We thank Professor Alfred Tissieres for his interest and support, and we acknowledge the help of Mrs M. Vollenweider and Mrs C. H. Tonka in the task of hand-dissecting salivary glands. This work was supported by a grant from the Swiss National Science Foundation.

Received 17 June; accepted 19 October 1983.

1. Painter, T. S. *Genetics* **19,** 179–188 (1934).
2. Sorsa, V. *Hereditas* **78,** 298–302 (1974).
3. Laird, C. D. *Cell* **22,** 869–874 (1980).
4. Laird, C. D., Wilkinson, L., Johnson, D. & Sandström, C. *Chromosomes Today* Vol. 7 (eds Bennet, M., Bobrov, M. & Hewitt, G.) 74–83 (George, Allen & Unwin, Hemel Hempstead, 1981).
5. Beermann, W. (ed.) *Results and Problems in Cell Differentiation* Vol. 4, 1–33 (Springer, New York, 1972).
6. Bender, W., Spierer, P. & Hogness, D. S. *J. molec. Biol.* **168,** 17–33 (1983).
7. Spierer, P., Spierer, A., Bender, W. & Hogness, D. S. *J. molec. Biol.* **168,** 35–50 (1983).
8. DuPraw, E. J. & Rae, P. M. M. *Nature* **212,** 598–600 (1966).
9. Lis, J. T., Prestidge, L. & Hogness, D. S. *Cell* **14,** 901–919 (1978).
10. Endow, S. A. & Glover, D. M. *Cell* **17,** 597–605 (1979).
11. Lyfschitz, E. *J. molec. Biol.* **164,** 17–34 (1983).
12. Bender, W. *et al. Science* **221,** 23–29 (1983).
13. Hilliker, A. J., Clark, S. H., Chovnick, A. & Gelbart, W. M. *Genetics* **95,** 95–110 (1980).

Human hepatitis B vaccine from recombinant yeast

William J. McAleer, Eugene B. Buynak, Robert Z. Maigetter, D. Eugene Wampler, William J. Miller & Maurice R. Hilleman*

Division of Virus and Cell Biology Research, Merck Institute for Therapeutic Research, Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486, USA

The worldwide importance of human hepatitis B virus infection and the toll it takes in chronic liver disease, cirrhosis and hepatocarcinoma, make it imperative that a vaccine be developed for worldwide application[1]. Human hepatitis B vaccines[2–6] are presently prepared using hepatitis B surface antigen (HBsAg) that is purified from the plasma of human carriers of hepatitis B virus infection. The preparation of hepatitis B vaccine from a human source is restricted by the available supply of infected human plasma and by the need to apply stringent processes that purify the antigen and render it free of infectious hepatitis B virus and other possible living agents that might be present in the plasma. Joint efforts between our laboratories and those of Drs W. Rutter and B. Hall led to the preparation of vectors carrying the DNA sequence[7,8] for HBsAg and antigen expression in the yeast *Saccharomyces cerevisiae*[9]. Here we describe the development of hepatitis B vaccine of yeast cell origin. HBsAg of subtype adw was produced in recombinant yeast cell culture, and the purified antigen in alum formulation stimulated production of antibody in mice, grivet monkeys and chimpanzees. Vaccinated chimpanzees were totally protected when challenged intravenously with either homologous or heterologous subtype adr and ayw virus of human serum source. This is the first example of a vaccine produced from recombinant cells which is effective against a human viral infection.

Several alternative approaches to a hepatitis B vaccine are being developed. HBsAg has been expressed by several transformed mammalian cell lines, such as the human hepatoma line, PLC/PRF/5 (refs 10, 11), simian virus 40-infected monkey kidney cells[12] and mouse L cells[13]. These sources are of some concern, however, because the cell lines may be neoplastic. Although HBsAg has been cloned in bacteria[7,14], expression was very weak. Other laboratories[15–19] have described the synthesis of oligopeptides that carry antigenic determinants of HBsAg but their potency in animals is low and much work will need to be done to potentiate antigenicity. Smith and collaborators[20] have described the construction of a recombinant vaccinia virus which expresses HBsAg and have proposed its use as a live attenuated vaccine; its antigenic potency has been demonstrated but whether such a vaccine would be safe and effective in man is still unknown.

