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# Comparison of the immunogenicity, efficacy and safety of 10 μg and 20 μg of a hepatitis B vaccine: a prospective randomized trial

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## SUMMARY

Four thousand and one hospital staff were screened for hepatitis B virus (HBV) markers in a vaccination programme in Hong Kong. The seropositivity rate for HBsAg, anti-HBs and anti-HBc were significantly higher in the 3160 existing hospital staff than in 841 new recruits. Of the subjects negative for HBV markers, 605 were randomized to receive three doses of either 10 or 20 μg of the Merck Institute vaccine (HB-VAX). Compared with the 20 μg dose, vaccination with the 10 μg dose results in equal immunogenicity and efficacy at the completion of the three injections but induced a slower response rate and lower anti-HBs titres with the first two doses. The commonest side-effect of local soreness was less with the 10 μg dose. We conclude that (1) hospital staff working in high endemic areas should be vaccinated on recruitment and (2) the 10 μg dose of HB-VAX can replace the recommended 20 μg dose for adults, being cheaper and as efficacious.

## INTRODUCTION

Since the development of the hepatitis B vaccine (HB-VAX) (Hilleman *et al.* 1975), the safety, immunogenicity and efficacy of the vaccine has been proven by various clinical trials using 20 and 40 μg doses (Szmuness *et al.* 1980; Szmuness *et al.* 1981; Krugman *et al.* 1981; Papaevangelou *et al.* 1982).

The high cost of the vaccine is one of the main factors preventing widespread use of the vaccine, especially in the Third World where the hepatitis B carrier rate is high (5–20%) (Lai *et al.* 1984). A hepatitis B vaccine with recombinant DNA in yeasts (McAlur *et al.* 1984) is now under clinical trial (Scolnick *et al.* 1984). However, this second generation vaccine will only become commercially available in 1–2 years' time, and there is no guarantee that the initial cost will be less than the currently licenced plasma-derived vaccine. The most immediate method to minimize the cost of mass vaccination is to find the lowest efficacious dose of HB-VAX. Preliminary studies by Deinhardt *et al.* (1983) and by Papaevangelou *et al.* (1983) using 10 μg dose of HB-VAX in 80 and 20 recipients respectively showed similar immunogenicity to that induced with the 20 μg dose. A further

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study in 100 subjects in Nigeria also gave the same result (Ayoola, 1984). The titres of antibody induced against hepatitis B surface antigen (anti-HBs) were not mentioned in the latter two studies. However, these encouraging results required further confirmation before the widespread use of 10 $\mu$g can be recommended as a standard dose in adults.

The aim of this study was to compare the immunogenicity, efficacy and safety of 10 and 20 $\mu$g dose of HB-VAX in healthy adults in Hong Kong, an area of high endemicity for hepatitis B infection (Yeoh, Chang & Kwan, 1984).

## MATERIALS AND METHODS

### *Screening of subjects*

From April 1983 to January 1984, serum samples from 4001 medical, dental and para-medical staff from the regional hospitals of the Medical and Health Department and of the University Department of Medicine, Hong Kong (MHD and UDM, HK) were screened for the following within 2 weeks of entrance into the study: (*a*) serum alanine transaminase level (ALT), (*b*) hepatitis B surface antigen (HBsAg) and (*c*) anti-HBs, using reverse passive haemagglutination (RPHA) and enzyme-linked immuno-absorbant essays (ELISA) respectively (Auscell and Ausab – EIA, Abbott Laboratories). In the initial screening, 500 each of the existing staff and of the new recruits were tested for antibody to hepatitis B core antigen (anti-HBc) by ELISA (Corzyme, Abbott Laboratories).

Those subjects fulfilling the following criteria were admitted to the study: (*a*) ALT levels of less than 50 $\mu$mol/min $l^{-1}$, (*b*) no history of receiving hepatitis B immune globulin within 4 months of screening, (*c*) no serious systemic illness or previous allergic reactions, (*d*) for females, willingness to remain not pregnant during the course of vaccination, (*e*) written informed consent and (*f*) serum negative for HBsAg and anti-HBs. These subjects were also tested for anti-HBc if this had not been already tested during the initial screening.

### *Protocol*

Subjects fulfilling the above criteria were allocated by a randomization table to receive either 10 or 20 $\mu$g of HB-VAX at 0, 1 and 6 months.

