JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1997, p. 2807–2809 Vol. 35, No. 11
0095-1137/97/$04.00+0

Molecular Epidemiology of Varicella-Zoster Virus in East London, England, between 1971 and 1995

K. HAWRAMI,[1] I. J. HART,[1] F. PEREIRA,[2] S. ARGENT,[1] B. BANNISTER,[3] B. BOVILL,[3] D. CARRINGTON,[4] M. OGILVIE,[5] S. RAWSTORNE,[6] Y. TRYHORN,[4] AND J. BREUER[1]*

Department of Medical Microbiology and Virology[1] and Department of Medical Epidemiology and Statistics,[2] St. Bartholomew's and Royal Hospital School of Medicine and Dentistry, Queen Mary Westfield College, Infectious Diseases Unit, Coppetts Wood Hospital,[3] and Department of Virology, St. Georges Hospital,[4] London, Department of Medical Microbiology, University of Edinburgh, Edinburgh,[5] and The Practice, Stow on the Wold, Gloucestershire,[6] United Kingdom

Received 13 May 1997/Returned for modification 22 July 1997/Accepted 22 August 1997

The molecular epidemiology of varicella-zoster virus in London, England, between 1971 and 1995 was examined by using two informative polymorphic markers, variable repeat region R5 and a *Bgl*I restriction site in gene 54. Viruses from 105 cases of chickenpox and 144 of zoster were typed. Two alleles of R5, A and B, were found at prevalences of 89 and 6%, respectively. No difference in allele frequency between the zoster and chickenpox cases was found, and no change in the frequencies of these alleles was observed to occur over time. By contrast, a *Bgl*I restriction site (*Bgl*I$^+$) was found with increasing frequency over time among cases of varicella ($P < 0.005$) and, to a lesser extent, cases of zoster. The *Bgl*I$^+$ polymorphism was strongly associated ($P < 0.0005$) with zoster in subjects who had immigrated to the United Kingdom from countries with low adult immunity to varicella (LAIV). Sixty-three percent of the subjects with zoster who had emigrated from countries with LAIV carried the *Bgl*I$^+$ virus, in contrast to 10% of adults who had grown up in countries with high adult immunity to varicella. The significance of these data, in view of the changing epidemiology of chickenpox, is discussed.

Varicella-zoster virus (VZV) causes chickenpox infection in susceptible individuals, following which the virus remains latent in the dorsal root ganglia until reactivation to cause zoster occurs in up to 25% of the population (3). There is apparently little variation of the virus, and one serotype appears to be responsible for VZV disease worldwide (3). Limited genetic differences between VZV isolates are found, and restriction analysis of American and Japanese wild-type viruses has identified distinct American and Japanese restriction fragment length polymorphisms (1, 6, 14). VZV isolates from the same geographical area are very similar (14). Restriction fragment length polymorphisms and PCR across informative restriction sites can differentiate between the Oka vaccine strain, derived from a Japanese virus, and wild-type American isolates (11, 14).

Recent reports from the United States and the United Kingdom suggest that the epidemiology of VZV infection may be changing. In both countries, the incidence of chickenpox in adults (over the age of 15 years) has risen over the past 20 to 30 years (3, 7). The reasons for this change are not clear. However, chickenpox in adults is known to be more common in certain tropical countries (3, 5). It has been suggested that immigrants from these areas may contribute to the changing epidemiology in the western hemisphere by increasing the numbers of susceptible adults (4) or by importing new strains of VZV. To investigate the latter possibility, we typed VZVs by using a system combining amplification across polymorphic regions and restriction sites of viral DNA (8, 11). VZVs collected predominantly from patients living in eastern and central London, England, between 1971 and 1995 were analyzed in this way to determine whether the molecular epidemiology of the virus was changing over time.

* Corresponding author. Mailing address: The Medical Colleges of The London and St. Barts, Queen Mary Westfield College, 37 Ashfield St., London E1 1BB, England. Phone: 0011-44-171-377-7141. Fax: 0011-44-171-377-5784.

