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RESEARCH ARTICLE

Comparative Evaluation of Alum and MPL as Adjuvants Toxicity and Related Immune Potential of Adjuvanted Rabies Vaccine

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ABSTRACT

Rabies is a dangerous and preventable viral disease that is mainly transmitted through a rabid animal bite. Human rabies has been considered a problem of great concern due to high death rate that is usually associated with rabies infection. Rabies vaccine is an active immunizing agent directed to protect against infection caused by rabies virus. Current study aimed to evaluate the potentials of Alum and Monophosphoryl lipid A (MPL) as adjuvants in enhancing the immune response of rabies vaccine. Therefore, anti- rabies total IgG, IFN- γ and Il-6 were determined post mice immunization with alum adjuvanted, MPL adjuvanted and non adjuvanted rabies vaccine. The adverse reactivity to the tested adjuvants was considered via evaluating their effects on liver regarding the apoptotic pattern through monitoring the cell cycle profile in addition to histopathological examination. It was observed that total IgG was significantly elevated in case of MPL adjuvanted vaccine than alum adsorbed and non- adsorbed one. In the same time, pro-inflammatory mediators (IFN- γ and Il-6) were elevated post different vaccine formulae administration and their levels were time dependent. Liver cells were arrested during the G2/M phase post vaccination with both alum and MPL adsorbed vaccine, while the percentage of apoptotic cells was insignificantly influenced ($P<0.05$). Histopathological examination post administration of MPL adsorbed vaccines showed significant pathological changes in liver and kidney tissues than those induced post administration of alum adsorbed one. Finally, it could be concluded that both adjuvants are promising immune enhancers to human rabies vaccine.

Introduction

Rabies is a major zoonotic disease, which remains a serious world public health problem. It is one of the most recognizable diseases that has been well known for more than 4300 years [1]. While rabies has been controlled throughout most of the developed world, it remains a significant burden in developing countries, causing many animal and human deaths [2]. According to WHO estimates, 55,000 annual human deaths are reported worldwide and more

than 10 million people undergo post-exposure prophylaxis every year. A vaccine is a biological preparation that provides active acquired immunity to a particular disease [3]. A vaccine typically contains an agent that resembles a disease-causing microorganism and is often made from weakened or killed microbe. It stimulates the immune system to recognize this agent as a threat [4] and consequently destroy it. Rabies vaccine is an active immunizing agent used to prevent infection caused by the rabies virus. Rabies vaccine enhances production of antibodies against rabies virus [5]. Vaccine adjuvants have empirically been identified for their ability to enhance the adaptive immune response to a co-administered antigen. The innate response induced by the adjuvant is important for the type and strength of the subsequent adaptive response [6]. The induction of immune responses in vivo is typically performed with antigens administered in external adjuvants, like alum and monophosphoryl lipid A (MPL). Aluminum-containing adjuvants induce strong innate immune responses that consist of an influx of neutrophils, eosinophils, NK cells, monocytes and dendritic cells (DCs) to the site of injection [7]. Besides the influx of cells, mast cells and macrophages quickly disappear after alum injection. The disappearance of macrophages is probably due to their activation and their subsequent adherence to the peritoneal wall, making it impossible to recover them in the peritoneal cavity [8]. Tissue-resident macrophages are considered the first cells to sense a disturbance in tissue homeostasis. Through their rapid production of cytokines and chemokines, they alert the immune system and recruit other cells of the innate immune system [9], like neutrophils. Indeed, neutrophils are also attracted rapidly after alum injection. Mast cells can directly sense alum and are amongst other cells responsible for the release of IL-1b and IL-5 [10-11]. MPL as adjuvant is a chemically modified derivative of lipopolysaccharides that greatly exhibited reduced toxicity while maintaining most of the immune stimulatory activity of lipopolysaccharides [12]. MPL has been used widely in clinical trials in prophylactic and therapeutic vaccines targeting infectious disease, cancer and allergies. MPL is a potent stimulator of T cell and antibody responses. MPL is the first and only Toll-Like Receptor (TLR) ligand in licensed human vaccines [13-14]. MPL is based on the Toll-Like Receptor-4 (TLR4)-active element of Salmonella lipopolysaccharides. MPL is less toxic than lipopolysaccharides by approximately 1000 fold. It is also active and non-toxic component of vaccines against hepatitis B virus as well as human papilloma virus [15]. MPL has been shown to be capable of binding and activating the TLR-4, present on antigen-presenting cells, which play a critical role in the induction of the innate immune response in addition to stimulation of the adaptive immune responses as a major consequence. It was reported that MPL directly affect adaptive immune responses via specific interactions with B cells [16]. It was also demonstrated that Virus Like Particles (VLPs) complement the ability of MPL to enhance the humoral immune responses [17]. Accordingly, present study aimed to evaluate the role of MPL as rabies vaccine immune enhancer compared to the currently used alum. In addition to monitoring the related immune mediators released post vaccination as well as evaluating the histopathological drawbacks of both alum and MPL on different organs post adjuvanted vaccine administration compared to non-immunized negative control mice.

