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Suppression of humoral immune response to hepatitis B surface antigen vaccine in BALB/c mice by 1-methyl-tryptophan co-administration

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ABSTRACT

Background and the purpose of the study: Indoleamine 2,3-dioxygenase (IDO) suppresses adaptive immune response. The purpose of this study was to determine the effect of the IDO inhibitor namely 1-methyl-DL-tryptophan (DL-1-MT) on antibody production after vaccination with hepatitis B surface (HBs) antigen.

Methods: Four groups of BALB/c mice were immunized with a HBs antigen vaccine. In the first group the vaccine had no DL-1-MT, whereas in the other three groups the vaccine contained 1 mg , 10 mg and 20 mg DL-1-MT. Blood samples were collected 5 weeks post-vaccination and anti-HBs antibodies in the serum were measured by ELISA.

Results: Compared to the three groups of mice that were immunized with the vaccines containing DL-1-MT, serum anti-HBs level was much higher in the mice that were immunized with the vaccine with out DL-1-MT.

Conclusions: Inhibition of IDO at the time of vaccination decreased humoral immune response to HBs antigen vaccine. The idea that IDO activity is simply immunosuppressive may need to be re-evaluated.

Keywords: DL-1-MT, IDO, Serum anti-HBs.

INTRODUCTION

Indoleamine 2,3-dioxygenase (IDO) is a 45 kDa enzyme that catalyses the initial rate-limiting step of tryptophan degradation along the kynurenine pathway. IDO is inducible by various inflammatory stimuli, mainly interferon-γ, and also IFNs type I, tumor necrosis factor-α, and lipopolysaccharide. This enzyme is widely distributed in various cell types including the antigen presenting cells (APCs) monocytes, macrophages and dendritic cells. Its expression in APCs is accompanied by impaired adaptive immune response because tryptophan depletion and kynurenine pathway products in local microenviroment decrease T-cell proliferation, increase T-cell apoptosis and induce the emergence of regulatory T-cells (Tregs) from naïve T-cells (1, 2). IDO mediated immunosuppression reduces graft rejection (3), and ameliorates the clinical course of experimental autoimmune diseases (4). We have recently shown that in hemodialysis patients, characterized by impaired adaptive immunity, IDO expression is increased and is further increased in the non-responders to hepatitis B virus vaccination (5).

Inhibition of T-cell function via IDO is also mediated by non-APC cell types. Expression of IDO in paternally derived placental trophoblast contributes to success of semi-allogenic pregnancy (6), while IDO expressed by tumor cells contributes to escape of tumors by immunosurveillance (7).

In the light of the above data, IDO inhibition seems to be attractive in cases where enhancement of adaptive immunity is beneficial. Clearly, such a case is the immune response to vaccines against various infectious agents. In the present study such an approach was tested experimentally by immunizing BALB/c mice with hepatitis B surface (HBs) antigen and 1-methyl-DL-tryptophan (DL-1-MT) was co-administered as an adjuvant. DL-1-MT is a competitive, non-toxic IDO inhibitor (8) that has been successfully used in vivo to break the immune privilege of placenta and tolerance against grafts, autoantigens, and tumors (3, 6, 9, 10).

MATERIAL AND METHODS

Animals

Twelve-week old female BALB-c mice bred and maintained in the animal facilities of the Research Institute at the Theagenion Anticancer Hospital of Thessaloniki. All studies were performed in

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accordance with the procedures issued by the Institutional Animal Care and Use Committee.

Immunization

Initially, suspensions of DL-1-MT (Sigma-Aldrich, St. Louis, MO, USA) in incomplete Freund's adjuvant (Sigma-Aldrich) and solutions of HBs antigen protein (Adw) (Abcam, Cambridge, UK) in phosphate buffer saline (Gibco BRL, Grand Island, NY, USA) were prepared. Then equal volumes of suspensions and solutions were mixed vigorously for making the final water-in-oil emulsions, which were injected in mice intraperitoneally at a volume of 200 µl.