MATERIALS AND METHODS

Two hundred sixty-one virus samples from cases of VZV infection stored from 1971 to 1995 were available for typing. In 12 (4.5%) of the samples, no virus could be detected. Of the remaining 249 virus samples, 105 came from cases of varicella occurring in the United Kingdom and 144 were from cases of zoster. The year of sampling and the age, sex, and ethnic origin of the patient were ascertained when possible. In cases of zoster, the country in which the patient had acquired chickenpox or, if the history was negative, the country in which the patient had grown up was also established. Africa, the Indian subcontinent, the People's Republic of China and other Asian countries, South America, and the West Indies were classified, for this analysis, as areas with low adult immunity to varicella (LAIV). Patients from Europe, the Middle East, and the United States were classified as coming from areas with high adult immunity to varicella (HAIV). Thirty percent of the patients with chickenpox and 35% of the patients with zoster were immunosuppressed, predominantly as a result of lymphoproliferative disease, chemotherapy, bone marrow or renal transplantation, or infection with human immunodeficiency virus. Eleven percent of the persons with varicella were pregnant.

Most of the viruses were collected at random from hospitalized patients in a multiethnic area of London; however, 51 (31%) of the 163 samples collected after 1993 came from patients seen by family doctors in practices around the United Kingdom. Fifty-two (21%) of the virus samples were isolates. The remainder were primary virus samples in viral transport medium frozen at −70°C or in vesicle fluid which had been air dried onto slides (8).

Primers spanning seven putative polymorphic loci were used to amplify directly from virus in tissue culture medium or viral transport medium or from the vesicle fluid on slides in accordance with published methods (8, 11). Five of the loci, variable repeat regions R1 to R5, were examined for length polymorphism by separation on 4% metaphore agarose gel, and results were confirmed by direct sequencing of the PCR products on an ABI 377 automated sequencer using dye terminators (8). In addition, two polymorphic restriction sites, a *Pst*I site in gene 38 and a *Bgl*I site in gene 54 (11), were amplified and, following restriction endonuclease digestion, the products were separated on 2% agarose. In both cases, a restriction site in the forward primer was used to control for the enzyme reaction.

The data were analyzed with the Stata statistical package (13). The two-sample *t* test, the chi-square test, and the chi-square tests for trends were used with the appropriate data. Regression analysis was carried out to adjust for potentially confounding variables.

TABLE 1. Characteristics of the persons with varicella and zoster sampled

Disease (no. of persons)	% (no.) of persons[a]									
	Male	Female	White	Nonwhite[b]	From country with:		Young[c]	Older[d]	R5A	*Bgl*I$^+$
					HAIV	LAIV				
Varicella (105)	51.4 (54)	44.8 (47)	67.6 (71)	28.5 (30)	68.6 (72)	27.6 (29)	25.7 (27)	61 (64)	90 (95)	29.5 (31)
Zoster (144)	46.5 (67)	34.7 (50)	73.6 (106)	8.3 (12)	71.5 (103)	7.6 (11)	37.5 (54)	43.8 (63)	89 (128)	13.9 (20)

[a] Missing values, where the data are not known, are not shown but were included in the statistical analysis.
[b] Includes Asian Indians (60%), blacks (30%), and Chinese and others (10%).
[c] Persons with varicella, <14 years old; persons with zoster, <50 years old.
[d] Persons with varicella, >15 years old; persons with zoster, >50 years old.

RESULTS

The age, ethnic origin, gender, and immune status of each of 249 persons with chickenpox or zoster are shown in Table 1. Also shown is the classification of the country of origin of each patient according to the pattern of varicella infection (LAIV or HAIV). Zoster patients in this study were younger than would be expected; this reflects the fact that 35% were immunosuppressed. There was no difference in the prevalence of different virus strains among immunocompetent and immunosuppressed patients. Similarly, the prevalences of different strains did not differ between community and hospitalized patients sampled over the same time period, i.e., 1993 onward.

