in Missouri and other areas where the disease is not endemic needs review. The national Lyme disease surveillance case definition was developed nearly 10 years ago [3], and a great amount of relevant new scientific evidence regarding Lyme disease and southern tick–associated rash illness has accumulated since that time. The findings of Wormser et al. [6, 9] argue in favor of establishing more rigorous inclusion criteria for patients who present with erythema migrans–like lesions in areas where Lyme disease is not endemic.

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References


Pneumocystis jiroveci Dihydropteroate Synthase Gene Mutations at Codon 171 but Not at Codons 55 or 57 Detected in Germany

Sir—After the introduction of HAART and of trimethoprim-sulfamethoxazole (TMP-SMZ) for Pneumocystis pneumonia prophylaxis, the incidence of Pneumocystis pneumonia in patients with AIDS decreased from 5.5 cases per 100 person-years in 1989 to 0.6 cases per 100 person-years in 1997 [1]. However, Pneumocystis jiroveci is still a major opportunistic pathogen in immunocompromised patients [2, 3].

The rate of TMP-SMZ treatment and prophylaxis failure for Pneumocystis pneumonia among patients with AIDS ranges from 1% to 30% per year, and treatment failure is associated with point mutations in the dihydropteroate synthase (DHPS) gene [4, 5]. Such point mutations have been reported at DHPS codons 23, 55, 57, 60, 111, 171, and 248 (table 1) [5].

The frequency of DHPS mutations at codons 55 or 57, which result in TMP-SMZ resistance [4], ranges from 7%–25% in Europe and Asia and 70% in the United States [6–9]. DHPS mutations at codons other than 55 and 57 have rarely been reported [6].

In this study, P. jiroveci DNA was detected by mitochondrial-transcribed large subunit nested PCR (mtLSU nPCR) [2] of bronchoalveolar lavage and induced sputum specimens obtained from 74 subjects. Seventeen patients had clinically proven Pneumocystis pneumonia, and another 57 subjects were identified as P. jiroveci carriers.

For all specimens, we performed a DHPS nPCR, with primers PK95 and PS634 for the first PCR step and PK160 and Bnest for the second PCR step, followed by sequence analysis. The DHPS gene fragment was successfully amplified in samples from 10 of 17 patients with Pneumocystis pneumonia and in samples from 15 of 57 P. jiroveci carriers.

We detected point mutations at DHPS codon 171 in samples obtained from 3 patients with Pneumocystis pneumonia, with TCA being replaced by TCG [10]. Because both TCA and TCG code for serine, there is no amino acid alteration in the DHPS enzyme.

Two of the 3 patients with the mutation at DHPS codon 171 were HIV infected, and P. jiroveci was successfully eradicated by TMP-SMZ therapy after 9 days, as demonstrated by follow-up mtLSU nPCR, which was performed every other day. The first patient had a favorable outcome. The second patient became coinfected with Staphylococcus aureus and cytomegalovirus and died on day 18 after commencement of TMP-SMZ treatment.

Table 1. Dihydropteroate synthase gene mutations in Pneumocystis jiroveci isolates.

<table>
<thead>
<tr>
<th>Codon</th>
<th>Amino acid</th>
<th>TMP-SMZ resistance</th>
<th>Association with TMP-SMZ prophylaxis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>Phe → Leu</td>
<td>Suspected</td>
<td>No</td>
<td>[5]</td>
</tr>
<tr>
<td>55</td>
<td>Thr → Ala</td>
<td>Yes</td>
<td>Yes</td>
<td>[5]</td>
</tr>
<tr>
<td>57</td>
<td>Pro → Ser</td>
<td>Yes</td>
<td>Yes</td>
<td>[5]</td>
</tr>
<tr>
<td>60</td>
<td>His → Asp</td>
<td>Suspected</td>
<td>No</td>
<td>[5]</td>
</tr>
<tr>
<td>111</td>
<td>Ile → Thr</td>
<td>Suspected</td>
<td>No</td>
<td>[5]</td>
</tr>
<tr>
<td>171</td>
<td>Ser → Ser</td>
<td>No</td>
<td>No</td>
<td>PR</td>
</tr>
</tbody>
</table>

NOTE. PR, present report; TMP-SMZ, trimethoprim-sulfamethoxazole.
The third patient had highly malignant T cell lymphoma. Owing to poor clinical response, after 7 days, intravenous TMP-SMZ therapy was switched to intravenous pentamidine therapy. *P. jiroveci* PCR results became negative for the first mtLSU nPCR step at day 9, but the results remained positive for the second step until day 17, when the patient died.

Other point mutations of the DHPS gene were not detected in this study. Because the 74 subjects had never received TMP-SMZ prophylaxis before presentation, this might explain the lack of mutations at codons 55 and 57 in our study.

The mutation at DHPS codon 171 has been only reported once (in 1999), and clinical data were not provided [4]. Because the amino acid serine remains unaffected at codon 171, and because eradication was achieved in 2 patients with TMP-SMZ, this mutation seems to be a spontaneous mutation and may not confer TMP-SMZ resistance. However, studies involving larger numbers of patients would be necessary to prove the suggested lack of clinical relevance.

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**Clinical Significance of Occult Hepatitis B Virus Infection Cannot Be Overlooked**

*SIR*—In their recent article, Kempinska et al. [1] reported a case of reactivation of hepatitis B virus (HBV) infection following an allogeneic bone marrow transplant in a hepatitis B–immune patient. They pointed out that, although this event is rare, it is important to understand the clinical features associated with the development of HBV reactivation, as well as to avoid the development of such a potentially fatal complication [1]. We agree that reactivation of HBV infection is a serious event in this special clinical instance, and that it deserves much attention. Nevertheless, several critical points should be further addressed.

First, reactivation of HBV infection is not rare in areas where hepatitis B is endemic, such as the Asia-Pacific region [2, 3]. For example, a previous study in Taiwan revealed that among 388 bone marrow transplant recipients, nearly 100 had preexisting hepatitis B surface antigen carriage [2]. After transplantation, abnormal liver function was the most common complication, and 2 patients died of HBV-associated hepatic failure [2]. Consistent findings were reported by Lau et al. [3] from Hong Kong.

Second, in addition to overt HBV infection, occult HBV infection (defined as the presence of HBV DNA in blood or liver tissues in patients who are negative for hepatitis B surface antigen with or without any HBV antibodies [4]) was also not rare in areas where hepatitis B is endemic. It has been reported that the prevalence of occult HBV infection was quite high in these areas (4–25% in the hepatitis B surface antigen–negative and hepatitis B core antibody–positive population) [5, 6].

Furthermore, as reviewed by Kempinska et al. [1], reactivation of HBV infection after transplantation can be potentially fatal. Unfortunately, this issue is often neglected clinically. Therefore, it is important for practicing physicians to identify recipients with pretransplantation occult HBV infection, which can be done with the use of sensitive assays to detect HBV DNA in serum or in liver tissue. A recent review shows that, in patients with occult HBV infection, serum HBV DNA levels range from 400 to $10^4$ copies/mL in patients positive for isolated hepatitis B core antibody and from 10 to $10^4$ copies/mL in patients positive for both hepatitis B surface antibody and hepatitis B core antibody [5]. Therefore, instead of insensitive hybridization-based assays, more-sensitive PCR-based or transcription-mediated amplification–based methods should be used [7]. Finally, the prior studies by us and our colleagues have suggested that hepaa-