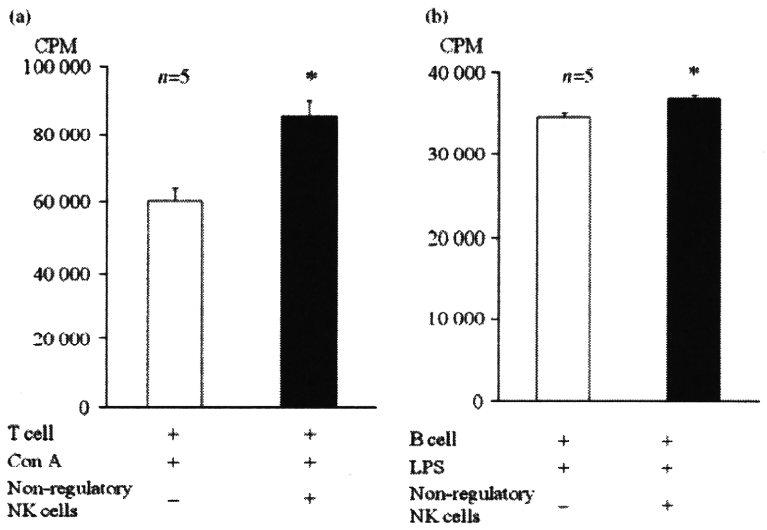

Fig. 5. Accentuation of T cell and B cells proliferation by non-IL-10-producing hepatic natural killer (NK) cells. T cells and B cells were cultured with concanavalin A (Con A) (a), lipopolysaccharides (LPS) (b), and with or without non-IL-10-producing NK cells (immunogenic NK cells). Increased T cell and B cell proliferation was observed due to the addition of non-IL-10-producing NK cells. Data of five separate experiments have been shown. $^*P < 0.05$, compared with controls.

Natural killer cells have many kinds of receptors including immunoglobulin-like receptors lectin-like receptors. NK cells also express toll-like receptors, TLR-2, TLR-3, TLR-4, TLR-7, TLR-8 and TLR-9 (22). To prepare regulatory NK cells, we took the advantage of the expression of TLR-9 on NK cells. In this study, we have shown that a population of NK cells in the liver produced abundant amounts of IL-10 in response to stimulation with CpG ODN. In fact, most of the immune activators induced IL-10 from liver NK cells; however, CpG ODN was the most potent stimulant. Interestingly, CpG ODN induced very low levels of IFN-γ from NK cells in this study. Our observations might come from the tolerogenic aspect of CpG ODN. CpG ODN is known to induce IL-10 production from B cell and macrophage and is now used for the treatment of allergy and asthma (23).

The frequencies of regulatory NK cells were significantly higher in the liver compared with those in the spleen. Functionally, regulatory NK cells of the liver had immunosuppressive properties in the context of mitogen-induced T and B cell proliferation.

Most of the NPCs of the liver, such as DCs, liver sinusoidal endothelial cells, Kupffer cells, hepatic stellate cells, and regulatory T cells are responsible for the tolerogenic properties of the liver (24, 25). One of the important factors that endow tolerogenic property of these cells is their capacity to produce significant amounts of IL-10. This study has shown that regulatory NK cells represent another NPC, capable of producing abundant amounts of IL-10. We have also shown another population of IFN-γ-producing NK cells in the liver. Several immunocytes have shown both immunogenic and tolerogenic potentials; (i) immunogenic DCs and regulatory DCs and (2) effecter T cells and regulatory T cells. Further study will be required to assess whether regulatory NK cells regulate the functional capacities of immunogenic NK cells or whether they are endowed with wide variety of immune suppressive functions.

This is the first study that showed the presence and functional features of regulatory NK cells in healthy murine liver. However, many aspects of this unique population of NK cells could not be properly addressed by our study. We had to stimulate liver NK cells with an immunostimulator to obtain IL-10-producing regulatory NK cells. This raises a question about the presence of these cells *in vivo* in pathogen-free conditions in the healthy liver. Decidual NK cells are thought to migrate from peripheral blood and differentiate into decidual NK cells; they then obtain their regulatory function because of the decidua's tolerogenic microenvironment (26). Liver is also considered to be a tolerogenic organ and it is possible that peripheral NK cells differentiate into regulatory NK cells and obtain immunosuppressive functions in the liver microenvironment.

In addition to their role in maintaining homeostasis, regulatory NK cells may also play a role in the pathogenesis of different liver-related diseases. Inadequate immune responses are detected in patients with chronic hepatitis B virus and hepatitis C virus (HCV) infections. On the other hand, distorted immune responses are seen in subjects with autoimmune liver diseases. Patients infected with HCV had more IL-10-producing NK cells compared with healthy controls and IL-10-producing regulatory NK cells might be involved in HCV persistence (27). Also, NK cells are suspected to be related with

autoimmunity and autoimmune diseases. Disruption of the critical balance between immunogenic NK cells and regulatory NK cells may regulate the autoimmune process of the liver. However, further studies about regulatory NK cells are likely to answer some queries regarding these conditions.

In conclusion, this study has shown that regulatory NK cells are present in the liver, just as their existence has been reported in placenta and peripheral blood. Also, their possible immunosuppressive role has been elucidated by this study. Further study will unveil the real implications of these cells in physiological and pathological conditions.

Acknowledgements

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Impaired dendritic cell functions disrupt antigen-specific adaptive immune responses in mice with nonalcoholic fatty liver disease

Teruki Miyake · Sheikh Mohammad Fazle Akbar · Osamu Yoshida · Shiyi Chen · Yoichi Hiasa · Bunzo Matsuura · Masanori Abe · Morikazu Onji

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Abstract
Background/aims The magnitude of antigen-specific immunity was assessed in a murine model of nonalcoholic fatty liver diseases (NAFLD). Because antigen-specific immunity was diminished in NAFLD mice, the underlying mechanisms were evaluated through analysis of the functions of antigen-presenting dendritic cells (DC) and other immunocytes.
Methods For 12 weeks, NAFLD mice received a high-fat (60%) and high-calorie (520 kcal/100 g) diet. C57BL/6 mice (controls) received a standard diet. NAFLD mice and control mice were immunized with hepatitis B vaccine containing hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg). Antibody to HBsAg (anti-HBs), HBsAg and HBcAg-specific cellular immune response and functions of whole spleen cells, T lymphocytes, B lymphocytes and spleen DCs of NAFLD and control mice were assessed in vitro.
Results Levels of anti-HBs and the magnitude of proliferation of HBsAg and HBcAg-specific lymphocytes were significantly lower in NAFLD mice than control mice ($P < 0.05$). The spleen cells of NAFLD mice produced significantly higher levels of inflammatory cytokines ($P < 0.05$) and exhibited significantly increased T cell proliferation compared with control mice ($P < 0.05$). However, the antigen processing and presenting capacities of spleen DCs were significantly decreased in NAFLD mice compared with control mice ($P < 0.05$). Palmitic acid, a saturated fatty acid, caused diminished antigen processing and presenting capacity of both murine and human DCs.
Conclusions Nonalcoholic fatty liver disease mice exhibit decreased magnitudes of antigen-specific humoral and cellular immune responses. This effect is mainly, if not solely, due to impaired antigen processing and presentation capacities of DC.

Keywords NAFLD · Adaptive immunity · Dendritic cell · HB vaccine

T. Miyake · S. M. F. Akbar · O. Yoshida · S. Chen · Y. Hiasa · B. Matsuura · M. Abe · M. Onji (✉)
Department of Gastroenterology and Metabology, Ehime University Graduate School of Medicine, Toon, Ehime 791-0295, Japan
e-mail: onjimori@m.ehime-u.ac.jp

S. M. F. Akbar
Department of Medical Sciences, Toshiba General Hospital, Tokyo, Japan

Introduction

Obesity and its associated conditions, including nonalcoholic fatty liver disease (NAFLD), have reached worldwide epidemic proportions. The pathological spectrum of NAFLD extends from simple hepatic steatosis to nonalcoholic steatohepatitis to liver cirrhosis. In addition to liver-related complications, patients with NAFLD are more prone to develop insulin resistance, type 2 diabetes mellitus and coronary heart disease [1, 2].

Different metabolic factors, such as over-nutrition, oxidative stress, mitochondrial injury and fatty acid lipotoxicity, are related to the pathogenesis of NAFLD [3]. Recent studies have shown the role of the immune system in NAFLD because some comorbidities associated with NAFLD, such as insulin resistance and type 2 diabetes mellitus, may be triggered by increased activation of the

cells of the immune system [4, 5]. Excessive production of tumor necrosis factor alpha (TNF-α) and interferon-gamma (IFN-γ), and activation of cells of innate immune responses have been reported in NAFLD [4–11].

However, little has been published about antigen-specific adaptive immunity in NAFLD, although epidemiologic data indicate that adaptive immunity may be compromised in these subjects. NAFLD patients are susceptible to infection and exhibit reduced responses to vaccinations [12, 13]. In addition, many NAFLD patients are also chronically infected with hepatitis B virus and hepatitis C virus [14, 15]. Because impaired virus-specific adaptive immunity is responsible for chronic infection with these viruses [16], NAFLD may also be characterized by distorted antigen-specific adaptive immunity.

