The Molecular Epidemiology of Varicella-Zoster Virus: Evidence for Geographic Segregation

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Of 75 varicella-zoster virus (VZV) isolates obtained from patients in Africa, Asia, and the Far East, 74 (98.6%) were found to be positive for a BglII restriction site in gene 54. By contrast, <22% of strains from patients in the United Kingdom and in North and South America were positive for the BglII restriction site. Viruses positive for BglII were significantly more common in zoster occurring in patients of nonwhite origin (P < .05). Irrespective of the country in which the sample was obtained, 98% of strains positive for BglII clustered within a single phylogenetic group, which we termed “group A”; the exception was 1 strain that appeared to be recombinant genotype C/A. We used the BglII site to examine both the spread of type A viruses in the United Kingdom and the patterns of VZV infections within persons from different ethnic groups who grew up in the United Kingdom or abroad.

Primary infection with varicella-zoster virus (VZV) causes chicken pox, after which immunity to clinical reinfection is usually lifelong [1]. The virus remains latent in the dorsal root ganglia,reactivating to cause shingles (zoster) under conditions of declining cellular immunity, most commonly in association with age [2]. Many studies have shown the virus to be genetically stable. Low rates of mutation do occur in tissue culture, as evidenced by differences between the live attenuated vOka vaccine strain and the parental wild-type virus from which it is derived [3]. However, strains recovered after low passage are

indistinguishable, at several loci, from the parent strain [4], as are viruses transmitted directly from one person to another [5]. The strains of virus causing varicella and, subsequently, zoster in 1 patient were found to be identical by restriction-enzyme analysis [6]. Geographically related strain variation, however, has been well described, and differences between Japanese and US genotypes have been used to distinguish between vOka, which is derived from a Japanese strain, and circulating wild-type US and UK viruses [7–10]. All US and UK strains were positive for the PstI site (PstI1) in gene 38, which distinguishes them from the Oka vaccine strain [9, 10]. In addition, the Oka strain is positive for a BglII site (BglI1) in gene 54, which is present in only 20% of US and UK strains overall [10, 11]. More recently, polymorphisms unique to vOka have been identified, and a SmaI restriction site in open reading frame (ORF) 62 has been used to distinguish between the vaccine strain and all wild-type strains, including Japanese strains [3, 12]. In a previous study, we had shown that BglI1 strains were present in 60% of patients with zoster whose primary infection had occurred in either the Indian subcontinent, Africa, or the Caribbean [13], compared with a prevalence of 10% in patients of white origin who had grown up in the United Kingdom. Moreover, among varicella cases in east London, an area of high immigration from these areas, there had been a significant in-
crease in $Bgl I^+$ strains during the preceding 25 years. At the time of that previous study, we had hypothesized that the $Bgl I^+$ allele was likely to be prevalent among circulating wild-type strains in these areas and that this virus may have been imported into the United Kingdom.

More recently, the mapping of single-nucleotide polymorphisms (SNPs) across the VZV genome has been used to differentiate at least 3 major clades of VZV [14]. Strains belonging to 2 of the clades, which we have termed “B” and “C,” appear to be common in subjects living in Europe, whereas strains from Asia, the Far East, and Africa group within a third large clade, termed “A.” Preliminary data had suggested that clade A strains were positive for the $Bgl I^+$ allele, whereas clades B and C were negative for it ($Bgl I^-$) [14]. To investigate this finding and to examine more closely (1) the distribution of the $Bgl I^+$ and $Bgl I^-$ alleles in relation to disease (varicella or zoster), (2) geographic origin of the sample, and (3) demographic characteristics of the host, we typed >300 virus strains collected within the United Kingdom and 98 virus strains collected from around the world, for the presence or absence of the $Bgl I^+$ allele. The relationship of the $Bgl I$ allele to phylogenetic subtype was examined in 33 UK strains and in 40 strains from around the world.

