Anti—Hepatitis B Serum Production in Laboratory Animals

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OBJECTIVES: To produce antisera to hepatitis B antigen (HBsAg) to use for the determination of antigen subtypes in immunodiffusion tests.

BACKGROUND: An ability to detect HBsAg subtypes is essential for studies of the transmission and clinical expression of hepatitis B virus (HBV). The immunodiffusion (ID) method (1) is the most specific method for detecting differences in the antigenic subtypes of HBV. Antigen subtypes are determined by comparing the precipitin lines and spurs of test sera to those of reference antigens after they react with anti—HBs sera. Antisera that react with the subtype specific surface determinants are necessary for this test.

DESCRIPTION: Rabbits of 2.5 to 4.0 kilograms body weight were used to produce antiserum. These rabbits were bled prior to immunization to test for anti—HBs activity by immunoelectroosmophoresis (IEOP) and radioimmune assay (RIA).

Cesium chloride purified selected sucrose fractions of HBsAg/adr, adw and ayw subtypes were used as immunizing antigens. The antigens were prepared by Electronucleonics, Inc. (Bethesda, Maryland) from human plasma previously tested at Walter Reed Army Institute of Research (WRAIR). Each antigen was diluted 1:2 with Freund's complete adjuvant (Difco Laboratory) and thoroughly emulsified with a Vortex super mixer. (2)

Five or six rabbits free of anti—HBs were immunized for each subtype of antigen. Each rabbit was inoculated with 0.25 ml of antigen—adjuvant emulsion intradermally into each of 4 sites on the thighs and back. An identical dose of the same antigen was given four weeks later. Test bleeding was done six weeks after the first dose of antigen. Sera were tested for the presence of anti—HBs by IEOP and ID. Rabbits whose sera gave subtype specific precipitin lines were exsanguinated. Rabbits with sera that formed weak precipitin lines were boosted with another identical dose of antigen and bled out two weeks later. All of the rabbit antisera were tested again by ID and complement fixation (CF) to determine the anti—HBs titer. Each rabbit serum was also tested for antibody to normal human serum proteins.

PROGRESS: Of five rabbits immunized with HBsAg/adw (EH—016), three produced sera that gave specific reactions with reference antigens (Table 1 and Figure 1). Two other rabbit sera formed definite precipitin lines with reference antigens but weak spurs were observed, suggesting they contained little subtype specific antibody. The anti—HBs CF titers varied from 1:512 to 1:1024.

Of six rabbits immunized with HBsAg/adr (EH—017), only three showed sharp precipitin lines and spurs with reference antigens (Table 1 and Figure 1). In addition, R 07 and R 14 contained anti—normal human serum reactions in low CF titer ($\leq$ 1:2).

Among the anti—ayw rabbit sera, two formed strong precipitin lines and spurs with reference antigens. The anti—HBs CF titers were 1:128 to 1:1024. Unfortunately, all of the anti—ayw rabbit sera contained anti—human serum reactivity.

DISCUSSION: The method of immunization by HBsAg in rabbits proved to be very satisfactory for the production of subtype specific antisera. The reactivity to HBsAg and normal serum protein were different among these rabbits. The anti—adw sera with no anti—human serum response indicated that the antigen

Table 1. Production of Anti–HBs in Rabbits

Rabbit No.	Immunizing Antigen	Anti–HBs			Anti–normal human serum	
		Immunodiffusion		CF Titer	ID	CF
		Spur formation	Optimum dilution			
R 01	adw EH–016 Fraction 16	Strong	1 : 2	1 : 512	Neg**	<1 : 2
R 02		Strong	1 : 2	1 : 512	Neg	<1 : 2
R 03		Strong	1 : 2	1 : 1024	Neg	<1 : 2
R 04		Weak	Und.*	1 : 512	Neg	<1 : 2
R 05		Weak	1 : 2	1 : 1024	Neg	<1 : 2
R 07	adr EH–017 Fraction 16	Strong	Und.	1 : 128	Neg	≤1 : 2
R 08		Weak	Und.	1 : 64	Neg	<1 : 2
R 09		Medium	Und.	1 : 128	Neg	<1 : 2
R 13		Weak	Und.	1 : 128	Neg	<1 : 2
R 14		Strong	1 : 2	1 : 1024	Pos***	≤1 : 2
R 15		Weak	Und.	1 : 128	Neg	<1 : 2
R 10	ayw EA–047 Fraction 10–14 pooled	None	—	1 : 1024	Pos	1 : 2
R 11		Weak	—	1 : 256	Pos	1 : 2
R 12		Weak	—	1 : 128	Pos	1 : 2
R 16		Medium	1 : 2	1 : 128	Pos	≤1 : 2
R 17		Strong	1 : 2	1 : 128	Pos	1 : 4