Exacerbated innate immunity and possible impaired adaptive immunity in NAFLD led us to assess antigen-specific immune responses in a murine model of NAFLD. Our study showed that in comparison to control mice, antigen-specific humoral and cellular immune responses were significantly reduced in NAFLD mice. Interestingly, impaired antigen-specific immune responses in NAFLD mice were detected in the presence of increased inflammatory cytokines and exacerbated T and B lymphocyte function. Because antigen-presenting dendritic cells (DC) are initiators and regulators of the antigen-specific immune responses [17–19], we examined the functional capacities of DCs in NAFLD mice. Finally, we also checked the impacts of fatty acids on the functional capacities of human peripheral blood mononuclear cells (PBMCs) and DCs to evaluate clinical implications of our study in NAFLD mice.

Materials and methods

Mice

Seven-week-old male C57BL/6J mice were purchased from Nihon Clea (Tokyo, Japan). Mice were housed individually in polycarbonate cages in our laboratory facilities and maintained in a temperature- and humidity-controlled room (23 ± 1°C) with a 12-h light/dark cycle. After 1 week, one group of mice was given a high-fat diet (HFD) that consisted of 20% protein, 20% carbohydrate and 60% fat with an energy density of 520 kcal/100 g (D12492, RESEARCH DIETS, Inc., New Brunswick, NJ). Control mice were fed a standard laboratory chow that contained 26% protein, 60% carbohydrate and 13% fat with an energy density of 360 kcal/100 g. All mice received humane care, and the study protocol was approved by the Ethical Committee of the Graduate School of Medicine, Ehime University, Japan.

Human volunteers

Ten normal human volunteers (age range, 26–55 years) were enrolled in this study. They were healthy and free from any evidence of liver disease, autoimmune disease and infectious disease. Informed consent was obtained from each volunteer included in the study, and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by the institution's human research committee (no. 16-1, dated 2004-02-26).

Assessment of biochemical and histological parameters

Blood glucose levels (Glucose PILOT; Aventir Biotech, LLC, Carlsbad, CA), serum insulin concentrations (Insulin kit, Morinaga, Yokohama, Japan), and serum triglyceride and cholesterol levels (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were measured by commercially available kits. We measured fatty acid concentrations in mice sera by liquid chromatography (Shikoku Chuken, Matsuyama, Japan).

Mice were anesthetized with diethyl ether and killed by cervical dislocation. The weight of the liver, spleen and fatty tissue were measured. Histological assessment of fatty liver was done by histochemistry.

Based on body weight, extent of fatty liver, levels of blood glucose, serum cholesterol and insulin levels, a mice model of NAFLD occurred 12 weeks after study start.

Immunization schedule

Nonalcoholic fatty liver disease mice and control mice were immunized once with intraperitoneal hepatitis B (HB) vaccine containing hepatitis B surface antigen (HBsAg, 1, 2 and 4 μg) (Heptavax-II, subtype adw, Banyu Pharmaceutical). Mice were also injected with hepatitis B core antigen (HBcAg; 1, 2 and 4 μg) (Tokyo Institute of Immunology, Tokyo, Japan).

Assessment of humoral immune response to HBsAg

The levels of antibodies to HBsAg (anti-HBs) in sera were measured 4 weeks after immunization by chemiluminescence enzyme immunoassay method (Shikoku Chuken, Matsuyama, Japan) as described previously [20]. Values were expressed as mIU/ml.

Isolation of T lymphocytes, B lymphocytes and DC

Different immunocytes from mice spleen were isolated as described previously [21–23]. In short, single cell suspensions of spleen were prepared and suspended in RPMI 1640

(Iwaki, Osaka, Japan) containing 10% fetal calf serum (Filtron PTY LTD, Brooklyn, Australia). T lymphocytes and B lymphocytes were purified from single cell suspensions of spleen using T lymphocyte and B lymphocyte isolation kits (Miltenyi Biotec, GmbH, Bergisch Gladbach, Germany) as described previously [20]. DCs were isolated from single cell suspensions of spleen by density column (specific gravity 1.082), plastic adherence, re-culture on plastic surface, and depletion of macrophages and lymphocytes [21].

Peripheral blood mononuclear cells were isolated from human blood by density centrifugation on Ficoll-hypaque (specific gravity 1.077). DCs were also isolated from PBMC by plastic adherence and culturing with granulocyte-macrophages colony-stimulating factor and interleukin (IL)-4, as described in our previous report [24].

Cytokine production by different immunocytes

Immunocytes from mice were cultured with concanavalin A (Con A, Sigma Chemical, St. Louis, MO), lipopolysaccharides (LPS, Sigma Chemical), cytosine-phosphate-guanosine oligodeoxynucleotide (CPG-ODN) (InvivoGen, San Diego, CA) and polyinosinic polycytidylic acid (poly I:C) (Sigma Chemical) for 48 h to assess production of cytokines.

Measurements of palmitic acid and oleic acid for culture experiments

In some experiments, palmitic acid and oleic acid were added to cultures to assess their impact on cytokine production from human PBMC and also to assess if these fatty acids have any role on antigen processing and presentation capacities of DC. In short, fatty acids were solubilized in ethanol with albumin as stock solution of 20 mM and stored at −20°C, as described previously [25, 26]. Fatty acid-albumin complex solutions were freshly prepared before each experiment. The pH was adjusted to 7.4. Subsequently, we performed preliminary experiments using 10–1000 μM of fatty acids to assess the concentrations of fatty acids that would not compromise cell viability. As cell viability was not compromised when 50–100 μM of fatty acid was used in any of the 10 preliminary experiments, we used 50 μM of fatty acid for assessing the effect of palmitic acid and oleic acid for co-culture experiments.

Preparation of HBsAg-specific and HBcAg-specific memory lymphocytes

Eight-week-old male C57BL/6 mice were immunized twice with HB vaccine containing 10 μg of HBsAg at an interval of 4 weeks or 10 μg of HBcAg. Serial evaluation revealed that at 7–8 months after immunization, antigen-specific lymphocytes in these mice were in a memory state, because these lymphocytes proliferated after stimulation with DCs and antigen, but not with antigen alone [27].

Preparation of HBsAg-pulsed DC

Spleen DCs or human blood DCs (1–2 million) suspended in 1.0 ml of RPMI 1640 plus 10% fetal calf serum were cultured with 50 μg of HBsAg or 50 μg of HBcAg (Institute of Immunology) for 48 h [24, 27]. DCs were recovered from the cultures and washed five times with phosphate-buffered saline. In some studies, palmitic acid and oleic acid (suspended in albumin) were added to cultures during preparation of antigen-pulsed murine spleen DCs and human blood DCs.

Lymphoproliferative assays

We conducted a series of experiments to optimize the protocols for the lymphocyte proliferation assays as described previously [20–24, 27]. T lymphocytes and B lymphocytes were cultured with or without polyclonal activators for 72 h to assess the proliferative capacities of these immunocytes.

Dendritic cells from NAFLD mice and control mice were cultured with T cells from these mice to assess the proliferative capacities of autologous and syngenic lymphocytes in vitro.

Lymphocytes from HBsAg-based vaccine-injected mice and HBcAg-immunized mice were cultured in the presence or absence of recombinant HBsAg or HBcAg for 120 h to evaluate antigen-specific cellular immune responses. Unpulsed DCs or antigen-pulsed DCs were cultured with antigen-specific memory lymphocytes for 120 h to assess the functional capacity of DC.

All cultures were performed in 96-well U-bottom plates (Corning, Tokyo, Japan). [^{3}H]-Thymidine (1.0 mCi/L, Amersham Biosciences UK LTD, Buckinghamshire, UK) was diluted in sterile phosphate-buffered saline and added to the cultures. Cells were harvested automatically by a multiple cell harvester (LABO MASH, Futaba Medical, Tokyo, Japan) after 16 h onto a filter paper (LM 101–10, Futaba Medical). The level of incorporation of ^{3}[H]-thymidine was determined in a liquid scintillation counter (Beckman-LS 5000, Beckman Instruments INC, Fullerton, CA). Data were expressed as count per minute (CPM) or stimulation index. Stimulation index was counted by dividing the levels of CPM in culture containing antigen with the levels of CPM in control cultures.

Estimation of cytokines

Levels of different cytokines in culture supernatants were estimated using a commercial kit for the cytometric bead array method as described previously [20, 27]. Levels of cytokines in culture supernatants were calibrated from the mean fluorescence intensities of the standard negative control, standard positive control and samples by Cytometric Bead Array software (BD Biosciences Pharmingen, San Jose, CA) using a Macintosh computer (SAS Institute, Cary, NC).

Statistical analysis

Values are presented as mean ± standard error of mean (SEM). Data were analyzed by unpaired *t* tests if data were normally distributed and by Mann-Whitney rank-sum test if they were skewed. Differences were considered significant at $P < 0.05$.

