statement[19] that the body reacts in a similar manner to malarial parasites as it would to inflammatory reactions.
A parallel finding to that of immune blotting was observed in a detailed study of IFA for *B. bovis*[6]. The latter conclusively demonstrated that sera of all cattle from tick free areas would stain both parasites and infected erythrocytes but would not stain uninfected erythrocytes. This staining disappeared at dilutions greater than ~ 1:50 in most but in some individuals reactions were obtained at 1:400. Hence a second serological assay complements the immunoblotting results presented here and is additional proof that most normal sera recognize antigens in *B. bovis* infected erythrocytes. The results therefore demonstrate that the antibody response to *B. bovis* antigens is in part paralleled by an increase in host isoantibody. As such the latter may erroneously target for *B. bovis* antigens when immunoblotting techniques are used. The results thus are in accord with a recent finding that many people, including babies, who had no record of parasitic infections, contained antibodies which reacted in immunoblots with major antigens of a spectrum of protozoan and helminthic parasites[20]. The accordance thus raises the question whether decoy antibody and antigen mimicry in parasite infections are more prevalent than suspected.

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Cytotoxicity of human peripheral blood T-lymphocyte clones activated by hepatitis B virus surface antigen

A.A. Hakim[1]

Department of Medicine, Stritch School of Medicine and Department of Histology, School of Dentistry Box 50, Loyola University Medical Center, Maywood (Illinois 60901, USA), 25 July 1984

Summary. The present studies examined the cytotoxic activities of peripheral blood lymphocytes (PBL) from volunteers with (sero-positive) and without (sero-negative) circulating antibodies to hepatitis B virus surface antigen before and 30 days after vaccination with hepatitis B virus surface antigen (HBsAg). Long-term culture of monospecific hepatitis B surface (HBsAg)-responsive T-lymphocytes were isolated and grown in large numbers. The mechanism of T-cell mediated cytolysis, and the identification of the carbohydrate determinants on the surface of these effector cells responsible for the killing effect, are being examined.
Key words. T-lymphocytes; natural killer cells; killer cells; cytotoxicity; hepatitis B virus surface antigen; hepatitis B virus-induced immunity.

Peripheral blood T-lymphocytes regulate various immune responses to most antigens[2,3]. They recognize antigens only in association with the major histocompatibility gene complex encoded molecules (Ia or DR) present on the surface of accessory cells[4,5] known as antigen presenting cells. When triggered by the antigen, the T-lymphocytes proliferate and secrete factors that enhance or suppress the production of immunological effector molecules (antibodies) and/or cells (cytotoxic lymphocytes).
When incubated in vitro, PBL obtained from hepatitis B vaccine recipients respond to HBsAg[6-8]. In presence of the antigen, T-cell growth factor (i.e. Interleukin-2) and Solcoseryl, peripheral blood mononuclear cells from vaccine recipients develop HBsAg-specific T-lymphocyte clones. In response to HBsAg, these clones proliferate, develop into helper T-cells and secrete immunoregulatory factors, interferon and B-cell growth factors[10].
Earlier studies from these laboratories[11] examined the biosynthesis of tumor associated antigen (TAA) in a cell-free system. They demonstrated that glycosylation of the TAA-protein moieties alters the interaction of TAA with its antibodies, and constitutes a post-translation modification of gene expression. The availability of purified glycosidases which permit the stepwise removal of each carbohydrate component[12,13] have prompted the use of HBsAg-specific T-lymphocyte clones as a model system in studying the characteristics of the cell-surface glycoproteins in effector-target cell interactions.
Materials and methods. Heptovax® (Merck Sharp Dohme, Product no. 38676) highly purified, sterile filtered solution 40 µg/ml. Vibrio Cholera neuraminidase (VCN), *E. coli* β-galactosidase (β-Galase), almond fucosidase (Fucase) and endo-β-N-acetylglucosaminidase were purchased from Sigma Chemical Co. Absence of proteinase activity in these glycohydrolase preparations was determined colorimetrically after incubation of 0.1 unit of either enzyme with each of the following substrates: Azocoll, casein yellow, denatured hemoglobin, and azocasein in 1.0 ml of 0.5 M NaAc, pH 5.5 at 37 °C for 24 h.
Solcoseryl (Solco, Basel, Switzerland), a deproteinized extract of calf blood containing 45 mg dry substance of which approximately 70% consists of inorganic salts, and the remainder contains amino acids, hydroxy and keto acids, deoxyribose, purines and acid and alkaline polypeptides[14] was used at 1% as a supplement in the culture media.

Serum-containing standard culture medium. The culture medium consisted of RPMI-1640 enriched with 10% fetal calf serum (Gibco Laboratories), 1% Solcoseyl, 4 mM glutamine, 100 µg/ml penicillin, 100 µg/ml streptomycin and 2 µg/ml amphotericin B. This medium was used to maintain the various target cell lines.

