

## Epidemics of two Victoria and Yamagata influenza B lineages in Yamagata, Japan

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(Accepted 25 January 2004)

### SUMMARY

We attempted to predict epidemics of influenza B, focusing on B/Victoria/2/87-like (V) and B/Yamagata/16/88-like (Y) lineages, in Yamagata, Japan. We collected 9624 nasopharyngeal swabs for virus isolation from patients with respiratory infections between 1996 and 2003 and 237 sera for seroepidemiological analysis by haemagglutination–inhibition test in 2001. We isolated 424 V-lineage and 246 Y-lineage viruses during the study period. Three herald viruses in the 2000–2001 season enabled us to predict a V-lineage epidemic in the following season. However, another V-lineage epidemic occurred in the 2002–2003 season, although we caught four herald Y-lineage viruses, whose antigenic drift was suggested by seroepidemiological study, at the end of the previous season. Since the epidemiology of the two influenza B lineages remains unclear, a careful watch should be kept on these lineages in order to provide effective public-health strategies against future epidemics.

### INTRODUCTION

Influenza viruses are the most important cause of acute respiratory infections. In temperate regions, influenza occurs in winter epidemics that affect 1–5% of the entire population and the total estimated cost of welfare may reach approximately US\$ 10–60 million per million population in developed countries [1]. The strategy of the World Health Organization (WHO) against influenza is primarily to utilize vaccines in large-scale immunization programmes. Therefore, an analysis of antigenicity among circulating viruses is crucial and WHO has established a Global Influenza Surveillance Network to enable better vaccines to be produced. On the other hand, protective herd immunity against influenza viruses is an important

factor in limiting the size of epidemics [1]. Thus both the antigenic analysis of circulating viruses and monitoring of herd immunity against them are important in realizing an effective anti-influenza programme.

The regular surveillance of influenza viruses in Japan has revealed that two or three subtypes of influenza A (H3), A (H1) and B have been co-circulating in recent years. Since 1996, H3 viruses have been circulating in every season and H1 viruses were associated with epidemics between the 1999–2000 and 2001–2002 seasons in Japan. Major epidemics of influenza B have occurred almost every 2 years; in 1996–1997, 1998–1999, 2000–2001, 2001–2002 and 2002–2003 seasons [2, 3]. A similar epidemic pattern of influenza B was seen in Yamagata, which is located in the north-eastern part of Japan.

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It is known that influenza B viruses can be divided into two antigenically distinct lineages, represented by B/Victoria/2/87 (V-lineage) and B/Yamagata/16/88 (Y-lineage), based on haemagglutination–inhibition (HI) tests using animal antisera and on genomic differences [4–9]. The former lineage has been circulating in Japan since at least 1976 and the latter was recognized for the first time in a school outbreak in the spring of 1987 [4]. V-lineage viruses circulated mainly in Asian countries in the 1990s and have spread worldwide since the 2001–2002 influenza season. The V-lineage strain has been recommended as vaccine antigen for use in the 2003–2004 influenza season [9–11].

Although we have tried to predict the characteristics of influenza epidemics based on virus isolation and seroepidemiological analysis at a public-health laboratory, we have not yet succeeded in producing a perfect prediction. However, we have accumulated useful data on influenza B epidemiology, especially on the two influenza B lineages. The epidemiology of these two lineages still remains poorly understood. Herein, we describe our results of virus isolation since the 1996–1997 season and seroepidemiological data using sera collected in 2001 in Yamagata.

## METHODS

### Collection of specimens

Between January 1996 and June 2003, 9624 nasopharyngeal swab specimens were obtained from patients with acute respiratory infection (ARI) at paediatric clinics collaborating with the local health authority of Yamagata Prefecture for the surveillance of viral diseases in Japan. Patients were clinically diagnosed as having ARI with fever and/or cough and/or rhinorrhoea. The specimens were collected and placed immediately in tubes containing a transport medium, and transported to the Department of Microbiology, Yamagata Prefectural Institute of Public Health for virus isolation.