Table 1 Antigenic potency in mice of HBsAg purified from yeast and from human plasma

Vaccine source	Antigen dose per injection (μg protein)	Anti-HbsAg response after vaccination No. positive/total	GMT
Human plasma	10	9/10	563
(lot 799-2)	2.5	10/10	2,235
	0.625	4/9	32
	0.156	0/10	4
ED_{50}	0.639		
Yeast	40	10/10	5,432
(lot 81-4)	10	10/10	3,400
	2.5	8/10	673
	0.625	8/10	967
ED_{50}	<0.625		

Groups of 10 5-week-old ICR/Ha mice propagated in our laboratories were given a single 1-ml injection intraperitoneally of serial fourfold dilutions of yeast or human plasma vaccine in alum diluent. The mice were bled individually and tested for serum antibody level 4 weeks later. Human plasma vaccine, lot 799-2, was prepared in these laboratories[2–4]. Yeast-derived vaccine, lot 81-4, was purified as described in Fig. 1 legend and adsorbed to alum. GMT, geometric mean titre, expressed in AUSAB units; ED_{50}, dose required to seroconvert 50% of the mice.

Valenzuela *et al.*[9] originally reported that yeast cells are able not only to express the HBsAg gene but also to assemble the polypeptides into particles that have much the same appearance as particles isolated from human plasma and which are immunogenic in mice. Since then, other laboratories[21,22] have shown that HBsAg produced in yeast is antigenic in rabbits and guinea pigs. With such progress, recombinant yeast has become an attractive alternative to human plasma as a source of antigen for hepatitis B vaccine.

For vaccine preparation, the HBsAg used was of subtype adw and was produced in fermentation cultures of *S. cerevisiae* carrying an expression vector using yeast alcohol dehydrogenase I as a promoter. The yeast strain used in these studies was obtained from G. Ammerer (University of Washington) and is similar to the strain described by Valenzuela *et al.*[9] in which the production of HBsAg in yeast was first reported.

Cells were collected by centrifugation and broken by homogenization with glass beads[23]. HBsAg particles were purified from the clarified extract by immune affinity chromatography using goat antibody to human HBsAg. Electron microscopy (Fig. 1) revealed a homogeneous array of particles free of extraneous morphological entities. The UV absorption pattern was the same as for the plasma antigen, with an $E^{1\%}$ of 45. SDS-polyacrylamide gel electrophoresis (Fig. 2) in reducing conditions revealed a major band at molecular weight 23,000 (23K) corresponding to the non-glycosylated polypeptide which is the major polypeptide of the viral envelope. In this respect it differs from the plasma antigen which has, in addition to the 23K polypeptide, a glycosylated derivative which migrates at 27K. The yeast and plasma antigens differ also in their reactivity in the radioimmunoassay (RIA) (AUSRIA II, Abbott). RIA reactivity of purified yeast-derived HBsAg varied from preparation to preparation in the range 20–50% of the reference human antigen.

Because of this reduced radioimmune reactivity, and because the yeast antigen is not glycosylated, it was important to determine whether the antigen was immunogenic. To test both antigenicity and immunogenicity in animals, purified antigen was formulated into a vaccine by adsorbing on alum adjuvant to contain 40 μg HBsAg protein and 0.5 mg aluminium (hydroxide) per 1 ml dose.

* To whom reprint requests should be addressed.

