Immunoprophylaxis to limit a hepatitis B epidemic among women undergoing *in vitro* fertilization

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Women (175) who participated in an in vitro fertilization (IVF) programme were possibly exposed to hepatitis B virus. Later it became evident that 79 women had a hepatitis B infection, 49 were exposed but not infected and 47 were not exposed. Hepatitis B immunoglobulin (HBIg) and recombinant hepatitis B vaccine (HBvaxDNA) were offered to all women and partners except for those with established hepatitis B. Women were given an 'intensive' schedule of HBIg (0, 1 months) and vaccine (0, 1, 2, 6 months). Spouses received HBIg (0, 1 months) and vaccine (0, 1, 6 months). Blood samples were taken at the time of diagnosis of the HBV epidemic and at regular intervals. Hospital personnel immunized according to the regular schedule (0, 1 and 6 months) with HBvaxDNA but without HBIg served as controls. During the observation period of 7 months anti-HBc seroconversion was not observed. At month 7 the seroconversion rate for males was 89%, significantly lower than that for females (100%). Intermediate rates were found for the control groups (94%). Significant differences in geometric mean titre between IVF-treated patients and controls were, however, observed at month 7 (551 mIU ml^{-1} for female patients versus 1582 mIU ml^{-1} for their controls and 171 mIU ml^{-1} for males versus 899 mIU ml^{-1} for their controls). Possible explanations for the low reactivity to HBsAg vaccine are discussed.

Keywords Hepatitis B postexposure prophylaxis passive active immunization

INTRODUCTION

In early March 1988 the University Hospital Dijkzigt was confronted with a hepatitis B epidemic in women who had undergone *in vitro* fertilization[1,2]. All women ($n=175$) who had undergone *in vitro* fertilization (IVF) since November 1987 but were without signs of hepatitis B virus (HBV) infection at first screening, as well as their sexual partners, were offered passive active immunization[3].

The rationale for starting passive active immunization 6–18 weeks after HBV-exposure was to modify infection in females and to prevent infection in males. All women followed an 'intensive' immunization scheme consisting of hepatitis B immunoglobulin (HBIg) on day 1 and 1 month later and recombinant vaccine on day 1 and 1, 2 and 6 months later. Male partners who may have been exposed to HBV for several weeks also received HBIg on day 1 and 1 month later and recombinant vaccine on day 1 and 1 and 6 months later.

In this study the levels of HBV protective antibodies (anti-HBs ≥ 10 mIU ml^{-1}) induced by passive active immunization were examined. An explanation was sought for the relatively poor immune response in the IVF population.

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MATERIALS AND METHODS

Patients and controls

From 2 November 1987 until 21 January 1988 175 women undergoing IVF might have been exposed to hepatitis B virus. Tissue culture medium supplemented with 7.5% heat-inactivated (30 min 56°C) human serum, which was used for pre-embryo cultures or other procedures during *in vitro* fertilization, seemed to be the most probable source of infection. The human serum was regularly pooled from blood samples of 15–20 pregnant women. All donors were individually screened for hepatitis B virus. Due to a breakdown in communication between the outpatient clinic of the Department of Obstetrics and the IVF laboratory, the results of the routine screening of sera from pregnant women for HBsAg temporarily did not reach the IVF laboratory and an HBV-positive serum was apparently included in the pooled serum. On 2 March 1988 the epidemic was discovered by reports on three cases of jaundice. All women at risk and their partners were immediately requested to visit the University Hospital Dijkzigt for medical examination. Blood samples were obtained from 174 potentially exposed women and 167 sexual partners and preventive measures were advised. These measures

Table 1 Overview of the vaccination groups after possible exposure to hepatitis B