Human sera were collected between August and September 2001 for the national epidemiological surveillance of vaccine-preventable diseases led by the Ministry of Health, Labour and Welfare of Japan [12]. From residents who agreed to enrol in the programme in Yamagata we collected 31 sera from the 0–4 years age group, 25 from 5–9 years, 21 from 10–14 years, 39 from 15–19 years, 33 from 20–29 years, 24 from 30–39 years, 22 from 40–49 years,

21 from 50–59 years, and 21 from the  $\geq 60$  years age group.

### Virus isolation and identification

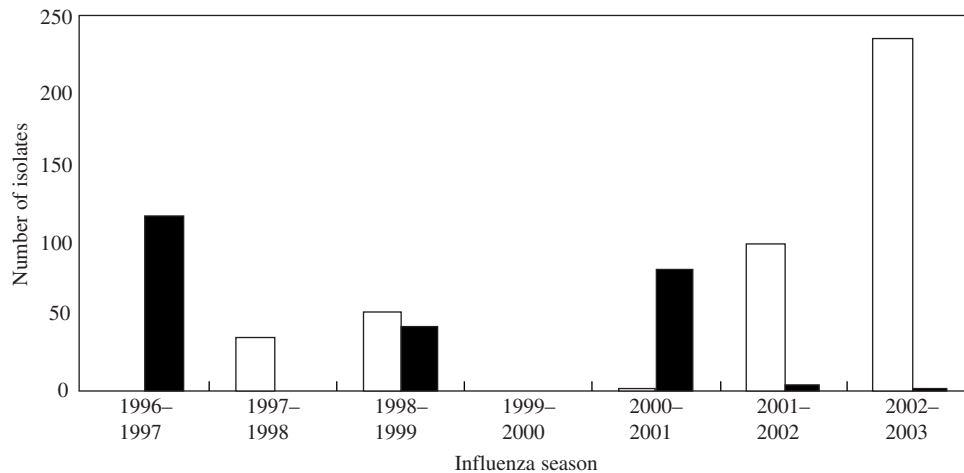
Virus isolation was carried out using a microplate method. Briefly, human embryonic fibroblast (HEF), HEP-2 cell line of human larynx carcinoma, Vero line from African green monkey kidney, Madin–Darby Canine Kidney (MDCK), RD-18S line of a human rhabdomyosarcoma and GMK line from green monkey kidney origin, were prepared on the wells of a 96-well microplate [13, 14]. After centrifugation at 3000 rpm for 15 min, 75  $\mu$ l of the supernatant was inoculated on to two wells of each cell line. The inoculated plates were incubated at 33 °C in an incubator. When a typical influenza virus cytopathic effect was observed, viral identification was carried out by means of a HI test. We used post-infection ferret sera against B/Guangdong/5/94, B/Beijing/243/97, B/Shangdong/7/97, B/Akita/27/2001 (anti-AK27-01) and B/Kagoshima/11/2002 for the V-lineage and B/Mie/1/93, B/Harbin/7/94, B/Yamanashi/166/98, B/Johannesburg/5/99 (anti-JOHA5-99) and B/Hiroshima/23/2001 for the Y-lineage. These reagents were obtained from either the National Institute of Infectious Diseases, Tokyo, Japan (NIIDJ) or purchased from Denka Seiken Co. (Tokyo, Japan). For identification, 1% guinea-pig red blood cells and 0.5% chicken red blood cells were used and the latter was also used for the seroepidemiological study.

### Seroepidemiological study

Sera were treated with Receptor Destroying Enzyme (RDE; Denka Seiken Co.) according to the manufacturers instructions. HI antibody titres were measured by a standard method [12]. Standard antigens of AK27-01 and JOHA5-99 viruses were provided either by NIIDJ or purchased from Denka Seiken Co. We used B/Yamagata/861/2001 (YA861-01) and B/Yamagata/222/2002 (YA222-02) as the V-lineage representative antigens in each season. We also used B/Yamagata/1045/2002 (YA1045-02) as the Y-lineage antigen.