Of the five polymorphic regions, only the noncoding R5 region was informative (8). Of the three putative R5 polymorphisms described (15), two alleles, A and B, were found in the population of viruses studied. Ten nucleotide sequences with polymorphism A and five with polymorphism B were virtually identical to the original Dumas sequence; there was a single nucleotide change of G to T in position 4 of the second repeat which was present in all 15 viruses (9). The prevalences of alleles A and B were 89 and 6%, respectively, among both chickenpox and zoster viral strains. In 5% of the cases (12 samples), this marker could not be typed because of lack of DNA. No association with age, ethnicity, area of origin, or gender was found. All viruses were positive for the *Pst*I site in gene 38, while the *Bgl*I site was present in 29.5% of the chickenpox strains and 14% of the zoster strains. Sixty-three percent of the patients with zoster who had spent their childhoods and had had chickenpox in a country with LAIV carried the *Bgl*I$^+$ strain, compared with 10% of patients who had grown up in an area with HAIV. This difference was highly significant (*P*, 0.0005). Only one person with zoster, originating in an area with LAIV, was white. It is therefore impossible to determine whether the *Bgl*I site was associated with ethnicity or country of origin. There was no association of the *Bgl*I$^+$ polymorphism with age or gender.

The prevalences of the R5 and *Bgl*I$^+$ polymorphisms in 223 samples for which the year of sampling was known were tabulated over time (Table 2) and compared with changes in the population characteristics over the same period. Results suggest that, after controlling for other variables, there was a significant increase in the prevalence of *Bgl*I$^+$ among patients with chickenpox during the period 1991 to 1996 compared with 1971 to 1985 (Table 2). No significant change in the prevalence of *Bgl*I$^+$ strains was seen among patients with zoster over the same period. Furthermore, the spread of *Bgl*I$^+$ strains did not appear to be epidemic, as their prevalence remained the same during consecutive years (Table 3).

The characteristics of the populations during these periods were similar, apart from a significant increase with time in the numbers of nonwhite patients (Asian Indian, 60%; black, 30%; Chinese and other, 10%) from areas with LAIV in both the chickenpox ($P = 0.001$) and zoster ($P = 0.007$) patient populations. There was no statistical association between *Bgl*I$^+$ strains from chickenpox patients and ethnicity (nonwhite or white), despite a significant increase in both with time. However, the proportion of nonwhite chickenpox patients with *Bgl*I$^+$ strains (43%) was higher than the proportion of whites with chickenpox (29%), albeit not significantly ($P = 0.185$).

DISCUSSION

The data presented here suggest that the population of VZV infecting residents of central and eastern London is different from that causing infection in Japan but similar to that in the United States. All United Kingdom and United States strains of VZV are positive for the *Pst*I restriction site in gene 38 (11). By contrast, 30% of Japanese strains, including the Oka strain, are *Pst*I site negative (10, 15). The *Bgl*I site in gene 54 is found

TABLE 2. Ages, genders, and areas of origin of patients sampled from 1971 to 1996 compared with prevalence of R5A and *Bgl*I$^+$ VZV polymorphisms[a]

Group	% (no.) of persons with:					
	Varicella			Zoster		
	1971–1985 ($n = 21$)	1986–1990 ($n = 14$)	1991–1996 ($n = 70$)	1971–1985 ($n = 38$)	1986–1990 ($n = 14$)	1991–1996 ($n = 66$)
R5A	95.2 (20)	92.9 (13)	87.1 (61)	86.8 (33)	92.9 (13)	87.9 (58)
*Bgl*I$^+$[b]	4.8 (1)	21.4 (3)	38.6 (27)	10.5 (4)	7.1 (1)	18.2 (12)
Male	61.9 (13)	42.9 (6)	50.0 (35)	42.1 (16)	78.6 (11)	60.6 (40)
LAIV[b]	4.8 (1)	14.3 (2)	37.1 (26)	0	7.1 (1)	15.2 (10)
Adult varicella (>15 yr old)	42.9 (9)	42.9 (6)	70.0 (49)			
Onset of zoster, <50 yr of age				47.3 (18)	50 (7)	43.9 (29)

[a] Results are from multiple logistic regression.
[b] Significant increase over time ($P < 0.005$).