Materials and Methodology

1. Swiss mice

Albino Swiss mice 18-20 gm body weight was kindly supplied from Helwan animal house – VACSERA –Egypt. Mice were kept one week before the vaccination for detection of mortality and morbidity. Mice were vaccinated subcutaneously using the test vaccine as 0.2 ml/mice

2. Rabies vaccine

VeroRab was kindly supplied from VACSERA R&D sector. The protein concentration was 35 mg/dose.

3. Aluminum hydroxide gel (Alum)

Aluminum chloride (0.63 M) was added to sodium phosphate (0.3 M)in 40 ml saline. Contents were stirred continuously at 40 to 60 rpm. Sodium phosphate (0.3 M) stock solution was added to the mixing bottle followed by addition of 300 ml normal saline and pH was adjusted to 6.8 ±0.2. Alum was added to vaccine as

0.35 mg/ml final concentration. Vaccine –Alum mixture was stirred over night at 37 oC prior to administration

4. Monophosphoryl lipid (MPL)

MPL was kindly purchased from Sigma–Aldrich-USA. It was prepared and added to Rabies vaccine according to the manufacturer's instruction.

5. ELISA Kits

Evaluation of pro-inflammatory mediators namely Interferon-γ (IFN-γ) and Interleukin-6 (IL-6) were carried out using ELISA kits (Bio-science-USA)

6. Evaluation of anti-rabies total IgG

Anti-rabies total IgG was evaluated using direct ELISA according to [18]. Polystyrene micro titer plates (96-flat bottomed wells, M 129A - Dynatech) were coated with 100 µl/well of 1 µg/ml rabies antigen in carbonate - bicarbonate buffer, pH 9.6. Plates were incubated overnight at room temperature. Plates were washed 3 times using washing buffer (PBS + 0.05/% Tween 20) as 0.1 M PBS, pH 7.4 and blocked with 100 µl/well 4% BSA (Sigma–Aldrich-USA). Plates were washed 3 times as previous. Animal anti-rabies immune sera developed post vaccination with Alum, MPL adjuvanted and non adjuvanted rabies vaccine candidates were 2-fold serially diluted and dispensed to the pre-coated plates starting as 1/50 in dilution buffer. Plates incubated for 1 h at 37°C (Jouan –France) then washed as previous. Anti-mouse conjugate (Peroxidase labeled) (Sigma-Aldrich-USA) was dispensed to the plates as 1/1000 final dilution in dilution buffer. Plates were incubated for 1 h at 37°C. Plates were washed as previous, inoculated with100 µl/well of substrate solution (O-phenylene diamine dihydrochloride (OPD) buffer (Sigma–Aldrich-USA) and incubated in the dark at room temperature for 30 min. Hundred µl/well of 2 N H2SO4 were added to stop the reaction. The absorbance was measured at 450 nm using ELISA reader (Bio-Rad micro plate reader, Richmond, Co).

7. Cellular immunity

Sera samples were collected from each mice group post immunization using alum and MPL adjuvanted rabies vaccine as well as non-adjuvanted vaccine. Evaluation of both IFN-γ and IL-6 was performed by dispensing the collected serum samples to the anti IFN and IL:-6 pre-coated ELISA plates as 100µl/well. Plates were incubating overnight at room temperature. Test sera solutions was decanted and washed as previous. Plates were incubated for at least 1 hour at room temperature. Plates were washed as previous. Immediately, 100µl of standard or sample were added to each well. Plates were incubated at room temperature for at least 2 h and plates were washed as previous. Detection antibody was added as 100 µl /well. Plates were incubated at room temperature for 2 h and washed as previous. Anti-mouse conjugate was added and plates were incubated for 30 min at room temperature. Plates were washed as previous and 100µl of substrate solution were added to each well. Plates incubated at room temperature for color development and the absorbance was recorded using ELISA plate reader (ELx-800- Biotek, USA) at 450 nm wavelength [19].

Histological Studies

Fresh liver and kidney specimens were exited from the control and treated groups and fixed in 10% neutral buffer formol and Carnoy's fluid for the histological studies. Specimens were washed and dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Sections were cut at 5µm thickness and stained with hematoxylin & eosin stain for histological studies according to [20].