	Group				
	1	2	3	4	
	Females		Males		Total
Total	128 (27)	47 (14)	79	96	350
Excluded at presentation[a]	32 (13)	0	1	8	41
Vaccination started	96 (14)	47 (14)	78	88	309
Excluded after screening[b]	58 (8)	6 (1)	4	11	79
Vaccination continued	38 (6)	41 (13)	74	77	230
Vaccination complete	29 (6)	30 (8)	56	65	180
Blood sample month 7	24 (3)	29 (8)	43	52	148
Evaluated month 7[c]	22 (3)	28 (8)	43	52	145

Figures in parentheses indicate number of women pregnant
[a]Clinical hepatitis pregnant and HBsAg+ known immune response after vaccination no serum available, [b]markers of past or present HBV infection with or without confirmation by pre-treatment sera, [c]see text for reasons for exclusion

included hygiene, use of condoms during sexual intercourse and passive active immunization

Analysis of the incidence of HBV infection in 7 day cohorts of *in vitro* fertilization treatment finally revealed that the period of infectious exposure only extended from 2 November to 13 December 1987 (period 1), this period coincided with the use of one of the two batches of pooled serum used in tissue culture None of the women treated from 14 December 1987 to 21 January 1988 (period 2) developed signs of a hepatitis B infection

Of the total of 175 women, 128 were exposed to the infectious batch of pooled serum, the remaining 47 women were not exposed to infectious culture medium Of the 128 women exposed in period 1, 30 had clinical signs of HBV or were HBsAg-positive and pregnant at first presentation One woman did not deliver serum and another woman was protected by previous vaccination Therefore immunization was started in 96 women Due to the presence of markers of hepatitis B in the pre-IVF serum (9 cases) or in serum taken at first presentation (49 cases), immunization was stopped in 58 of the 96 women (*Table 1*) The remaining 38 women continued immunization and may be characterized as exposed but not infected (group 1) A second group of vaccinees (group 2) is composed of women who received IVF treatment in period 2, the information that the culture medium used in period 2 was not infectious became available about 2 months after immunization had started

Immunization was completed in the large majority of women The other immunization groups comprised the partners The spouses of 79 HBV infected women by IVF treatment were labelled as group 3 The spouses of 49 non-infected women from period 1 together with the spouses of 47 women treated in period 2 comprised group 4 Further details of the four immunization groups are given in *Tables 1* and *2* The control groups, hospital personnel immunized in the same period, consisted of 84 females (group 5) and 53 males (group 6)

Immunization

Hepatitis B immunoglobulin (5 ml, 100–200 IU anti-HBs ml^{-1}, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB)) and hepatitis B recombinant vaccine (10 μg, HBvaxDNA, Merck Sharp & Dohme, West Point, PA, USA) were administered to all women except those with clinical hepatitis or pregnant women with HBsAg-positivity, as determined by rapid blood testing with the reversed passive haemagglutination assay (Hepatest, Wellcome Laboratories, Beckenham, UK) at first presentation A second dose of HBIg was given 1 month later Additional doses of vaccine were administered 1, 2 and 6 months later HBIg and vaccine were discontinued as soon as HBV markers not due to HBIg or vaccine were detected in the serum

Male partners received HBIg on day 1 and 1 month later and HBvaxDNA (10 μg) on day 1 and 1 and 6 months later Immunization was discontinued when vaccinees were found to be positive for HBsAg, anti-HBc or anti-HBs at screening After 4 months, partners of HBV-positive women (group 3) received an additional dose of HBIg if the anti-HBs titre was below 20 mIU ml^{-1} and likely to fall below 10 mIU ml^{-1} before the booster vaccine injection at 6 months

Hospital personnel, who served as controls, received HBvaxDNA (10 μg) from the same lot according to the regular schedule on day 1 and 1 and 6 months later without HBIg (*Table 2*) HBIg was administered by injection i m into the buttock, HB-vaccine was injected i m into the deltoid muscle

Blood sampling and laboratory methods

Pre-immunization serum samples were obtained in March 1988 For females follow-up blood samples were drawn 1, 2, 6 and 7 months after the first vaccine injection For males, blood samples were taken after 1, 4, 6 and 7 months All pre-immunization samples from groups 1–4 as well as follow-up samples were tested for HBsAg, anti-HBc and anti-HBs Blood samples from controls were tested for anti-HBc and anti-HBs after 7 months

