CLINICAL RESEARCH

Antibody responses to recombinant and plasma derived hepatitis B vaccines

SHEILA E BROWN, CAROLYNNE STANLEY, COLIN R HOWARD, ARIE J ZUCKERMAN, MICHAEL W STEWARD

Abstract

The antibody response to hepatitis B surface antigen (anti-HBs) induced in 25 recipients of a recombinant hepatitis B vaccine derived from yeast was compared with that induced in 25 recipients of a vaccine prepared from hepatitis B surface antigen (HBsAg) derived from plasma. Anti-HBs affinity and specificity were compared using assays of antibody affinity with two different antigens, a complex of the major polypeptide of HBsAg (p25; molecular weight 25 000 daltons) covalently linked to its glycosylated form (gp30) prepared from native purified HBsAg, and a cyclical synthetic peptide representing amino acid residues 139-147 of the major polypeptide of HBsAg and known to represent a major part of an *a* determinant. There was no difference in anti-HBs affinity or molar antigen binding sites of the antibody measured with either antigen between the two groups. All subjects in both groups produced antibody that bound to the gp30/p25 complex antigen, whereas 22 of the recipients of the plasma derived vaccine compared with 24 of those receiving the yeast derived vaccine produced antibodies that bound to the cyclical synthetic peptide 139-147. These results support the finding of similar levels of anti-HBs, measured by commercial solid phase radioimmunoassay, in the two vaccine groups after three doses of vaccine.

These results show no significant difference in the quantity, quality, or specificity of the anti-HBs response induced by the recombinant hepatitis B vaccine and the plasma derived hepatitis B vaccine.

Department of Medical Microbiology, London School of Hygiene and Tropical Medicine, London WC1E 7HT

SHEILA E BROWN, PHD, BSC, research fellow
CAROLYNNE STANLEY, senior technician
COLIN R HOWARD, MRCPATH, DSC, reader in virology
ARIE J ZUCKERMAN, FRCP, FRCPATH, professor of microbiology
MICHAEL W STEWARD, FRCPATH, DSC, professor of immunology

Correspondence to: Dr Brown.

Introduction

The most important immunological response to hepatitis B virus is directed towards antigenic determinants on the surface of the virus particle, hepatitis B surface antigen (HBsAg). Antibody to HBsAg (anti-HBs) is known to protect against reinfection[1] with any HBsAg subtype if the antibody is directed towards the group specific *a* determinant present on all subtype variants of HBsAg.[2,3] This has led to the development of a hepatitis B vaccine, which consists of highly purified and inactivated HBsAg particles isolated from the plasma of persistently infected subjects.[4] The safety and efficacy of this vaccine in various population groups at risk of acquiring hepatitis B has been established.[1,2] The current vaccine, separated from the plasma of asymptomatic carriers, is, however, expensive and requires extensive purification and inactivation by at least two different procedures and vigorous safety testing to ensure freedom from host proteins and contaminating transmissible agents. Moreover, production of antibodies, assessed by radioimmunoassay, is poor in patients receiving maintenance haemodialysis[5,6] and immunocompromised patients.[7,8] There is, therefore, a need to develop safe and effective vaccines from alternative sources of HBsAg.

In the absence of a cell culture for propagating hepatitis B virus, recombinant deoxyribonucleic acid (DNA) methods provide a good alternative for the production of HBsAg for immunisation purposes. Cloning of the hepatitis B virus genome and determination of its primary sequence have identified a continuous 892 base pair sequence, which constitutes the gene coding for HBsAg (the S gene).[9-11] The cloned S gene has been introduced into yeast (*Saccharomyces cerevisiae*), and the expressed HBsAg was shown to elicit specific anti-HBs antibodies in mice, rabbits, chimpanzees, and humans.[3,12-18]

We have previously shown the importance of determining the affinity of the anti-HBs response using synthetic peptides representing the amino acid sequence of part of an *a* determinant of HBsAg.[19,20] The quantitative commercial antibody assays are inadequate because they provide no information on the epitope specificity and quality of the antibody response. In this context it should be noted that several antibody assays, particularly the enzyme linked immunosorbent assay, are more sensitive to high affinity antibodies than low.[21,22] We compared the amount, affinity, and specificity of the anti-HBs responses in subjects who had

received the current vaccine containing HBsAg isolated from plasma of persistently infected donors and in those who had received a vaccine containing HBsAg produced by the recombinant DNA method in yeast.