### Statistical analysis of antigenicity among influenza B viruses based on seroepidemiological study

HI titres for YA861-01, AK27-01, YA222-02, JOHA5-99 and YA1045-02 were compared. Titres



**Fig. 1.** Number of influenza B isolates from children with acute respiratory infections between the 1996–1997 and 2002–2003 influenza seasons in Yamagata, Japan. □, Victoria lineage; ■, Yamagata lineage.

were recorded as 0, 10, 20, 40, 80, 160, 320 and 640, and compared using the Friedman test (a non-parametric test for related samples). If the Friedman test was significant, *post-hoc* tests were conducted (multiple comparison, the related Wilcoxon test with Bonferroni correction). Additionally, correlation coefficients (Spearman's rank correlation) were estimated. *P* values less than 0.05 (two-tailed) were regarded as statistically significant. All statistical analysis was performed using the SPSS for Windows (version 10.7J) software (ref. SPSS Base 10.0 Application Guide, SPSS Inc., Chicago, IL, USA, 1999).

## RESULTS

### Influenza virus isolation in Yamagata

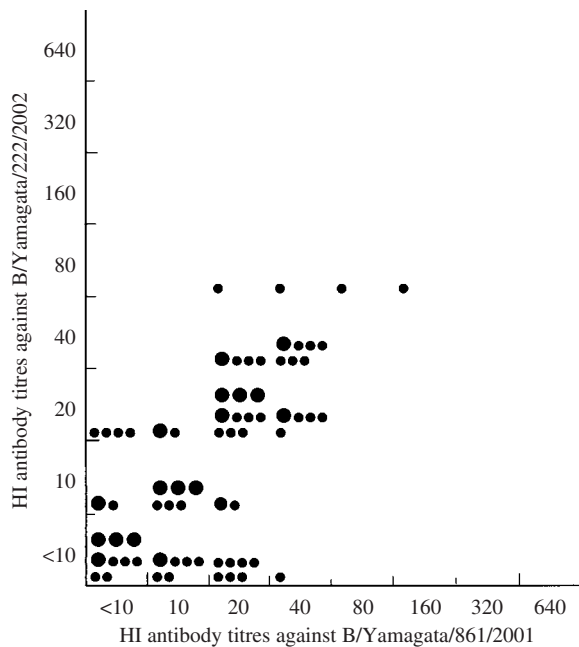
During the study period, we isolated 1124 influenza A (H3), 388 influenza A (H1) and 670 influenza B viruses. Among the influenza B viruses, 424 out of 670 strains were identified as V-lineage and 246 as Y-lineage (Fig. 1). While Y-lineage viruses were a major cause of the influenza B epidemic in the 1996–1997 and 2000–2001 seasons, the V-lineage viruses predominated in 1997–1998, 2001–2002 and 2002–2003. In the 1998–1999 season, both lineages co-circulated almost equally. Patients, from whom influenza B viruses were isolated, were under 16 years old except for 21 cases during the study period.

For the 2000–2001 influenza season, 80 Y-lineage viruses and three V-lineage viruses were isolated. All three V-lineage strains, which were isolated in May at the end of the 2000–2001 season, had an HI titre of

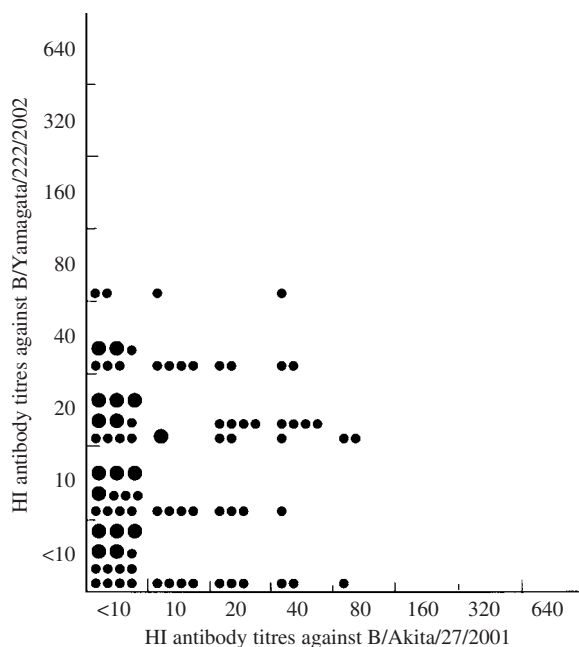
40 with anti-B/Shangdong/7/97. Thus, one of them, YA861-01 was chosen as the representative strain of V-lineage for that season. For the 2001–2002 season, 98 V-lineage viruses and four Y-lineage viruses were isolated. The HI titres of the V-lineage viruses with anti-AK27-01 were 10 for nine isolates, 20 for 58, 40 for 29 and 80 for two isolates. YA222-02 (HI titre 20) was chosen as the representative strain of the V-lineage for this season. Four Y-lineage viruses had HI titres of 40 and 80 with anti-JOHA5-99, and YA1045-02 (HI titre 80) chosen as the representative Y-lineage virus for the 2001–2002 season. We chose representative strains from ones which were isolated the earliest or in the earlier period and which also had an intermediate HI titre in each season.

