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Comparative Evolution of Immune Enhancing Potentialof AlumandMPL asRabies Vaccine Adjuvants and Related Adverse Events

Mohamed Ayman Mohamed[1], Doaa Abd El Aziz Badr[2], Rania I. Shebl[3]and Aly Fahmy Mohamed[2]

[1]*Faculty of Biotechnology, MSA Universityand*
[2]*The Holding Company for the Production of Vaccines, Sera and Drugs (VACSERA-Egy Vac) and* [3]*Microbiology and Immunology Department, Faculty of pharmacy, Ahram Canadian University (ACU).*

Abstract: *Rabies is a preventable viral disease of mammals most often transmitted through the bite of a rabid animal. The vast majority of rabies cases reported to the Centers for Disease Control and Prevention(CDC). Rabies vaccine is an active immunizing agent used to prevent infection caused by the rabies virus. Alum and MPL are two adjuvantsenhance the immune response post vaccination. Therefore, the anti- rabies total IgG was evaluated to determine the immune response to alum and MPL adjuvanted and nonadjuvanted rabies vaccine. The adverse reactivity to the used alum and MPL adjuvanted rabies vaccine was considered via evaluation of its effect on liver regarding the apoptotic pattern via monitoring the cell cycleprofile,it was noticed that the cells were arrested during the G2/M phase post vaccination with both alum and MPL adsorbed vaccine.Sole vaccine administration showed an arrest during the G0-G1 Phase. Also, the apoptosis pattern of cell significantly elevated than negative control. Pathological changes related to administration of MPL adsorbed vaccines showed a significantpathological changes in liver and kidneytissues than those induced post administration of Alum 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 depended. Total IgG was significantly elevated in case of MPL adjuvanted vaccine than alum adsorbed and non- adsorbed one.*

Keywords: *Rabies vaccine, Alum & MPL adjuvants, Histopathology, Antibodies, IFN and IL-6*

I. INTRODUCTION

Rabies is a major zoonotic disease, which remains a serious world public health problem. It is one of the most recognizable zoonosis and 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. The antigen stimulates the immune system to recognize the agent as a threat, master it, recognize [4] and destroy any of these microorganisms that it later encounters therefore, Rabies vaccine is an active immunizing agent used to prevent infection caused by the rabies virus. The 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, CD11b+ 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 amongst 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 to produce IL-1b, IL-5, CCL2 and RANTES [10-11]. MPL as adjuvant is a chemically modified derivative of lipopolysaccharide that displays greatly reduced toxicity while maintaining most of the immune stimulatory activity of lipopolysaccharide [12]. MPL adjuvant has been used extensively in clinical trials as a component 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 TLR ligand in licensed human vaccines, in the form of AS04[13-14]. MPL has been shown to be capable of binding and activating the so-called Toll-like receptor-4 (TLR-4), present on key antigen-presenting cells, which play a key role in the induction of the innate and subsequent adaptive immune responses. Recent observations suggest that TLR4 agonist, such as MPL, directly affect adaptive immune responses via specific interactions with B cells [15]. Based on the current data demonstrating similar relative boost ability of total antibody responses with Alum and AS04 formulations it is very likely that VLPs complement the ability of MPL to enhance the humoral immune responses [16]. So, the aim of the present work is to evaluate the role of MPL as rabies vaccine immune enhancer compared with the current used alum Also, mentoring the related immune, mediators released post vaccine relative time and finally evaluate the histopathological drawbacks of both alum and MPL on different organs post adjuvanted vaccine administration compare with non-immunized negative control mice

II. MATERIALS AND METHODOLOGY:

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

Rabies vaccine:
VeroRab was kindly supplied from VACSERA R&D sector. the protein concentration was 35 mg/ dose

Alum

Aluminum hydroxide gel (Alum):
Prepared by preparing Aluminum chloride as 0.63 Min and Sodium phosphate 0.3 M in 40 ml saline solution each. Contents were stirred continuously during the procedure at 40 to 60 rpm. 0.3 M sodium phosphate stock solution was added to a mixing bottle. 300 ml normal saline was added.pH was adjusted to 6.8 ±0.2. Alum was added to vaccine as 0.35 mg / ml final concentration. Vaccine –Alum mix was stirred over night at 37 ^{o}C hrs pre administration

Monophosphoryl lipid (MPL):
Kindly purchased from (Sigma – Aldrich, USA). Itwas preparedaccording to the producer instruction. Rabies vaccine was added and kept stirred over night at 37 ^{o}C pre administration