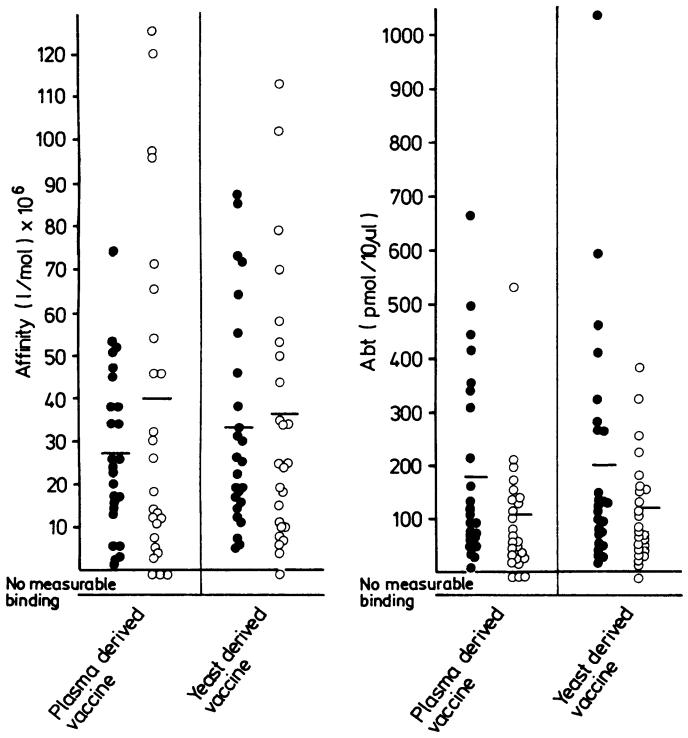
Methods

Serum samples were obtained from 50 volunteers who had received three doses of hepatitis B vaccine. Of these, 25 had received vaccine prepared by Merck Sharp and Dohme research laboratories from HBsAg expressed in yeast by the Merck process[3] and the remaining 25 had received vaccine prepared from human plasma positive for HBsAg (Hep-B Vax, Merck Sharp and Dohme). Both sets of subjects belonged to similar age and risk groups and were treated with identical regimens of vaccine. All serum samples were taken at seven months, one month after the third dose of vaccine. Serum titres of anti-HBs were measured by solid phase radioimmunoassay (Ausab, Abbott Laboratories, Chicago).

Affinities of antibodies to HBsAg in the serum were determined as described previously, using two different antigens labelled with iodine-125.[19,20] The first was a Triton X-100 solubilised complex (gp30/p25) of the major HBsAg polypeptide, p25, with its glycosylated form, gp30, prepared from purified 22 nm HBsAg particles,[23] and the second was a cyclical synthetic peptide representing amino acid residues 139 to 147 of the major polypeptide of HBsAg and known to represent part of a group specific *a* determinant of HBsAg present on all subtype variants.[19,24,25]

Results and comment

The figure shows the affinity values and the molar antigen binding sites of the antibody (Abt values) obtained with the serum samples from both groups, using as antigens either the gp30/p25 complex or the cyclical synthetic peptide 139-147. There was significant correlation of the Abt values between the two antigens (yeast vaccine $r=0{\cdot}6129$, plasma vaccine $r=0{\cdot}8904$; $p<0{\cdot}01$). There was no significant correlation, however, between the antibody affinity values using the gp30/p25 complex and the cyclical peptide (yeast vaccine $r=0{\cdot}2407$, plasma vaccine $r=0{\cdot}0404$; $p>0{\cdot}1$). Serum samples from all 50 subjects yielded positive results on solid phase radioimmunoassay for anti-HBs, and all contained detectable antibody that bound to the gp30/p25 complex. However, three subjects in the group who had received vaccine made from HBsAg isolated from human plasma and one subject in the group who had received yeast derived HBsAg vaccine had no detectable antibody specific for the cyclical synthetic peptide 139-147.



Affinity values and Abt values (molar antigen binding sites of the antibody) of anti-HBs antibodies in recipients of three doses of plasma derived hepatitis B vaccine and yeast derived hepatitis B vaccine measured with gp30/p25 complex (●) and cyclical synthetic peptide 139-147 (○). Mean values are indicated by bars.

The mean affinity values and mean Abt values for antibodies to both antigens and the mean titres for the two groups were not significantly different (Student's *t* test, $p>0{\cdot}3$). Jilg *et al* reported slightly lower seroconversion rates and mean anti-HBs levels, particularly in male patients, in recipients of a recombinant vaccine compared with those vaccinated with a plasma derived vaccine.[14] They suggested that this may have been because of the lower dose of recombinant vaccine used (10 µg) compared with the dose of plasma derived vaccine (20 µg). Our data confirm the preliminary results reported by two other groups, showing similar seroconversion rates and anti-HBs titres with both recombinant and plasma derived vaccines.[15,16,18]

To assess the anti-HBs responses in the two groups more fully specificity and quality of the anti-HBs were measured using the gp30/p25 complex and the cyclical synthetic peptide 139-147. The gp30/p25 complex is derived from native purified HBsAg and is considered to bear the major antigenic determinants of the native protein.[26,27] In contrast, synthetic peptide 139-147 corresponds to a major part of an *a* group determinant of HBsAg.[19,24,25] All subjects in both groups produced antibodies that bound to the gp30/p25 complex. In addition, 24 of the 25 subjects who had received yeast derived vaccine and 22 of the 25 who had received the plasma derived vaccine produced antibodies that bound to the synthetic peptide 139-147. This does not necessarily mean that the four subjects who did not have antibody that bound synthetic peptide 139-147 did not have antibody directed to part of the *a* determinants, because peptide 139-147 does not represent all of the *a* determinants. In the study of Jilg *et al* all subjects who seroconverted had antibodies against the *a* determinants in both the recombinant and plasma derived groups.[14]