0028-0836/84/020178—03$01.00

Table 2 Antigenic potency in grivet monkeys of HBsAg purified from yeast and from human plasma

Vaccine source	Antigen dose per injection (μg protein)	Anti-HBsAg response after initial vaccine dose (geometric mean titre) Week 4	Week 8	Week 12	Week 52
Human plasma	10	36	213	170	127
(lot 86016)	2.5	343	6,227	17,348	9.924
	0.625	53	4,642	3,164	5,688
	0.156	15	128	83	358
Yeast	40	88	1,078	7,103	11,554
(lot 81-4)	10	184	877	8,489	4,984
	2.5	225	1,168	6,361	10,868
	0.625	109	925	518	313

A group of four initially seronegative grivet monkeys (*Cercopithecus aethiops*), weighing 3–5 kg, were each given two 1-ml intramuscular (i.m.) doses of yeast or human plasma vaccine 4 weeks apart. Dilutions of antigen were made in alum placebo of the same composition as the vaccine. Animals were bled at biweekly intervals for 1 yr and tested for antibody to HBsAg by using a commercial RIA kit (AUSAB, Abbott). Protein was measured by the method of Lowry[29]. Human plasma lot 86016 was prepared in these laboratories[2–4].

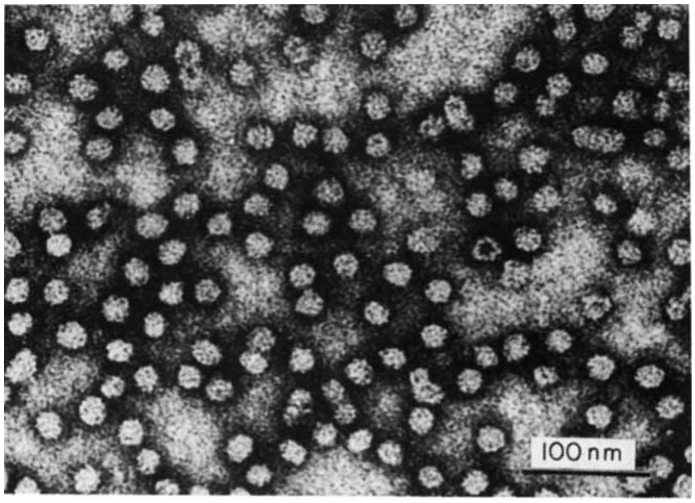

Fig. 1 Electron micrograph of HBsAg particles from recombinant yeast. Cells were grown in a 335-l fermentation vessel, collected by centrifugation, resuspended in an equal volume of 0.01 M sodium phosphate *p*H 7.5, containing 0.01% Triton X-100, and disrupted by rapid stirring with glass beads in a Dyno-Mill (Impandex; see ref. 23). The resulting extract was clarified by centrifugation for 90 min at 10,000*g*. The clarified yeast extract was applied to a column of Sepharose 4B to which had been attached goat antibody to human HBsAg. The column was developed at a flow rate of 2 column vol per h. Extraneous protein was washed away with 5 column vol of buffer A and the HBsAg was eluted with 3 M NH_4SCN. Fractions containing HBsAg were pooled and thiocyanate was removed by dialysis against 0.01 M sodium phosphate *p*H 6.8, containing 0.15 M NaCl. Dialysed antigen was diluted to 40 μg ml^{-1} and visualized by negative staining with 2% phosphotungstic acid.