Four groups of animals, 10 per each group, were immunized. In all groups the injected emulsions contained 2 µg of HBs antigen protein (Adw). In the first control group the emulsion had no DL-1-MT, whereas in the other 3 groups the amount of DL-1-MT was 1 mg, 10 mg and 20 mg respectively.

Determination of antibodies against hepatitis B surface antigen

Five weeks after immunization blood samples were collected from the heart of anaesthetized animals and serum was stored at -20°C.

Antibodies against HBs antigen (anti-HBs) in the serum were measured by means of ELISA (Mouse Anti-HBsAg IgG ELISA Assay, Express Biotech International, Thurmont, MD, USA) according to the manufacturer instructions with the exception of serum dilution, which had to be higher.

Statistical analyses

Comparison of means among the four groups of animals was assessed by one-way analysis of variance (ANOVA) followed by Bonferroni's test. Results were expressed as mean±SD and $p<0.05$ considered statistically significant and 95% confidence intervals of difference were also calculated.

RESULTS

ANOVA followed by Bonferroni's test revealed that three groups of mice that were immunized with vaccines containing DL-1-MT, had serum anti-HBs level much higher than those that were immunized with the vaccine without DL-1-MT. Interestingly, no statistically significant differences were detected in the serum anti-HBs levels among the three groups of mice that were immunized with the vaccines containing DL-1-MT at amounts of 1 mg, 10 mg and 20 mg respectively.

More precisely, serum anti-HBs level in the group of mice that did not receive DL-1-MT was 40445±32866 U/ml and in the groups that received 1 mg, 10 mg and 20 mg of DL-1-MT were 2740±1999 U/ml, 3151±3131 U/ml and 7089±2488 U/ml. Results are presented graphically in figure 1 and the p values and the 95% confidence intervals of difference among the groups of mice are provided in table 1.

DISCUSSION

While, effective vaccines for two of the world's leading killers, HIV and malaria, remain in the research stage, effective vaccines against tuberculosis, hepatitis C and other common infectious agents are still expected and there are many conditions in which the efficacy of the already available vaccines is diminished. Thus the effort for enhancement of the immunogenicity of vaccines continues.

In the present study it was investigated whether inhibition of IDO enhance the immunogenicity of a vaccine containing a HBs protein antigen. IDO activity is considered to be immunosuppressive, reduces graft rejection (3), ameliorates the clinical course of experimental autoimmune diseases (4), contributes to success of semi-allogenic pregnancy (6) and escape of tumors by immunosurveillance (7). Although many studies have confirmed that IDO inhibits cell-mediated adaptive immune response (1, 2), less is known about the effect of IDO on humoral adaptive immunity.

Surprisingly, in the present study inhibition of IDO by DL-1-MT did not increase serum anti-HBs levels. On the contrary, compared to the three groups of mice that were immunized with the vaccines containing DL-1-MT, serum anti-HBs level was much higher in the mice that were immunized with the vaccine without DL-1-MT. Interestingly, the effect of DL-1-MT was strong regardless of the concentration, since no statistically significant differences were detected in serum anti-HBs levels among the three groups of mice that received 1 mg, 10 mg or 20 mg DL-1-MT in the vaccines. This could be due to the strong inhibitory effect of DL-1-MT on IDO where a small dose strongly inhibits IDO activity.

Thus in the simple experimental model used in the present study, inhibition of IDO at the time of vaccination decreased humoral adaptive immune response. Although the effect of IDO on cell-mediated adaptive immunity has been studied to a great extent, its' effect on humoral immunity is much less studied. In a model of nephrotoxic serum glomerulonephritis, although DL-1-MT exacerbated the disease by enhancing Th1 response, it significantly decreased antigen specific IgG1 levels (11). The effect of IDO inhibition on humoral immunity has been evaluated in more detail in an elegant study that used the K/BxN murine rheumatoid arthritis model and it has been shown that IDO drives B-cell-mediated autoimmunity (12). In the reported study IDO inhibition by administration of DL-1-MT did not exacerbate arthritis symptoms, but ameliorated symptoms. Alleviation of arthritis was not due an altered T-cell response, since Th1, Th2, Th17 and Tregs were unaffected, but autoreactive B-cell response was diminished, demonstrating a

Table 1. p values and 95% confidence intervals of difference of serum anti-HBs levels in groups of mice treated with different doses of DL-1-MT.