### Seroepidemiological study using standard and circulating viral antigens

To compare antigenicity among standard and circulating viral antigens in view of herd immunity, we measured HI antibody titres among people in Yamagata. The scatter plots of the HI antibody prevalence against YA861-01, AK27-01 and YA222-02 (V-lineage) are shown in Figures 2 and 3. The results showed that the antibody-positive rate to YA222-02 was similar to that of YA861-01 ( $r=0.72$ ,  $P=0.000$ ) (Fig. 2), but different from that of AK27-01 ( $r=0.15$ ,  $P=0.19$ ) (Fig. 3). In other words, the herd immunity recognized a similar antigenicity between V-lineage representative isolates between the 2000–2001 and 2001–2002 influenza seasons and a different one between the standard antigen and circulating antigen in the 2001–2002 influenza season.

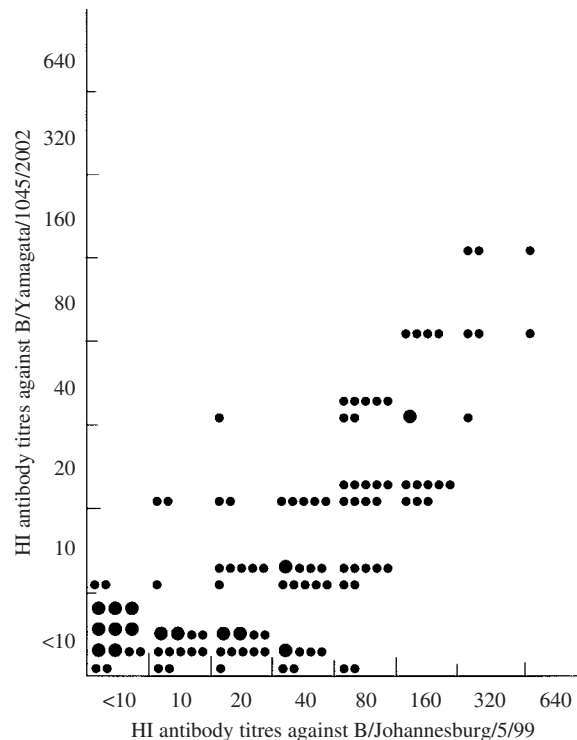


**Fig. 2.** HI antibody titres against B/Yamagata/861/2001 and B/Yamagata/222/2002 (Victoria lineage) among 237 people in Yamagata, 2001. ●, Ten persons; ●, 1 person.



**Fig. 3.** HI antibody titres against B/Akita/27/2001 and B/Yamagata/222/2002 (Victoria lineage) among 237 people in Yamagata, 2001. ●, Ten persons; ●, 1 person.

The HI antibody-positive rates to JOHA5-99 and to YA1045-02 (Y-lineage) are shown in Figure 4. The results indicated that the herd immunity levels seem likely to be proportional, however, herd immunity (antibody-positive rate) against YA1045-2 was lower than that of JOHA5-99 ( $r=0.77$ ,  $P=0.000$ ). That is



**Fig. 4.** HI antibody titres against B/Johannesburg/5/99 and B/Yamagata/1045/2002 (Yamagata lineage) among 237 people in Yamagata, 2001. ●, Ten persons; ●, 1 person.

the herd immunity identified the antigenic difference between the standard antigen and the circulating antigen in the 2001–2002 influenza season by approximately four-fold HI titre.