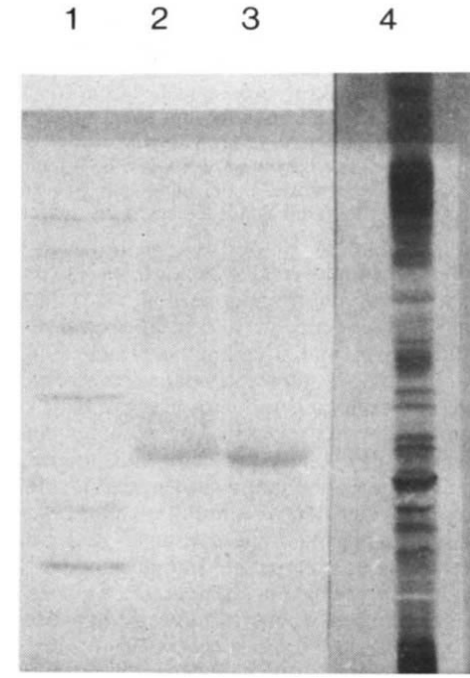

Fig. 2 SDS-polyacrylamide gel electrophoresis of cell culture and yeast-derived HBsAg. All samples were reduced, denatured and electrophoresed as described by Laemmli[30]. After electrophoresis, polypeptides were visualized with Coomassie brilliant blue (lanes 1–3) or with the silver stain procedure described by Morrissey[31] (lane 4). Lane 1, molecular weight standards (3 μg each): phosphorylase *b* (94K), bovine serum albumin (68K), ovalbumin (43K), carbonic anhydrase (30K), soybean trypsin inhibitor (21K) and lysozyme (14.3K). Lane 2, 30 μg of HBsAg from the human hepatoma cell line PLC/PRF/5 (ref. 10), also purified from yeast as described in Fig. 1 legend. Lane 3, 30 μg of HBsAg purified from yeast as described in Fig. 1. Lane 4, 10 μg of clarified yeast extract as described in Fig. 1 legend.

Studies in mice (Table 1) showed the yeast-derived antigen to be at least as antigenic as the antigen purified from human plasma. Grivet monkeys also developed antibody following vaccination with the yeast-derived antigen (Table 2). A single injection of the vaccine at all dose levels resulted in seroconversion of all the animals in both vaccine groups. These results were important as they showed that high antibody titres were maintained for at least a year.

Protective efficacy was tested for by using susceptible chimpanzees. The four chimpanzees that received the recombinant vaccine developed antibody in substantial titre following vaccination (Table 3). Following challenge with infectious human plasma, all four vaccinated animals were protected. By contrast, all four unvaccinated animals developed hepatitis B virus infection with positive antigenaemia, antibody to hepatitis B core antigen (anti-HBcAg), elevation of serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT), and liver histopathology. It is important to note that the animals were protected against both subtype adr and ayw challenge even though the vaccine is of the adw subtype.

Yeast fermentation technology is well established and we have shown that HBsAg can be isolated from yeast extracts in a highly purified form by a single application of immune affinity chromatography. Vaccine made from this antigen is equally as potent as human plasma-derived vaccine in stimulating antibodies in mice, and is protective in challenge experiments in chimpanzees. Antibodies raised by yeast-derived vaccine persisted for at least a year in monkeys, showing no important deviation from that of the plasma vaccine.

Table 3 Protective efficacy in chimpanzees of HBsAg purified from yeast and from human plasma

Injection	Chimp no.	Before challenge: Anti-HBsAg titre (at 12 weeks)	Before challenge: Antigen subtype	After challenge (week of onset or weeks of duration): HBsAg Onset	HBsAg Duration	Anti-HBcAg Onset	Anti-HBcAg Duration	SGOT elevation Onset	SGOT elevation Duration	SGPT elevation Onset	SGPT elevation Duration	Liver pathology onset
Yeast vaccine (lot 81-4)	110	1,830	adr	–	–	–	–	–	–	–	–	–
	138	540	adr	–	–	–	–	–	–	–	–	–
	103	18,300	ayw	–	–	–	–	–	–	–	–	–
	120	7,200	ayw	–	–	–	–	–	–	–	–	–
Unvaccinated controls	111	<8	adr	10	10	15	9	17	3	17	6	20
	128	<8	adr	8	11	12	12	17	3	16	5	20
	127	<8	ayw	6	14	12	12	13	3	13	7	16
	130	<8	ayw	6	18	10	14	22	1	14	10	24