1. Cell cycle analysis

Adverse effect of test adjuvants on the liver cells was evaluated through determination of the related cell cycle profile using cell cycle analysis. Liver tissues were treated with lyses buffer and processed according to the manufacturer's procedure. Cells were harvested and fixed gently with 70% (v/v) ethanol in PBS, maintained at a temperature of 4°C overnight then re-suspended in PBS containing 40 µg/ml PI and 0.1 mg/ml RNase and 0.1% (v/v) Triton X- 100 in a dark room. Post 30 min at 37°C, the cells were analyzed using a flow-cytometer (Becton-Dickinson, San Jose, CA, USA) equipped with an argon ion laser at a wavelength of 488 nm [21].

Results

1. Evaluation of anti-rabies total IgG

Rabies vaccine induced antibody level was significantly elevated in case of Alum and MPL adjuvanted vaccine candidates compared the non-adjuvanted one (P<0.05). Also, MPL adjuvanted vaccine showed a long-last ingreleased antibody, while the alum adjuvanted and non-adjuvanted vaccine showed a faster declining phase of antibody level (Figure 1).

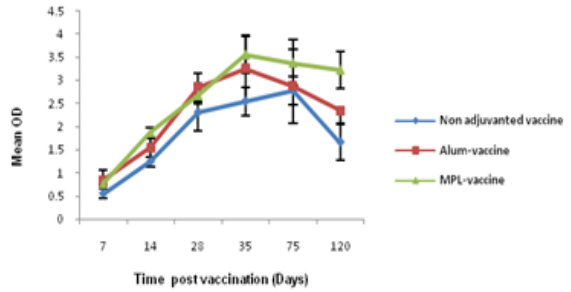

Figure 1: Comparative evaluation of total antibody concentration in serum samples of mice groups treated with alum and MPL adjuvanted and non-adjuvanted vaccines. Antibody titer was determined at different time interval (7, 14, 28, 35 and 75 and 120 days). Results were expressed as mean optical density ± SD.

2. Cellular immunity

Regarding the cellular immune response, it was noted that both IL-6 and IFN-γ were detected 3 days post vaccination and their level was subsequently elevated relative to time. IL-6 level was significantly elevated and maintained almost stationary phase in case of MPL adjuvanted vaccine (P<0.05), while a noticed depletion of IL-6 in case of alum adjuvanted and non-adjuvanted vaccine were recorded (Figure 2). In the mean time, IFN-γ level developed post immunization with alum and MPL adjuvanted vaccines was significantly elevated than in case of non-adjuvanted vaccine (P<0.05). However, IFN-γ level in case of alum adsorbed vaccine was maintained longer than in case of MPL adjuvanted vaccine and non adjuvanted one (Figure 3).

3. Cell cycle analysis

Data recorded revealed that liver cells of immunized mice were arrested in G2-M phase was significantly elevated post administration of alum and MPL adjuvanted vaccines (P>0.05). However, cells in S and G0-G1 phase were insignificantly changed than in control non treated cells (P>0.05). Also, statistically

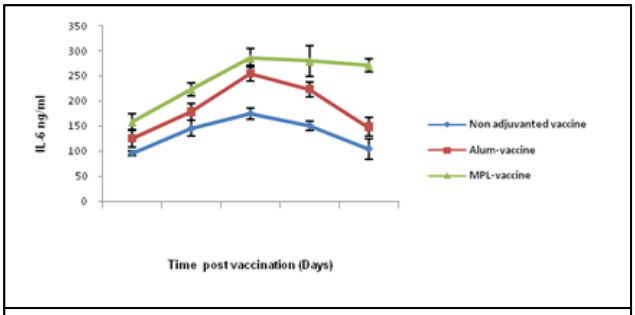

Figure 2: Assessment of IL-6 in sera samples post mice vaccination with alum and MPL adjuvanted as well as non-adjuvanted vaccines showing an increase in IL-6 level in MPL adjuvanted vaccine compared to alum adjuvanted and non-adjuvanted vaccines. Test was carried out in independent triplicates.

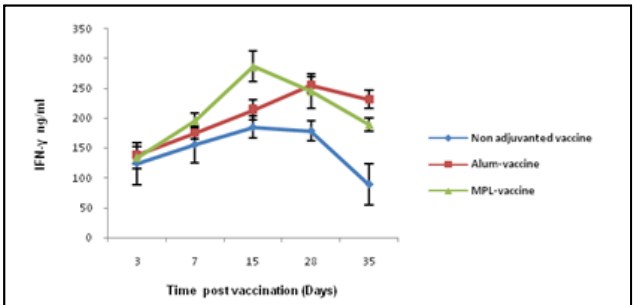

Figure 3: IFN-γ in sera samples of vaccinated mice groups showing an elevation in IFN-γ level in case of alum and MPL adjuvanted vaccine compared to non adjuvanted one. However, alum adjuvanted vaccine recorded maintenance in IFN-γ level than in alum adjuvanted vaccine relative to time. Results were presented as mean ± SD of three independent tests.