Blood samples were tested for HBsAg and anti-HBc by enzyme immunoassay (Abbott Laboratories, Chicago, IL, USA) Anti-HBs was tested by radio immunoassay (Ausab, Abbott Laboratories) and quantified using the WHO standard preparation[4] A protective anti-HBs level was defined as anti-HBs concentration ≥ 10 mIU ml^{-1}, in the absence of other HBV markers

Statistical calculations

Statistical differences in protective anti-HBs levels were

Table 2 Characteristics of IVF treated females, partners and controls completing hepatitis B vaccination

			Vaccination schedule in months		
			HBIg	HBvaxDNA	
Group	No	Mean age (range)	0, 1	0, 1 6	0, 1 2 6
1 Women exposed	22	35 (28 39)	×		✗
2 Women not exposed	28	35 (24–40)	×		✗
3 Partners HBV infection	43	39 (32–57)	×[a]	✗	
4 Partners no HBV infection	52	38 (29–48)	×[b]	×	
5 Female controls	84	34 (22–57)		✗	
6 Male controls	53	35 (22 61)		×	

[a]Males with anti-HBs levels below 20 mIU ml^{-1} at month 4 received an additional HBIg injection
[b]Sexual partner exposed but not infected ($n=20$) and sexual partner not exposed ($n=32$)

calculated with 95% confidence intervals for the difference between two proportions The geometric mean titres (GMTs) for all vaccinees in various groups were compared, after logarithmic transformation by the Student's *t*-test

RESULTS

Of the 230 individuals who continued immunization after initial screening (79 females, 151 males), a total of 180 vaccinees (59 females, 121 males) completed the immunization programme Postimmunization blood samples were obtained in 148 cases (53 females, 95 males) at month 7 Three women (2 in group 1 and 1 in group 2) were excluded from evaluation because they showed more than 10 mIU ml^{-1} anti-HBs on day 1, although anti-HBc tests were negative

Of the 38 females exposed to HBV (group 1), three did not show up for further treatment after month 1 At that time, they were all negative for both HBsAg and anti-HBc Two females did not return for follow-up at month 6 but were HBsAg-negative and anti-HBs-positive at month 4, another nine women who were HBsAg-negative at month 6 with anti-HBs $\geqslant 10$ mIU ml^{-1} did not appear for follow-up at month 7 Of the 37 women who delivered healthy living children[5], nine received one dose of HBIg and HBvaxDNA during pregnancy and 14 received full immunization during pregnancy No adverse reactions to HBIg or vaccine were reported during the observation period of 7 months for vaccinees with or without HBV markers at the time of first presentation, except for one pregnant vaccinee who had a spontaneous abortion two days after initial immunization

The rate of development of protective antibody levels ($\geqslant 10$ mIU ml^{-1}) is shown in *Table 3* One month after the first dose of HBIg and vaccine, protective antibodies were found in 99% (203/205) of the vaccinees Anti-HBs levels were inadequate in two cases (one female in group 2 with 8 mIU ml^{-1} and one male in group 3 with no detectable anti-HBs) At month 2 protective antibody levels were demonstrated in all females tested At month 4 23% of the men of group 3 and 20% of those of group 4 were unprotected (anti-HBs <10 mIU ml^{-1}) At month 6 protective anti-HBs levels were measured significantly less often in group 4 males (48%, 95%CI 36–60%) compared with both groups 1 and 2 (87%, 95%CI 75–99%) and group 3 (81%, 95%CI 71–91%) due to the fact that the anti-HBs level of the majority of the vaccinees in group 4 remained just below the arbitrarily chosen level of 10 mIU ml^{-1} (see GMTs at month 6 in *Table 4*) For group 3 the higher rate of 81% was probably due to the extra dose of HBIg given at month 4 to 25 spouses with anti-HBs titres <20 mIU ml^{-1} Protective anti-HBs levels after completion of vaccination at month 7 were found in all women of groups 1 and 2 (100%) whereas the percentage of males in groups 3 and 4 together ($n=95$) with protective antibodies at month 7 was 89% (95%CI 83–95%)