Eight chimpanzees, each weighing 40–60 kg, were selected for study based on negative findings in tests for HBsAg, anti-HBsAg, anti-HBcAg, elevation in transaminase, liver histopathology and tuberculin reaction. The animals were separated into two groups, four test animals and four controls. Each of the four test animals was given three 40-μg doses of yeast-derived HBsAg vaccine in 1 ml volume i.m. at 4-week intervals. All eight animals were then challenged by intravenous injection of 1,000 chimpanzee infectious doses of subtype adr or ayw virus in 1 ml of human hepatitis B plasma. Antigen and antibody titres were measured by commercial (Abbott) RIA kits (AUSRIA, AUSAB and CORAB for HBsAg, anti-HBsAg and anti-HBcAg, respectively). SGOT and SGPT assays were performed by the Sigma-Frankel (no. 505) and by the UV absorption (Boehringer-Mannheim) procedures, respectively. SGOT titres >40 and SGPT titres >30 were considered elevated. The subtype adr and ayw human plasmas used for challenge were obtained from Drs R. Gerety and E. Tabor of the Office of Biologics, US Food and Drug Administration; they were of measured viral infectiousness for chimpanzees and were subtyped serologically. The animals were bled at weekly intervals during the 36-week period of observation, covering 12 weeks before virus challenge and 24 weeks after. Liver biopsies were taken at 4-week intervals using a Menghini 16T needle. The tissues were fixed in 10% buffered formalin solution and the haematoxylin/eosin-stained sections were prepared by Dr A. Phelps of these laboratories under blind code number. The tests were carried out in animals that were held in isolation in the facilities of Dr William E. Greer at the Gulf South Research Institute, New Iberia, Louisiana. –, All remained negative.

Human HBsAg is composed of a sequence of 226 amino acids of which the a antigen determinant is dominant. Small differences in amino acid sequence may occur at several positions in the polypeptide chain and are responsible for the subtype specificities[24]. In previous studies, chimpanzees that were cross-challenged with heterologous subtypes of hepatitis B virus after recovery from infection or vaccination with human plasma-derived antigens, were solidly protected due to the common group specificity of the dominant a antigen that is present in all HBsAg subtypes[25]. A protective efficacy trial in man of subtype ad vaccine of human plasma origin has shown strong protection against the homologous subtype[26,27] and, most recently, against the heterologous subtype ay[28] in studies carried out on the staffs of renal dialysis centres where subtype ay hepatitis is most common. The positive cross-protection afforded against heterologous subtype ayw virus challenge in chimpanzee immunized with type adw vaccine of yeast origin, indicates that the a antigen remains dominant in the recombinant-produced antigen obtained from human plasma.

We thank Dr C. E. Carty and F. X. Kovach for assistance in fermentation, B. J. Harder, N. Grason and J. Bailey for assistance in antigen purification, Dr B. Wolanski and R. Ziegler for electron microscopy, and H. E. Darmofal, J. T. Deviney, K. I. Guckert, R. R. Roehm and L. W. Stanton for assistance in the animal tests.

Received 11 July; accepted 16 November 1983.