Serum-free chemically-defined medium. To avoid the complex effects of serum proteins in examining target cell specificity and enzyme effects on cell membrane oligosaccharides, a chemically defined medium was used. The medium consisted of RPMI-1640 supplemented with transferrin (35 µg/ml), human serum albumin HSA (2.5 mg/ml); Interleukin-2 (2 units/ml); Solcoseryl (1%) and antibiotics (penicillin 100 units/ml, streptomycin 100 µg/ml and gentamicin).

Target cell lines. Human embryonic liver (HEL); kidney (HEK) and lung fibroblast (WI-35). Human neoplastic cell lines: human hepatocellular carcinoma (PLC/PRF/5) which expresses, and Chang and Mahlavu liver cell lines which do not express HBsAg; and leukemia-derived K-562; human amelanotic and melanotic (HMMC-ShA and HMMC-RW) and mammary carcinoma HMCC-1, HMCC-2, and HMCC-3 cell lines.

Isolation and cloning of HBsAg-reactive T-lymphocytes. 75–100 ml of peripheral blood from each volunteer who was vaccinated with hepatitis B vaccine 'Heptovax®' were collected. Peripheral blood monocytes (PBM) were isolated by centrifuging the heparinized blood on a Ficoll/Hypaque gradient (d = 1.007). The live cells from the interface were resuspended at 1×10^5/ml in the above serum-free medium supplemented with 1% of lectin-free T-cell growth supplement (TCGS) from Meloy Laboratories, Springfield, VA. TCGS is a concentrated culture supernatant of activated human PBM and Interleukin-2, and other factors required for the continuous propagation of T-cells[15–17]. After incubation in 24-well plates (Costar, Cambridge, MA) for 1 week, the cells were resuspended again at 1×10^8/ml and restimulated with 20 µg/ml of HBsAg in the presence of 5×10^8/ml autologous irradiated (2000 rad) PBM and 5% TCGS for 3 days.

Table 1. Effect of enzyme treatment on pre-vaccination peripheral blood lymphocyte (PBL) cytotoxicity (% toxicity)

Effector: target cell ratio	Trypsin Untreated	Treated	Vibro cholera neuraminidase (VCN) Untreated	Treated	VCN followed by trypsin Untreated	Treated
Sero-negative						
100	6.4 ± 0.5	1.6 ± 0.3	6.3 ± 0.5	24.2 ± 1.9	6.4 ± 0.5	12.3 ± 0.9
50	5.6 ± 0.4	1.4 ± 0.3	5.7 ± 0.4	21.6 ± 1.7	5.5 ± 0.5	10.7 ± 0.9
25	4.3 ± 0.4	0.6 ± 0.1	3.1 ± 0.4	18.5 ± 1.4	3.0 ± 0.4	9.7 ± 0.8
12.5	2.2 ± 0.2	0.5 ± 0.1	1.8 ± 0.2	15.7 ± 1.3	2.2 ± 0.2	9.5 ± 0.7
6.25	1.1 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	12.9 ± 1.2	1.4 ± 0.2	9.7 ± 0.7
3.125	0.6 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	12.1 ± 1.1	0.3 ± 0.1	9.4 ± 0.5
1.56	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	10.2 ± 0.9	0.1 ± 0.1	6.4 ± 0.4
Medium (spontaneous)	0.5 ± 0.1		0.4 ± 0.1		0.7 ± 0.1	
Sero-positive						
100	22.0 ± 2.1	12.8 ± 0.8	22.2 ± 2.2	33.8 ± 3.4	22.1 ± 2.1	18.4 ± 1.6
50	17.6 ± 1.8	11.4 ± 0.6	17.8 ± 1.8	26.4 ± 2.1	18.2 ± 2.0	16.5 ± 1.7
25	16.2 ± 1.5	9.5 ± 0.4	16.6 ± 1.6	24.5 ± 1.9	16.1 ± 1.5	14.2 ± 1.3
12.5	15.3 ± 1.3	7.3 ± 0.3	15.5 ± 1.4	21.8 ± 1.7	13.2 ± 1.2	10.4 ± 0.6
6.25	14.4 ± 1.1	7.0 ± 0.2	14.3 ± 0.9	19.7 ± 1.5	10.6 ± 0.7	10.1 ± 0.5
3.125	7.93 ± 0.8	6.9 ± 0.2	8.1 ± 0.4	12.2 ± 1.1	8.0 ± 0.4	10.0 ± 0.4
1.56	3.85 ± 0.1	6.4 ± 0.1	3.52 ± 0.1	6.7 ± 0.6	3.6 ± 0.3	9.2 ± 0.4
Medium (spontaneous)	0.5 ± 0.1		0.4 ± 0.1		0.7 ± 0.2	

The above data represent the mean ± SD of % toxicity calculated as described in text. Trypsin 2100 BAEE units/2 × 10^5 lymphocytes. VCN 0.5 unit/2 × 10^5 lymphocytes, incubated for 30 min with gentle stirring at 37°C in 0.5% CO_2-air humidified atmosphere, then immediately assayed for cytotoxicity as described in text.

