Liver and biliary

Enhancement of antibody production to hepatitis B surface antigen by anti-idiotypic antibody

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SUMMARY Studies were undertaken to determine whether anti-idiotypic antibody (anti-Id) against antibody to hepatitis B surface antigen (anti-HBs) could modulate *in vitro* anti-HBs production by human peripheral blood mononuclear cells stimulated with pokeweed mitogen. Peripheral blood mononuclear cells from patients positive for serum anti-HBs produced significantly increased amounts of anti-HBs by the addition of IgG fraction of anti-anti-HBs as well as purified HBsAg in a soluble form when compared to those in cultures with pokeweed mitogen alone. F(ab')₂ but not Fc fragments of anti-anti-HBs significantly enhanced anti-HBs levels in cultures. Anti-anti-HBs or HBsAg alone, however, did not induce anti-HBs production. Anti-HBs production was not observed by the additions of these additives when peripheral blood mononuclear cells from chronic HBsAg carriers and control individuals were used. These findings indicate that anti-Id modulates the immune response to HBsAg.

Jerne¹ proposed that the immune response to an antigen can be regulated by a series of idiotype (Id)-anti-Id reactions. Ids, which are located on or close to the antigenbinding site of both antibody molecules and antigen receptors on lymphocytes, are components of this network. Anti-Id can limit the immune response to an antigen^{2,3} or, enhance the immune response to it.^{4,5}

Kennedy *et al*⁶ found that prior injection of antibodies to the idiotype of antibody to hepatitis B surface antigen (anti-HBs) into mice markedly increased the number of spleen cells secreting immunoglobulin M (IgM) anti-HBs when the mice were subsequently inoculated with HBs antigen (HBsAg). Anti-HBs secreting spleen cells were induced as well by the injection of the anti-Id alone without antigen.⁷ These findings suggest that the immune response to anti-HBs, at least in mice, is regulated through an Id-anti-Id network.

Studies were undertaken to evaluate whether this system of immunoregulatory control is operative in man. *In vitro* culture of human peripheral blood lymphocytes stimulated with anti-anti-HBs or purified HBsAg in the presence of pokeweed mitogen was used.

Methods

PATIENTS

We studied four patients positive for serum anti-HBs, three chronic HBsAg carriers and three control individuals. All patients with anti-HBs and chronic carriers showed features of chronic persistent hepatitis or minor, non-specific changes in liver histology. The three controls were all negative for HBsAg and anti-HBs serology. A preliminary study showed that peripheral blood lymphocytes from all four patients positive for anti-HBs secreted detectable amounts of anti-HBs into culture medium when cultured with pokeweed mitogen.

SEPARATION OF LYMPHOCYTES

Peripheral blood mononuclear cells were isolated from heparinised venous blood by Ficoll-Hypaque gradient sedimentation. The interphase peripheral blood mononuclear cells were suspended at 2.5×10^6 cells/ml in RPMI 1640 (GIBCO) culture medium (RPMI 1640 supplemented with 15% heat inactivated fetal bovine serum, glutamine and antibiotics).

ADDITIVES

The HBsAg (serotype adr) prepared from pooled human plasma was kindly provided by Dr Takashi Takahashi of Kitasato Institute, Tokyo, Japan.

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Received for publication 29 November 1985.

Purification was carried out by sequential density gradient separations using zonal centrifugation procedures.

Monoclonal antibody against the above HBsAg obtained from ascitic mice⁸ was kindly supplied by Dr Namiko Yoshihara, National Institute of Health, Tokyo, Japan. IgG proteins of ascitic fluids were obtained from saturated $(\text{NH}_4)_2\text{SO}_4$ precipitates and by subsequent DEAE-cellulose column chromatography.

F(ab')_2 and Fc fragments of IgG were isolated by pepsin digestion and isolated by Sephadex G150 gel chromatography. F(ab')_2 fragments (1 mg) were injected into rabbits weighing approximately 2 kg together with complete Freund's adjuvant. After 14 days, two booster injections of the F(ab')_2 fragments (0.5 mg) in complete Freund's adjuvant were given at weekly intervals. Rabbits were bled and killed 10 days after the last booster injection. Rabbits antiserum was extensively absorbed with mouse Ig. Aliquots of antiserum were passed through five times on a CNBr-activated Sepharose 4B column coupled with normal mouse serum IgG. The isolation of IgG and preparation of F(ab')_2 of affinitypurified antiserum were carried out as described above. Antibody against monoclonal antibody (6D6) to human liver specific membrane lipoprotein (LSP) was raised in rabbits using an identical procedure as the above,¹⁰ and served as a control. An IgG fraction obtained from rabbit antiserum to normal mouse serum was also provided as a control. We determined the antibody concentration in a spectrophotometer at 280 nm using an extinction coefficient of 15 for a 1% preparation.