ELISA KITS
Evaluation of pro-inflammatory mediators namely Interferon –γ (IFN-γ) and Interleukin -6 (IL-6) were kindly supplied from Bio-science –USA

Evaluation of anti-rabies total IgG
Anti-rabies total IgG was evaluated using direct ELISA according to [17]. 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, then blocked with 100 µl/well of 4% BSA (Sigma-Aldrich, USA). Plates were washed 3 times as previous. Animal anti-rabies immune sera developed post vaccination with MPL, Alum 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 and 100 µl/well of substrate solution (O-phenylene diamine dihydrochloride (OPD) buffer (Sigma- Aldrich, USA).Plates were incubated in the dark at room temperature for 30 min. Hundred µl/well of 2 N H_2SO_4 were added to stop the reaction. The absorbance was measured at 450 nm using ELISA reader (Bio-Rad micro plate reader, Richmond, Co).

Cellular immunity
Evaluation of both IFN and IL-6 was performed by dispensing of 100µl both capture antibodies to each ELISA plate/well. Plates were incubating overnight at room temperature. Coating buffer was decanted and wash as previous. 200µl blocking buffer were dispensed/well.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 hours, and thenwashed as previous. 100 µl of detection antibody per well were added. Plates were incubation at room temperature for 2 hours. Plates were washed as previous. Anti mouse conjugate was added and plates were incubatedfor 30 minutes at room temperature. Plates were washed as previous. 100µl of substrate solution were added to each well. And plates incubated at room temperature for color development. Developed color was recorded using ELISA plate reader (ELx-800- Biotek, USA) at 450 nmwavelength [18].

Histological Studies:

Fresh liver and kidney specimens were exited from the control and treated groups. The specimens were 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 5µ thickness and stained with hematoxylin & eosin stain for histological studies according to [19].

Cell Cycle Analysis

Adverse effect of test adjuvants on the livers apoptoticand related cell cycle profile weretested where the liver tissues were treated with lyses buffer and processed according to the protocol of manufacturer where 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. After 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. The cell cycle and sub-G1 group were determined and analyzed, as described previously [20].

III. RESULTS

Evaluation of anti-rabies total IgG

The Ab level was significantly elevated in case of Alum and MPL adjuvantedvaccine candidates compared the non-adjuvanted one ($P<0.05$). Also, MPL adjuvant vaccine showed a long-lastingreleasedAb, while the alum adjuvanted and non-adjuvanted vaccine showed a somewhat faster declining phase of Ab level [Fig.1].

Cellular immunity

Regarding the cellular immuneresponse, it was noted that both IFN-γ and IL-6 were detected 3 days post vaccination and their level sowed a subsequent elevation relative to time and the IFN-γ level was significantly elevated and maintained almost stationary phase in case of MPL adjuvanted vaccine ($P<0.05$) while a noticed depletion of IFN-γ in case of alum adjuvanted and non-adjuvanted vaccine. Also, the IFN-γ level developed post immunization with alum and MPL adjuvanted vaccines was significantly elevated than in case of non-adjuvanted vaccine ($P<0.05$). In the mean-time the IL-6 level showed almost a nearpattern to IFN-γ despite the level in case of alum adsorbed vaccine was maintained longer than in case of MPL adjuvanted vaccine and non- adjuvanted one [Fig 2-3]

Cell Cycle Analysis

Data recorded revealed that the there was a mild toxicity of adjuvants used in vaccine enhancement compared to the control. And the late apoptotic % was decreased in MPL adsorbed rabies vaccine than in Alum adsorbed one. Also, there was a mild % of apoptotic cells that was significantly($P<0.05$) elevated in adsorbed and non-adsorbed vaccine compared to control mice tissue. The interrelation ship of apoptosis % was non-significant in case of the vaccine formulae used ($P>0.05$). Although, data recorded revealed that cells arrested in G2-M phase was elevated insignificantly elevated post administration of alum and MPL adsorbed vaccine ($P>0.05$), Also, cell arrested in the S and G0-G1 phase were insignificantly changed than in control nontreated cells ($P>0.05$) and an insignificant apoptosis was noticed indicating that the adjuvants used induced little toxicity to liver tissues and that apoptosis is G2-M phase related. [Fig.4-5].