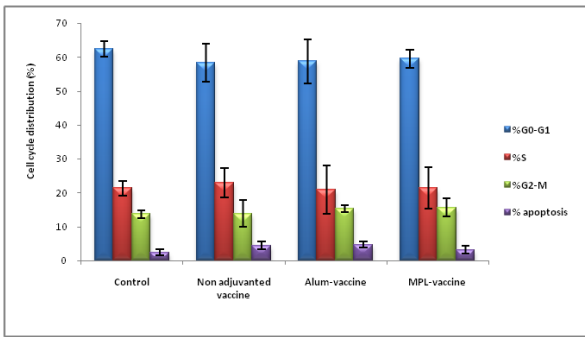

Figure 4: Evaluation of cell cycle analysis of affected liver cells post administration of Alum and MPL adjuvanted and non-adjuvanted vaccine compared to control. The analyses were carried out in independent triplicates. Data were expressed as the mean percentage of cells in each phase ± SD. (*): Statistically significant difference compared to control (P < 0.05).

insignificant difference in the percentage of apoptotic cells were recorded in groups injected with different formulations of vaccines compared to control group indicating that tested adjuvants induced little toxicity to liver tissues and this toxicity is associated with G2-M phase arrest (Figure 4,5).

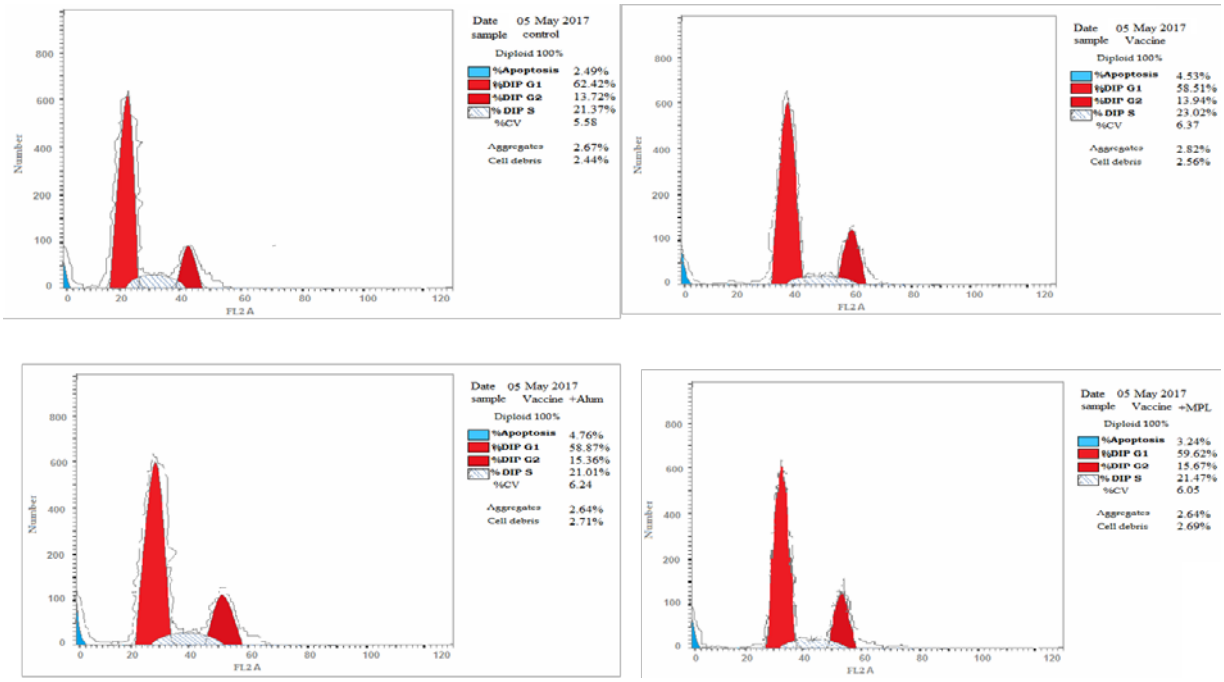

Figure 5: Cell cycle analysis profile [A]: Control sample; [B] liver cells in non adjuvanted vaccine; [C]: alum adjuvanted vaccine; [D]: MPL adjuvanted vaccine.