1. Deinhardt, F. & Gust, I. D. *Bull. Wld Hlth Org.* **60,** 661–691 (1982).
2. Hilleman, M. R. *et al. Viral Hepatitis* (eds Vyas, G. N., Cohen, S. N. & Schmid, R.) 525–537 (The Franklin Institute Press, Philadelphia, 1978).
3. Hilleman, M. R. *et al.* in *Viral Hepatitis 1981 int. Symp.* (eds Szmuness, W., Alter, H. & Maynard, J.) 385–397 (The Franklin Institute Press, Philadelphia, 1982).
4. Buynak, E. B. *et al. J. Am. med. Ass.* **235,** 2832–2834 (1976).
5. Adamowicz, P. *et al.* in *INSERM Symp.* No. 18 (eds Maupas, P. & Guesry, P.) 37–49 (Elsevier, Amsterdam, 1981).
6. Coutinho, R. A. *et al. Br. med. J.* **286,** 1305–1308 (1983).
7. Valenzuela, P. *et al. Nature* **280,** 815–819 (1979).
8. Edman, J. C., Hallewell, R. A., Valenzuela, P., Goodman, H. M. & Rutter, W. J. *Nature* **291,** 503–506 (1981).
9. Valenzuela, P., Medina, A., Rutter, W. J., Ammerer, G. & Hall, B. D. *Nature* **298,** 347–350 (1982).
10. Alexander, J. J., Bey, E. M., Geddes, E. W. & Lecatsas, G. *S. Afr. med. J.* **50,** 2124–2128 (1976).
11. Barin, F., Maupas, P., Coursaget, P., Goudeau, A. & Chiron, J. P. in *INSERM Symp.* No. 18 (eds Maupas, P. & Guesry, P.) 263–266 (Elsevier, Amsterdam, 1981).
12. Moriarty, A. M., Hoyer, B. H., Shih, J. W-K., Gerin, J. L. & Hamer, D. H. *Proc. natn. Acad. Sci. U.S.A.* **78,** 2606–2610 (1981).
13. Dubois, M. F., Pourcel, C., Rousset, S., Chany, C. & Tiollais, P. *Proc. natn. Acad. Sci. U.S.A.* **77,** 4549–4553 (1980).
14. Burrell, J. C., Mackay, P., Greenway, P. J., Hofschneider, P. H. & Murray, K. *Nature* **279,** 43–47 (1979).
15. Lerner, R. A. *et al. Proc. natn. Acad. Sci. U.S.A.* **78,** 3403–3407 (1981).
16. Dreesman, G. R. *et al. Nature* **295,** 158–160 (1982).
17. Bhatnagar, P. K. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 4400–4404 (1982).
18. Prince, A. M., Ikram, H. & Hopp, T. P. *Proc. natn. Acad. Sci. U.S.A.* **79,** 579–582 (1982).
19. Gerin, J. L. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 2365–2369 (1983).
20. Smith, G. L., Mackett, M. & Moss, B. *Nature* **302,** 490–495 (1983).
21. Miyanohara, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 1–5 (1983).
22. Hitzeman, R. A. *et al. Nucleic Acids Res.* **11,** 2745–2763 (1983).
23. Deters, D., Muller, U. & Homberger, H. *Analyt. Biochem.* **70,** 263–267 (1976).
24. Ono, Y. *et al. Nucleic Acids Res.* **11,** 1747–1757 (1983).
25. Gerety, R. J., Tabor, E., Purcell, R. H. & Tyeryar, F. J. *J. infect. Dis.* **140,** 642–648 (1979).
26. Szmuness, W., Stevens, C. E., Zang, E. A., Harley, E. J. & Kellner, A. *Hepatology* **1,** 377–385 (1981).
27. Francis, D. P. *et al. Ann. intern. Med.* **97,** 362–366 (1982).
28. Szmuness, W. *et al. New Engl. J. Med.* **307,** 1481–1486 (1982).
29. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. *J. biol. Chem.* **193,** 265–275 (1951).
30. Laemmli, U. K. *Nature* **227,** 680–685 (1970).
31. Morrissey, J. H. *Analyt. Biochem.* **117,** 307–310 (1981).

Antisera to a synthetic peptide of the *sis* viral oncogene product recognize human platelet-derived growth factor

Henry L. Niman

Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California 92037, USA

It has recently been reported that the sequences of the *sis* oncogene of simian sarcoma virus (SSV) and of human platelet-derived growth factor (PDGF) are very similar[1,2], establishing the most solid link yet between the mitogenic actions of growth factors and the transforming proteins of retroviruses. To investigate molecular mechanisms of transformation I have produced antisera against synthetic peptides corresponding to segments of the protein sequences predicted by the nucleotide sequences of viral oncogenes. Applying this approach to the case of *sis* and PDGF, I report here the results of probing outdated human platelets with an antiserum directed against a synthetic peptide representing residues 139–155 of the predicted sequence of the SSV transforming protein, p28^{sis} (ref. 3). I detected peptides of apparent molecular weights (MWs)

0028–0836/84/020180—04$01.00