Results

Characteristics of NAFLD mice

On the basis of data from preliminary experiments, we assessed different parameters of HFD-consuming mice and control mice 12 weeks after study commencement. As shown in Fig. 1a, the body weight of both groups of mice was similar at the start of experiments. However, 12 weeks after study commencement, the body weight of HFD mice was significantly higher than that of control mice ($P < 0.05$). Concentrations of fasting blood sugar, blood insulin and serum cholesterol levels, as well as the weights of total fatty tissues, were also significantly ($P < 0.05$) increased in HFD mice compared with control mice (Fig. 1b–e). In addition, the weights of the liver, spleen, and subcutaneous and visceral fat were significantly increased in HFD mice compared with control mice ($P < 0.05$) (data not shown). Levels of different fatty acids were significantly ($P < 0.05$) increased in the sera of HFD-consuming mice 12 weeks after study commencement. Levels of palmitic acid and oleic acid are shown in Fig. 2a. Histological assessment revealed severe fatty liver at 12 weeks after study commencement in HFD mice (Fig. 2b).

Decreased antigen-specific humoral immune responses to HBsAg in NAFLD mice

Levels of anti-HBs in the sera were significantly lower in NAFLD mice compared with control mice due to immunization with 1 μg (levels of anti-HBs in the sera, NAFLD mice versus control mice; 14 ± 6 mIU/ml versus 205 ± 66 mIU/ml, $n = 10$, $p < 0.05$), 2 μg (levels of anti-HBs in the sera, NAFLD mice versus control mice; 17 ± 8 mIU/ml versus 440 ± 72 mIU/ml, $n = 10$, $P < 0.05$) and 4 μg (levels of anti-HBs in the sera, NAFLD mice versus control mice; 107 ± 21 mIU/ml versus 524 ± 99 mIU/ml, $n = 10$, $P < 0.05$).

Decreased antigen-specific cellular immune responses to HBsAg and HBcAg in NAFLD mice

Lymphocytes from all HBsAg and HBcAg-immunized control mice showed significant proliferation when cultured

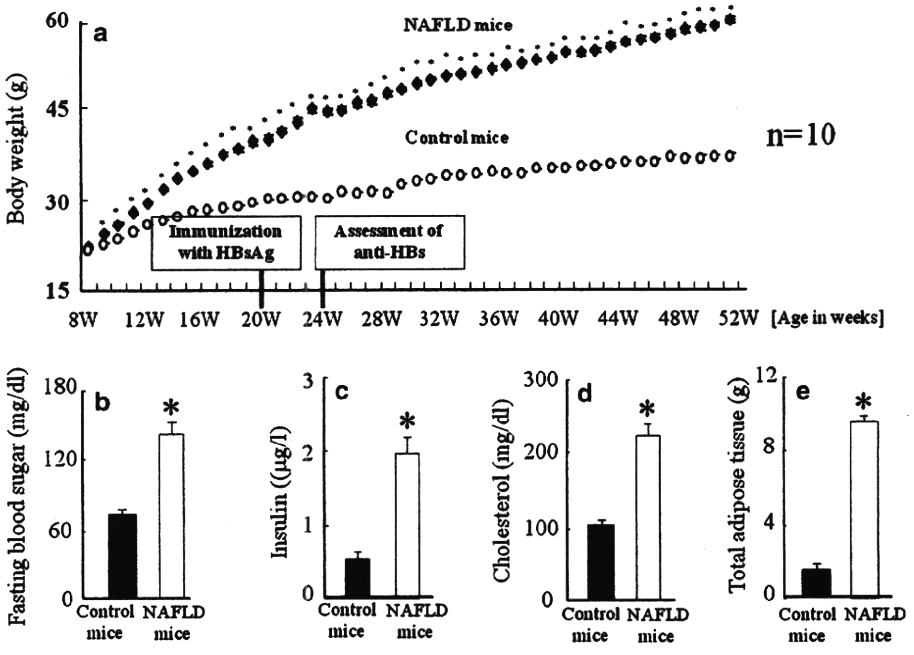

Fig. 1 Characterization of mice with nonalcoholic fatty liver disease (NAFLD). Eight-week-old male C57BL/6 (control) mice were given either a high-fat diet containing 60% fat and 520 kcal/100 g or a standard laboratory chow. The kinetics of weight gain of NAFLD mice and control mice are shown in **a**. Levels of blood sugar, serum insulin and cholesterol, and amounts of total adipose tissues were significantly higher in NAFLD mice compared with control mice ($P < 0.05$) (**b–e**). Data are mean and SEM of body weight of 10 mice in each group. *$P < 0.05$ compared with control mice of same duration

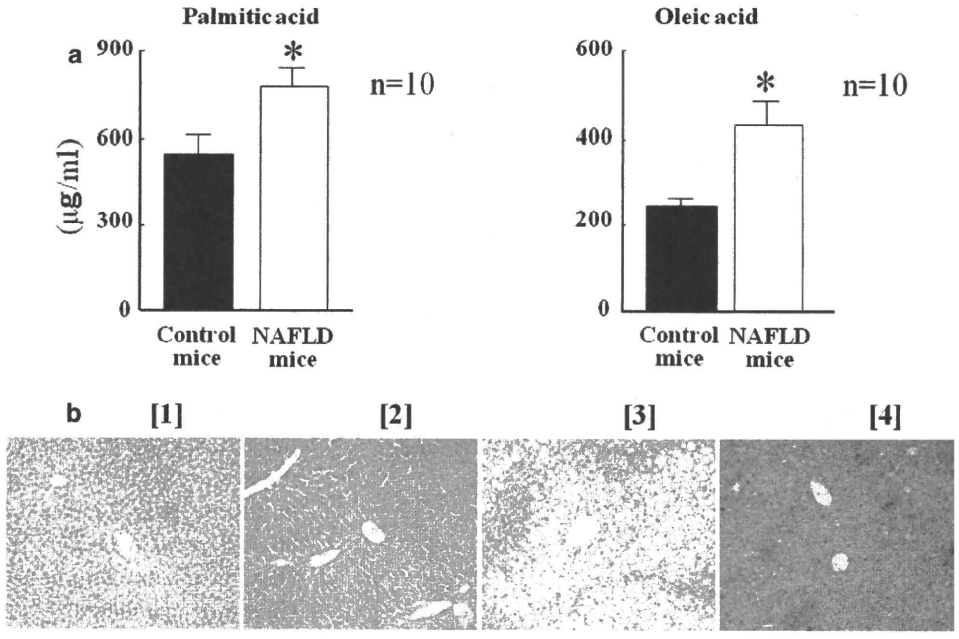

Fig. 2 **a** Increased levels of palmitic acid and oleic acid in the sera of nonalcoholic fatty liver disease (NAFLD) mice. Data are mean and SEM of different parameters of 10 mice in each group. $*P < 0.05$ compared with control mice of same duration. **b** Severe fatty liver of NAFLD mice. Figures in [1] and [2] represent liver specimens from control mice by hematoxylin eosin stain and Sudan III stain, respectively. Figures in [3] and [4] represent hematoxylin eosin stain and Sudan III stain of NAFLD mice, respectively

with different doses of HBsAg (1, 10, and 100 μg/ml) (Fig. 3a) and HBcAg (1, 10, and 100 μg/ml) (Fig. 3b). In contrast, lymphocytes from HBsAg and HBcAg-immunized NAFLD mice exhibited significantly ($P < 0.05$) lower levels of proliferation in cultures stimulated with 1, 10 and 100 μg/ml of HBsAg or 1, 10 and 100 μg/ml of HBcAg compared with control mice (Fig. 3a, b). In fact, lymphocytes from some HBsAg-immunized and HBcAg-immunized lymphocytes did not show significant proliferation due to stimulation by HBsAg or HBcAg.

Increased proliferative capacities of lymphocytes from NAFLD mice

Initially, we assumed that the impaired antigen-specific humoral and cellular immune responses of NAFLD mice might be responsible for the decreased proliferative capacity or cytokine production of lymphocytes of these mice. However, levels of proliferation of T lymphocytes to Con A (Fig. 4a) and B lymphocytes to LPS (Fig. 4b) from NAFLD mice were significantly higher than those from control mice ($P < 0.05$).

In addition, spleen T and B cells from NAFLD mice produced significantly higher levels of inflammatory cytokines (IL-6, TNF-α and IFN-γ) due to stimulation with different polyclonal immune stimulators (Con A, LPS, CPG -ODN and poly I:C) (Fig. 5) compared with those from control mice ($P < 0.05$).