Groups	Groups	Sig.	95% Confidence Interval	
			Lower Bound	Upper Bound
No DL-1-MT	1 mg DL-1-MT	.001	12180.10	63230.37
	10 mg DL-1-MT	<0.001	15189.08	59399.92
	20 mg DL-1-MT	.001	11250.68	55461.52
1 mg DL-1-MT	No DL-1-MT	.001	-63230.37	-12180.10
	10 mg DL-1-MT	1.000	-25935.87	25114.40
	20 mg DL-1-MT	1.000	-29874.27	21176.00
10 mg DL-1-MT	No DL-1-MT	<0.001	-59399.92	-15189.08
	1 mg DL-1-MT	1.000	-25114.40	25935.87
	20 mg DL-1-MT	1.000	-26043.82	18167.02
20 mg DL-1MT	No DL-1-MT	.001	-55461.52	-11250.68
	1 mg DL-1-MT	1.000	-21176.00	29874.27
	10 mg DL-1-MT	1.000	-18167.02	26043.82

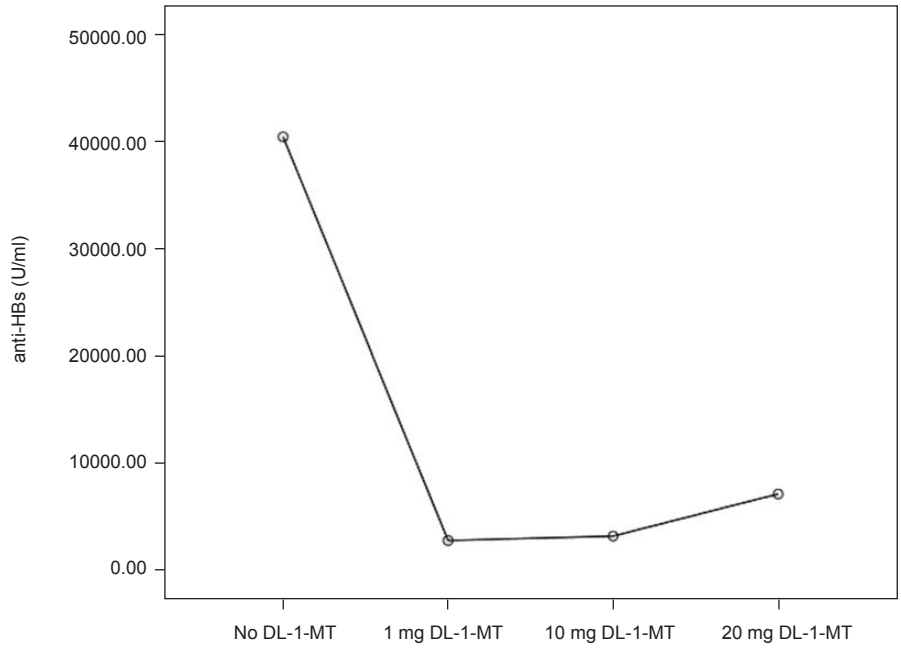

Figure 1. Serum anti-HBs levels in groups of mice treated with different doses of DL-1-MT.

role for IDO in stimulating B-cell response. DL-1-MT reduced the number of reactive to the self-antigen antibody-secreting cells leading to a great decrease of autoantibody titers. Importantly, DL-1-MT exposure only during the initiation of arthritis diminished the subsequent pathogenic B-cell response and alleviated arthritis. These results are in accordance with findings of this study, where it was found that early administration of DL-1-MT, within the vaccine, decreased the antibody response to the injected antigen. The exact molecular mechanisms remain to be elucidated.

In conclusion, inhibition of IDO at the time of vaccination decreases humoral immune response to HBs antigen protein vaccine in BALB/c mice. It seems that inhibition of IDO is not a proper approach for enhancement of the adaptive immune response to vaccines against infectious agents and the idea that IDO activity is simply immunosuppressive may need to be re-evaluated.

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