Table 3 Development of protective antibody levels (anti-HBs $\geqslant 10$ mIU ml^{-1}) after vaccination

		Percentage with anti-HBs $\geqslant 10$ mIU ml^{-1}			
		Month			
Group	Sex	1	2/4[a]	6	7
1	Female	100 (33)	100 (33)	87 (31)	100 (22)
2	Female	97 (39)	100 (39)	87 (31)	100 (28)
3	Male	98 (61)	77 (57)	81 (58)	93 (43)
4	Male	100 (72)	80 (64)	48 (68)	86 (52)
5	Female				94 (84)
6	Male				94 (53)

[a]Blood sample females at month 2, males at month 4
Figures in parentheses indicate number of blood samples tested

Table 4 Levels of anti-HBs after HB-vaccination expressed as geometric mean titre in mIU ml^{-1} calculated for vaccinees with anti-HBs $\geqslant 1$ mIU ml^{-1}

		GMT in mIU ml^{-1}			
		Month			
Group	Sex	1	2 4[a]	6	7
1	Female	35 (33)	45 (33)	30 (29)	396 (22)
2	Female	35 (38)	47 (39)	44 (30)	715 (28)
3	Male	34 (60)	16 (56)	16 (56)	150 (43)
4	Male	37 (72)	19 (65)	9 (65)	190 (50)
5	Female				1582 (80)
6	Male				899 (50)

[a]Blood sample females at month 2, males at month 4 Figures in parentheses indicate number of blood samples with anti-HBs $\geqslant 1$ mIU ml^{-1} anti-HBs
p values for differences in GMT at month 7 group 1 versus 2, not significant, group 3 versus 4, not significant, group 5 versus 6, not significant, group 1+2 (GMT=551 mIU ml^{-1}) versus 3+4 (GMT=171 mIU ml^{-1}), $p<0.002$, group 1+2 versus 5, $p=0.001$, group 3+4 versus 6, $p<0.001$

Table 4 shows the geometric mean titres of anti-HBs for the various groups Antibody levels of ≈ 35 mIU ml^{-1} at month 1 probably reflect the first dose of HBIg in groups 1–4 Titres rose to about 45 mIU ml^{-1} at month 2 for women receiving two doses of HBIg and vaccine

At month 4, after two doses of HBIg and vaccine, the GMTs for groups 3 and 4 were 16 and 19 mIU ml^{-1} anti-HBs, respectively A significant increase in anti-HBs was not observed until after the booster dose was given At month 7 the geometric mean titres of anti-HBs were significantly lower for all IVF groups compared with their control groups (group 1 and 2 versus 5 $p<0.05$, group 3 and 4 versus 6 $p<0.001$)

Since selection of vaccinees due to loss to follow-up may have occurred, the GMT levels of all vaccinees per group were compared with the GMTs of those vaccinees who completed the full immunization scheme in each IVF group No differences in GMTs could be shown at any moment

At month 7 an effect of sex on the immune response to hepatitis B vaccine was indicated by a difference between the female and male groups (IVF groups 1+2 versus 3+4) The influence of sex was not exhibited by control groups 5 and 6 During the observation period of maximal 7 months anti-HBc seroconversion did not occur in IVF groups All controls were also anti-HBc-negative after immunization

DISCUSSION

Hepatitis B immunoglobulin given after exposure to HBV results in prolongation of the incubation period of the virus and partial protection[6–8] Beneficial effects of

postexposure prophylaxis with hepatitis B plasma vaccine have also been demonstrated in adults[9–11]