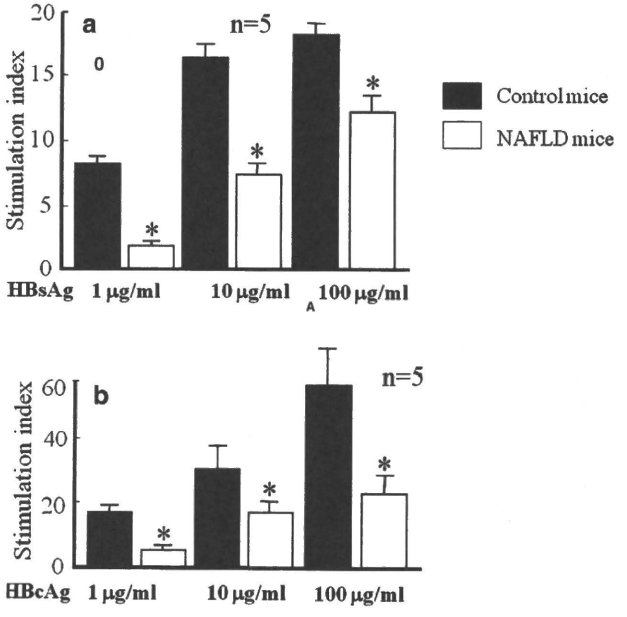

Fig. 3 Decreased antigen-specific immune responses in mice with nonalcoholic fatty liver disease (NAFLD). NAFLD mice ($n = 5$) and control mice ($n = 5$) were immunized with a hepatitis vaccine containing 4 μg of hepatitis B surface antigen (HBsAg) and 4 μg of hepatitis B core antigen (HBcAg). Four weeks after immunization, spleen lymphocytes (2×10^5) were cultured with different recombinant HBsAg and HBcAg for 5 days. Levels of blastogenesis were assessed from incorporation of ^{3}H-thymidine as count per minute (CPM). Data are presented as stimulation index, which was calculated by dividing the levels of CPM in cultures containing HBsAg (**a**) and HBcAg (**b**) with cultures containing no HBsAg or HBcAg. Mean ± SEM of five separate experiments are shown. $*P < 0.05$ compared with control mice

Impaired functional capacities of spleen DCs from NAFLD mice

As the functional capacities of lymphocytes were mostly higher in NAFLD mice, we evaluated the antigen processing and presentation capacities of DCs because they regulate both the induction and effecter phases of antigen-specific immune responses. Spleen DCs from NAFLD mice had significantly reduced capacities to stimulate HBsAg-specific memory lymphocytes (levels of blastogenesis; 3480 ± 67 CPM) compared with DCs from control mice (15823 ± 134 CPM, $n = 5$) ($P < 0.05$). This indicated that DCs of NAFLD mice were less able to process and present HBsAg in culture.

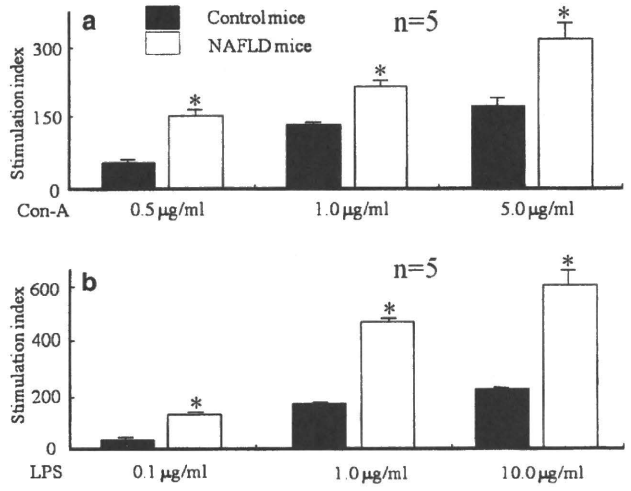

Fig. 4 Increased non-antigen-specific proliferation of T and B lymphocytes from nonalcoholic fatty liver disease (NAFLD) mice to polyclonal antigens. T and B lymphocytes from NAFLD mice and control mice were stimulated with concanavalin A (Con A) (**a**) and lipopolysaccharides (LPS) (**b**), respectively. Levels of proliferation of lymphocytes were assessed from the levels of count per minute in cultures and expressed as the stimulation index, as described in the legend of Fig. 3. Mean and SEM of five separate experiments are shown. $^*P < 0.05$ compared with control mice

Defective loading of DCs from NAFLD mice with soluble antigen

To further assess the function of DCs, we produced HBsAg-pulsed DCs and HBcAg-pulsed DCs. These DCs are supposed to activate antigen-specific immunocytes directly. In our study, HBsAg-pulsed DCs and HBcAg-pulsed DCs from NAFLD mice had a significantly lower capacity to stimulate HBsAg-specific (Fig. 6a) and HBcAg-specific lymphocytes (Fig. 6b) compared with those from control mice ($P < 0.05$).

Assessment of impaired DC function of NAFLD mice

The impaired DC function of NAFLD mice could have resulted from negative feedback of increased lymphocyte activity. In order to assess this phenomenon, we cultured DCs from NAFLD mice and control mice with T cells from these mice. The levels of blastogenesis of cultures containing DCs from NAFLD mice with T cells from NAFLD mice and control mice were 35.5 ± 3.2 and 37.4 ± 4.3, respectively

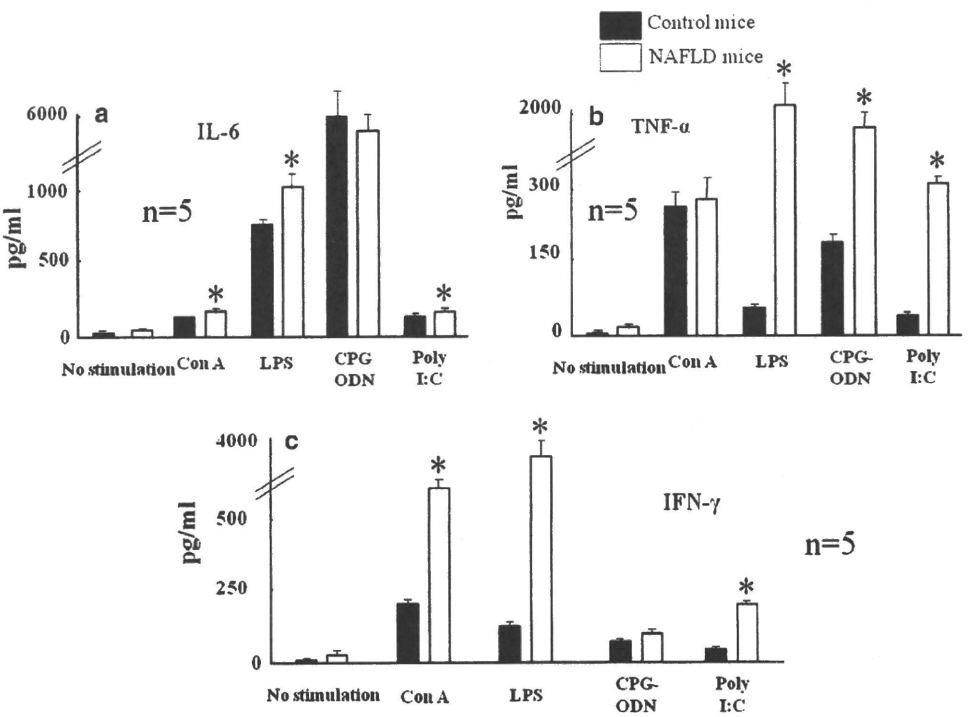

Fig. 5 Increased production of inflammatory cytokines from spleen cells of nonalcoholic fatty liver disease (NAFLD) mice due to stimulation with polyclonal mitogens. Spleen cells from NAFLD mice and control mice were stimulated with concanavalin A (Con A), lipopolysaccharides (LPS), cytosine-phosphate-guanosine oligodeoxynucleotide (CPG-ODN) and polyinosinic polycytidylic acid (poly I:C). Levels of IL-6 (**a**), TNF-α (**b**) and IFN-γ (**c**) were measured in the culture supernatants by the cytometric bead array method. Mean and SEM of five separate experiments are shown. $^*P < 0.05$ compared with control mice

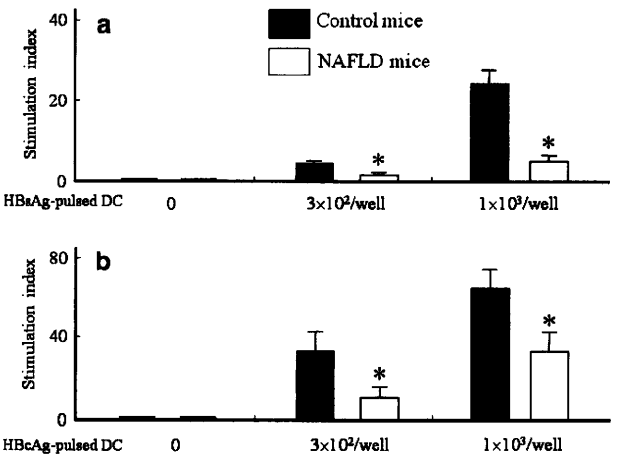

Fig. 6 Decreased capacity of hepatitis B surface antigen (HBsAg)-pulsed (**a**) and HBcAg-pulsed dendritic cells (DCs) (**b**) from nonalcoholic fatty liver disease (NAFLD) mice to activate HBsAg and HBcAg-specific memory lymphocytes in vitro. HBsAg-pulsed DCs and HBcAg-pulsed DCs were challenged to induce proliferation of antigen-specific lymphocytes in vitro. Levels of proliferation of HBsAg-specific and HBcAg-specific lymphocytes without DCs were regarded as a stimulation index of 1.0. Mean and SEM of five separate experiments are shown. *$P < 0.05$ compared with HBsAg-pulsed DC from control mice

(stimulation index of mean and SEM of 5 separate experiments) ($p > 0.05$). On the other hand, the levels of blastogenesis of cultures containing DCs from control mice with T cells from NAFLD mice and control mice were 41.4 ± 2.9 and 46.2 ± 2.2, respectively (stimulation index of mean and SEM of 5 separate experiments) ($p > 0.05$).