Table 2. Effect of enzyme treatment on post-vaccination peripheral blood lymphocyte (PBL) cytotoxicity (% toxicity)

Effector: target cell ratio	Trypsin Untreated	Treated	Vibro cholera neuraminidase (VCN) Untreated	Treated	Sequential VCN then trypsin Untreated	Treated
Sero-negative						
100	42.3 ± 2.9	34.7 ± 2.2	42.5 ± 3.0	63.2 ± 3.1	43.0 ± 3.1	51.2 ± 3.5
50	39.5 ± 2.6	31.3 ± 1.9	39.1 ± 2.6	53.3 ± 2.8	40.1 ± 2.9	46.9 ± 3.2
25	29.8 ± 2.2	23.4 ± 1.4	30.1 ± 2.7	40.2 ± 2.7	29.8 ± 1.9	35.4 ± 2.8
12.5	23.9 ± 1.9	16.8 ± 0.9	24.2 ± 1.6	36.3 ± 2.5	23.9 ± 1.6	24.4 ± 1.5
6.25	17.3 ± 1.4	13.1 ± 0.8	17.4 ± 1.4	17.4 ± 1.8	17.2 ± 1.6	19.2 ± 1.3
3.125	12.9 ± 1.0	10.4 ± 0.7	13.1 ± 1.2	16.2 ± 1.6	12.9 ± 0.8	15.6 ± 0.9
1.56	6.5 ± 0.1	6.4 ± 0.1	7.0 ± 0.2	9.4 ± 1.0	7.3 ± 0.7	9.4 ± 0.8
Medium (spontaneous)	0.3 ± 0.1		0.7 ± 0.2		0.6 ± 0.1	
Sero-positive						
100	34.1 ± 3.3	26.4 ± 2.3	33.9 ± 3.5	61.9 ± 4.3	62.5 ± 5.1	59.5 ± 4.9
50	29.9 ± 2.9	21.9 ± 1.9	30.1 ± 2.9	58.4 ± 3.9	58.6 ± 3.9	31.4 ± 3.1
25	28.8 ± 2.8	20.7 ± 1.7	28.5 ± 2.6	48.1 ± 2.8	49.0 ± 2.2	30.9 ± 3.0
12.5	24.7 ± 2.4	16.8 ± 1.5	25.1 ± 2.1	33.5 ± 2.3	33.1 ± 2.3	25.3 ± 2.7
6.25	22.3 ± 1.9	15.4 ± 1.3	22.6 ± 1.9	27.8 ± 1.8	28.1 ± 1.5	22.6 ± 2.5
3.125	18.0 ± 1.4	10.4 ± 0.8	18.4 ± 0.8	13.7 ± 1.2	14.1 ± 0.8	15.2 ± 1.4
1.56	9.8 ± 0.7	6.4 ± 0.1	9.4 ± 0.4	6.5 ± 0.6	9.3 ± 0.4	9.9 ± 0.6
Medium (spontaneous)	0.4 ± 0.1		0.5 ± 0.1		0.7 ± 0.1	

The above data represent the mean ± SD of % cytotoxicity calculated as described in text. Trypsin 2100 BAEE units/2 × 10^5 lymphocytes. VCN 0.5 unit/2 × 10^5 lymphocytes, incubated for 30 min with gentle stirring at 37°C in 0.5% CO_2 humidified atmosphere, then immediately assayed for cytotoxicity. Viability of the lymphocytes after treatment was greater than 96% as determined by Trypan Blue exclusion.

Then 1 vol. of complete medium with 10% TCGS was added and the cells were incubated for 4 additional days. The HBsAg-reactive cells were cloned[10].

After cloning, aliquots of 1×10^6 lymphocytes were washed and suspended in 5 ml of prewarmed RPMI-1640-2% FCS containing various concentrations of trypsin (300, 600, 1200, 2400 ... units), VCN (0.05, 0.1, 0.5, 1.0, 1.5 ... units), galactosidase (1, 2, 4, 8, 10, 12 ... units), fucosidase (0.5, 1.0, 3, 6, 9 ... units) and β-N-acetylglucosaminidase (0.2, 0.4, 0.8, 1.0, 2.0, 4.0 ... units). The cell suspensions were incubated for 30 min at 37°C and repeatedly shaken. The cells were then washed and viable cells counted (viability was greater than 96% by the trypan blue exclusion test). For cytotoxicity, the cell concentration was adjusted to 2×10^6 cells/ml and diluted with RPMI-1640 serum-free medium.