The combination of HBIg and hepatitis B vaccine for postexposure prophylaxis has been shown to increase protection, meaning fewer clinical and HBsAg-positive infections, compared with HBIg or HB-vaccine alone[12–14] Still, although the spouses of acute hepatitis B patients received HBIg (5 ml) on day 1 and plasma vaccine on day 1 and 1 and 6 months later (20 μg, Merck Sharp & Dohme), 11% exhibited signs of subclinical infection (anti-HBc-seroconversion) at the 3 month examination[12] Repeated injections of HBIg can, however, in the case of accidental exposure to HBV maintain protective levels of anti-HBs throughout the incubation period[7 15] To accelerate the active immune response, a vaccination schedule with short injection intervals has been proposed for postexposure prophylaxis[16]

At the discovery of the HBV epidemic the large majority of patients who had undergone *in vitro* fertilization were offered postexposure prophylaxis In order to modify the course of the disease an 'intensive' schedule of two doses of HBIg and four doses of vaccine was offered to the females To prevent infection in males the standard immunization schedule was used with an additional dose of HBIg at month 1[12] No HBV infections, in particular no anti-HBc-seroconversion, were encountered after the start of passive active immunization, however, the protective effect of passive active immunization in this setting cannot be proven in view of the absence of a control group with comparable exposure The absence of infections in partners after the start of immunization is remarkable since they may have been exposed to HBV for several additional days or weeks because 34 women were still HBeAg-positive at the start of immunization Sexual contact is said to be an important mode of transmission[17 18] However sexual exposure appears to be a less efficient mode of transmission compared with the percutaneous route and the chance that men will contract hepatitis B virus during a heterosexual relationship has been estimated to be $<5\%$[12]

The overall compliance of couples possibly exposed to HBV appeared to be poor The loss to follow-up may have been due to lack of motivation since exposure to HBV in IVF treatment period 2 had not occurred (groups 2 and 4) and vaccinees were regularly informed about their immune status

Passive active immunization of HBsAg-positive or HBV-negative and pregnant vaccinees was practically devoid of side-effects It is doubtful whether the abortion 2 days after initial immunization was indeed a direct adverse effect of immunization The first trimester abortion rate after IVF did not differ from that found for the period before HBV contamination of the culture medium was discovered[5] In fact, immunization of pregnant women in case of high risk for hepatitis B infection is advocated Despite statements of changes in immunity during pregnancy there is little evidence of impairment of cellular immunity in pregnancy The ability to respond to HBsAg vaccine appeared to be adequate[19]

This study provided the opportunity to determine whether administration of HBIg and recombinant HB-vaccine to adults in a high risk situation consistently led to protective levels of anti-HBs soon after immunization The first dose of HBIg resulted in a protective level of anti-HBs in all but two cases Subsequent HBIg and vaccine doses did not produce a major increase in anti-HBs levels, except for the booster dose of vaccine at month 6 In fact, administration of a supplementary dose of HBIg at month 4 was thought to be necessary for the majority of the spouses of HBsAg-positive women

The overall response rate to recombinant HB-vaccine at month 7 was comparable to the response rate for hospital personnel Protective levels of anti-HBs before the booster dose were, however, relatively low (87% for both female groups and 48% and 81% (mean 63%) for groups 3 and 4, respectively, at month 6) compared with other studies of recombinant vaccine[20–22] in which $\geqslant 93\%$ of healthy adults had anti-HBs $\geqslant 10$ mIU ml^{-1} at the time of the booster dose The 'intensive' immunization schedule with an extra dose of vaccine after 2 months did not appear to accelerate the immune response in females In fact, as far as postexposure prophylaxis is concerned, immunogenicity after administration of recombinant vaccine was very disappointing in this study

Recently, Iwarson recommended an accelerated immunization schedule that would yield a more rapid antibody response[16 23], such a schedule might be an alternative to HBIg in combination with HB-vaccine in the postexposure situation This study, with a vaccine schedule of 0, 2 and 6 weeks, did not yield satisfactory antibody levels at all, thus emphasizing the need for HBIg in postexposure prophylaxis