CELL CULTURE CONDITIONS

Peripheral blood mononuclear cells ($2 \times 10^6/\text{ml}$) were cultured in a 1.0 ml-final volume RPMI 1640 culture medium in the presence of HBsAg (100 ng–1 $\mu\text{g}/\text{ml}$) or anti-anti-HBs (20–200 ng/ml) with 20 μg pokeweed mitogen in 12×75 mm culture tubes. The cells were cultured at 37°C in a moist atmosphere of 5% CO_2 . The cells were then washed extensively to remove the additives and cultured in 1 ml culture medium without any additives for another seven days at 37°C in a 5% CO_2 atmosphere. At the termination of the culture period, the cells were centrifuged and the culture supernatants were stored at -20°C .

HEPATITIS B VIRUS SEROLOGIC TESTING

HBsAg, anti-HBs, anti-HBc, HBe antigen and anti-HBe were determined using commercially available radioimmunoassay kits (Abbott).

IMMUNOGLOBULIN MEASUREMENTS

Total IgG and IgM concentrations in culture super-

natants were measured with a microtitre plate enzyme linked immunoassay (ELISA).^{11,12} Microplates (Dynatec) were coated with 100 μl of 10 $\mu\text{g}/\text{ml}$ rabbit anti-human IgG or IgM (Cappel Lab.) in carbonate buffer (pH 9.6) to each well. The plates were incubated overnight at 4°C and then washed with 0.05% Tween-20 in phosphate buffered saline (pH 7.4). One hundred microlitres of appropriately diluted test samples and standard human IgG or IgM were added in duplicate and incubated for three hours at 4°C . After washing, 100 μl rabbit-human IgG- or IgM-conjugated peroxidase (Cappel Lab.) were added to each well and incubated for two hours at room temperature. The enzyme reaction was undertaken with orthophenylene-diamine dissolved in substrate buffer (pH 5.0) and 33% H_2O_2 . Light absorbance was measured at 492 nm in a spectrophotometer. Absorbance by wells of test samples was expressed as nanograms of IgG or IgM by reference to a standard curve constructed by linear regression analysis. Standard curves were linear in the range of 5 to 80 ng/ml.

STATISTICAL ANALYSIS

The significance of differences between groups were determined by Student's *t* test.

Results

Peripheral blood mononuclear cells from four patients positive for anti-HBs released significantly increased amounts of anti-HBs into culture medium when purified HBsAg or IgG fraction of anti-anti-HBs was added as compared with the anti-HBs levels in culture with pokeweed mitogen alone (Table 1). The enhancement of anti-HBs production occurred in a dose dependent manner and the similar amounts of anti-HBs were produced in the presence of HBsAg and anti-anti-HBs. These additives alone, however, did not induce the production of detectable amounts of anti-HBs in cultures. The addition of HBsAg or anti-anti-HBs did not affect the levels of anti-HBc, IgG and IgM.

Peripheral blood mononuclear cells from three chronic HBsAg carriers and three control individuals did not produce detectable amounts of anti-HBs although similar amounts of IgG were found in the presence of HBsAg or anti-anti-HBs together with pokeweed mitogen (data not shown).

In another set of experiments, we examined the effect of IgG fragments of anti-anti-HBs on anti-HBs production in cultures by peripheral blood mononuclear cells. The peripheral blood mononuclear cells from three patients with anti-HBs showed significantly enhanced anti-HBs production on the addition of F(ab')_2 fragments (radioimmunoassay

Table Effects of anti-anti-HBs or HBsAg on the productions of anti-HBs, anti-HBc and immunoglobulins by PWM stimulated human peripheral blood mononuclear cells (PBMC)