The ^{51}Cr-chromium release assay for NK and K cell activity was performed simultaneously by using the same effector cell preparations[18,19]. The assay was that described by Kraft et al.[18]. The following mixture were incubated for 4 h: a) 200 µl RPMI-1640-FCS+200 µl of effector cells (1×10^6 cells/ml) and its dilution+200 µl target cells (5×10^4/ml for measurement of NK cell activity. b) 200 µl post-immunization serum serially diluted+200 µl effector cells (1×10^6 cells/ml) +200 µl target cell (5×10^4/ml) for measurement of ADCC (K cell activity). c) 200 µl RPMI-FCS+200 µl RPMI-FCS or post-immunization serum+200 µl target cell (5×10^4/ml) for measurement of spontaneous ^{51}Cr-release. After incubation, the cells were resuspended to ensure dispersal of released isotype and centrifuged at 250 × g for 5 min, then counted in a gamma counter.

Results. The present studies examined peripheral blood lymphocytes (PBL) from volunteers who had no circulating antihepatitis B virus surface antigen antibodies (HBsAb) (sero-negative) and who had HBsAb (sero-positive) before and 30 days after vaccination with the hepatitis B virus vaccine (HBsAg).

The data summarized in table 1 indicate less than 5.6% cytotoxicity for PBL from sero-negative (effector cells) against ^{51}Cr-PLC/PRF/5 cells (target cells). After vaccination the lymphocytes had around 42.3% cytotoxicity (table 2), a statistically significant ($p < 0.001$) increase. The prevaccination cytotoxic activity of PBL from sero-positive (effector cells) against the ^{51}Cr-PLC/PRF/5 target cells was approximately 22.0% (table 1). At 30 days post-vaccination the PBL attained 34.1% cytotoxicity, also a statistically significant ($p < 0.005$) increase.

Therefore, the first finding is that PBL from sero-negative individuals had much lower cytotoxicity than PBL from sero-positive healthy adults. Vaccination with HBsAg increased significantly the cytotoxic activities of PBL from both groups.

Trypsin treatment of the cloned cytotoxic lymphocytes reduced the cytotoxicity of PBL from sero-positive individuals by 41.6% and that of PBL from sero-negative ones by 75%. These results indicate that the cytotoxic activity against ^{51}Cr-PLC/PRF/5 is caused by more than one type cytotoxic determinant, i.e. both trypsin-resistant and a trypsin-sensitive ones.

Treatment with neuraminidase increased the cytotoxic activity of PBL from sero-negative adults before vaccination, from 6.3 to 24.2% that of PBL from sero-positive adults from 22.2 to 33.9% (table 1). The cytotoxic activity of PBL post-vaccination from sero-negative and sero-positive healthy adults was 42.5 and 33.9% respectively. In vitro treatment of these lymphocytes with neuraminidase increased the cytotoxicity from 42.5 to 63.2% of PBL after vaccination from sero-negative, and from 33.9 to 61.9% for PBL after vaccination from sero-positive adults (table 2). Therefore, the data indicate that vaccination with HBsAg and in vitro incubation with neuraminidase increased the PBL cytotoxicity; this suggests that terminal (i.e. neuraminidase-sensitive) sialic acid modulates the cytotoxic lymphocyte activity. Hydrolysis of these sialic acid terminal residues increased, i.e. activated cytotoxic lymphocytes.

Table 3 summarizes the PBL cytotoxicity against a number of ^{51}Cr-labeled target cells. Cytotoxicity of PBL from HBsAb-sero positive was significantly enhanced after vaccination, not only against PLC/PRF/5 cells, but also against melanoma, mammary carcinoma, Chang, Mahlavu and K-562 cells. The lymphocytes from HBsAb-sero-positive differed from PBL of vaccine recipients and chronic active hepatitis B by their target cells. The PBL from HBsAb-sero-positive had a statistically nonsignificant effect against Chang and Mahlavu cells, which significantly increased after vaccination to become comparable to the activity of PBL from chronic active hepatitis B patients (CAH-B). The PBL from CAH-B patients differed from PBL of both groups by their greater ability of kill Chang, Mahlavu, K-562 and especially HEL cells. The PBL from CAH-B patients appears to have its killing ability against cells expressing HBsAg and liver membrane antigens. The killing ability of K-562 is a characteristic of Natural Killer cells. The PBL from vaccine recipients had stronger killing ability against K-562 cells than PBL at prevaccination suggesting that vaccination with HBsAg increased the Natural Killer cytotoxic activity. Further studies using monoclonal antibodies are needed to confirm this observation.