Known factors that influence the immune response to vaccine include age, sex, antigen dose and number of doses given, site of injection and freezing of the vaccine[24 25] Sex-related differences in immune response were found for the combined IVF groups but not for controls Age, dose, site of injection and vaccine were the same for the IVF groups and controls Could the large doses of HBIg have interfered with the development of anti-HBs after immunization? Studies of the concurrent administration of HBIg and plasma derived vaccine at one or two occasions showed that the results were similar to those obtained with vaccine alone[9 26–28] Szmuness *et al* [28] found that injection of 300 IU of HBIg did not inhibit the anti-HBs response to 20 μg doses of HB-vaccine (Heptavax B, Merck Sharp & Dohme) in adults In neonates, two major studies have yielded conclusive evidence in favour of the dual approach of postexposure prophylaxis by combination of passive active immunization[13 29] The attack rate for development of the persistent carrier state in neonates of HBeAg-positive HBsAg carrier mothers was 90% in untreated groups Both HBIg alone or vaccine alone had 70–80% efficacy while efficacy rates in the groups given dual prophylaxis were above 90%[13] There was no statistically significant advantage from multiple doses as opposed to a single dose of immune globulin in conjunction with hepatitis B vaccine for efficacy nor for immune response (GMT)[29]

Yet, it cannot be excluded with certainty that an inhibiting effect of HBIg was masked by the high dose of the vaccine used in these studies To investigate this possible interference, Lelie *et al* [30] compared the anti-HBs response to a low dose (0 6 μg) of HB heat-inactivated vaccine (CLB, 3 μg per dose) in health-care workers with and without a single dose simultaneously administered HBIg (500 IU) The anti-HBs titres of recipients of vaccine alone compared with those with

HBIg and vaccine were slightly but significantly higher at 3 and 5 months after the first injections but this difference was no longer significant at the time of the booster dose (month 8) and thereafter

The use of higher doses of HBIg and the recombinant vaccine with a limited epitope range compared with plasma HBsAg justifies re-examination of a possible interaction between doses of HBIg and the immune response to recombinant vaccine Studies of children receiving recombinant vaccine however did not reveal significant differences when supplemental HBIg was administered[31]

Environmental factors have never been known to influence the immune response to immunization Nevertheless, emotions may have an important effect on the immune system In rats stress has been shown to suppress cellular and humoral immunity[32–34] For humans *in vitro* and *in vivo* studies have demonstrated a direct effect of psychological stress on parameters of immune function[34 35] Several diseases caused by viruses, such as Epstein-Barr virus, herpes simplex virus type 1 and cytomegalovirus, may be stress-related[36] The ability of human lymphocytes to respond to an activating agent declined significantly within 1–2 months of a serious psychological event In some people the responses remained low for an entire year[37]

Without doubt participation in an *in vitro* fertilization programme and the subsequent discovery of a possible infection with hepatitis B virus can be considered an episode of stress For the majority of vaccinees the period of stress lasted until several months after the first injection of vaccine Therefore the effect of psychological stress on the human immune response may not be ruled out as a possible factor influencing the reactivity to HBsAg