Patients, Materials, and Methods

Patients and samples. Viruses were collected opportunistically from patients with varicella ($n = 105$) or zoster ($n = 151$) who presented to general practitioners and university hospitals during 1979–1996 (table 1). The origins of these samples have been described elsewhere [13]. In brief, 69% of samples were from patients presenting to university hospitals in London, Edinburgh, and Belfast. Of these patients, 40% were female, and 85% were adult (44% from London and 21% from east London during 1997–2000 (table 1). In both studies, detailed demographic data were obtained—including ethnic origin, country of birth, age at which chicken pox was diagnosed, country in which chicken pox was contracted, and, when appropriate, age at which immigration to the United Kingdom occurred. These strains were analyzed to determine whether there was any association between genotype and the four variables of sex, ethnicity, immunosuppression, and age. In addition, the relationship between $Bgl I$ genotype, ethnic origin, and geographic origin of the sample was evaluated. Samples were divided by ethnic origin (white [$n = 160$] or nonwhite [$n = 23$, comprising 10 Afro-Caribbean, 8 Asian from the Indian subcontinent, and 5 Far East Asian from China/Hong Kong and Vietnam] and by country (United Kingdom [UK] or non-UK) in which the patient had contracted varicella or zoster. Of the 23 nonwhite patients, 2, who had been born in Hong Kong and Vietnam and who had emigrated to the United Kingdom at ages 9 and 11 years, were excluded from this analysis, because neither could remember having contracted varicella and because there was uncertainty regarding their location at the time of VZV infection. To increase the numbers, an additional 21 samples from nonwhite patients (11 Afro-Caribbean and 10 Asian [6 from the Indian subcontinent and 4 from the Far East]), who presented during the subsequent year (2000–2001) of the east London zoster study, were included in the analysis. Thus, a total of 202 (160 white and 42 nonwhite) patients in the United Kingdom who presented with zoster were available for this analysis. The data on these samples and on 38 samples collected from nonwhite patients outside the United Kingdom who presented with zoster were compared.

A total of 98 strains collected from patients with varicella or zoster were obtained from collaborators in Africa (Zambia and Guinea Bissau), Asia (Bangladesh and India), the Far East (Hong Kong, Singapore, and Japan), and North and South America.

<table>
<thead>
<tr>
<th>Origin</th>
<th>No. of patients</th>
<th>Immunocompromised$^a$, %</th>
<th>Male$^a$, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chicken pox</td>
<td>Zoster</td>
<td>Total</td>
</tr>
<tr>
<td>United Kingdom</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General</td>
<td>105</td>
<td>151</td>
<td>256</td>
</tr>
<tr>
<td>London</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>East</td>
<td>0</td>
<td>106</td>
<td>106</td>
</tr>
<tr>
<td>South</td>
<td>0</td>
<td>77</td>
<td>77</td>
</tr>
<tr>
<td>Additional (nonwhite)</td>
<td>0</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Zambia</td>
<td>0</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Guinea Bissau</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Singapore</td>
<td>14</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>Japan</td>
<td>2</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>India</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>9</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Brazil</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>United States</td>
<td>18</td>
<td>0</td>
<td>18</td>
</tr>
</tbody>
</table>

$^a$ —, Unknown.
Restriction fragment-length–polymorphism analysis of polymerase chain-reaction (PCR) products from strains of varicella-zoster virus that are either negative (lanes S1–S6) or positive (lane P) for BglI. PCR amplification was performed with a forward primer into which, to control for the enzyme reaction, a BglI restriction site had been engineered. Each product was digested with BglI endonuclease and was analyzed by electrophoresis on 4% NuSieve agarose gel. M, 100-bp marker; NTC, nontemplate control; P, positive control; Undig, undigested product; S1–S6, digested products.

Figure 1.
UK strains: 31% of strains from patients with varicella were \( BgI \)\(^{+} \), compared with 15% of strains from patients with zoster (\( P < .01 \)) (table 2).