**DISCUSSION**

In terms of the prediction of influenza B epidemics, three influenza B V-lineage isolates from the end of the 2000–2001 season were considered to be herald viruses for the following 2001–2002 season [15, 16]. The representative isolates in 2000–2001 and in the following season had a similar antigenicity as shown by HI analysis with ferret standard antisera. Sero-epidemiological study in Yamagata also supported the notion that these viruses had a similar antigenicity as shown in Figure 2. We then postulated that the primary reason for the influenza B V-lineage epidemic in the 2001–2002 season was the existence of the herald virus at the end of the previous season. Although the herald wave phenomenon is not always observed [16–18], this observation is useful information for predicting forthcoming influenza epidemics. Based on the same theory, we predicted that there would be a Y-lineage epidemic in the 2002–2003 season, as we detected four Y-lineage viruses in

March and April 2002 and we observed an antigenic drift in these strains compared to the standard antigen in human sera as shown in Figure 4. However, our prediction was wrong and V-lineage viruses predominated again in the 2002–2003 season. Worldwide, influenza B viruses antigenically closely related to the B/Hong Kong/330-like virus (V-lineage) also predominated in both the 2001–2002 and 2002–2003 seasons [10, 19].

For the 2000–2001 season, V-lineage viruses accounted for approximately 8% of all influenza virus isolates in Japan [20]. Isolates in the 2000–2001 season from several areas in Japan showed HI titres of 10–20 with anti-AK27-01 (homologous titre 320) according to NIIDJ [20]. V-lineage isolates in Yamagata for the 2001–2002 season also largely showed similar HI titres with anti-AK27-01. Thus, most V-lineage isolates in Japan between the 2000–2001 and 2001–2002 seasons might have HI titres of 10–20 with anti-AK27-01. Furthermore, herd immunity in Yamagata showed that HI titres against YA222-02 were not proportional to those against AK27-01 as shown in Figure 3. These results indicated that AK27-01 was not the representative strain of circulating V-lineage viruses in the community for the 2000–2001 and 2001–2002 seasons. Several previous studies have indicated such discrepancies in antigenicity between egg-grown influenza antigens (standard antigens in this study) and tissue-cultured antigens [21–25]. We need to resolve such technical issues in order to build an effective surveillance system with better predictability.

In conclusion, we report herein our success and failure in predicting the main lineage of influenza B epidemics in the 2001–2002 and 2002–2003 seasons. Currently we are not sure whether Y-lineage isolates at the end of the 2002–2003 season are the herald viruses for the next influenza season. The epidemiology of the two influenza B lineages still remains unclear. Therefore, a careful watch is required to distinguish these two lineages in order to provide more effective public-health strategies against influenza B epidemics. To implement this objective, it is necessary to continue with the epidemiological study of influenza B lineages, using virus isolation, serological analysis and modern molecular technology, without being discouraged by errors in prediction [26].

#### ACKNOWLEDGEMENTS

We thank the doctors, nurses, and people of Yamagata Prefecture for their assistance and collaboration

in the surveillance of viral infectious diseases. This work was partially supported by Daido Life Welfare Foundation.