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REFERENCES

1 Alberda, A T, van Os, H C, Zeilmaker, G H, Rothbarth, Ph H, Heijtink, R A and Schalm, S W Hepatitis B-virusinfectie bij vrouwen behandeld met in vitro fertilisatie *Ned Tijdschr Geneesk* 1989, **133**, 20–25
2 Quint, W G V, de Bruijn, I, Kruining, J and Heijtink, R A HBV-DNA detection by gene amplification in acute hepatitis B *Hepatology* 1990, **12**, 653–656
3 CDC-ACIP Update on hepatitis B prevention *Morbid Mortal Week Rep* 1987, **36**, 353–361
4 World Health Organization Biological Substances *International Standards, Reference Preparations and Reference Reagents* Geneva, Switzerland 1982, pp 70–71
5 Van Os, H C, Drogendijk, A C, Fetter, W P F, Heijtink, R A and Zeilmaker, G H The influence of contamination of the culture-medium with hepatitis B virus on the outcome of IVF-pregnancies *Am J Obstet Gynecol* 1991, in press
6 Krugman, S, Overby, L R, Mushawar, I K, Ling, C M, Frosner, G G and Deinhardt, F Studies on natural history and prevention re-examined *N Engl J Med* 1979, **300**, 101–106
7 Grady, G F, Lee, V A, Prince, A M *et al* Hepatitis B immune globulin for accidental exposures among medical personnel final report of a multicenter controlled trial *J Infect Dis* 1978, **138**, 625–638
8 Hoofnagle, J H, Seeff, L B, Bales, Z B, Gerety, R J and Tabor, E Passive-active immunity from hepatitis B immune globulin re-analysis of a Veterans Administration Cooperative study of needle-stick hepatitis *Ann Intern Med* 1979, **91**, 813–818
9 Szmuness, W, Stevens, C E, Zang, E A, Harley, E J and Kellner, A A controlled clinical trial of the efficacy of the hepatitis B vaccines (Heptavax B) A final report *Hepatology* 1981, **1**, 377–385
10 Francis, D P, Hadler, S C, Thompson, S E *et al* The prevention of hepatitis B with vaccine report of the Centers of Disease Control multi-center efficacy trial among homosexual men *Ann Intern Med* 1982, **97**, 362–366
11 Szmuness, W, Stevens, C E, Harley, E J *et al* Hepatitis B vaccine in medical staff of hemodialysis units efficacy and sub-type cross-protection *N Engl J Med* 1982, **307**, 1481–1487
12 Papaevangelou, G, Roumeliotou-Krayannis, A, Richardson, S C and Krugman, S Postexposure immunoprophylaxis of spouses of patients with acute viral hepatitis B *Viral Hepatitis B and Liver Disease* (Ed Zuckerman, A J) Alan R Liss, Inc, New York, 1988, pp 992–994
13 Beasley, R P, Hwang, L Y, Lee, G C Y, Lan, C C, Roan, C H, Huang, F Y and Chen, C L Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immunoglobulin and hepatitis B vaccine *Lancet* 1983, **ii**, 1099–1102
14 Mitsui, T, Iwano, K, Suzuki, S *et al* Combined hepatitis B immune globulin and vaccine for postexposure prophylaxis of accidental hepatitis B virus infection in hemodialysis staff members comparison with immune globulin without vaccine in historical controls *Hepatology* 1989, **10**, 324–327
15 Beasley, R P, Hwang, L Y, Lin, C C *et al* Hepatitis B immune globulin (HBIG) efficacy in the interruption of perinatal transmission of hepatitis B virus carrier state initial report of a randomised double-blind placebo controlled trial *Lancet* 1981, **ii**, 388–393
16 Wahl, M, Hermodsson, S and Iwarson, S Hepatitis B vaccination with short dose intervals – a possible alternative for post-exposure prophylaxis? *Infection* 1988, **16**, 229–232
17 Darani, M and Gerber, M Hepatitis B antigen in vaginal secretions *Lancet* 1974, **ii**, 1008
18 Papaevangelou, G, Roumeliotou-Karayannis, A, Tassopoulos, N Kolaitis, N and Stathopoulou, P Source of infection due to hepatitis B virus in Greece *J Infect Dis* 1983, **147**, 987–989
19 Stirrat G M Immunology of diseases of pregnancy *Immunology of Pregnancy and its Disorders* (Ed Stern, C M H) Kluwer Academic London 1989, pp 115–143
20 Hollinger, F B, Troisi, C L and Pepe, P E AntiHBs responses to vaccination with a human hepatitis B vaccine made by recombinant DNA technology in yeast *J Infect Dis* 1986, **153**, 156–159
21 Jilg, W, Schmidt, M, Zoulek, G, Lorbeer, B, Wilske, B and Deinhardt, F Clinical evaluation of a recombinant hepatitis B vaccine *Lancet* 1984, **ii**, 1174–1175
22 Heijtink, R A, Kruining, J, Bakker, M and Schalm, S W Immune response after vaccination with recombinant vaccine *Antiviral Res* 1985, Suppl **I**, 281–288
23 Iwarson, S Post-exposure prophylaxis for hepatitis B active or passive? *Lancet* 1989, **ii**, 146–148
24 Dienstag, J L, Werner, B G, Polk, B G *et al* Hepatitis B vaccine in health care personnel safety, immunogenicity and indicators of efficacy *Ann Intern Med* 1984, **101**, 34–40
25 Shaw, F E Jr, Guess, H A, Roets, J M *et al* Effect of anatomic injection site, age and smoking on the immune response to hepatitis B vaccination *Vaccine* 1989, **7**, 425–430
26 Zachoval, R, Jilg, W, Lorbeer, B, Schmidt, M and Deinhardt, F Passive-active immunization against hepatitis B *J Infect Dis* 1984, **150**, 112–117
27 Goudeau, A, Coursaget, P, Barin, F, Dubois, F, Chiron, J-P, Denis, F and Diop Mar, I Prevention of hepatitis B by active and passive-active immunization *Viral Hepatitis 1981 International Symposium* (Eds Szmuness W Alter H J and Maynard, J E) Franklin Institute Press, Philadelphia, 1982 pp 509–525
28 Szmuness, W, Stevens, C E, Oleszko, W R *et al* Passive-active immunization against hepatitis B immunogenicity studies in adult Americans *Lancet* 1981, **ii**, 575–577
29 Wong, V C W Ip H M H Reesink, H W *et al* Prevention of the HBsAg carrier state in newborn infants of mothers who are chronic carriers of HBsAg and HBeAg by administration of hepatitis B vaccine and hepatitis B immunoglobulin *Lancet* 1984, **i**, 921 926
30 Lelie, P N, Reesink, H W, Grijm, R, de Jong-van Manen, S Th and Reerink-Brongers, E E Simultaneous passive and active immunization against hepatitis B Non-interference of hepatitis B immune globulin with the antiHBs response to reduced doses of heat-inactivated hepatitis B vaccine *Hepatology* 1986, **6**, 971–975
31 Stevens, C E, Taylor, P E, Tong, M J *et al* Yeast-recombinant hepatitis B vaccine Efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B virus transmission *J Am*