Discussion

We found no significant difference in the affinity of the anti-HBs that bound to the gp30/p25 or the cyclical synthetic peptide 139-147 between the two groups. The cyclical form of peptide 139-147 was used in preference to a linear form as we have previously shown that anti-HBs serum samples always bind to the cyclical form of the peptide with a higher affinity.[19,20] The cyclical form therefore probably corresponds more closely than the linear form of the peptide to the conformation of this amino acid sequence in the native HBsAg. In this context it should be recognised that peptide conformation is important for interaction with antibodies raised against native HBsAg.[28] The affinity values of anti-HBs induced in both the recombinant and plasma derived vaccine groups reported here are similar to those previously reported in a group of 19 subjects who received the currently licensed plasma derived vaccine.[20] The affinity of anti-HBs in these subjects for the cyclical synthetic peptide 139-147 was shown to be similar to the high affinity of anti-HBs antibody in a group of 13 patients who had recovered from acute hepatitis B.[19] There is considerable evidence to suggest that antibody affinity influences the biological properties of antisera,[29] and, in particular, high affinity antibody has been shown to be superior to low affinity antibody in several functions mediated by antibodies such as immune elimination of antigen, virus neutralisation, and, in man, the prevention of Rh immunisation by the use of passively transferred anti-D antibodies.[30-32]

Our results show that the yeast recombinant HBsAg vaccine induced the production of high affinity anti-HBs in man. The affinity, titre, and specificity of this antibody response were similar to those obtained after immunisation with the plasma derived HBsAg vaccine.

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Lactose absorption, milk consumption, and fasting blood glucose concentrations in women with idiopathic osteoporosis

G FINKENSTEDT, F SKRABAL, R W GASSER, H BRAUNSTEINER

Abstract

Lactose tolerance tests were performed in 33 women with osteoporosis and 33 control women matched for age. A questionnaire was used to elicit any history of milk intolerance and the subjects' daily intake of calcium derived from milk and dairy products. Eleven patients and four controls gave a history of milk intolerance ($p<0.01$); 13 patients had lactose malabsorption compared with four controls ($p<0.01$). The daily intake of calcium derived from milk was significantly lower in patients (125 (SEM 20) mg *v* 252 (43) mg; $p<0.05$). Curves of blood glucose concentrations during the lactose tolerance test in subjects with lactose malabsorption were significantly flatter in patients than controls ($p<0.05$). The fasting blood glucose concentration was higher (5·44 (0·17) mmol/l (98 (3) mg/100 ml)) in the patients than the controls (4·88 (0·11) mmol/l (88 (2) mg/100 ml); $p<0.05$), although body weight was significantly lower (61·6 (2·2) kg *v* 66·3 (1·6) kg; $p<0.05$).

Absorption of lactose is significantly impaired in women with "idiopathic" osteoporosis; this combined with low consumption of milk and a subclinical disorder of glucose metabolism may be a major factor in the development of idiopathic osteoporosis in women.

Department of Internal Medicine, University Hospital, University of Innsbruck, 6020 Innsbruck, Austria

G FINKENSTEDT, MD, senior registrar
F SKRABAL, MD, professor, chief of division of endocrinology and hypertension
R W GASSER, MD, senior registrar
H BRAUNSTEINER, MD, professor of internal medicine and head of department

Correspondence to: Dr Finkenstedt.

Introduction

The contribution of malabsorption of lactose to the development of osteoporosis is not well documented. Birge *et al* and Newcomer *et al* found an increased incidence of lactose intolerance in patients with osteoporosis,[1,2] while Alhava *et al* could not show any difference in bone mineral content between subjects with and without lactose intolerance.[3] We determined the prevalence of lactose malabsorption and the amount of calcium consumed that was derived from milk and dairy products in 33 women with osteoporosis compared with 33 control women matched for age.

Patients, methods, and results

Thirty three women aged under 65 with "idiopathic" osteoporosis were compared with 33 women without osteoporosis (Singh index >4) of the same ethnic origin matched for age. We excluded patients with endocrine disorders, liver and renal disease, postgastrectomy states, malabsorption syndromes, rheumatoid arthritis, osteomalacia, and malignancy and patients receiving corticosteroids. Patients and controls were not taking drugs that influenced calcium or bone metabolism. Osteoporosis was confirmed by the presence of reduced bone mineral density in plain *x* ray films and either a femoral trabecular index $<5^{4}$ or the presence of spontaneous fractures of vertebrae or long bones, or both.

Malabsorption of lactose was defined as a rise in glucose concentration of <1·11 mmol/l (20 mg/100 ml) in capillary blood samples taken at 30 minute intervals after the ingestion of 50 g lactose dissolved in water.[5] Patients were given a questionnaire asking about their mean daily or weekly ingestion of milk, yoghourt, cottage cheese, and other cheese and about tolerance to