Histological Studies:

Regarding the adverse events related to the administration of Alum and MPL adjuvanted rabies vaccine. The histopathological studies were performed on kidney and liver of the mice tissues, whereas the effect of the rabies without any adjuvant in the kidney cortex of mice that croup up well-developed architecture for Glomerular, Proximal convoluted tubule with the brush borders and distal convoluted tubule. Further, the effect of vaccine on liver tissue of mice appears well-proportioned of the central area which contain central vein hepatocytes, sinusoidal spaces, Kupffer cellshepatic vein branch of hepatic artery and bile duct. Although the effect of alum adjuvanted rabies vaccine in the kidney and liver that appearance significant effect on kidneys in

the area in 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 ,lobulated encase with numerous hemorrhagic areas which contains hemosiderin .Further, the compelling effect on the liver in the portal area showed a highly increase Kupffer cells with some normal architecture hepatocytes and central vein but few hepatocytes contain pyknotic nuclei. The liver present expressive effect for MPL adjuvanted rabies vaccine in the kidney and liver in the mice tissue that attend effect in kidney as highly dilated and corrugated arterial wall which contains hemolyzed blood cells, numerous degenerated areas which contain debris of degenerated, some neurotic areas which have lots of pyknotic nuclei and atrophied glomerulus also present expressive effect on the liver tissue such portal area showing 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. Also, showing numerous degenerate changes in cytoplasm and nuclei of hepatocytes which include vacuolation in cytoplasm [Figs.6-7].

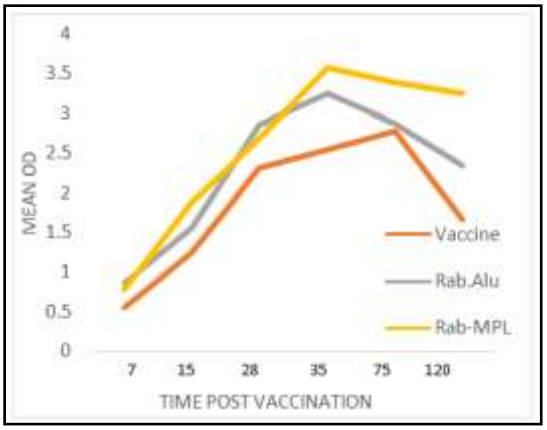

Fig.1: comparative evaluation of antibodies concentration for mice that treated with Alum and MPL adjuvanted and non-adjuvanted vaccine compared to control samples and Samples were taken on several sporadic days (4, 14, 21, 28 ,35 and 70 days).

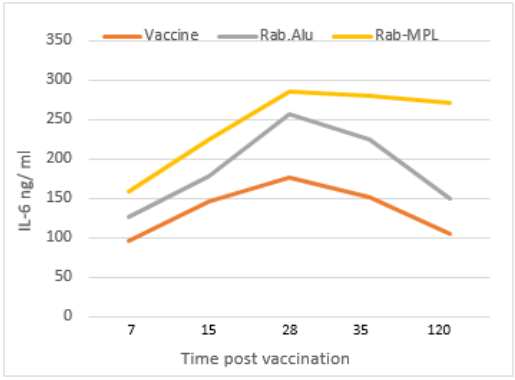

Fig.2: Assessment of IL-6 in sera samples post vaccination mice tissue treated with Alum and MPL adjuvanted and non-adjuvanted vaccine.

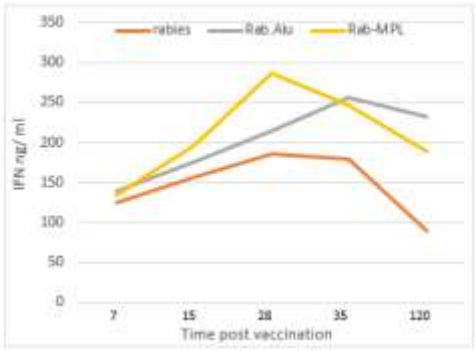

Fig.3: Assessment of IFN in sera samples post vaccination mice tissue treated with Alum and MPL adjuvanted and non-adjuvanted vaccine.

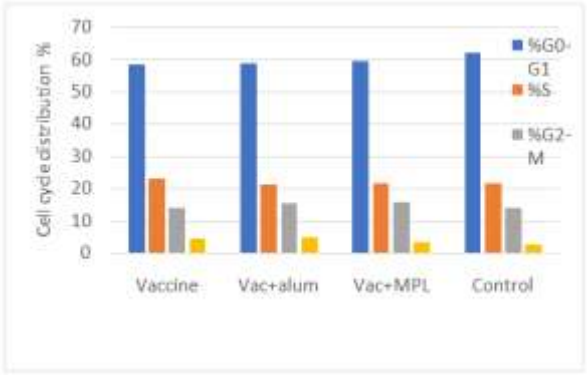

Fig.4: Evaluation of cell cycle analysis of affected liver cells post administration of Alum and MPL adjuvanted and non-adjuvanted vaccine compared to control. The analyses were carried out in (independent) triplicates, and the data were expressed as the mean percentage of cells in each phase ± SD

