

## IMMUNIZATION AGAINST HEPATITIS B IN CHILDREN FROM ENDEMIC ZONE: EVALUATION OF THE ANTIBODY RESPONSE AGAINST THE DNA RECOMBINANT VACCINE (ENGERIX B-20 MCG)

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### SUMMARY

A previous seroepidemiological study in the rural zone of Vargem Alta (ES) SouthEast of Brazil, showed a prevalence of up to 9% of hepatitis B surface antigen (HBsAg) in some areas.

One hundred susceptible children aging 1 to 5 years old were selected and immunized with a recombinant DNA hepatitis B vaccine (Smith-Kline 20 mcg) using the 0-1-6 months vaccination schedule. Blood samples were collected at the time of the first vaccine dose (month 0) in order to confirm susceptible individuals and 1,3,6 and 8 months after the first dose, to evaluate the antibody response. Our results showed that two and five months after the second dose, 79% and 88% of children seroconverted respectively, reaching 97% after the third dose.

The levels of anti-HBs were calculated in milli International Units/ml (mIU/ml) and demonstrated the markedly increase of protective levels of antibodies after the third dose. These data showed a good immunogenicity of the DNA recombinant hepatitis B vaccine when administered in children of endemic areas.

**KEY WORDS :** DNA recombinant vaccine; Immune response-children; hepatitis B recombinant vaccine; Anti-HBs

### INTRODUCTION

Hepatitis B infection is a severe and worldwide problem for public health. The only practical solution for this problem is a large scale immunization of high risk populations<sup>(1,2)</sup>. Although safe and effective vaccines manufactured from the plasma of chronic carriers of hepatitis B virus (HBV) have been available since the early 1980s, only a small proportion of candidates for vaccination have, in fact, been immunized. The reasons for this unsatisfactory situation include high cost and limited capacity for production of plasma-derived vaccines and individuals reluctance to be treated with products manufactured from human blood. The recent development of vaccines produced from genetically engineered yeast cell<sup>(7,11)</sup> has considerably improved the prospects for control of hepatitis B, since they can be produced in unlimited quantities at reasonable cost without re-

sorting to the use of human blood for their manufacture.

Our study was accomplished in the rural zone of Vargem Alta(ES), in the SouthEast of Brazil. A seroepidemiological survey conducted by our group in 8000 serum samples collected from 1985 to 1987 in this area, demonstrated a high prevalence up to 9% of hepatitis B surface antigen (HBsAg) in some communities (unpublished data). Because of the high circulation of the Hepatitis B virus, we made a schedule using the children to look for their vaccine response. A group of children were selected for the study of the immunological response against the recombinant DNA vaccine, which was evaluated through the detection of anti-HBs antibodies in serially collected serum samples.

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The purpose of this study is to show the immunogenicity of the DNA recombinant vaccine, after 3 doses of 20 mcg in a group of children selected from an endemic area.

## MATERIALS AND METHODS

### Vaccine

Recombinant DNA vaccine using Hepatitis B surface antigen (HBsAg) expressed in yeast (Engerix B) was kindly provided by Smith Kline Biologicals, Belgium.

### Participants and Screening

One hundred children (54 males and 46 females) from the rural zone of Vargem Alta ranging from 1 to 5 years old were studied. These children were selected based on their negative results for serological tests of hepatitis B infection markers (HBsAg, anti-HBs and anti-HBc) and normal values for aminotransferases levels.

### Laboratory Methods

HBsAg, anti-HBs anti-HBc were determined using enzyme immunoassays produced in our laboratory<sup>(2,3)</sup>. Serum aminotransferases activity was measured using Reitman and Frankel method, with normal value ranging from 4-32 RFU/ml for Alanine aminotransferase and from 4-36 RFU/ml for

Aspartate aminotransferase.

Determination of milli International Units/ml was done by radioimmunoassay (Ausab- Abbott Laboratories) using a WHO International Reference for anti-Hepatitis B immunoglobulin, kindly supplied by the International Laboratory for Biological Standard (CLB, Amsterdam, The Netherlands).

### Immunization protocol and Immunity determination

Three vaccine doses of 20 mcg (1ml) were administered intramuscularly (deltoid region) using the usual schedule of 0,1 and 6 months.

Blood samples were collected at month 0 (susceptibles selection) and evaluation of the vaccine response was done at months 1,3,6 and 8. The antibody levels were expressed in mIU/ml and only titres equal or greater than 10 mIU/ml were considered as positive.

## RESULTS

From 54 children of the male group, 42 (77.7%) and 45 (83.3%) developed anti-HBs response two and five months after the second dose respectively, and 53 (98.1%) reached seroconversion two months after the third dose. From 46 children of the female group, 37 (80.4%) and 43 (93.4%) developed anti-HBs response two and five

Table 1  
Results of anti-HBs antibodies in milli International Units/ml after two and three doses (0,1,6 months schedule vaccination).