Med Assoc 1987, **257**, 2612–2616
32 Keller, S E, Weiss, J M, Schleifer, S J, Miller, N E and Stein, M Suppression of immunity by stress effect of graded series of stress on lymfocyte stimulation in the rat *Science* 1981, **213**, 1397–1400
33 Ballieux, R E Stress and immune response parameters and markers *Breakdown in human adaptation to "stress Towards a multidisciplinary approach* (Eds Ballieux, R E, Fielding, F J and l'Abbate, A) Martinus Nijhoff Publishers, The Hague, 1984, pp 732–739
34 Rose, N R, Friedman, H and Fahey, J L (Eds) *Manual of clinical laboratory immunology*, American Society (Microbiology) Washington, DC, 1986
35 Kiecolt-Glaser, J K, Garner, W, Speicher, C, Penn, G M, Holliday, J and Glaser, R Psychosocial modifiers of immunocompetence in medical students *Psychosom Med* 1984, **46**, 7–14
36 Glaser, R, Kiecolt-Glaser, J K, Speicher, C E and Holliday, J E Stress, loneliness and changes in herpes virus latency *J Behav Med* 1985, **8**, 249–260
37 Marx J L The immune system 'belongs in the body" Pervasive anatomical and biochemical links between the immune and nervous systems help explain how mood might influence disease susceptibility *Science* 1985, **227**, 1190–1193