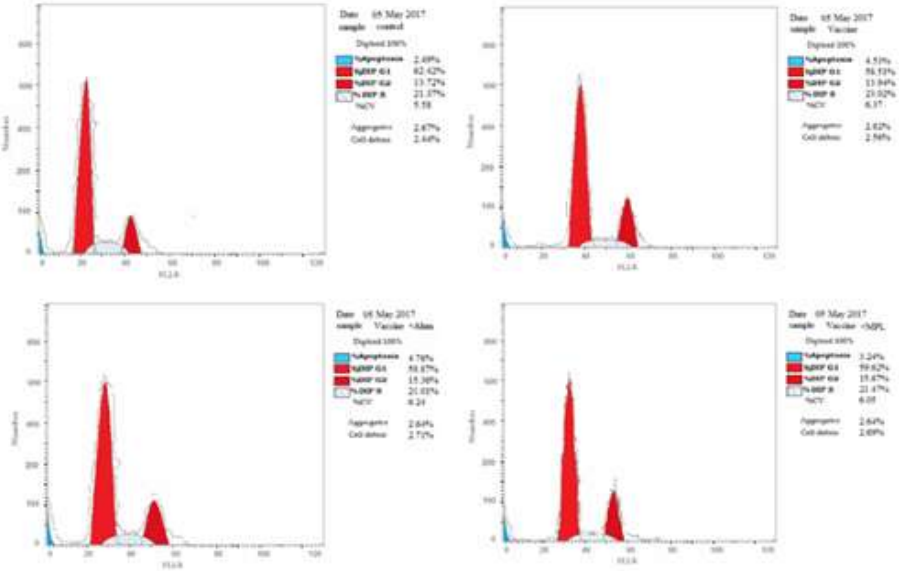

Fig.5: Cell cycle analysis of. [A]; Control sample; [B] tissue treated with vaccine; [C], tissue/cells treated with vaccine and Alum [D]; tissue / cells treated with vaccine and MPL, respectively.

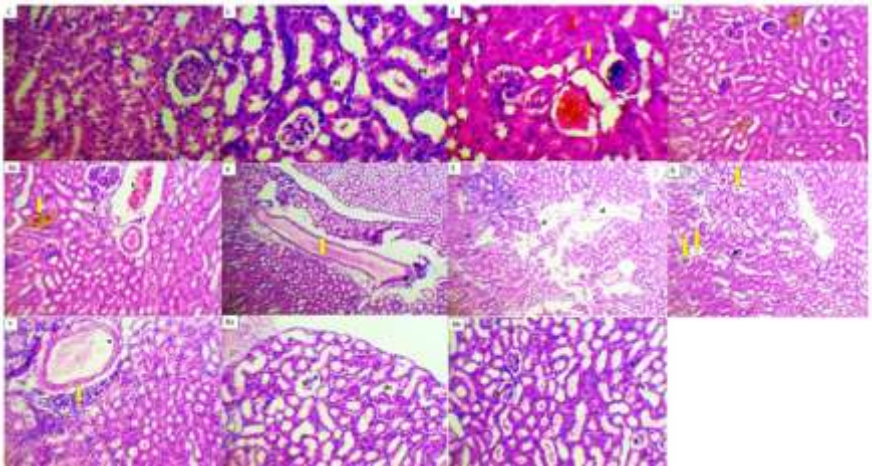

Fig.6: photomicrographs of the kidney cortex of mice of the control and all treated groups is stained with Hematoxylin and Eosin (H&E stain). (C): showing well developed architecture of the kidney cortex of control mice. Notice: Glomerular (G), Proximal convoluted tubule (PX) with the brush borders (bd) and distal convoluted tubule (ds) (x400). (1): showing well developed architecture of the kidney cortex of control mice. Notice: 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 vaccine plus Alum adjuvant.