Blood was taken to check for anti-HBs titre and ALT level at 0, 1, 6, 8, 36 and 60 months after the first vaccination. The anti-HBs titre was obtained by ELISA using Hollinger 's formula (Hollinger *et al.* 1982) and a WHO reference standard. Anti-HBc was tested in all subjects at 8 months, i.e. 2 months after the last vaccination. For subjects positive for anti-HBc at 8 months, anti-HBc was also tested in the sera taken at 1 and 6 months. Any side-effects that might be related to the vaccine were noted during each follow-up.

### *Definition of the occurrence of hepatitis events during the course of vaccination and follow-up*

Any subjects with symptoms suggestive of ' hepatitis ' or abnormal ALT during follow-up were recalled and blood taken for further examination within 1 week. The previous blood samples of these subjects were re-checked for HBsAg and anti-HBc (Ausria and Corab, Abbott Laboratories) as well as IgM antibody to

Table 1. *Hepatitis B status of existing hospital staff and new recruits in Hong Kong regional hospitals*

| | Existing hospital staff (%) | New recruits (%) | All staff screened (%) |
|---|---|---|---|
| Number of subjects | 3160 | 841 | 4001 |
| Median age in years (range) | 34 (20–56) | 20 (18–22) | — |
| HBsAg positive | 297* (9·4) | 53* (6·3) | 350 (8·7) |
| Anti-HBs positive | 1051** (33·3) | 168** (20·0) | 1219 (30·5) |
| Anti-HBc positive alone (in the first 500 subjects) | 16*** (8·0) | 6*** (1·2) | 22 (2·2) |

* $P < 0{\cdot}01$, $\chi^2 = 7{\cdot}9801$; ** $P < 0{\cdot}0001$, $\chi^2 = 55{\cdot}3216$; *** $P < 0{\cdot}05$, $\chi^2 = 4{\cdot}6663$.

hepatitis A virus (IgM anti-HAV), cytomegalovirus (CMV) and Epstein–Barr virus (EBV) antibody titres.

Subjects were defined as having (*a*) HBV infections if at least two sequential samples of their sera became positive for anti-HBc and/or HBsAg, (*b*) HAV infection if their sera were positive for IgM anti-HAV, (*c*) CMV or EBV infection if rising antibody titres to the relevant virus were found or (*d*) non-A non-B (NANB) hepatitis if (*a*), (*b*) and (*c*) were negative in the presence of a persistently raised ALT level of over 100 $\mu$mol/min $l^{-1}$. Subjects diagnosed as having HBV infection were withdrawn from the trial. Those suffering from other forms of hepatitis were witheld from further vaccination until the ALT levels had returned to normal for at least 2 weeks.

*Details of vaccinees and results of initial screening*

Of the 4001 subjects screened, 3160 were existing hospital staff and 841 were new recruits to the MHD and UDM, HK. The hepatitis B status of the existing staff and new recruits are tabulated in Table 1.

Six hundred and thirty subjects fulfilled the criteria for inclusion and agreed to join the vaccine programme. Twenty-five subjects defaulted (12 from the 10 $\mu$g regime, 13 from the 20 $\mu$g regime). Six hundred and five vaccinees completed the third dose of vaccination with blood taken at 8 months. Table 2 shows the data of the two groups of vaccinees. The two groups were comparable in age, sex and the ratio of existing staff to new recruits.

The results of the two groups of vaccinees up to eight months after vaccination were compared using the $\chi^2$ test and Fisher 's exact test.

## RESULTS

*Hepatitis events during the course of vaccination and follow-up*

One of the 297 recipients receiving 10 $\mu$g HB-VAX developed HBV infection according to the definition given above. The patient was a new recruit who was asymptomatic but had become positive for IgM anti-HBc when her serum was checked at 8 months. The earlier specimens of sera were then rechecked for this patient. At 1 month, she was negative for all HBV markers. At 6 months, her IgM anti-HBc cut-off ratio was 1·55, anti-HBc showed 94 % blocking, and her anti-HBs

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Table 2. *Details of the 605 vaccinees*

| | 10 μg dose | 20 μg dose |
|---|---|---|
| No. of subjects | 297 | 308 |
| Male : female | 65 : 232 | 92 : 216 |
| Median age in years (range) | 20 (18–62) | 20 (18–53) |
| No. of existing staff | 113 | 132 |
| No. of new recruits | 184 | 176 |

level was 311 milli-International units (mi.u.)/ml. At 8 months, the values for the above tests were 1·12, 98% and 4662 mi.u./ml respectively. At no time was she positive for HBsAg.