Additives (µg/ml)	Anti-HBs (RIA ratio)	Anti-HBc (% inhibition)	IgG (ng/ml)	IgM (ng/ml)
PWM alone (20)	5.2±2.0	23.2±4.3	18750±11153	5130±1771
PWM+anti-anti-HBs (0.2)	19.5±6.9†	24.0±12.9	20766±11465	4940±1362
(0.05)	23.9±8.7†	23.2±7.9	21787±12894	4927±1334
(0.02)	13.6±5.7*	23.9±7.0	20000±11100	5147±1619
PWM+HBsAg (1)	13.9±6.6*	20.7±12.5	18850±10061	5147±1114
(0.4)	24.6±10.2†	22.6±8.1	21550±11050	4697±1408
(0.1)	18.8±5.7†	25.1±9.4	20533±11656	5090±1526

Peripheral blood mononuclear cells from four patients positive for serum anti-HBs were cultured with IgG fraction of anti-anti-HBs or purified HBsAg in the presence of pokeweed mitogen for three days. After extensive washing, the cells were cultured without any additives for another seven days. After the culture, the supernatants were obtained. Results were expressed as mean±SD from four different experiments. *:p<0.05, †:p<0.01.

ratio=21.7±6.3, mean±SD, p<0.05) or the IgG fraction (16.7±5.5, p<0.05) when compared with the cultures in pokeweed mitogen alone (4.3±1.4) (Figure). On the other hand, anti-HBs levels in the presence of Fc fragments did not differ from those of pokeweed mitogen alone. The addition of an acid elution fraction of anti-anti-HBs antiserum from affinity column coupled to serum IgG of normal mouse did not affect anti-HBs production by peripheral blood mononuclear cells.

Anti-HBs production by peripheral blood mononuclear cells was not increased or decreased in the presence of IgG fraction of anti-anti-LSP monoclonal antibody or antiserum to normal mouse serum used as control additives (data not shown).

Figure Studies were undertaken to determine which fragments of IgG of anti-anti-HBs could modulate anti-HBs production. Peripheral blood mononuclear cells (PBMC) from three patients positive for serum anti-HBs were cultured with fixed amounts (50 ng/ml) of F(ab')₂ or Fc fragments of anti-anti-HBs in the presence of pokeweed mitogen (PWM). Results were expressed as mean±SD. *:p<0.05.

Discussion

In the present study we have shown that anti-anti-HBs as well as HBsAg generates an enhanced anti-HBs response in cultures in the presence of pokeweed mitogen when peripheral blood mononuclear cells from patients positive for serum anti-HBs are used as effector cells. The specificity of anti-anti-HBs for enhanced anti-HBs production was indicated by the following: anti-anti-HBs antiserum was obtained by immunisation with F(ab')₂ fragments of anti-HB, an anti-HBs response was increased by F(ab')₂ fragments of anti-anti-HBs but not by Fc fragments or acid elution of anti-anti-HBs from affinity column coupled to normal mouse IgG, anti-HBs production was not affected by rabbit antiserum to unrelated control antibodies. These findings suggest that an immunomodulatory control by anti-Id to Id of anti-HBs is also operative in man as others have already shown in mice.^{6,7}

If the paratope and Id represent the same structural region on the antibody molecule, then the conformational fit of the antigen and anti-Id to the sites on the antibody should be similar though a mirror image.¹³ It should be possible to utilise the anti-Id for antigen mimicry and trigger the host's immune response into producing antibody by administering anti-Id.

We used rabbit anti-anti-HBs antiserum generated against anti-HBs monoclonal antibody raised in mice. A common Id was defined by a rabbit anti-Id antiserum generated against human anti-HBs.¹⁴ This common Id associated with anti-HBs was also identified in sera from rabbits, mice, guinea pigs, swine, goats, and chimpanzees that had been immunised with HBsAg.¹⁵ It is possible therefore that rabbit anti-anti-HBs antiserum induces an

enhanced anti-HBs response by human peripheral blood mononuclear cells. In addition, the anti-Id-induced anti-HBs recognised the group-specific a determinant of HBsAg that is responsible for inducing protective immunity against HB virus, suggesting the potential for the use of anti-Id vaccines for HB virus infections.⁷ Such success has been reported with anti-Id-induced protection in experimental trypanosomiasis.¹⁶ It is indicated, however, that this anti-Id-induced protective effect is genetically restricted.¹⁷ This is important because man represents a genetically outbred population, which may severely limit the effectiveness of anti-Id vaccines. The problems associated with using idiotype antibodies produced in a heterologous species for injection into humans may be circumvented by the use of human hybridoma antibodies.

Although we have shown that the immune response to HBsAg in man is regulated through an Id-anti-Id network, whether the regulation of this compartment of the immune system plays any role in determining the outcome of HB virus infection remains to be confirmed.

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