(2): showing large hemorrhagic area in the cortex (h), 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&3b): (3a) showing atrophied glomerulus (2)or lobulated encase(2) with numerous hemorrhagic areas (h) which contains hemosiderin granules. (3b): Showing large hemorrhagic granules area (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 vaccine plus MPL adjuvant. (4) showing highly dilated and corrugated arterial wall (a) which contains hemolyzed blood cells (Yellow arrow) (x200). (5): showing numerous degenerated areas (d). which contain debris of degenerated convoluted tubule (CT), some neurotic areas which have lots of pyknotic nuclei (p). (6): showing highly destructed convoluted tubule (CT) specially in the outer cortical layer, numerous atrophied glomerulus (Yellow arrow) which contain pyknotic nuclei (p) (x200). (7): showing lymphocytic infiltration (Yellow arrow) around the highly dilated arterial which contains hemolyzed blood cells (x400). (8a&8b): showing highly destructed and atrophied glomerulus (G). highly widened convoluted tubule (CT) specially the distal ones (ds). Notice: lots of pyknotic nuclei (P) while numerous convoluted tubule (CT) and glomerulus show normal architecture (x400).

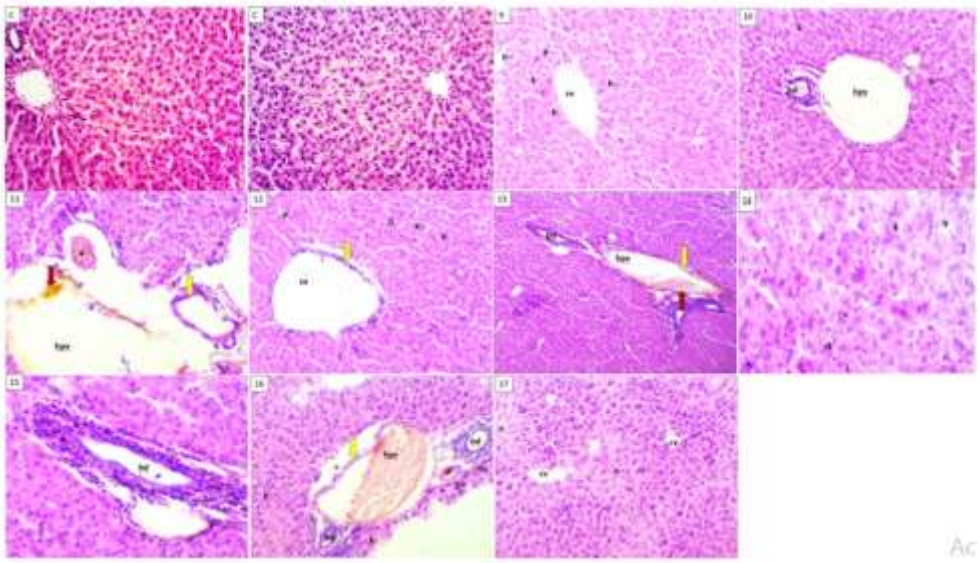

Fig.7: a photomicrographs of liver tissue of a central mice: showing central area of the hepatic tissue of control mice which contain central vein (cv), hepatocytes (h), sinusoidal spaces (s) and Kupffer cells (k) (x250).(C): a photomicrograph of the portal area of hepatic tissue of a control: showing hepatic vein (hp), branch of hepatic artery (a) and bile ducts (bd) (x250). (9): a photomicrograph of the portal area of hepatic tissue of mice treated by rabies vaccine: showing, highly increase Kupffer cells (k) with some normal architecture hepatocytes and central vein (cv) but few hepatocytes contain pyknotic nuclei (p)(x250). (10): showing highly increase Kupffer cells (k), highly widened hepatic portal vein, destructed bile ducts (bd), with lymphocytic infiltration in the portal area and HPV. (11): showing, highly dilated arterial, thickened and congested arterial (a) which contain hemolyzed blood cells, highly dilated and corrugated well of hepatic portal vein (hpv) which contain 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): showing the central area of liver tissue of mice treated by Alum adjuvanted vaccine, delaminated central and highly endothelial lining (Yellow arrow)of central vein (cv) which contain hemolyzed blood cells, some hepatocytes contain pyknotic nuclei or karyolytic nuclei (k) (x200). (13): portal area showing highly dilated hepatic portal vein (hpv) which contains hemolyzed blood cells (Red arrow) with delaminated endothelial lining (Yellow arrow), highly thickened and completely destructed wells of bile ducts (bd) with lymphocytes infiltration. (14): showing numerous degenerate changes in cytoplasm and nuclei of hepatocytes which include vacuolation (v) in cytoplasm, karyolytic nuclei (k), with highly increase Kupffer cells (x400). (15): showing highly destructed, elongated well of bile ducts, which is surrounded by numerous lymphocytes (x250). (16): showing many dystrophic changes in the portal area. These changes include highly dilated or 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 shows highly increase lymphocytes and Kupffer cells (k) with numerous pyknotic nuclei (p) or karyolytic nuclei of hepatocytes are detected (x250). (17): the central area showing corrugated (cv) which is surrounded by numerous necrotic area (n) with numerous degenerated changes in nuclei of hepatocytes and increase Kupffer cells (x250).