Lymphocyte spontaneous cytotoxic activity was examined according to the method of Schneimer et al.[18]. Using PBL from sero-negative donors, the data in table 4 indicate that with the exception of C-014, the prevaccination cytotoxicity varied between 0.23 and 2.9% compared to 18.3–49.2% at 30 days post vaccination, a statistically significant ($p < 0.001$) increase.

In PBL from the HBsAb-sero-positive group, the data in table 5 indicate that the prevaccination cytotoxicity varied between 11.5

Table 3. Peripheral blood lymphocyte cytotoxic activities against ^{51}Cr-labeled target cells

Effector cells PBL	Target cells	Cytotoxicity (%)
HBsAb-sero positive donors, pre-vaccination	WI-38	2.2 (0.1)
	HEL	2.0 (0.4)
	HEK	0.2 (0.01)
	PLC/PRF/5	18.9 (1.9)
	HMMC-ShA	11.5 (0.9)
	HMMC-RW	19.2 (1.8)
	HMCC-1	21.5 (1.9)
	HMCC-2	16.4 (1.3)
	HMCC-3	12.5 (1.2)
	Chang	3.2 (0.2)
	Mahlavu	2.7 (0.1)
	K-562	13.8 (1.1)
HBsAb-sero positive donors, post-vaccination	WI-38	2.7 (0.1)
	HEL	2.3 (0.4)
	HEK	0.2 (0.02)
	PLC/PRF/5	62.3 (4.8)
	HMMC-ShA	24.4 (2.1)
	HMMC-RW	49.6 (3.1)
	HMCC-1	68.5 (5.2)
	HMCC-2	60.4 (5.0)
	HMCC-3	48.5 (3.0)
	Chang	14.5 (0.9)
	Mahlavu	10.4 (0.8)
	K-562	28.7 (1.9)
CAH-B patients	WI-38	9.4 (0.8)
	HEL	23.3 (1.7)
	HEK	1.2 (0.1)
	PLC/PRF/5	8.5 (1.1)
	HMMC-ShA	3.6 (0.1)
	HMMC-RW	4.1 (0.2)
	HMCC-1	11.2 (0.9)
	HMCC-2	8.2 (0.7)
	HMCC-3	6.3 (0.6)
	Chang	19.4 (1.2)
	Mahlavu	15.8 (1.1)
	K-562	34.5 (2.7)

The above data represent the mean ± SD in parenthesis between three separate experiments; each was carried out in triplicate. The cell lines used as target cells are explained under the experimental section. Effector: Target cell ratio was 50.

and 25.9%, compared to 27.8–40.2% post-vaccination, a significant ($p < 0.001$) increase. Interestingly, the post-vaccination cytotoxicity values were not significantly different for each of the two groups.

To identify the carbohydrate determinant at the cytotoxic PBL surface responsible for the killing effect, the isolated cloned PBL were preincubated for a brief period with trypsin and with a series of glycohydrolases, washed and then assayed for cytotoxicity. The data in table 6 indicate differences in the stability of the PBL cytotoxicity from pre- and post-vaccination donors to various enzyme treatments. For example, after vaccination, 82% of the cytotoxic activity of PBL from the sero-negative (–) donors was stable to trypsin, compared with 25% for the PBL from the same donor before vaccination. PBL from sero-positive donors after vaccination had 77.5% of the cytotoxic activity stable to trypsin; whereas only 22.5% of the PBL cytotoxicity from the same donor at prevaccination was stable to trypsin. Therefore, vaccination with HBsAg increases the trypsin-stable cytotoxic lymphocytes, i.e. Natural Killer-like cells.

Pre-incubation of the PBL with either neuraminidase or fucosidase significantly ($p < 0.001$) increased the cytotoxicity of pre- and post-vaccination PBL from both the HBsAb-sero-negative and sero-positive groups. On the other hand, β-galactosidase and β-N-acetylglucosaminidase reduced significantly ($p < 0.005$) the cytotoxicity of pre- and post-vaccination PBL from both HBsAb-sero-negative and the sero-positive groups. These data indicate that the splitting of neuraminic acid and/or fucose from the cell surface increased, whereas the splitting of galactose and/or glucose reduced the cytotoxicity. Therefore, it is suggested that β-galactose and/or glucose terminal residues of the surface glycoproteins modulate PBL-cytotoxic acticity.

Discussion. The results described indicate significant increases in PBL cytotoxicity at 30 days post-vaccination in both HBsAb-sero-negative ($p < 0.001$) and sero-positive individuals ($p < 0.005$). Several factors could be responsible for the increase in PBL cytotoxicity, and current studies are examining some of these factors.