Effect of fatty acid on antigen-specific immunity

In spite of an inflammatory microenvironment, DCs from NAFLD mice could not induce proliferation of antigen-specific T cells in vitro. We assumed that some fatty acid may be related to impaired DC function. Levels of different fatty acids in the sera were significantly increased in the sera of NAFLD mice ($P < 0.05$). We decided to assess the effects of palmitic acid and oleic acid on DC function because studies have documented diverse effects of these fatty acids on immune responses [28]. HBsAg-pulsed DCs prepared in the presence of palmitic acid showed significantly lower levels (stimulation index; 1.4 ± 0.3, $n = 3$) of stimulatory capacity compared to HBsAg-pulsed DCs prepared in the presence of oleic acid (stimulation index; 15.8 ± 2.2, $n = 3$) ($p < 0.05$).

Increased cytokine production by human PBMC in presence of palmitic acid

Human PBMCs were cultured with palmitic acid and oleic acid, and levels of cytokines were assessed. Palmitic acid, but not oleic acid, induced significantly higher levels of IL-1β (palmitic acid vs. oleic acid, 1615 ± 300 pg/ml vs. 365 ± 141 pg/ml, $n = 5$) and TNF-α (palmitic acid vs. oleic acid, 828 ± 253 pg/ml vs. 260 ± 97 pg/ml, $n = 5$) from human PBMCs in vitro ($P < 0.05$).

Disruption of antigen processing and presenting capacities of human HBsAg-pulsed DCs cultured in presence of palmitic acid

Human HBsAg-pulsed DCs prepared in the presence of palmitic acid induced significantly lower levels of antigen-specific T cell proliferation in vitro compared with those prepared in the presence of oleic acid (stimulation index, 12.3 ± 1.2 vs. 1.9 ± 0.4, DCs cultured in presence of oleic acid vs. DCs cultured with palmitic acid, $P < 0.05$, $n = 5$).

Restoration of antigen-specific immunity of NAFLD mice after consuming control diet for 8 weeks

To assess if it was possible to reverse the impaired antigen-specific immunity of NAFLD mice by providing them with a control diet, we fed the control diet to NAFLD mice for 8 weeks and immunized them with HBsAg to assess antigen-specific immunity. Providing a normal diet for 8 weeks caused decreased body weight and normalization of different biochemical parameters of NAFLD mice. Interestingly, these mice also produced similar levels of anti-HBs compared with age- and sex-matched control mice (406 ± 93.4 mIU/ml vs. 441 ± 109 mIU/ml, $n = 5$, $P > 0.05$) due to immunization with a HB vaccine containing 2 μg of HBsAg.

Discussion

To develop insights about the nature and magnitudes of adaptive immune responses in NAFLD, we prepared a murine model of NAFLD because many immune-related cellular and molecular events cannot be properly examined in humans due to ethical and technical concerns. Providing a HFD that contained high levels of fat (60%) and a high calorie density (520 kcal/100 g), we realized a murine model of NAFLD after 12 weeks. Spleen cells of NAFLD mice in this study also produced significantly higher levels of proinflammatory cytokines (IL-6, IFN-γ, and TNF-α) compared with control mice that consumed normal laboratory chow. In addition, T and B lymphocytes from NAFLD mice exhibited increased proliferative capacities in response to polyclonal immune modulators compared with control mice.

In spite of an increased activated state of cells with innate immunity, NAFLD mice were almost incapable of

responding to the antigenic challenge for induction of antigen-specific humoral and cellular immune responses. In this study, we used HBsAg and HBcAg for induction of adaptive immunity. Subjects with NAFLD may also induce impaired levels of adaptive immunity in response to challenge with microbes and cancer cells. Presumptive data supporting this have been cited in the literature [29, 30].

In spite of harboring an activated population of lymphocytes and an inflammatory mucosal milieu, impaired antigen processing and presentation of DCs in NAFLD subjects hindered their antigen-specific immunity. This condition is comparable to chronic infections and cancers in which impaired functions of DCs compromise an effective antigen-specific immunity. The functional anomaly of DCs in NAFLD may be induced by various factors. One is related to diet. Palmitic acid, but not oleic acid, caused down-regulation of HBsAg processing and presentation of DCs. Some other fatty acids, especially a saturated fatty acid, may be responsible for impaired DC function in NAFLD. A second possibility of impaired DC function in NAFLD mice may be due to the non-antigen-specific maturation of DCs in these mice. In order to process and present antigen to T cells, DCs need to recognize, internalize, process and present antigens to clonally selected immunocytes. In an inflammatory microenvironment, DCs may undergo activation and maturation prior to recognition, internalization and processing of antigens. Although these DCs produce abundant amounts of cytokines, they are unable to induce activation of antigen-specific immunocytes because of their nature of maturation in a non-antigen-specific manner [17–19]. In fact, diminished antigen-specific immunity due to non-antigen-specific maturation of DCs has been reported by us in a model of Con-A-induced hepatitis [31]. Although we have shown that the saturated fatty acid, palmitic acid, can induce impaired function of DCs, NAFLD mice also had impaired glucose tolerance. This may also contribute to impaired immune responses [32]. In fact, the role of impaired glucose intolerance on adaptive immunity or on the functional capacity of DCs should be well addressed in the future.

Similar results to those in this study in NAFLD mice were also found in humans, as palmitic acid caused increased inflammatory cytokine production and impaired DC function in human DCs in this study. However, further study is needed to confirm these findings in patients with NAFLD.

Interestingly, the immune anomaly of the NAFLD condition was reversed in our model by providing a control diet for 8 weeks. This emphasizes the importance of diet therapy in NAFLD.

In this study, we used two well-characterized antigens of hepatitis B virus (HBV) to induce adaptive immunity in NAFLD mice. Obesity and NAFLD have reached worldwide epidemic proportions. In addition, HBV infection represents a major global public health problem, with 2 billion HBV infections and 350 million chronic HBV carriers. A protective vaccine against the HBV is now available and used extensively around the world. However, little has been explored about the protective capacity of this vaccine in subjects with obesity and NAFLD. Our study indicates the need to re-visit the ongoing protocol of a prophylactic vaccine against HBV. In addition, NAFLD mice also exhibited impaired DC function and adaptive immunity to HBcAg. Taken together, it appears that NAFLD may be related to impaired adaptive immunity to various antigens, mainly if not solely because of defective antigen processing and presentation capacities of DCs.

This is one of the first approaches to dissect the mechanisms underlying immune anomaly in NAFLD. Additional insights into the cellular and molecular mechanisms underlying antigen-specific immunity in NAFLD will help with the development of strategies to induce proper antigen-specific immunity in NAFLD subjects to tackle their susceptibility to infection and low responses to vaccination.

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LETTER TO THE EDITOR

COMBINATION THERAPY OF LAMIVUDINE AND INTERFERON-ALPHA IN PEDIATRIC PATIENTS WITH CHRONIC HEPATITIS B IN BANGLADESH: A SAFE AND EFFECTIVE THERAPEUTIC APPROACH FOR PEDIATRIC CHB PATIENTS IN DEVELOPING COUNTRIES

M. AL-MAHTAB[1,4], S. RAHMAN[1,4], S.M. AKBAR[2], S.I. KHAN[3], H. UDDIN[4], M.F. KARIM[5] and F. AHMED[5]

[1]Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh; [2]Department of Medical Sciences, Toshiba General Hospital, Tokyo, Japan; [3]Department of Animal Science, Bangladesh Agricultural University, Mymensingh, Bangladesh; [4]Viral Hepatitis Foundation, Dhaka, Bangladesh, [5]Department of Hepatology, Dhaka Medical College, Bangladesh

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Hepatitis B virus (HBV) is mainly transmitted during birth or perinatal period, however, treatment is not usually recommended for pediatric patients with chronic hepatitis B (CHB). Twelve pediatric patients with CHB in Bangladesh were treated with both lamivudine and interferon. Lamivudine was given at a dose of 3 mg/kg, daily for 12 months. Two months after commencement of lamivudine therapy, all patents were given interferon-α (3 million IU/square meter of body surface area) three times weekly, subcutaneously for 10 months. Combination therapy was safe for all pediatric CHB patients. The levels of serum HBV DNA became undetectable (<500 copies/ml) in 8 patients and reduced in 4 patients after the end of therapy. Anti-HBe was detected in 10 of 12 patients at this time point. The levels of serum alanine aminotransferase (ALT) were significantly reduced in these patients ($p<0.05$) due to therapy. Neither flare of HBV DNA nor elevation of serum ALT were detected during follow-up. In conclusion, combination therapy with lamivudine and interferon-α represents a new and novel therapeutic option for treatment of pediatric CHB patients.