#### REFERENCES

1. World Health Organizations. Influenza vaccines. *Weekly Epidemiol Rec* 2002; **28**: 230–239.
2. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare, Japan. *Influenza 1997/98. Infect Agents Surveill Rep* 1998; **19**: 272–273.
3. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare, Japan. *Influenza in 2001/02 season, Japan. Infect Agents Surveill Rep* 2002; **23**: 307–308.
4. Kanegae Y, Sugita S, Endo A, et al. Evolutionary pattern of the hemagglutinin gene of influenza B viruses isolated in Japan: cocirculating lineages in the same epidemic season. *J Virol* 1990; **64**: 2860–2865.
5. Rota PA, Wallis TR, Harmon MW, Rota JS, Kendal AP, Nerome K. Cocirculation of two distinct evolutionary lineages of influenza type B virus since 1983. *Virology* 1990; **175**: 59–68.
6. Rota PA, Hemphill ML, Whistler T, Regnery HL, Kendal AP. Antigenic and genetic characterization of the haemagglutinins of recent circulating strains of influenza B virus. *J Gen Virol* 1992; **73**: 2737–2742.
7. Kinnunen L, Ikonen N, Pöyry T, Pyhälä R. Evolution of influenza B/Victoria/2/87-like viruses: occurrence of a genetically conserved virus under conditions of low epidemic activity. *J Gen Virol* 1992; **73**: 733–736.
8. Lindstrom SE, Hiromoto Y, Nishimura H, Saito T, Nerome R, Nerome K. Comparative analysis of evolutionary mechanisms of the hemagglutinin and three internal protein genes of influenza B virus: multiple cocirculating lineages and frequent reassortment of the NP, M, and NS genes. *J Virol* 1999; **73**: 4413–4426.
9. Nerome R, Hiromoto Y, Sugita S, et al. Evolutionary characteristics of influenza B virus since its first isolation in 1940: dynamic circulation of deletion and insertion mechanism. *Arch Virol* 1998; **143**: 1569–1583.
10. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2003–2004 influenza season. *Weekly Epidemiol Rec* 2003; **78**: 58–62.
11. Chi XS, Bolar TV, Zhao P, Rappaport R, Cheng S. Cocirculation and evolution of two lineages of influenza B viruses in Europe and Israel in the 2001–2002 season. *J Clin Microbiol* 2003; **41**: 5770–5773.
12. Tuberculosis and Infectious Diseases Control Division, Health Service Bureau, Ministry of Health, Labour and Welfare, Government of Japan and Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Japan: Annual Report 2000 National Epidemiological Surveillance of Vaccine-preventable Diseases, 2002: 15–82.

13. Numazaki Y, Oshima T, Ohmi A, et al. A microplate method for isolation of viruses from infants and children with acute respiratory infections. *Microbiol Immunol* 1987; **31**: 1085–1095.
14. Mizuta K, Abiko C, Goto H, Murata T, Murayama S. Enterovirus isolation and presumptive identification by a modified microplate method from children with acute respiratory infections (ARI). *Int J Infect Dis* 2003; **7**: 138–142.
15. Glezen WP, Couch RB, Taber LH, et al. Epidemiologic observations of influenza B virus infections in Houston, Texas, 1976–1977. *Am J Epidemiol* 1980; **111**: 13–22.
16. Glezen WP, Couch RB, Six HR. The influenza herald wave. *Am J Epidemiol* 1982; **116**: 589–598.
17. Sahara K, Sugieda M, Nagaoka H, et al. Epidemiological analysis of influenza B virus belonging to B/Victoria/2/87 lineage isolated in off-season of 1998 and late epidemic season in Shizuoka Prefecture. *Kansenshogaku Zasshi* 2000; **74**: 481–485.
18. Nakajima S, Nishikawa F, Nakamura K, Nakajima K. Comparison of the HA genes of type B influenza viruses in herald waves and later epidemic seasons. *Epidemiol Infect* 1992; **109**: 559–568.
19. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2003 influenza season. *Weekly Epidemiol Rec* 2002; **77**: 344–348.
20. Odagiri T, Saito T, Saito T, et al. Analysis of influenza isolates in 2000/2001 season. *Infect Agents Surveill Rep* 2001; **22**: 252–259.
21. Katz JM, Naeye CW, Webster RG. Host cell-mediated variation in H3N2 influenza viruses. *Virology* 1987; **156**: 386–395.
22. Katz JM, Webster RG. Antigenic and structural characterization of multiple subpopulations of H3N2 influenza virus from an individual. *Virology* 1988; **165**: 446–456.
23. Pyhälä R, Pyhälä L, Valle M, Aho K. Egg-grown and tissue-culture-grown variants of influenza A (H3N2) virus with special attention to their use as antigens in seroepidemiology. *Epidemiol Infect* 1987; **99**: 745–753.
24. Lathey JL, Van Voris LP, Belshe RB. Superiority of tissue-culture-grown antigens over egg-grown antigens for serologic diagnosis of influenza B virus infections. *J Med Virol* 1986; **19**: 155–159.
25. Robertson JS, Naeye CW, Webster RG, Bootman JS, Newman R, Schild GC. Alteration in the hemagglutinin associated with adaptation of influenza B virus to growth in eggs. *Virology* 1985; **143**: 166–174.
26. Bush RM, Bender CA, Subbarao K, Cox NJ, Fitch WM. Predicting the evolution of human influenza A. *Science* 1999; **286**: 1921–1925.