 27% (14/52), respectively [4]. The prevalence rates in both groups were higher than our subsequent observations. However, there was no intention to “create an image” of 100% vs. 0%, because we showed in the same article that magA prevalence rates in strains from outside our hospital were 75% (6/8) and 3% (3/113), respectively [4]. The difference was noted. However, with only 8 PLA strains from outside our hospital, we could not judge whether there was a real bias in our strains. (Some colleagues in Taiwan were reluctant to provide us with PLA strains for comparison.) Hence, we kept monitoring the prevalence rates and collected strains more carefully or even prospectively. We concluded that our earlier prevalence rates were actually too high. We could not exclude errors that occurred before or during sampling for some strains and, therefore, decided not to use strains from 1996, to exclude several less well documented strains from 1997–2001, and to obtain more carefully collected strains from 2002. The prevalence rate of magA after resampling was 83% (35/42) in PLA strains. The new prevalence rate was presented immediately in 2005 [6] and then again in 2006 [1], and it is very consistent with the results in strains that have been collected prospectively to date.

The difference in prevalence rates for magA between strains from our hospital and those of Yeh’s group [7, 8] (the 2 other cited studies analyzed non-PLA strains [3] or strains from patients with ruptured PLA [9]) probably occurred because we excluded patients with secondary cases before enrollment and because there might have been more patients with metastatic infections in our hospital (magA prevalence rates in patients with PLA plus complications has been reported as 100% [6] and 92% [10]). It is also possible that geographic differences between hospitals played a role [9].

A detection method with a sensitivity of 83% (or 63% for Yeh et al.) and a specificity of 97% for PLA should be helpful [1]. For severe cases of metastatic infection, it should be very helpful (sensitivity, 100% or 92%).

It is unfair to say that magA is not a specific virulence gene for PLA or is not a virulence gene per se just because it is located in the K1 cps loci. If magA is unique to the K1 strain, then why is serotype K1 important for PLA, and why is magA nonspecific? By making such statements, Yeh et al. seem to neglect all of the bacterial genetics and immunological work that we have done. If their perspective were right, it would also be debatable whether the K1 capsule per se is the virulence factor for PLA (especially
for metastatic infection) [10], because the K1 cps region is located in the genome of a specific genotype of a *K. pneumoniae* strain that contains other regions involved in virulence [6, 11]. Can we say that the type of genome, rather than K1 capsule per se, is important for PLA with metastatic infection? This would be unfair for Fung et al.’s work as well [10]. The per se argument is actually a debate about definitions, not an interpretation of scientific findings.

Yi-Ping Chuang,1 Chi-Tai Fang,2 and Jin-Town Wang1,2
1Department of Microbiology, National Taiwan University College of Medicine, and 2Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan

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Reprints or correspondence: Prof. Jin-Town Wang, Dept. of Microbiology, National Taiwan University College of Medicine, 1, Sec. 1, Jen-Ai Rd., Taipei, Taiwan (wangjt@ntu.edu.tw).

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