Epidemiological studies indicate that encounter with the hepatitis B virus (HBV) during neonatal and perinatal period induces chronic infection in about 90% subjects (1). This is especially relevant in the developing countries of the world that harbor more than 90% of the total bulk of approximately 350 million global chronic HBV carriers. In most developing countries, HBV infection is mainly transmitted to the offspring from the mother during birth because of unsafe delivery, lack of implementation of universal immunization program against the HBV, and inadequate HBV screening of pregnant women (2). Pediatric patients with chronic HBV infection harbor HBV for the rest of their life with or without clinical symptoms of liver damage, but all of them remain at risk of developing HBV-related complications during their lifetime. Accordingly, these patients should be treated so that HBV-related complications can be minimized.

There are some controversies about therapeutic

Key words: antiviral drug, interferon, combination therapy, chronic hepatitis B, pediatric patients

Mailing address: Dr. Sheikh Mohammad Fazle Akbar, MD, Ph. D. Principal Investigator, Department of Medical Sciences, Toshiba General Hospital, Higashi Oi 6-3-22, Shinagawa, Tokyo 140-8522, Japan
Tel: ++81 3 3764 0511 Fax: ++81 3764 8992
e-mail: sheikh.akbar@po.toshiba.co.jp

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efficacies of different antiviral drugs in pediatric patients with chronic hepatitis B (CHB). A study from Taiwan concluded that real advantage of interferon (IFN) therapy was not observed in pediatric and young CHB patients because the response rate was almost the same between the treated and the control group (3). On the other hand, Kuloglu et al. showed that IFN treatment caused sustained control of HBV replication and histological improvement of liver biopsy specimens in about 40% of Turkish children with CHB (4). Similar data have been reported on the therapeutic efficacy of nucleoside analogs in pediatric patients (5-8). The diverse outcome of antiviral agents in different clinical trials may reflect the differences in therapeutic protocol, characteristics of the patients, genotypes of the HBV, and method of therapeutic assessment (3-8).

Bangladesh is a South-Asian country with a population of more than 140 million. HBV infection is endemic in Bangladesh and mother-to-child transmission common. The health care delivery system is not properly organized in Bangladesh. There are no treatment guidelines for treating HBV infection and other infectious diseases in Bangladesh and most other developing countries. In fact, few clinical trials or even pilot studies have been accomplished in these countries to develop treatment recommendations that may be applicable in the context of their socio-economic conditions. Accordingly, treatment guidelines organized by the professional organizations of developed and rich countries are usually followed by these countries. Although various antiviral drugs are now available for treatment of CHB patients, most of these drugs have limited efficacy and profound side effects. In addition, patients receiving these drugs should be properly followed for prolonged duration (7). Due to these facts, antiviral drugs are not used in pediatric patients with CHB in Bangladesh.

On the basis of various realities, we assumed that a combination of antiviral and immune modulator drugs may provide a better therapeutic outcome because patients with CHB require both control of the HBV and also restoration of host immunity (9).

In this study, we treated 12 pediatric patients with CHB with lamivudine and interferon for 12 months and followed them for further 6 months. The findings of the pilot study in the context of therapy of pediatric CHB patients in Bangladesh and other developing countries will be discussed.

MATERIALS AND METHODS

Patients' profiles

Twelve pediatric patients with CHB, aged 4-14 years, were enrolled in this study. The profiles of the patients are summarized in Table I. Chronic HBV infection in these cases was ascertained by the presence of hepatitis B surface antigen (HBsAg) in the sera in two consecutive samples taken 6 months apart. Five of these patients had one or more chronic HBV carriers in their family. All patients were checked for parameters of routine blood tests, alanine aminotransferase (ALT), hepatitis B e antigen (HBeAg), and antibody to HBeAg (anti-HBe) in the sera. Liver biopsy could be performed in 3 of 12 patients. The patients were free from hepatitis C virus infection. HBV DNA was assessed in all cases one week before treatment commencement. All patients were positive for hepatitis B e antigen (HBeAg) in the sera. The levels of ALT was variable in these patients (34-123 IU/L; upper limit of normal value of ALT; 42 IU/L). Six of these patients had elevated serum ALT (above upper limit of normal), whereas, the levels of ALT were within normal range in the other 6 patients.

Clinical investigation and laboratory tests

Blood samples were collected from each patient and sera was preserved at -20°C before estimation. Complete blood count along with assessment of parameters of liver and kidney functions were carried out for all patients at enrollment and once every month thereafter. In addition, all patients were asked to contact the controller of the clinical trial (MA) if they noticed any anomaly in their physical condition. Serum ALT levels and prothrombin time were assessed commercially. Cut off value for abnormal ALT was 42 IU/L. HBeAg in the sera was checked commercially by ELISA using a commercial kit (Abbott Labs, Chicago, IL, USA). Anti-HBe was estimated by passive hemagglutinin method (Tokyo Institute of Immunology, Tokyo, Japan). HBV DNA was quantified by polymerase chain reaction method using a commercial kit (Amplicon HBV Monitor Assay, RT-PCR, Roche Molecular Systems, CA, USA) which allowed determining as low as 500 copies/ml of HBV DNA. The study was carried out at the Lab-Aid specialized Hospital, Dhaka, Bangladesh, and was approved by the hospital. All patients or their guardians gave informed consent after explaining the nature and purposes of the pilot study.

Therapeutic regimen

These patients did not receive any antiviral or immune

modulator drugs 6 months before therapy commencement. All patients were given lamivudine at a dose of 3 mg/kg, daily for 12 months. Two months after starting oral intake of lamivudine, all patients were given IFN (Roche, Switzerland) at a dose of 3 million IU/square meter of body surface area, three times a week, subcutaneously. The treatment was continued for 12 months (all patients received lamivudine for 12 months and Lamivudine and IFN for 10 months). The patients were followed-up for an additional 6 months after termination of the study (Fig. 1).

Assessment of safety of combination therapy

Total blood counts and parameters of liver and kidney functions were measured on a monthly basis for 18 months to assess the safety of combination therapy. Also, physical conditions of the patients were examined during their visit to the hospital. We maintained active telephone contact concerning their physical conditions.

Assessment of efficacy of combination therapy

To assess therapeutic efficacy of combination therapy, ALT, HBV DNA, HBeAg, and anti-HBe in the sera were checked in all patients before, and 6, 12, and18 months after therapy commencement.

RESULTS

Satisfactory compliance of therapy in all patients

All patients took both drugs according to the protocol for the entire duration of 12 months. This is an important aspect of this study because drug compliance rate is poor in most developing countries. However, all patients attended hospital during follow up.

Safety of combination therapy in pediatric CHB patients

After receiving IFN some patients reported malaise and a slight feverish condition, which were controlled without any specific therapy. Abnormality in liver and kidney functions or features of exacerbated liver damages were not detected in any patient. The levels of ALT were not elevated 1.5 times the pre-treatment levels in any patient. Also, the levels of blood creatinine were within normal range in all patients. Again, flare up of HBV DNA was not recorded after cessation of therapy in any patient. There were no drop outs in this pilot study due to safety concern.

Reduced HBV DNA due to combination therapy

Most patients had very high levels of HBV DNA in the sera prior to the start of therapy (Table I). Significant reduction of HBV DNA was seen in all cases 6 months after therapy commencement. Further reduction of HBV DNA was seen at 12 months after start of therapy. HBV DNA became undetectable (<500 copies/ml) in 8 of 12 patients after the end of therapy (Fig. 1). Also, HBV DNA reduced in 4 patients with CHB.