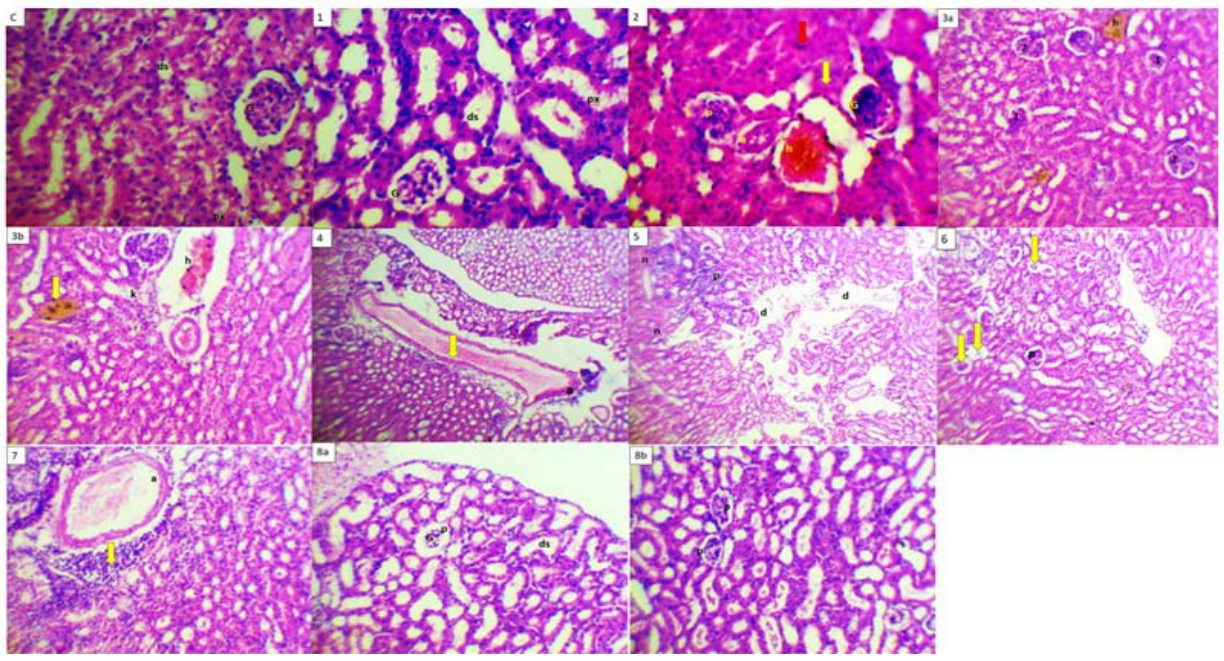

Figure 6: Photomicrographs of the kidney cortex of control mice and all treated groups post staining with hematoxylin and eosin (H&E) stain.

4. Histological Studies

Regarding the adverse effects related to the administration of Alum and MPL adjuvanted rabies vaccine. Histopathological studies were performed on kidney and liver of the mice tissues, whereas the effect of rabies vaccine without any adjuvant was recorded as well. The effect of vaccine on liver tissue of mice appears well-proportioned of the central area which contains central vein hepatocytes, sinusoidal spaces, Kupffer cells hepatic vein branch of hepatic artery and bile duct.

The effect of alum adjuvanted rabies vaccine in the kidney and liver indicated the appearance significant effect on kidneys in the area of the cortex, arterial wall, highly distorted glomerular which contains many pyknotic nuclei or karyolytic nuclei and highly stratified cuboidal

epithelial cells of the convoluted tubule, with numerous hemorrhagic areas which contains hemosiderin. Further, the compelling effect on the liver in the portal area showed an increase in Kupffer cells with some normal architecture in hepatocytes and central vein but few hepatocytes contain pyknotic nuclei.

there is well developed architecture of the kidney cortex of mice showing Glomerular (G), Proximal convoluted tubule (PX) with the brush borders (bd) and distal convoluted tubule (ds) (x400). (2&3): Photomicrographs of kidney cortex of mice treated with Alum adjuvanted vaccine. (2):