The SNP pattern and the phylogenetic tree based on the genotyping of 73 strains by the SNP methodology are shown in figures 2 and 3, respectively. Of the 73 virus strains genotyped in this study, 41 were found to be genotype A (figure 3). All were \( BgI \)\(^{+} \) (figure 2). Seven strains were genotype B, and 22 were genotype C. None of the genotype B and genotype C strains were \( BgI \)\(^{+} \) (figure 2). One genotypically mixed, C/A strain from Brazil was found to be \( BgI \)\(^{+} \) (figure 2). An additional 2 recombinant, B/C strains were identified (figures 2 and 3). Using these results, we calculated that 41 (98%) of 42 strains that were \( BgI \)\(^{-} \) belonged to genotype A and that all strains that were \( BgI \) negative belonged to either genotype B or genotype C; the 1 \( BgI \)\(^{-} \) strain that was not genotype A was, in fact, a recombinant, C/A strain.

Of the 183 patients from the 2 prospective, community-based studies, 23 had \( BgI \)\(^{+} \) strains and 160 had \( BgI \)\(^{-} \) strains. Patients with \( BgI \)\(^{+} \) strains and patients with \( BgI \)\(^{-} \) strains were similar with respect to sex (male/female proportion, 10/23 vs. 80/160, respectively), immunosuppression (4/23 vs. 21/160, respectively), and mean age (56 vs. 53 years, respectively) (\( P > .05 \)). A total of 23 of the patients were nonwhite, and, among these, the proportion who reactivated a \( BgI \)\(^{+} \) strain was significantly higher than that in whites (8/23 vs. 15/160, respectively; \( P < .01 \)).

For the 202 UK patients, the results of analysis of VZV genotype in relation to patient ethnicity and country where varicella or zoster were acquired are shown in table 3. Group 1 subjects were white and had lived their entire lives in either the United Kingdom or Ireland. None had lived abroad or had contracted varicella abroad. Of the 160 patients in this group, 73% (117) remembered having contracted varicella in the United Kingdom, although most did not remember the age at which it had occurred. As expected, 91% of this group were infected with a \( BgI \)\(^{-} \) strain. Group 2 included 16 nonwhites who had been born and had lived their entire lives in the United Kingdom, and 14 of these 16 remembered having contracted varicella in the United Kingdom, although only 4 remembered the age at which it had occurred. In addition, 3 subjects who had immigrated at ages <3 years and who remembered having

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**Figure 2.** Genotyping of 33 United Kingdom (UK) and 40 non-UK strains of varicella-zoster virus, at 13 informative loci in open reading frames (ORFs) 1, 21, 50, and 54. Single-nucleotide polymorphisms (SNPs), compared with the reference strain, Dumas [15], are denoted by black boxes. Major genotypes (A–C), 3 putative recombinant strains (1 genotype C/A and 2 genotype B/C), and the \( BgI \) restriction site are shown.

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**Figure 3.** Phylogenetic tree of 33 United Kingdom (UK) and 40 non-UK varicella-zoster virus strains (parsimony method) [16]. Bootstrap values and genotypes A–C are shown; countries of origin of strains are indicated. Numerals (e.g., \( \times 2 \)) indicate the number of viruses from a particular country that have the same single-nucleotide-polymorphism sequence.
contracted varicella in the United Kingdom were included in this group. Despite having contracted varicella in the United Kingdom, a significantly higher proportion of this group were infected with a BglI⁺ strain (P<.01) (table 3). Group 3 comprised 23 nonwhite subjects who had immigrated to the United Kingdom as adults (age >16 years). Of these 23 subjects, 20 (87%) remembered having contracted varicella as children in their country of origin, although only 5 remembered the exact age at which it had occurred. None remembered having contracted varicella since arrival in the United Kingdom. A total of 30% (7/23) of the subjects in group 3, despite remembering having contracted varicella in the United Kingdom were included in this group. None remembered having contracted varicella in another country, although only 5 remembered the exact age at which it had occurred. None remembered having contracted varicella since arrival in the United Kingdom. A total of 30% (7/23) of the subjects in group 3, despite remembering having contracted varicella in a country in which BglI⁺ genotype A strains apparently predominate, reactivated BglI⁻ strains (table 3). This finding is in line with the 40% reactivation of BglI⁻ strains among nonwhite immigrants with zoster who were sampled in our previous study of opportunistically collected strains [13]. By contrast, all strains from patients with zoster who were sampled in Africa (Zambia) and the Far East (Singapore, Japan, and Hong Kong) were BglI⁺, and, overall, only 1 of 75 strains sampled from Asia, Africa, and the Far East was BglI negative (table 2).