Effect of combination therapy on HBeAg seroconversion

Combination therapy also induced HBeAg sero-

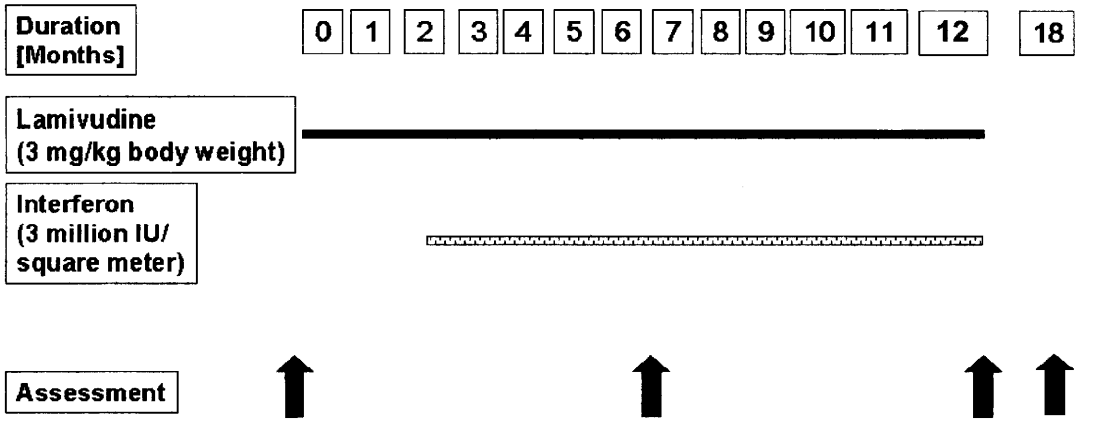

Fig. 1. *Design of combination therapy in pediatric patients with chronic hepatitis B. Lamivudine was administered to all patients for 12 months. Interferon was given two months after start of lamivudine administration. All patients received therapy for 12 months. The patients were followed-up for 18 months.*

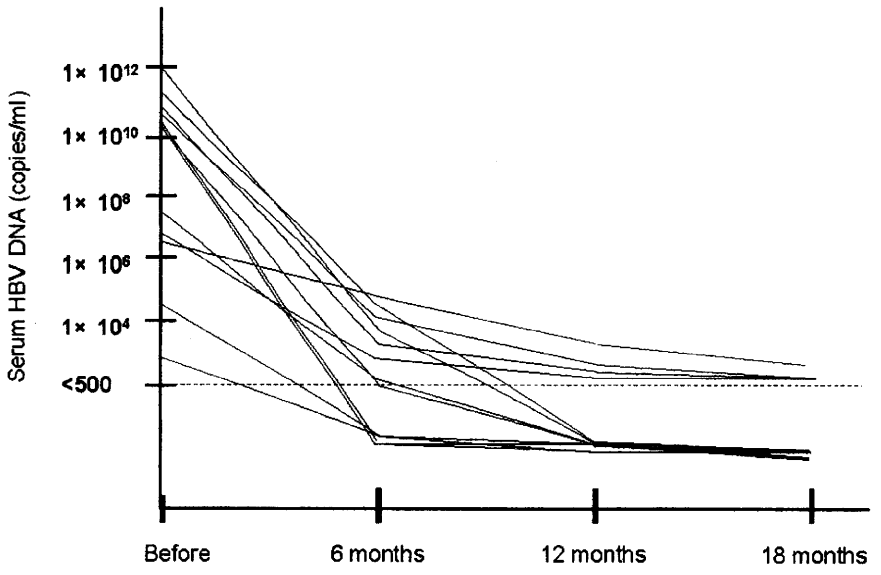

Fig. 2. *Down regulation of HBV DNA due to combination therapy in pediatric patients with chronic hepatitis B. HBV DNA was measured in all patients before start of therapy (0 month), 6, 12 and 18 months after therapy commencement.*

conversion in a considerable number of subjects. Although all patients were HBeAg positive before commencement of therapy, 3 of them became negative for HBeAg and developed anti-HBe 6 months after the initiation of therapy. At the end of 12 months, 10 of 12 patients developed anti-HBe in the sera

Impact of combination therapy on serum ALT levels

All six patients with elevated levels of ALT exhibited normal ALT 12 months after therapy commencement. The mean value of ALT before therapy was 51±7 IU/L. This was significantly reduced to 30±2 IU/L after therapy (N=12, $p<0.01$). Also, the levels of ALT in patients with normal ALT was reduced after therapy compared to their levels before therapy commencement. The levels of ALT in 6 patients with ALT below the upper limit of normal were 36±3 IU/L (N=6). The levels of serum ALT were reduced to 25±4 IU/L ($p<0.05$) after therapy.

No flare of HBV DNA and ALT during follow-up period

Flare up of HBV DNA or elevation of ALT (1.5 times the upper limit of normal value) could not be seen in any patient with pediatric CHB during the follow-up period. When the patients were followed-up at 18 months (6 months after therapy completion), HBV DNA remained undetected in 8 patients 12 months after therapy commencement. Also, the levels of HBV DNA were not increased in the remaining 4 patients that did not become HBV DNA negative due to therapy (Fig. 2). The levels of ALT above the upper limit of normal or above pre-treatment levels were not detected in any patient 18 months after the start of therapy.

DISCUSSION

Combination therapy is not a new concept for treating patients with chronic HBV infection because the HBV is a complex virus and persistent presence of this virus in CHB patients induces altered and distorted immunity (10-11). Accordingly, therapy of CHB patients is carried out to achieve sustained control of the HBV and restoration of host immunity (12). As one antiviral agent seems to be incapable of inducing sustained control of the virus, proper restoration of host immunity by combination of two antiviral drugs or combination of antiviral plus immune modulators have recently been used in adult CHB patients (13-15).

However, there are few clinical trials about combination therapy in pediatric patients with CHB.

Table I. *Clinical profiles of the pediatric chronic hepatitis B patients.*

Age	9±0.92 years*
Range	4-14 years
Sex (male:female)	7:5
Base Line HBV DNA (copies/ml)	
1×10^4-1×10^8	5 patients
1×10^9-1×10^{12}	7 patients
HBeAg-positive	12 patients
Alanine aminotransferase (IU/L)	51±7.6 IU/L
<42 IU/L	6 patients
>42IU/L	6 patients

** Data are shown as mean and standard error of mean*

In fact, therapy of pediatric patients is a matter of controversy in clinics because it has been assumed that these patients are immune tolerant, and antiviral drugs are either completely ineffective or less effective in pediatric CHB patients (3).

The combination therapy that we carried out with lamivudine and IFN was safe for all pediatric patients with CHB during treatment state and also during the follow-up period. The therapeutic efficacy of the combination therapy was inspiring and we detected both viral reduction and HBeAg sero-conversion in the majority of patients. It seems that the clinical efficacy of our combination therapy was better than that reported by others, which may reflect differences of protocol and study populations (16-17).

A notable part of the study lies in the fact that this was conducted in Bangladesh. The treatment persisted for 12 months and patients were followed for 6 months, and there were no drop-outs. The patients were provided with information about the nature and purpose of the study and we also explained the scope and limitations of the trials in details. We are optimistic that it will be possible to perform large scale randomized controlled trials with combination therapy in CHB patients in Bangladesh in the future.

There are some limitations to this study. The number of patients is limited. There is no comparable control group in this study. In fact, it is a pilot study to generate some insights for conducting a case control study in the future.

Pediatric patients with CHB are a permanent and living reservoir of HBV and considerable numbers of them could develop HBV-related complications in the future. They need therapy, but an infinite therapy with nucleoside analogs would not be a proper therapeutic regime for these patients. The duration of therapy should be finite, and Hartman et al. have reported that compliance of lamivudine therapy is extremely difficult for pediatric patients (18). Considering these facts, combination of lamivudine and IFN may be a practical therapeutic approach for pediatric CHB patients. However, further study is needed to optimize a therapeutic protocol for these patients.

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Review Article

Immune-mediated damages of the hepatocytes: mechanisms of destruction of hepatocytes and progression of liver diseases by non-cytopathic viruses

*Sheikh Mohammad Fazle Akbar[1], Mamun Al-Mahtab[2]
[1]Principal Investigator, Department of Health Sciences, Toshiba General Hospital, Tokyo, Japan, [2]Assistant Professor, Department of Hepatology, Banglabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

*Correspondence to
Department of Health Sciences, Toshiba General Hospital, Higashi Oi 6-3-22, Shinagawa-ku, Tokyo 140-8522, Japan,
Email <sheikh.akbar@po.toshiba.co.jp>, Tel: 81-3-3764-0511, Fax: 81-3-3764-8992

Abstract

The liver is a vital organ of the body that performs several critical functions on a regular basis for maintenance of homeostasis. It is also susceptible to be attacked by various noxious agents due to exceptional blood supply from the gut. Although several viruses are hepatotrophic nature, and localize and replicate in hepatocytes, most of them possess no cytopathic property or produce toxins. However, infection with hepatitis virus causes different types of liver diseases in human. In this study, a sketch will be provided about mechanism of liver damage induced by non cytopathic viruses in human.

Multivariable functional and regulatory potentials of the liver

The liver is one of the largest organs of human body that perform several vital functions. Its role is critical for survival and maintenance of homeostasis. It is unique because it receives blood supply from both portal and systemic circulation [1]. Thus, various products from the gut that include food ingredients, allergens, different metabolites, and various noxious materials enter the liver persistently on a routine basis. The hepatocytes, the building block of the liver, are capable of synthesizing various products such as enzymes and hormones. It also causes detoxification of various drugs and noxious materials. It is also an excretory organ. In addition, the liver also plays a critical role in shaping immunity [2]. It is regarded as a graveyard for lymphocytes, harbors increased proportions of cells of innate immunity (natural killer cells), and also contain regulatory immunocytes that control exacerbated immune responses. It is also well known for harboring various specialized cells that include Kupffer cells and other specialized cells. Liver sinusoids allow rolling of different cells and immunocytes and thus an intimate interaction between liver cells and invading agents become possible. It has an extreme potential to undergo regeneration. However, death of hepatocytes is followed by fibrogenesis [3].