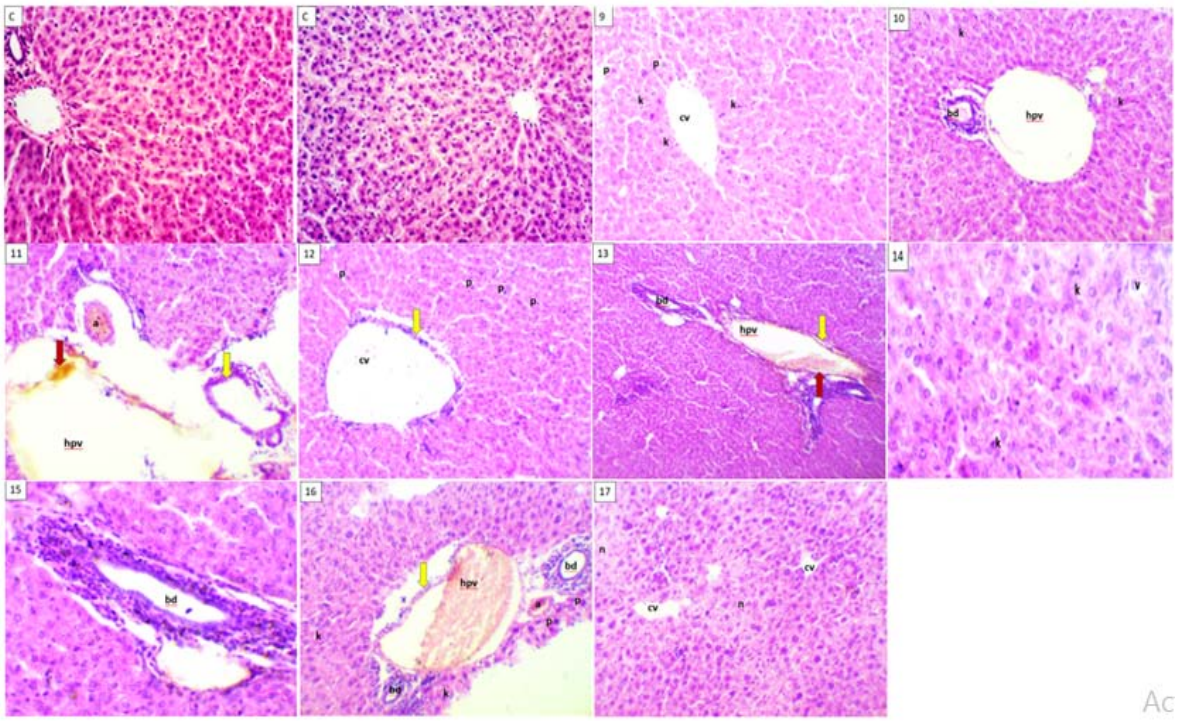

Figure 7: Photomicrographs of liver tissue of a mice showing central area of hepatic tissue with central vein (cv), hepatocytes (h), sinusoidal spaces (s) and Kupffer cells (k) (x250) of control mice group compared to other groups treated with non adjuvanted, aLum adjuvanted and MPL adjuvantedrabies vaccine.

MPL adjuvanted rabies vaccine in the kidney and liver tissues showed highly dilated and corrugated arterial wall containing hemolyzed blood cells, numerous degenerated areas. In addition to other neurotic areas which have lots of pyknotic nuclei and atrophied glomerulus indicating expressive effect on the liver tissues. Portal area exhibited highly dilated hepatic portal vein which contains hemolyzed blood cells with delaminated endothelial lining, highly thickened and completely destructed wells of bile ducts with lymphocytes infiltration. Numerous degenerate changes in nuclei and cytoplasm of hepatocytes as indicated by vacuolation in the cytoplasm were also recorded (Figures 6, 7).

[C] & [1]: Photomicrographs of kidney cells in control and non adjuvanted vaccine treated group , respectively, exhibited no signs of abnormality, where

Large hemorrhagic area in the cortex (h) with highly thickened arterial wall (a), highly distorted glomerular (G) which contains many pyknotic nuclei (p) or karyolytic nuclei (k) and highly stratified cuboidal epithelial cells of the convoluted tubule (CT) (Red arrow); They contains lots of karyolytic nuclei (k) (Yellow arrow)(x400). (3a): Atrophied glomerulus (2)or lobulated encase(2) with numerous hemorrhagic areas (h) which contains hemosiderin granules. (3b): Large hemorrhagic granules (h) with numerous hemosiderin granules (Yellow arrow), most convoluted tubule (CT) have karyolytic nuclei (k)(x400).

(4 to 8): Photomicrographs of kidney cortex of mice treated with MPL adjuvanted vaccine. (4): Highly dilated and corrugated arterial wall (a) which contains hemolyzed blood cells (Yellow arrow) (x200). (5): Numerous degenerated areas (d)which contain debris of

degenerated convoluted tubule (CT), some neurotic areas which have lots of pyknotic nuclei (p). (6): Highly destructed convoluted tubule (CT) especially in the outer cortical layer, numerous atrophied glomeruli (Yellow arrow) which contain pyknotic nuclei (p) (x200). (7): Lymphocytic infiltration (Yellow arrow) around the highly dilated arterial which contains hemolyzed blood cells (x400). (8a&8b): Highly destructed and atrophied glomerulus (G) with widened convoluted tubule (CT) especially the distal ones (ds). Lots of pyknotic nuclei (P) while numerous convoluted tubule (CT) and glomerulus showed normal architecture (x400) (Figure 7).