No subjects receiving 20 μg HB-VAX developed evidence of HBV infection. The number of hepatitis B events are not significantly different in the two groups of vaccinees (Fisher 's exact test).

One other recipient, who was symptomatic, and receiving 10 μg of HB-VAX, developed IgM anti-HAV at 6 months. His liver enzymes were raised for 4 weeks. He subsequently completed the course of vaccination.

The one subject who became positive for anti-HBc is excluded from the subsequent analysis of immunogenicity and anti-HBs titres.

*Frequency of side-effects*

The types and frequency of side-effects are listed in Table 3.

Local soreness decreased with the number of vaccine injections, and, although it could be severe, lasted for 12–24 h only.

The side-effects other than local soreness, pyrexia and fatigue included skin rash, headache, mild joint pain and nausea. They occurred in 5 episodes with 891 injections of the 10 μg dose and in 11 episodes with 924 injections of the 20 μg dose. None of the side-effects lasted for more than 36 h.

*Immunogenicity of 10 μg and 20 μg of HB-VAX*

(*a*) *Overall immunogenicity.* The overall immune response rate after the third dose of vaccine, i.e. at 8 months, was 93·6% for the 10 μg recipients, and 96·1% for the 20 μg recipients. There was no statistical difference between the two groups. Taking an anti-HBs level of ⩾ 10 mi.u./ml as a cut-off point, the percentages of subjects with an anti-HBs of over 10 mi.u./ml were 91·2% and 95·1% for those receiving the 10 and 20 μg dose respectively. There was again no statistical difference between the two groups. There was also no difference in response rate between the two groups of recipients when each group was sub-divided into existing hospital staff and new recruits. The persistence of the anti-HBs will be re-assessed at 36 months.

(*b*) *Antibody response – titre and rate of development.* The results are shown in Table 4. The percent of responders and their antibody titre after the first, second and third injections of vaccine were recorded at 1, 6 and 8 months after starting vaccination respectively.

In the vaccinees receiving the 10 μg dose, the anti-HBs seroconversion rate was lower after the first and the second injections when tested at 1 and 6 months respectively compared with those receiving 20 μg per dose. The proportion of

Table 3. *Frequency of side effects of the two dosages of HB-VAX*

| | 10 μg dose (%) | 20 μg dose (%) | *P* |
|---|---|---|---|
| No. of vaccinees | 297 | 308 | — |
| Local soreness | | | |
| First injection | 22·3[a] | 28·2[d] | N.S. |
| Second injection | 11·8[b] | 17·2[e] | $P < 0{\cdot}01$ $\chi^2 = 7{\cdot}9196$ |
| Third injection | 7·8[c] | 15·3[f] | $P < 0{\cdot}01$ $\chi^2 = 7{\cdot}932$ |
| Pyrexia (< 39 °C) | | | |
| First injection* | 1·7 | 3·0 | N.S. |
| Second injection* | 1·4 | 2·3 | N.S. |
| Third injection* | 1·7 | 0·3 | N.S. |
| Fatigue | 1·6 | 2·9 | N.S. |
| Others | 0·6 | 1·2 | N.S. |

* Calculations by Fisher's exact test. The rest of the calculations are done by $\chi^2$ test.

| | | |
|---|---|---|
| a vs b | $P < 0{\cdot}001$ | $\chi^2 = 12{\cdot}4482$ |
| a vs c | $P < 0{\cdot}001$ | $\chi^2 = 11{\cdot}2423$ |
| b vs c | N.S. | — |
| d vs e | $P < 0{\cdot}01$ | $\chi^2 = 8{\cdot}3036$ |
| d vs f | $P < 0{\cdot}001$ | $\chi^2 = 11{\cdot}9403$ |
| e vs f | N.S. | — |

Table 4. *The percentage of recipients becoming positive for anti-HBs and the anti-HBs titre following the two dosage regimes of HB-VAX*

| | 10 μg HB-VAX (296 vaccinees) | | | 20 μg HB-VAX (308 vaccinees) | | |
|---|---|---|---|---|---|---|
| Anti-HBs titre (mi.u./ml) | 1 month (%) | 6 months (%) | 8 months (%) | 1 month (%) | 6 months (%) | 8 months (%) |
| 3–9·9 | 5·7 | 9·1 | 2·4 | 9·1 | 7·1 | 1·0 |
| 10–99·9 | 6·1 | 33·4 | 6·8 | 13·6 | 26·6 | 7·8 |
| 100–999·9 | 0·3 | 40·2** | 32·4 | 1·6 | 49·0** | 27·9 |
| > 1000 | — | 3·4*** | 52·0 | — | 8·4*** | 59·4 |
| Total | 12·1* | 86·1**** | 93·6 | 24·3* | 91·1**** | 96·1 |

mi.u. = milli-international units.