Nature of liver diseases

As mentioned, liver is constantly exposed to gut-derived blood that contain a wide variety of noxious products, such as food products, allergens, alcohol and its metabolites, drugs and its metabolites, and abundant amounts of microbial agents. One of the primary functions of the liver in physiological conditions is to minimize the effects of these materials. These are primarily attained by metabolic activities, detoxification properties, secretory capabilities, and scavenging apparatuses of the liver. As a general rule, the liver effectively performs a combination of these activities and maintains homeostasis [4].

Although several noxious agents enter the liver and detoxified by the inherent capacities of hepatocytes, a group of microbial agents are capable of invading the liver to establish an infection. They replicate in the hepatocytes and establish a transient, semi-transient or persistent infection in the liver. Some of these microbial agents are also capable of integration in the host genome of the hepatocytes.

Hepatotrophic viruses of clinical importance

Many viruses may enter, localize and replicate in the liver, however, some viruses are either exclusively hepatotrophic or dominant part of their life cycle are accomplished in the liver. Here, we would discuss about hepatitis viruses that causes liver diseases or liver-related pathologies in human. The mechanisms underlying their liver damage-inducing capacities would be mainly discussed. In this context, we

will mainly discuss about hepatitis viruses, such as hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis E virus (HEV). Among these, HAV and HEV enter the body mainly by oral route, whereas, HBV and HCV infect the human through contaminate blood or body fluid or enter human body by penetration of body surfaces. Although the route of entry of these viruses is different, all of them localize and replicate in the liver. In addition, HBV also integrate into host genome.

Nature of pathogenesis of these viruses

After entry into the hosts, these viruses are localized in the liver. This seems to be a rapid process. The mechanisms underlying entry of these viruses into the liver is not well understood, however, it appears that the viruses use specific cell surface receptors for their entry into hepatocytes. Subsequently, the viruses establish infection in the liver and start to replicate. In some cases, it is also integrated into host genome.

Although HAV, HBV, HCV, and HEV are hepatotrophic in nature, none of them can induce damage or destruction of human hepatocytes directly. They do not have toxins or enzymes capable of destroying hepatocytes. In fact, these are noncytopathic virus (reviewed at reference 5-7). Due to these factors, infections with most of these viruses may remain unnoticed for prolonged period of time or even for whole life. Evidences of HAV and HEV infections are detected in about 70-90% of people of developing countries, where pure water supply and hygienic food supply is yet to be materialized. However, in most cases there is no subjective symptom suggestive of liver damages or laboratory evidences of hepatitis. Even if these subjects have minimum evidences of hepatitis that usually remain undetected because none attend physicians due to infection. This is specially true and relevant in the developing countries of the world. In some cases, features of liver diseases, such as hepatitis, malaise, nausea, loss of appetite and general weakness are detected in some patients. These patients also show abnormal liver functions with elevated levels of alanine aminotransferase (ALT) and bilirubin in the sera. Other abnormalities of liver functions depend on the extent of liver diseases. These patients may suffer from acute hepatitis, and some of them may develop severe hepatitis or even life-threatening fulminant hepatitis. However, the critical factor is that evidences of liver damages due to HAV or HEV usually develop 4-12 weeks after entry of viruses. Most importantly, liver damages are usually detected after the peak of viral replications indicating that these viruses are not direct inducers of liver damages.

This is also same or similar in the context of HBV and HCV infection. Although most of the HBV-infected subjects in the developing countries are infected with the virus either during or around birth or preschool period, evidences of damages of hepatocytes may become noticeable in only 10% HBV-infected subjects either at their thirties or forties or even later. However, high levels of HBV replication are detected in these subjects during their early ages. Similarly, liver damages in HCV-infected subjects are not related to the degree of viral replication and considerable time is required between liver damages after entry of HCV.

Mechanism of liver damages and viral control during infection with hepatitis viruses

It is presumed that the immune systems of the hosts play a cardinal role in initiating and maintaining liver damages in patients with hepatitis virus infection, however, a complete understanding about underlying molecular and cellular events has not been fully elucidated. On the other hand, host immune systems are mainly responsible for containment of these viruses. Accordingly, more insights are needed about the nature of immune responses in HAV and HEV infection that induces a self-limiting hepatitis in considerable numbers of subjects with development of protective immunocytes that can block infection with same virus in future. On the other hand, in case of HBV infections, host immune responses usually effective control virus-mediated liver damages for about 30 or 40 years or whole life in some individuals, whereas, progressive liver damages are seen in other subjects. Control of HBV replication is seen in some, whereas, that can not be achieved in others, although apparently all these subjects are immune competent. During HCV infection, diverse role of host immunity are also visible. We would discuss heterogeneous nature of host immune responses in different hepatitis virus infection to develop insights about mechanisms of liver damages and viral control.

Host immunity: HAV and HEV infections

Host immunity may be divided into two main categories; (1) innate immunity and (2) adaptive immunity. The cells of innate immunity promptly recognize microbial agents, undergo activation and produce a variety of immune modulators. The cells of innate immunity can also engulf or destroy the microbial agents or infected cells by their cytotoxic properties. Circumstantial evidences indicate that HAV and HEV may induce host immunity, however, cytopathic types of innate immune activation that can kill hepatocytes are not induced at early stages of HAV or HEV

infections. This is basically supported by the fact that visible evidences of damages and destructions of hepatocytes are not seen within days or weeks of entry of HAV or HEV. Also, elevation of ALT has not detected within weeks or months after entry of HAV or HEV.

In the next phase, some HAV or HEV-infected patients develop features of liver damages that become evident by increased levels of ALT and appearance of subjective symptoms of liver diseases. In some cases, the patients may develop severe acute hepatitis or fuliminant hepatitis. In others, these patients are cured after an acute episode of hepatitis. On the contrary, some patients never develop any features of apparent hepatitis and develop protective antibody or harbor markers of previous HAV or HEV infections. Studies have not addressed about cellular and molecular events of these diverse clinical and immunological features in HAV and HEV infections. From the accumulating data, it appears that adaptive immune responses that act via viral-specific cytotoxic T cells (CTL) and CD4+ helper T cells play regulatory role in this respect. Also, a population of T cells or regulatory natural killer (NK) cells that regulate or suppress exacerbated immunity may have a dominant role in this respect. The nature of adaptive immunity may also be different in different pathological conditions. Patients with acute episode of HAV and HEV and developing protective immunity harbor CTLs that destroy liver cells as well as induce protective immunity. Patients that develop protective immunity possibly harbor few liver damaging CTLs or other immunocytes. In case of fulminant hepatitis, regulatory T cells seem to fail to block progression of hepatitis effectively due to several factors that may include oxidative stress, platelet factors and intracellular matrix.

Host immunity: HBV and HCV infection

Host immunity during HBV and HCV infection is not only complex, but, poorly understood. HBV-infected patients with no feature of liver diseases seem to develop a mutually acceptable interactions with host in which the HBV is regarded as non danger or self entity. Neither the host is capable of discarding the HBV completely, nor the host immunity cause liver damages. A type of co-existence between HBV and host immunity prevails in these subjects. Asymptomatic HBV carriers fall in this category. These patients are usually infected with HBV during neonatal or pre-school period. In course of time, the host regains immunity to HBV and induces liver damages and develops HBeAg seroconversion. This is regulated by HBV-specific CTL and CD4+ T cells. In fact, balances between different types of immunocytes dictate the nature of HBV-related pathologies. When the activity of CTLs recall non HBV-specific immunocytes to the liver, a second cascade of liver cells injury and possibly exacerbation and remission of hepatitis, a typical feature of progressive liver disease that cause complication starts. In addition to T cells, various other immunocytes, especially macrophages and dendritic cells may play regulating roles in this respect.

In case of HCV infection, the nature of host immunity is comparable to that of CHB patients. Most of the HCV-infected subjects exhibit liver damages. It is still obscure why fibrosis is more pronounced in HCV infection. A role of iron and fatty acid has been predicted to control HCV-induced liver damages, but, these vents are yet to clarified in clinics.

Host immunity in case of hepatocellular carcinoma (HCC)

Genesis of HCC in HBV and HCV-infected subjects is usually a time consuming process. Although the process of carcinogenesis starts at an early stage of HBV and HCV infection, clinically apparent HCC nodules are seen long time after infection with these viruses. A critical balance between oncogenic and antioncogenic potentiality of host-derived factors regulates these events. In addition, HCC nodules at their primary state avoid recognition by antigen-presenting cells by modifying their receptors. Antigen-presenting cells may also have defective functions in this regard [8].

Host immunity in autoimmune hepatitis and primary biliary cirrhosis

Although two main liver diseases are regarded as autoimmune liver diseases, the antigen responsible for their autoimmunity could not be found out till now. Some antigens have been claimed to be related with autoimmune hepatitis (AIH), however, there is no direct evidences of their role during induction of liver damages.

Similarly, AMA is regarded as a marker of PBC, but, there is no role of AMA in inducing damages hepatocytes in PBC. AMA is merely a marker that helps diagnosis of PBC, but, is not related to pathogenesis of this disease. Thus, two major autoimmune diseases of the liver are regarded autoimmune liver diseases without knowing the nature and characteristics of responsible autoantigens.