(C): Photomicrograph of the portal area of hepatic tissue of a control group showing hepatic vein (hpv), branch of hepatic artery (a) and bile ducts (bd) (x250).

(9-11): Photomicrographs of liver tissue of mice treated with non-adjuvanted rabies vaccine. (9): Portal area of hepatic tissue showing increase in Kupffer cells (k) with some normal architecture hepatocytes and central vein (cv) but few hepatocytes contain pyknotic nuclei (p)(x250). (10): Increase in Kupffer cells (k), highly widened hepatic portal vein and destructed bile ducts (bd) with lymphocytic infiltration in the portal area and hpv. (11): Highly dilated, thickened and congested artery(a) containing hemolyzed blood cells, highly dilated and corrugated well of hepatic portal vein (hpv) showing nearly hemolyzed blood cells (Red arrow), wells of bile ducts are highly destructed or ruptured (Yellow arrow); the portal area is invaded by lymphocytes (x250).

(12-14): Photomicrographs of liver tissue of mice treated with alum adjuvanted rabies vaccine. (12): Liver tissue of mice showing delaminated central and highly endothelial lining (Yellow arrow) of central vein (cv) containing hemolyzed blood cells, some hepatocytes showed pyknotic nuclei or karyolytic nuclei (k) (x200). (13): Portal area showing dilated hepatic portal vein (hpv) with hemolyzed blood cells (Red arrow)and delaminated endothelial lining (Yellow arrow), highly thickened and completely destructed wells of bile ducts (bd) with lymphocytes infiltration. (14): numerous degenerate changes in cytoplasm and nuclei of hepatocytes including vacuolation (v) in cytoplasm, karyolytic nuclei (k) and increase in Kupffer cells (x400).

(15-17): Photomicrographs of liver tissue of mice treated with MPL adjuvanted rabies vaccine. (15): Highly destructed elongated well of bile ducts, which is surrounded by numerous lymphocytes (x250). (16): Various dystrophic changes in the portal area including highly dilated and congested hepatic portal vein (hpv) with delaminated endothelial lining (Yellow arrow). It contains hemolyzed blood cells, mail-farmed widened bile ducts, destroyed arterial well (a), lymphocytes infiltration in the portal area. The portal area showed also an increase in lymphocytes and Kupffer cells (k) with numerous pyknotic nuclei (p) or karyolytic nuclei of hepatocytes (x250). (17): Central area showing corrugated central vein (cv) which is surrounded by numerous necrotic area (n) with numerous degenerated changes in nuclei of hepatocytes and increase in Kupffer cells (x250).

Discussion

Despite the existence of safe and efficient prophylactic measures against rabies infection, rabies has been considered one of the significant issues of human and animal mortality. The success of post-exposure prophylaxis against rabies infection in the endemic countries is usually hindered by some obstacles such as the cost. Critical prophylactic regulations such as the massive vaccination of dogs usually don't reach its aim in rabies-endemic countries due to animal as well as vaccine-related issues [22].

Vaccine potency is critically influenced by many factors during its formulation. The criteria in selecting this formulation should consider the properties of the antigenic components, the required immune response, route of delivery, avoiding significant side effects as well as vaccine stability. An adjuvant is a chemical substance that is added during the formulation of the vaccine in order to elevate the immune response. Ideal adjuvant should be safe, stable, biodegradable, inexpensive, capable of stimulating an antigen specific immune response and ensuring the reproducibility of the vaccine potency during manufacturing [23]. It was also

reported that the safety measures of adjuvanted vaccines greatly related to the long persistence time of the adjuvant in the tissues as well as the ability of the adjuvant particles to accumulate in lymphatic system [24].

Aluminum was first used adjuvant in licensed human vaccines. However the extended and continuous use of aluminum salts as adjuvants, their immune mechanism of action is still incompletely understood in addition to their adverse effects [25]. Aluminum adjuvants mainly stimulate the production of antibodies and thus they are appropriate for triggering potential immune response towards killed microorganism. On the other hand, they are not effective in infections related to intracellular pathogens [26]. Despite that aluminum and MPL are used as adjuvants in vaccine, they exhibited many drawbacks such as their failure to elevate the immune response of weak antigen, carcinogenic effect as well as their strong stimulatory response in localized area [27]. It was reported that aluminum is a weak immuno-stimulant for the release of antibodies and it caused Th2 response instead of Th1 stimulation [28]. A study suggested that MPL could promote the neutralizing antibody response of rabies vaccine when used with the initial injection of DNA rabies vaccine [29].