* Und. = Undiluted
** Neg = Negative
*** Pos = Positive

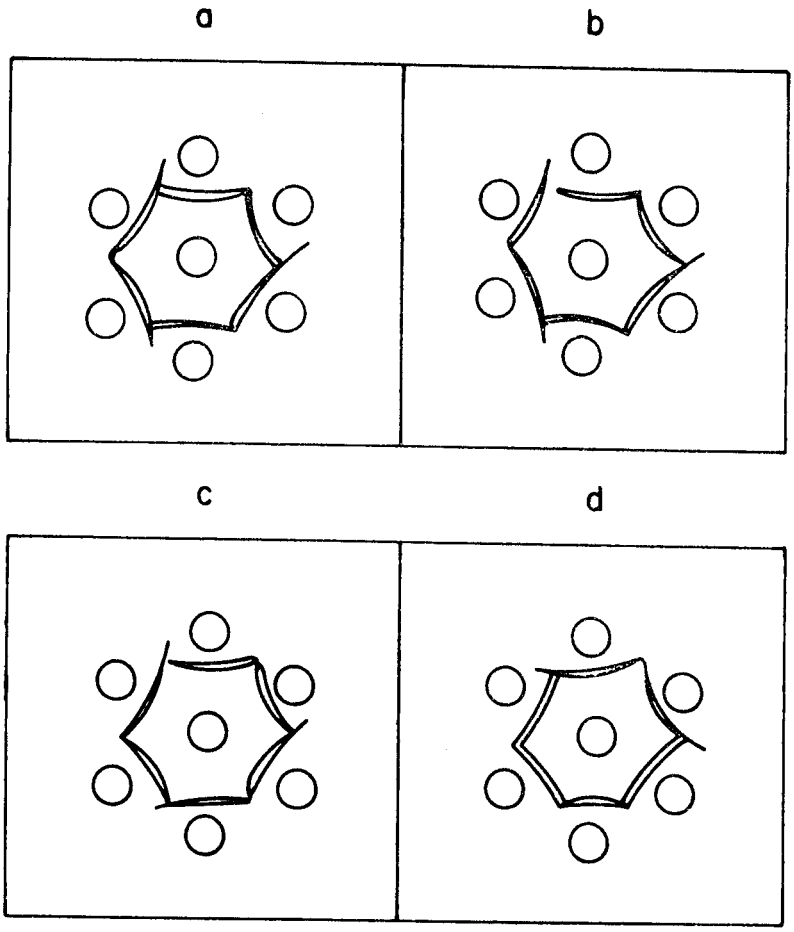

Figure 1. Patterns of immunodiffusion reactions observed with reference HBsAg. In each pattern, reference HBsAg/ayw were placed in the top and right upper wells, HBsAg/adr in right lower and bottom wells and HBsAg/adw in left upper and left lower wells. The central wells contain: a) R 01 anti-adw (1:2), b) R 02 anti-adw (1:2); c) R 09 anti-adr (undiluted); and d) R 17 anti-ayw (diluted 1:2 with normal human serum).

used, HBsAg/adw (EH–016), was essentially free of normal serum protein. The adw antiserum produced sharper precipitin lines and spurs with reference antigens than either adr or ayw antisera. The anti–d reactivity of both the adr and adw antisera was stronger than the anti–w and anti–r (Figure 1). Also the production of antibody to normal serum proteins by two rabbits immunized with HBsAg/adr and all rabbits immunized with HBsAg/ayw indicated that those antigens were incompletely purified. Nevertheless, these antisera could be absorbed sufficiently with normal human serum to allow their use for subtyping by ID.

REFERENCES:

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