* $P < 0{\cdot}001$, $\chi^2 = 14{\cdot}95$; ** $P < 0{\cdot}05$, $\chi^2 = 4{\cdot}76$; *** $P < 0{\cdot}01$, $\chi^2 = 6{\cdot}8993$; **** $P < 0{\cdot}05$, $\chi^2 = 3{\cdot}9119$.

vaccinees receiving the 10 μg dose with an anti-HBs titre of above 100 mi.u/ml was also significantly lower than that of the vaccinees receiving the 20 μg dose. However, by 8 months, i.e. 2 months after the third injection, the anti-HBs titres were not significantly different between those receiving the 10 and the 20 μg dose.

(c) *Antibody response related to age and sex.* The anti-HBs response rates after the third injection when put into different age groups, are given in Table 5. There was no difference in the immune response in all age groups between the 10 and 20 μg regimes. When the total study population was considered, the immune

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Table 5. *Immune response rate to 10 μg and 20 μg regimes of HB-VAX at 8 months in relation to age*

| Age in years | 10 μg | | 20 μg | | Overall |
|---|---|---|---|---|---|
| | No. of subjects | % of responders | No. of subjects | % of responders | % of responders |
| 18–30 | 261 | 94·3 | 262 | 97·3 | 95·8[a] |
| 31–40 | 22 | 90·0 | 28 | 89·3 | 90·0[b] |
| > 40 | 13 | 84·6 | 18 | 88·9 | 87·1[c] |
| | a vs b | $P = 0{\cdot}076$ (Fisher's exact test) | | | |
| | a vs c | $P = 0{\cdot}050$ (Fisher's exact test) | | | |
| | a vs b+c | $P = 0{\cdot}025$ (Fisher's exact test) | | | |

response rate of those above 30 years of age was significantly lower than those aged 30 or below. There was no difference in immune response between males and females.

## DISCUSSION

It has been well documented that seropositivity for HBV markers, especially for anti-HBs, increases with the years of service in a hospital environment in areas of low endemicity for HBV (Mosley *et al.* 1975; Koff, 1978). The data of the HBV status of the 4001 hospital staff screened for the vaccination programme in Hong Kong (Table 1) showed that the incidences of HBsAg, anti-HBs and anti-HBc positivity were all significantly higher in the existing staff than in the new recruits. The most important fact that requires emphasis is the higher proportion of those positive for HBsAg in the existing staff. Since we did not re-check the HBsAg status for all HBsAg-positive subjects after a 6-month interval, it is possible that some of the existing staff who were positive for HBsAg on screening were suffering (or recovering) from acute HBV infection, and were not chronic HBV carriers. However, the proportion of these HBsAg-positive subjects having acute HBV infection is probably low as these adult subjects were all asymptomatic at the time of screening and their ALT levels were all below 100 μmol/min $l^{-1}$. It is more likely that the majority of these was chronic HBV carriers. This higher incidence of chronic HBV carriers in the existing staff cannot be entirely attributed to the older age of these subjects since a previous survey of 16334 subjects in the general population of Hong Kong showed a decline in the prevalence of chronic HBV carriers after the age of 30, the incidence of HBsAg being 8·1 % at age 16–20, 11·4 % at age 21–30, 9·7 % at age 31–40, 9·6 % at age 41–50 and 8·7 % at the age of over 50 (Yeoh *et al.* 1984). Therefore, it appears that, although the chance of an adult acquiring HBV infection and becoming a chronic carrier is lower than that of children (Beasley, 1982), existing hospital staff compared with new recruits do have a higher prevalence of chronic HBV carriers. It can be concluded that vaccination of hospital staff who are negative for HBV markers should have a high priority, and should be carried out on recruitment into hospital service, in areas of high endemicity like Hong Kong. (Further details of the HBV status of hospital staff will be published in a separate paper.)