Investigating the clinical potential of many adjuvants is still of significant importance as the mechanism of action of adjuvants are still not fully recognized [30]. In addition, determining their effect on the immune response could aid in reaching optimum effective and safe immune response. For example, it was reported that the adjuvant induced inflammatory response at the injection site resulted in necrosis of muscle fibers which is accompanied by migration of monocytes followed by differentiation into dendritic cells which play an important role in the development of the immune response [31]. Cellular immunity that was recorded in the current study was in agreement with another study, where it was found that both alum and MPL adjuvants act as stimulators to release protective CD8 memory T cells. Alum played a critical role in the generation of population of CD8 memory T cells which is characterized by their long-lived nature. The released alum-primed CD8 T cells has a potential to be differentiated into IFN-γ–producing cells which justifies the recorded prolonged IFN-γ level in case of alum-adjuvanted rabies vaccine compared to MPL-adjuvanted candidate. In the mean time, it was reported that the released IL-6 has been considered a key factor in inducing the activation of CD8 T cells to be differentiated into cytotoxic T cells (CTLs) in case of MPL-adjuvanted vaccine. This also rationalized the recorded elevated IL-6 that was associated with MPL-adjuvanted rabies vaccine [32].

Concerning the influence of test adjuvants on cell cycle analysis, the recorded cell cycle profile indicated mild toxicity to liver cells in both alum and MPL adjuvanted vaccines compared to non adjuvanted vaccine. On the other hand, the apoptotic pattern wasn't significantly influenced in presence of both adjuvants. Meanwhile and to the best of our knowledge, the effect of tested adjuvants on the apoptotic pattern of cells was rarely reported.

In order to study the impact of MPL adjuvanted rabies vaccine on histopathology two organs; liver and kidney were used as they are commonly studied organs by other workers as well [33]. Current study revealed that both adjuvants showed pathological changes in both liver and kidney cells compared to control untreated group. Also, MPL adjuvanted vaccine exhibited more changes than that recorded in case of alum adjuvanted vaccine. On the contrary, non adjuvanted vaccine exhibited no signs of adverse effects. In agreement to current findings, other studies reported that alum exhibited obvious necrosis in muscle fibers [34] and could induce granulomas at the injection site [23]. Furthermore, the recorded infiltration of lymphocytes was matching that reported by [34] where it was demonstrated that immunization with alum adjuvanted vaccine resulted in attraction of cells of immune response such as macrophage, natural killer cell, neutrophil, eosinophil as well as immature dendritic cells at the site of injection. These dendritic cells up take the antigens that were released from alum at the injection site and are directed these antigens to the draining lymph node to be taken up by dendritic cells (DC) and presented to

T cells. Moreover, binding of these antigens to B cells through surface immunoglobulins resulted in differentiation of B cells into plasma cells(PCs). Plasma cells release low-affinity antibodies (LAb) and B cells are mobilized to B cell follicle. In the mean time, CD8 and CD4 T cell activation resulted in the production of effector CD8 (eCD8) T cells and effector CD4 (eCD4) T cells. The eCD4 activates the development of T helper into (Th) 1, 2 or T follicular helper (Tfh) cells but alum directed the development of Th2 and Tfh cells, which reach B cell follicle and activate the B cells to PCs resulting in the secretion of high-affinity antibody (HAb) [35].

Another study demonstrated that rats injected with aluminum demonstrated deformation of the cells of liver parenchyma, disturbance in the arrangement of sinusoids in the central vein of the liver and distortion of the normal shape of the hepatocytes compared to the control. Consequently, special care should be taken in consideration when this adjuvant is used [36]. On the other side, a study reported that intravenous administrations of MPL resulted in inflammatory changes in addition to elevated spleen weights [37].

Accordingly, current study highlights the potentials of alum and MPL in promoting the immune response of rabies vaccine despite the drawbacks detected in different tissues due to the pro-inflammatory reactions which may be related to their role in activating the immune response and especially it is well known that the relief of these cellular changes is time dependent.

Conclusion

It is important to point out that both alum and MPL are promising adjuvants of significant immune enhancing potentials that is based on the elevation of both cellular and humoral immune response despite the detected drawbacks. Cell cycle profile post exposure to alum and MPL adsorbed and non-absorbed vaccine candidates revealed that both adjuvants exhibited a mild toxic effect on liver cells which is related to G2/M phase arrest. However, histopathological examination indicated significant inflammatory reactions accompanied with MPL adsorbed vaccine than alum adsorbed one.

Recommendation

It is recommended to find out the role of in-vivo adjuvant concentration and related safety measures. In the mean time, the relief of the adjuvant toxic effect should be determined relative to time. Finally, deep monitoring of the immune response and related changed mediators in addition to the antioxidant level should be carried out as they may have a role in the tissue toxicity.

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