The major finding of the present investigation is the modulation fo PBL cytotoxic activity by certain cell surface carbohydrate determinants. Treatment with trypsin produced a decrease in PBL cytotoxicity, suggesting more than one determinant for cytotoxicity; i.e. a trypsin-sensitive receptor similar to that of

Table 4. Effect of vaccination with the hepatitis virus cell surface antigen (HBsAg) on cytotoxic lymphocytes. A) HBsAb and anti-HBc-negative volunteers

Volunteer code	Date of blood	Effector to target cell ratio 100	50	25	12.5	6.25	3.125	1.56	Spontaneous ^{51}Cr-release (cpm)	Total ^{51}Cr-release (cpm)
		Cytotoxicity (%)								
C-008	Pre-	2.30	3.20	2.37	1.20	0.84	0.94	ND	95	4427
	Post-	39.9	30.8	18.5	10.4	5.10	ND	ND	263	6963
C-009	Pre-	0.23	0.38	0.80	1.00	1.06	ND	ND	89	4816
	Post-	38.5	29.8	20.4	9.12	4.93	1.27	1.68	248	6711
C-010	Pre-	0.50	0.31	0.63	1.33	1.53	1.62	1.12	100	4816
	Post-	18.3	16.3	16.9	10.3	6.65	3.72	1.46	102	3722
C-011	Pre-	0.90	0.95	1.13	1.25	1.43	1.52	0.98	99	4816
	Post-	49.2	35.4	26.4	13.2	11.2	ND	ND	251	6575
C-012	Pre-	2.92	2.08	1.73	1.73	1.54	1.08	0.82	99	4816
	Post-	40.4	34.2	29.5	23.3	ND	ND	ND	104	3799
C-013	Pre-	2.14	1.80	1.61	1.25	0.84	0.38	0.19	102	4816
	Post-	32.5	30.25	27.0	23.0	20.9	16.4	4.70	100	3799
C-014	Pre-	22.0	17.6	18.2	14.1	15.3	7.93	3.85	92	4816
	Post-	34.1	29.9	28.8	24.7	22.3	18.0	ND	102	3722

The above data represent the average of a triplicate run. ND indicates not determined. Cytotoxicity was determined by the formula given in the text.

Table 5. Effect of vaccination with the hepatitis virus cell surface antigen (HBsAg) on cytotoxic lymphocytes. B) HBsAb and anti-HBc-positive volunteers

Volunteer code	Date of blood	Effector to target cell ratio 100	50	25	12.5	6.25	3.125	1.56	Spontaneous ^{51}Cr-release (cpm)	Total ^{51}Cr-release (cpm)
		Cytotoxicity (%)								
S-011	Pre-	22.5	22.7	20.1	17.1	15.6	13.2	10.7	89	4816
	Post-	38.8	31.8	21.0	9.03	5.50	ND	ND	257	6846
S-012	Pre-	25.9	22.3	10.4	12.6	2.00	4.62	1.4	83	4427
	Post-	40.2	31.8	18.8	13.03	5.41	ND	ND	237	6755
S-013	Pre-	17.8	17.5	15.1	13.2	11.4	10.1	10.1	95	3722
	Post-	34.1	27.9	25.1	23.4	14.8	5.99	8.17	96	2480
S-014	Pre-	11.5	9.08	7.82	6.10	4.39	1.98	0.74	82	4816
	Post-	27.8	24.3	20.1	19.7	17.4	12.1	8.88	107	3722
S-015	Pre-	19.4	16.7	16.6	12.5	12.9	7.84	7.15	103	3722
	Post-	39.4	34.0	29.7	23.5	22.3	17.3	7.65	114	4816
S-017	Pre-	18.3	16.3	16.9	10.3	6.65	3.72	1.46	102	3722
	Post-	33.4	30.3	26.6	24.1	21.1	16.5	4.97	96	2480
S-018	Pre-	22.0	17.6	18.2	14.1	15.3	7.93	3.85	92	4427
	Post-	34.1	29.9	28.8	24.7	22.3	18.0	ND	102	2480

The above data represent the average of a triplicate run. ND indicates not determined. Cytotoxicity was determined by the formula given in the text.

Table 6. Effect of glycohydrolases and trypsin on peripheral blood lymphocyte (PBL) cytotoxicity