TABLE 3. Numbers and percentages of *Bgl*I$^+$ and *Bgl*I$^-$ VZV strains sampled between 1994 and 1996

Strain type	No. (%) of strains sampled in:		
	1994 (n = 20)	1995 (n = 27)	1996 (n = 10)
*Bgl*I$^+$	9 (45)	9 (33)	4 (40)
*Bgl*I$^-$	11 (55)	18 (67)	6 (60)

in up to 20% of United States strains (11) and was present in between 14% (zoster) and 29.5% (varicella) of the virus samples in this study. Although the *Bgl*I$^+$ polymorphism is also found in the Japanese strains, for example, Oka, its prevalence is not known. In contrast to the Japanese findings (15), the R5B allele is rare and the R5C allele has not been found in Britain.

The origin of the *Bgl*I$^+$ strains in the United Kingdom is not clear. One possibility is that the *Bgl*I$^+$ variant is arising by point mutation (11). We found no alteration of the genotypes of viruses cultured to passage seven, and others have reported similar findings (1). The finding of mixed genotypes, i.e., *Bgl*I$^+$ R5A and *Bgl*I$^+$ R5B, does suggest that viral recombination, which has been observed in vitro (2) and in vivo (12), may be occurring. We did not find any evidence of mixed infections, even on limiting-dilution sequencing of viruses from immunocompromised patients.

Alternatively, the *Bgl*I$^+$ strains may have been imported from countries with LAIV. The highly significant association of *Bgl*I$^+$ strains with zoster in immigrants from areas with LAIV ($P < 0.0005$) and the significant rise in the prevalence of *Bgl*I$^+$ strains among cases of chickenpox, in parallel with the increase in the immigrant population, both point to zoster in subjects from areas with LAIV as the source of the *Bgl*I$^+$ strain. It is not clear from the data whether, in patients with zoster, there is an association between *Bgl*I$^+$ strains and persons of nonwhite ethnic origin which is occurring independently of the country of origin, as the nonwhite persons are the same as the persons from countries with LAIV. Most probably, however, the high prevalence of *Bgl*I$^+$ strains (63%) among persons with zoster who have migrated to the United Kingdom from countries with LAIV, compared with that among patients from countries with HAIV (10%), reflects differences in virus epidemiology in different countries rather than ethnic differences in susceptibility. This is supported by the finding that among cases of chickenpox in the United Kingdom, there is no significant association of any one strain with ethnicity or country of origin (P = 0.184). *Bgl*I$^+$ strains were, however, more common in nonwhites with chickenpox (43%) than in whites with chickenpox (29%), a finding which could result from the transmission of *Bgl*I$^+$ strains by index cases who are predominantly nonwhite to members of their families and communities. The possibility of ethnically determined differences in susceptibility to *Bgl*I$^+$ strains cannot, however, be ruled out.

The increase over time in the prevalence of *Bgl*I$^+$ strains causing chickenpox in the United Kingdom may simply reflect equilibration of a geographically distinct viral strain with the more prevalent *Bgl*I$^-$ strains. It is also possible that the prevalence of *Bgl*I$^+$ strains has increased because they are "fitter" than *Bgl*I$^-$ strains and spread more easily. *Bgl*I$^+$ strains differ from *Bgl*I$^-$ strains by a silent base change from T to C in position 95,241 within gene 54. Since no change in the protein sequence results, it is unlikely that *Bgl*I$^+$ strains differ biologically from *Bgl*I$^-$ strains unless the polymorphism is linked to another determinant of pathogenesis or virulence. These questions require further study.

In summary, our data suggest that the *Bgl*I$^+$ strain has been increasing in the population studied over the past 25 years. The most probable source of this strain is cases of zoster occurring in immigrants from countries with LAIV. These data are, however, based on a sample set which is not representative of all cases occurring in this community over the time period studied. Further work on a more representative sample set is needed to verify this result and to determine whether it is of biological significance.

ACKNOWLEDGMENTS

We thank the many general practitioners who contributed samples, in particular, Mike Wyndham and Mark Whittiker.

This work was funded by the London Hospital Special Trustees.

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