Discussion

Results from a previous study had led us to hypothesize that BglI⁻ strains might be prevalent in Africa, the Indian subcontinent, the Far East, and the West Indies and that immigration from these areas might have led to increased circulation of BglI⁺ strains in the United Kingdom [13]. These data were based on opportunistically collected samples, for which incomplete demographic data were available. More-recent data, from strains collected prospectively, confirm the prevalence of BglI⁺ strains found in that previous study (12%–13% vs. 15%, respectively). This suggests that, in the United Kingdom, opportunistically collected samples of virus strains are genetically representative of the population of circulating wild-type strains and that the prevalent strain of VZV in the United Kingdom is BglI negative. A similar distribution of genotypes was seen in North America, with 11% of the samples that we tested being BglI⁺. This is consistent with previous reports, which found, albeit in low numbers of subjects, that ~20% of North American strains were BglI⁺ [11]. Our data also showed that 4 of 5 samples from South America (Rio de Janeiro) were BglI negative. By contrast, all but 1 of the strains from Africa (Zambia and Guinea Bissau), the Indian subcontinent (India and Bangladesh), and the Far East (Japan, Hong Kong, and Singapore) were BglI⁻. The numbers of strains tested from each country were small and, in some cases (Guinea Bissau, Brazil, India, and Bangladesh), were obtained during a single outbreak of varicella, albeit from epidemiologically unrelated sources; thus, they may not be representative of the prevalent strain in each country.

Be that as it may, the viral genotypes causing varicella in Brazil were strikingly different from those sampled in varicella outbreaks in Guinea Bissau, India, and Bangladesh. Moreover, we have shown that the BglI genotype in varicella outbreaks in the United Kingdom is broadly similar from year to year [13]. Our data therefore support previous findings suggesting geographic segregation of VZV genotypes and indicate that viruses from the African and Asian subcontinents carry the BglI⁺ allele, whereas those from the United Kingdom and the Americas seem more likely to be BglI negative.

Previously published data have shown that the strain of virus causing zoster in a patient was identical to the primary varicella strain infecting that patient [5]. The similarity of viral genotypes causing varicella and zoster is supported by the similar pattern of BglI genotypes among virus strains from varicella and zoster cases in Singapore and Japan. However, in the United King-
dom, the prevalence of $Bgl^+$ strains is significantly higher in samples from cases of varicella. This probably reflects an increase in $Bgl^+$ strains from patients with varicella in east London since the early 1980s [13]. In an earlier study, we had speculated that this was due to these strains’ recent importation in immigrants from Africa and Asia [13]. The data generated by the presented study support this hypothesis. A total of 41 (98%) of 42 $Bgl^+$ strains tested belong to 1 major genotype, A; the remaining 1 strain was a mixed, C/A genotype. Genotype A strains appear to predominate among strains sampled in Africa, Asia, and the Far East (figures 2 and 3) [14]. Genotype B and genotype C $Bgl^-$ strains have been found in Europe, the United States, and South America [14]. Similar findings in 2 other studies (although each used different genotyping SNPs, as well as different nomenclatures to describe the genotypes) confirm the robustness of these data [14, 17–19]. Thus, the presence of a $Bgl^-$ restriction site in gene 54 appears to be a reliable marker of genotype A and may provide a useful screening tool for monitoring the spread of VZV globally. In keeping with this, we found that all 6 of the $Bgl^+$ strains from the United Kingdom were genotype A. Although it is not possible, on the basis of the few loci typed, to determine the exact geographic origin of the UK genotype A strains, preliminary analysis suggests that it is diverse. Figures 2 and 3 show that at least 1 of the UK genotype A strains groups closely with Japanese strains, whereas others cluster with African and Indian strains. Although 2 of the African/Asian strains were isolated from subjects of Bangladeshi origin and 1 strain was isolated from a subject of Nigerian origin, the Japanese-like strain and 2 of the African/Asian strains were isolated from whites who have lived their entire lives in the United Kingdom. This suggests that genotype A strains are circulating in the United Kingdom.