Enzyme	Concentration	Lymphocytes		Percent cytotoxicity at effector:target cell ratio 100	50	25
Trypsin	1200 units	Pre-sero(−)	UL	6.4 ± 0.5	5.6 ± 0.4	4.3 ± 0.4
			TL	1.6 ± 0.3	1.4 ± 0.1	0.6 ± 0.1
		Post-sero(−)	UL	42.3 ± 2.9	39.5 ± 2.9	29.8 ± 2.2
			TL	34.7 ± 2.2	31.3 ± 1.9	23.4 ± 1.4
		Pre-sero(+)	UL	22.0 ± 2.1	17.6 ± 1.8	16.2 ± 1.5
			TL	12.8 ± 0.8	11.4 ± 0.6	9.5 ± 0.4
		Post-sero(+)	UL	34.1 ± 3.3	29.9 ± 2.9	28.8 ± 2.8
			TL	26.4 ± 2.3	21.9 ± 1.9	20.7 ± 1.7
Vibrio cholera neuraminidase (VCN)	0.5 units	Pre-Sero(−)	UL	6.3 ± 0.5	5.7 ± 0.4	3.1 ± 0.3
			TL	24.2 ± 1.9	21.6 ± 1.7	18.5 ± 1.4
		Post-sero(−)	UL	42.2 ± 3.9	39.1 ± 3.6	30.1 ± 2.7
			TL	63.2 ± 3.1	53.3 ± 2.8	40.2 ± 2.7
		Pre-sero(+)	UL	22.4 ± 1.9	17.8 ± 1.5	16.6 ± 1.2
			TL	33.8 ± 2.4	26.4 ± 2.1	24.5 ± 1.9
		Post-sero(+)	UL	33.9 ± 3.1	30.1 ± 2.6	28.5 ± 2.4
			TL	61.9 ± 5.4	58.4 ± 3.9	48.1 ± 2.8
β-Galactosidase	10 units	Pre-sero(−)	UL	6.7 ± 0.5	5.8 ± 0.5	4.1 ± 0.3
			TL	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
		Post-sero(−)	UL	43.4 ± 3.9	40.1 ± 3.8	30.2 ± 2.6
			TL	5.7 ± 0.4	3.2 ± 0.1	1.1 ± 0.1
		Pre-sero(+)	UL	21.9 ± 1.7	17.5 ± 1.2	16.5 ± 1.2
			TL	3.1 ± 0.1	2.2 ± 0.1	0.9 ± 0.1
		Post-sero(+)	UL	34.5 ± 2.3	30.2 ± 2.4	29.4 ± 2.3
			TL	4.7 ± 0.1	3.2 ± 0.1	0.9 ± 0.1
Fucosidase	3 units	Pre-sero(−)	UL	6.9 ± 0.5	5.4 ± 0.4	4.3 ± 0.3
			TL	20.4 ± 1.8	19.4 ± 1.8	16.4 ± 1.4
		Post-sero(−)	UL	44.1 ± 3.6	40.3 ± 3.8	29.8 ± 1.4
			TL	62.5 ± 5.7	51.4 ± 4.9	39.6 ± 2.5
		Pre-sero(+)	UL	22.3 ± 1.6	17.4 ± 1.3	16.5 ± 1.3
			TL	29.8 ± 2.3	24.3 ± 1.1	22.3 ± 1.8
		Post-sero(+)	UL	34.9 ± 2.9	30.5 ± 2.5	26.4 ± 2.1
			TL	58.3 ± 4.7	55.4 ± 5.2	42.3 ± 3.9
β-N-Acetylglucos-aminidase	2 units	Pre-sero(−)	UL	7.2 ± 0.6	5.7 ± 0.3	4.5 ± 0.2
			TL	0.5 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
		Post-sero(−)	UL	43.9 ± 3.9	39.7 ± 3.5	30.4 ± 2.5
			TL	6.2 ± 0.5	4.4 ± 0.3	4.2 ± 0.2
		Pre-sero(+)	UL	22.9 ± 1.9	17.9 ± 1.2	17.3 ± 1.4
			TL	5.4 ± 0.3	4.3 ± 0.3	3.7 ± 0.2
		Post-sero(+)	UL	35.1 ± 3.1	31.6 ± 2.6	27.6 ± 2.1
			TL	8.2 ± 0.7	5.3 ± 0.4	4.1 ± 0.3

The above data are presented as mean ± SD. UL and TL indicate untreated and treated lymphocytes. The (−) and (+) indicate sero-negative and sero-positive donors respectively.

NK-cells, and trypsin stable receptors in antibody-dependent cell cytotoxicity. NK cells have been reported to lose their cytotoxic capacity when treated with trypsin[20–22]. Thus it could be concluded that the Fc-receptor involved in ADCC is resistant to trypsin treatment which confirms earlier findings[23–25].

Treatment with neuraminidase produced a 280% increase in the cytotoxicity of PBL from HBsAb-sero-negative donors compared to a 51% increase in the cytotoxic activity of PBL from HBsAb-sero-positive volunteers. Pretreatment of lymphocytes with Vibrio cholera neuraminidase (VCN) has been shown to produce increase in PBL proliferative response towards mitogens[26,27], antigens[28] in the mixed lymphocyte reaction[29], and the generation of cell-mediated cytotoxicity in vitro[30]. Evidence has been published that VCN enhances preferentially a T-cell response[31] and that the removal of peripheral sialic acid from the responding lymphocyte will enhance responsiveness. Neuraminidase acts not only by unmasking new antigenic sites, but also by altering the cell-membrane characteristics; it specifically the carbohydrate terminals needed for lymphocyte function. Fucosidase has been shown to act similar by to neuraminidase. Since both neuraminic acid and fucose terminate the carbohydrate chains in a glyco protein, their removal will reveal new galactose and/or glucosamine sites which may cause the enhancing effects. The splitting of galactose and/or glucosamine caused the inhibition of the PBL cytotoxic activities. Further studies are in progress to establish that galactose and/or glucosamine of the oligosaccharide moiety of the surface glycoprotein form the molecular basis for the NK and/or cytotoxic lymphocyte interaction with the target cell. Nevertheless, it can be stated that these cell-to-cell interactions are modulated by the presence of sialyl-, galactosyl- and/or glucosyl-transferase systems at the lymphocyte membrane.