Time	Evaluation of Response	Males	Females
2 months after the second dose	anti-HBs+/total	42/54	37/46
	geometric mean	52.8	50.5
	median	36.6	36.6
	(range)	(9.0-172.4)	(9.0-164.4)
5 months after the second dose	anti-HBs+/total	45/54	43/46
	geometric mean	123.9	122.6
	median	72.3	56.7
	(range)	(11.8-1045.4)	(9.1-835.4)
2 months after the third dose	anti-HBs+/total	53/54	44/46
	geometric mean	1124.7	1958.1
	median	184.8	206.6
	(range)	(13.7-9671.3)	(47.0-11175.0)

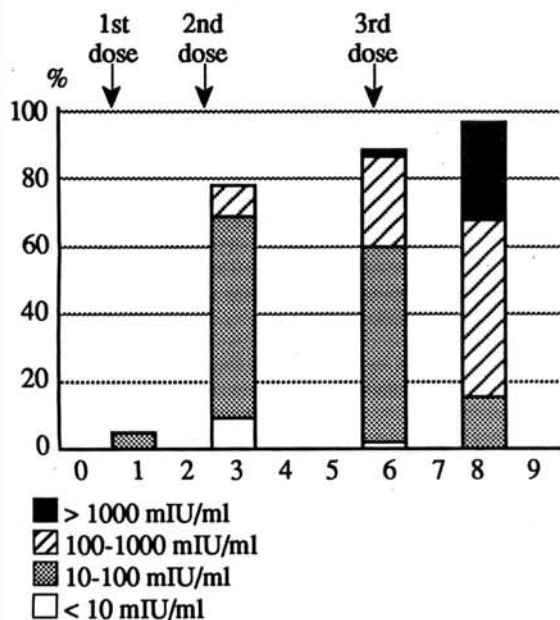


Figure 1. Anti-HBs response to recombinant HB vaccine (Smith-Kline 20mcg) in mIU/ml.

months after the second dose, respectively, and 44 (95.6%) reached seroconversion two months after the third dose. These results are shown in Table 1.

The geometric mean and the median observed two months after the third dose, was slightly higher in females (1958.1 and 206.6 respectively) than in males (1124.7 and 184.8 respectively).

Considering the overall group, we found that after the first dose, 4% of the children developed anti-HBs response. This value increased to 79 and 88% two and five months after the second dose, respectively. Two months after the third dose, the anti-HBs response reached 97%. The quantitative anti-HBs response is demonstrated in Figure 1, where a markedly increase in the antibody levels expressed in mIU/ml is observed after the third dose.

## DISCUSSION

Earlier studies have shown that both yeast and plasma-derived vaccines are equally efficient in eliciting an antibody response in adults and in children<sup>(5,9,10)</sup>.

Using a plasma derived vaccine (5mcg/ dose HEVAC B - France) in children patients undergoing hemodialysis treatment, we demonstrated

87.5% of seroconversion after four months after the third dose<sup>(13)</sup>.

ISAHAK et al.<sup>(8)</sup>, showed that medical students vaccinated with 1.0 ml of 20 mcg of Engerix B intramuscularly (deltoid region) according to the 0,1,6 month vaccination schedule reached 100% of seroconversion with high anti-HBs levels after the third dose.

In a endemic area of Amazon (Brazil) two children groups (Group A- 0,5 ml/10mcg and Group B- 1,0 ml/20 mcg), were both vaccinated intramuscularly (Engerix B) in the deltoid region, using the same above mentioned vaccination schedule. Six months after the third dose, the group A presented 97,7% of seroconversion and the group B reached 100%. These results suggest that the recombinant DNA Hepatitis B vaccine, in both doses is highly immunogenic for children living in endemic zones<sup>(6)</sup>. CASTRO & ROSA<sup>(4)</sup> also demonstrated that the dose of 10 mcg/0.5ml of recombinant vaccine (Engerix B) in children, induce 100% of seroconversion. Comparatively, ANDRÉ<sup>(11)</sup> demonstrated that the dose of 10 mcg of yeast derived vaccine, in newborns and children, provides anti-HBs titres equivalent to those obtained with the 20 mcg dose in adults.

The results obtained in our study showed that the schedule proposed by the manufacturer provided satisfactory immunological response after 8 months of study and that 97% of the children developed protective antibody levels against Hepatitis B. Two months after the last dose, the geometric mean values of mIU/ml in females was 1.7 times greater than in males.

Similar results were observed in adults by ANDRÉ<sup>(11)</sup> when the same schedule of vaccination was used.

We could observe that the qualitative seroconversion values are not so great between the second and third dose ( 88 and 97% respectively ). After the second dose, 28% had values equal or greater than 100 mIU/ml, increasing to 82% after the third dose, showing the importance of a three-dose vaccine schedule to elicit high concentrations of specific antibodies, also observed by ANDRÉ<sup>(11)</sup>.

Currently, the schedule of 0,1,6 months of 10 mcg/ml vaccine is being used in the National Immunization Program for endemic areas in Brazil.

## RESUMO

### Imunização contra Hepatite B em crianças de zona endêmica: avaliação da resposta de anticorpo à vacina HB recombinante (Engerix B- 20mcg).

Um estudo soroepidemiológico prévio na zona rural de Vargem Alta (ES) - Sudeste do Brasil, mostrou uma predominância de até 9% do antígeno de superfície da Hepatite B (HBsAg). Foram selecionadas 100 crianças com faixa etária entre 1 e 5 anos de idade, as quais foram imunizadas com vacina contra Hepatite B -DNA recombinante (Smith-Kline, 20 mcg) nos meses 0,1 e 6. Foram coletadas amostras de sangue antes da primeira dose da vacina (mês 0) para confirmação dos susceptíveis, e nos meses 1,3,6 e 8 após a vacinação para avaliação da resposta vacinal. Os resultados mostraram que 79 e 88% das crianças apresentaram soroconversão dois e cinco meses após a segunda dose respectivamente, atingindo 97% de soroconversão, após a 3a.dose.

Os níveis de anti-HBs foram calculados em miliunidades internacionais/ml (mUI/ml), demonstrando um considerável aumento dos níveis de anticorpos protetores após a 3a. dose. Os resultados demonstraram uma boa imunogenicidade da vacina de DNA recombinante contra hepatite B, quando administradas em crianças de áreas endêmicas.

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