*Immunogenicity and efficacy of 10 and 20 μg of HB-VAX*

In our study, about 300 subjects received either the 10 or the 20 μg dose of HB-VAX. This large sample size would enable us to detect differences in immunogenicity of the order of 10–15 % according to the figures quoted by Fleiss (1973). This is probably the reason why our study reveals some minor differences between the two groups of vaccinees that were not found in the previous three studies (Deinhart *et al.* 1983; Papaevangelou *et al.* 1983; Ayoola, 1984).

Table 4 shows that vaccinees receiving the 10 μg dose of HB-VAX had a significantly lower immune response rate after the first dose (at 1 month). It should be noted, however, that the percentage of responders after the first dose of 20 μg of HB-VAX was also quite low (24·3 % only).

After the second dose (at 6 months), vaccinees receiving the 10 μg dose had a lower immune response rate as well as a lower proportion of subjects with anti-HBs titres of above 100 mi.u./ml. But the actual percentage of responders to the 10 μg dose was 86·1 %, and is well within the acceptable response range.

By the eighth month (i.e. 2 months after the third dose), there was no difference in immune response rate or in the anti-HBs titres between the groups receiving the 10 or the 20 μg dose.

Only one subject, receiving the 10 μg dose, became positive for anti-HBc at 6 and 8 months. This subject 's IgM anti-HBc cut-off ratio was already low at 6 months (1·55) with a slight decline at 8 months (1·12). Her anti-HBs titre was also already moderately high at 6 months (311 mi.u./ml). From these serological data, it is likely that the subject acquired the HBV infection before or shortly after the second dose of vaccine, and that the infection was not due to an inadequate anti-HBs response to the 10 μg dose regime. The efficacy of the 10 and 20 μg dose in protecting vaccinees from acquiring HBV infection was therefore comparable. That HBsAg was not detected in the sera of the one subject who became positive for anti-HBc may be due to the fact that blood was taken only at 0, 1 and 6 months, with a gap of 5 months between the second and third blood-taking.

Our results show that, although the 10 μg dose gives a slower response rate as well as a slightly lower titre of anti-HBs after the first two doses of vaccine, the immunogenicity after the third dose is comparable to that found with the 20 μg dose. The efficacy of the 10 and 20 μg doses in prevention of hepatitis B infection is also comparable. This is of obvious significance in reducing the cost of mass vaccination.

When the total study population was considered, differences related to age in the immune response rate to the vaccine were observed. This finding is not unexpected, and is in accord with the results obtained by Deinhardt *et al.* (1983). Subjects in the age group of 18–30 years (group *a* in Table 5) had a better immune response rate than subjects in the age group of 31–40 years and subjects over the age of 40 (groups *b* and *c* respectively in Table 5). The difference was just significant when group *a* was compared with group *c* ($P = 0{\cdot}05$). However, when group *a* was compared with group *b*, the $P$ value was 0·076. This failure to reach statistical significance is probably due to a type II error since group *b* consisted of 50 vaccinees only, too small a number to detect small differences. When the immune response

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rate of group *a* was compared with group *b*+*c* (a study population of 81 vaccinees), it was markedly and significantly better ($P = 0{\cdot}025$).

*Frequency of side-effects*

From Table 3, it can be seen that for both the 10 and the 20 $\mu$g dose, the occurrence of local soreness decreased with each injection. This may be partly explained by the vaccinees becoming more psychologically prepared for the injections.

Of greater importance is that using the 10 $\mu$ dose, there is significantly less local soreness following the second and third injections. Thus as far as side effects are concerned, the 10 $\mu$g dose is more advantageous than the 20 $\mu$g dose, and may lead to better vaccinee compliance.

In summary, hospital staff working in high endemic areas have a high risk of becoming chronic HBsAg carriers and should preferably be vaccinated on recruitment into hospital service. In adults, vaccination with 10 $\mu$g dose of HB-VAX, when compared with the 20 $\mu$g dose, resulted in (i) equal immunogenicity and efficacy on completion of the course of three doses of vaccine, (ii) a slower response rate and a lower anti-HBs titre with the first two doses, and (iii) a lower frequency of local soreness as a side-effect. We conclude that the 10 $\mu$g dose of HB-VAX can be used with equal safety in adults as the usually recommended 20 $\mu$g dose. This would reduce the cost by half in a mass vaccination programme.