Since the cloned cytotoxic lymphocytes from HBsAb-vaccine recipients, i.e. post-vaccination donors, killed K-562 target cells, which are usually specific target cells for Natural Killer (NK) cells, but at the same time their major cytotoxicity was stable to trypsin, while that of the NK-cells is trypsin unstable, the cloned cytotoxic lymphocytes could be classified as Natural Killer-like cells. Further studies on the identity of these cells are in progress.

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Induction of IgG_1 and IgE responses to protein-conjugated and unconjugated β-lactam antibiotics in the mouse – efficacy of Freund's complete adjuvant

M. Harada, A. Watanabe, M. Matsumoto and M. Takeuchi

Shionogi Research Laboratories, Shionogi & Co. Ltd, Fukushima-ku, Osaka 553 (Japan), 1 October 1984

Summary. One or two injections two weeks apart of protein-conjugated penicillin G, cephalothin or cefmetazole emulsified with Freund's complete adjuvant were quite effective in producing anti-antibiotic antibodies of the IgE as well as of the IgG_1 class in mice. Long-lasting and boostable production of both antibody classes was also obtained against unconjugated cephalothin or cefmetazole, though the positivity depended on the mouse strain.
Key words. Freund's complete adjuvant; IgE response; IgG_1 response; β-lactam antibiotics; strain difference.

To analyze the allergic side-effects of β-lactam antibiotics in man, we first need to know the immunogenicity of these drugs in animals. Especially important is the ability to produce antibodies which mediate anaphylactic reactions, because the most prevalent and severe side effects belong to Type I of immediate hypersensitivity. Therefore, we examined the immunogenicity of some antibiotic agents in the mouse and found that not only IgG_1 but also IgE responses to both protein-conjugated and unconjugated antibiotics could be obtained in animals immunized using Freund's complete adjuvant.

Materials and methods. 1) Animals. C_3H/HeNShi and $C_{57}BL$/6JShi mice (female, 7–8 weeks old) were used as antibody producers, and DS/Shi mice (female, 7–8 weeks old) and Wistar/Shi rats (female, 8–9 weeks old) were employed as recipients in the PCA test.

2) Antibiotics. Cephalothin (CET, Shionogi), penicillin G (PCG, Meiji Seika) and cefmetazole (CMZ, Sankyo) were used.

3) Antibiotic-protein conjugates. The antibiotics were conjugated with bovine γ-globulin (BGG, Sigma) or guinea pig serum albumin (GpSA, Sigma) according to Levine et al.[1] and the epitope densities of the conjugates prepared were determined by the technique of Ebata et al.[6]. The conjugates used as immunogens were CET_{17}-BGG, BPO_{31}-BGG (BPO: benzylpenicilloyl, the main haptenic form of PCG) and CMZ_{17}-BGG, and those employed as PCA-elicitors were CET_{13}-GpSA, BPO_{17}-GpSA and CMZ_{16}-GpSA.

4) Immunization. A) Immunization with antibiotic-BGG conjugates: C_3H/HeNShi and $C_{57}BL$/6JShi mice were i.p. injected with 0.2 ml of an emulsion of Freund's complete adjuvant (FCA) containing 1 mg of an antibiotic-BGG conjugate once or twice at 2 weeks apart. C_3H/HeNShi mice were also immunized by three biweekly i.p. injections of alum-precipitated immunogen (1 µg of an immunogen plus 1 mg of aluminum hydroxide gel) prepared according to Katsura[4]. As negative controls, both strains of mouse were immunized to plain BGG in the same way. The immunized animals were bled by heart puncture 1 or 2 weeks later, and antisera of a group of five mice were pooled for the determination of antibody activity. B) Immunization with unconjugated antibiotics: C_3H/HeNShi and $C_{56}BL$/6JShi mice were i.p. injected with 0.2 ml of FCA emulsion containing 1 mg of each antibiotic agent three times a week for 2 weeks (a total of 6 times), and antisera were prepared 4 weeks after the last injection. Controls were injected with an emulsion of FCA and physiological saline solution.