Recombination between wild-type strains has not previously been described, although recombination has been observed in vitro [20]. In the present study, we found 3 putative recombinant strains, giving an overall recombination frequency of 4% (3/73). This figure is much higher than what normally would be expected for herpesviruses, even under in vitro conditions, and remains to be verified. All 3 of these strains were recovered from locations (London and Brazil) in which cocirculation of $>$1 genotype is apparently common. Because none of these strains 3 would have been detected by typing at the $Bgl^-$ locus alone, more-extensive genotyping may be required in areas of mixed genotypes, and this is likely to be particularly important in countries where vaccination with Oka is routine.

The data generated in the present study have enabled us to analyze more closely the relationship between viral genotype, patient ethnicity, and country in which the patient has become infected with VZV. Overall, $Bgl^+$ strains were significantly more common among nonwhites than among whites ($P < .01$). This remained true even when we analyzed only those who had contracted chicken pox in the United Kingdom; that is, 37% of UK nonwhites had $Bgl^+$ strains, compared with 9% of whites (table 3; group 1 vs. group 2). It is possible that this difference reflects high intrafamilial transmission of VZV, with nonwhites in the United Kingdom being more likely to acquire a $Bgl^+$ strain from family members who themselves were infected in a country with a high prevalence of circulating $Bgl^+$ strains. Alternatively, different ethnic groups may be more susceptible to a particular strain—or, within a given geographic area, 1 strain may be more virulent than the other.

Surprisingly, 30% (7/23) of nonwhites who were likely to have had varicella in a country with a high $Bgl^+$ prevalence reactivated $Bgl^+$ strains (table 3). Three explanations are possible. First, in Africa, Asia, and the Far East, $Bgl^+$ strains may be more common than previously had been thought but, because of biased sampling or random error, were not detected in the 38 zoster and 37 varicella strains. Sampling of greater numbers of prospectively collected strains will be necessary to exclude this possibility. Second, it is possible that the 7 subjects with $Bgl^+$ strains had not, in fact, had varicella in their country of origin but had contracted asymptomatic primary varicella in the United Kingdom; this is somewhat less likely, because varicella in adults is usually symptomatic [21] and because reactivation of having contracted varicella as an adult is good [22]. Third, it is possible that the patients positive for $Bgl^-$, despite preexisting immunity from primary varicella in their country of origin, became reinfected with a UK strain of virus, which then reactivated (table 3, group 3). Reinfection of latently infected persons has been well described [23, 24]. Replication and antigen presentation within local lymphoid tissue are likely to be important for boosting of cell-mediated immunity and consequent protection of latently infected persons against virus reactivation [23]. The notion that reinfecting wild-type strains might cause systemic infection with spread to ganglia is not really consistent with our current understanding of VZV pathogenesis. However, there is 1 scenario in which a similar phenomenon may have occurred, in a few vaccine recipients in whom wild-type zoster developed without any obvious breakthrough varicella after vaccination [25]. In vitro experiments support the notion that VZV latency may result from infection of sensory-nerve endings by free virus in the epithelium, which tracks in a retrograde fashion to infect dorsal root ganglia [26]. If this theory is true, then superinfecting virus, which does not produce a rash, is unlikely to lead to latent infection and subsequent reactivation. In monkeys, however, seeding of the ganglia with simian VZV has been shown to occur before rash development, suggesting that retrograde spread may not always be necessary for ganglionic infection [27]. In addition, autopsy reports from some immunocompromised children who died early during varicella infection have shown the presence of virus in ganglia in the absence of skin rash [1]. Further work, to examine our findings more closely, is now needed.

Our data demonstrate that the $Bgl^-$ site is a good screening marker for genotype A VZV strains and that it may prove to
be a powerful tool in the investigation of questions of molecular epidemiology. We have already shown that BglI* genotype A strains are spreading in parts of the United Kingdom and that these may have been imported from countries with a higher prevalence of genotype A strains. Screening for the BglI allele in different populations may also shed light on patterns of VZV infection, reinfection, and recombination. Such data may have important implications for the use and monitoring of VZV vaccines.

References