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## REFERENCES

Ayoola, E. A. (1984). The immune response of healthy Nigerian adults to small doses of hepatitis B vaccine: comparison of 10 and 20 $\mu$g doses. *Journal of Medical Virology* **13**, 223–225.

Beasley, R. P. (1982). Hepatitis B virus as the etiologic agent in hepatocellular carcinoma – epidemiologic considerations. *Hepatology* **2**, 21–26S.

Deinhardt, F., Zachoval, R., Jilg, W., Lorbeer, B. & Roggendorf, M. (1983). Immune responses to active and passive-active vaccination against hepatitis. *Journal of Infection* **7** (I), 21–25.

Fleiss, J. L. (1973). In *Statistical Methods for Rates and Proportions*. New York: John Wiley.

Hilleman, M., Buynak, E., Roehm, R., Tytell, A., Berthland, A. & Lampson, S. (1975). Purified and inactivated human hepatitis B vaccine: progress report. *American Journal of Medical Science* **270**, 401–404.

Hollinger, F. B., Adam, E., Heiberg, D. & Melnick, J. L. (1982). Response to hepatitis B vaccine in a young adult population. In *Viral Hepatitis* (eds. W. Szmuness, H. J. Alter & J. E. Maynard), pp. 451–466. New York: Franklin Institute Press.

Koff, R. S. (1978). Specific epidemiologic problems. In *Viral Hepatitis* (ed. R. S. Koff), *Clinical Gastroenterology Monograph Series*, pp. 115–119. New York: John Wiley.

Krugman, S., Holley, H. P., Davidson, M., Sunberkoff, M. S. & Matsariotis, N. (1981). Immunogenic effect of inactivated hepatitis B vaccine: comparison of 20 and 40 $\mu$g doses. *Journal of Medical Virology* **8**, 119–121.

Lai, C. L., Wu, P. C., Yeoh, E. K., Lok, A. S. F., Lin, H. J., Lam, S. K. & Todd, D. (1984). Hepatocellular carcinoma and the hepatitis B virus. In *Viral Hepatitis B Infection in the Western Pacific Region: Vaccine and Control* (ed. S. K. Lam, C. L. Lai & E. K. Yeoh), pp. 3–16. Singapore: World Scientific.

McAlur, W. J., Buynak, E. B., Maigetter, R. Z., Wampler, D. E., Miller, W. J. & Hilleman, M. R. (1984). Human hepatitis B vaccine from recombinant yeast. *Nature* **12**, 178–179.

Mosley, J. M., Edwards, V. M., Casey, C., Redeker, A. G. & White, E. (1975). Hepatitis B virus infection in dentists. *New England Journal of Medicine* **293**, 729–734.

Papaevangelou, G., Vissoulis, C., Roumeliotou-Karayannis, A., Kolaitis, N. & Krugman, S. (1982). Comparison of safety and immunogenicity of adw and ayw hepatitis B vaccines. *Journal of Medical Virology* **9**, 231–236.

Papaevangelou, G., Roumeliotou-Karayannis, A., Vissoulis, C., Stathopoulou, P., Kolaitis, N. & Krugman, S. (1983). Reduction of the dose of hepatitis B vaccine. *Journal of Infection* **7** (I), 69–70.

Scolnick, E. M., McLean, A. A., West, D. J., McAlur, W. J., Miller, W. J. & Buynak, E. B. (1984). Clinical evaluation in healthy adults of a hepatitis B vaccine made by recombinant DNA. *Journal of the American Medical Association* **251**, 2812–2815.

Szmuness, W., Stevens, C. E., Harley, E. J., Zang, E. A., Oleszko, W. R., William, D. C., Sadovsky, R., Morrion, J. M. & Kellner, A. (1980). Hepatitis B vaccine: demonstration of efficacy in a controlled clinical trial in a high risk population in the United States. *New England Journal of Medicine* **303**, 833–841.

Szmuness, W., Stevens, C. E., Zang, E. A., Harley, E. J. & Kellner, A. (1981). A controlled clinical trial of the efficacy of the hepatitis B vaccine (Heptavax B): a final report. *Hepatology* **1**, 377–385.

Yeoh, E. K., Chang, W. K. & Kwan, J. P. W. (1984). Epidemiology of viral hepatitis B infection in Hong Kong. In *Viral Hepatitis B Infection in the Western Pacific Region: Vaccine and Control* (ed. S. K. Lam, C. L. Lai & E. K. Yeoh), pp. 34–42. Singapore: World Scientific.