IV. DISCUSSION

The preponderance of infections that occur in the CNS are lethal, as it is an immune-privileged site, in which immune functions are limited [21]. Circulation in the cerebral tissue is characterized by the blood-brain barrier, which restricts diapedesis and therefore helps restrain lymphocytic infiltration and development of inflammatory responses in the brain, which would surely prejudice the integrity of functional connections between neurons [22]. Furthermore, some viruses have developed efficient mechanisms that allow them to adapt and escape the immune response. Rabies virus becomes invisible to the immune system after invading the CNS, probably consequently of apoptosis mechanisms [23]. Aluminum was first used in human vaccines and was the only adjuvant in use in licensed vaccines. Despite its extensive and continuous use, the immune mechanism of action of aluminum remains incompletely understood in addition to the adverse events of its use [24]. Aluminum adjuvants act primarily to increase antibody production and are therefore suitable for vaccines targeting primarily killed pathogens. Aluminum-adjuvanted vaccines have not been successful in preventing infection due to intracellular pathogens [25].Mostly aluminum and MPL adjuvants are used in vaccine, but these chemical adjuvants have many disadvantages, such as side effects, strong local stimulation and carcinogenesis, together with complicated preparations or failure to increase immunogenicity of weak antigen [26]. Comparative studies in humans and animals showed that aluminum is a weak adjuvant for antibody induction and induces a Th2, rather than a Th1 response [27]. In conjunction with MPL adjuvant has been evaluated and clinical trials are still under investigation, although the mechanism of action of adjuvants often remain poorly understood [28].Nevertheless, this study reported that antibodies concentration was time dependent.Regarding to pathological effect on different organs our data was matching that reported by [29] despite their examination of CNS pathological changes the detection of necrosis, gliosis and inflammatory infiltrates in parenchyma and perivascular space. The inflammatory infiltrates were mainly composed of lymphocytes and macrophages [30]. No significant pathology was observed in skin and salivary gland. No RABV antigens were detected in any specimens [31]. However, it was reported that alum alone group exhibited only small aggregates at that time. And the lymphoid tissue reaction was generally considered to represent a local manifestation of the immune response, the nonspecific reaction to aluminum adjuvant might have been augmented by the specific reaction to the toxoid adsorbed [32]. Also, the Alum showed hardly injection of aluminum caused marked necrotic changes in muscle fibers [33]. it has been suspected to occasionally cause delayed neurologic problems in susceptible individuals. Mainly, the long-term persistence of aluminic granuloma also termed macrophagic myofasciitis is associated with chronic arthromyalgias and fatigue and cognitive dysfunction [34]. Safety concerns largely depend on the long bio-persistence time inherent to this adjuvant, which may be related to its quick withdrawal from the interstitial fluid by avid cellular uptake, and the capacity of adjuvant particles to migrate and slowly accumulate in lymphoid organs and the brain [35]. Regarding the effect of Alum salt as adjuvant on liver tissueour record was in accordance to [36] recording that the aluminum treated rats showed distortion of the arrangement of parenchyma of the liver, loss of radial arrangement of sinusoids from the central vein of the liver and loss of hexagonal shape of the hepatocytes when compared with the control. Based on our histological observations, we therefore conclude that aluminum chloride exposure was detrimental to the liver of adult rats and hence caution should be taken in its usage. Our data was in accordance with [37].RATs immunized with IMT504adjuvanted rabies vaccine induceda highly effective immune response in rats, observed even after using of highly diluted rabies vaccine accompanied with protective effective vaccine potency (ED50) post challenging with CVS, as antibody titers developed faster and were significantly higher with IMT504-adjuvanted diluted vaccine vs non- adjuvanted vaccine [38]. All five administered IMT504- adjuvanted diluted vaccine reached protective antibodies ($\geq$ 0.5 IU/ml) after the second injection. After the third injection, individuals receiving IMT504-adjuvanted diluted vaccine reached levels approximately 10 times higher than controls (M ± SEM: 31.0 ± 10.9 vs 3.40 ± 0.99 IU/ml). Finally suggested that IMT504 may allow fewer inoculations, highly significant dose-sparing of vaccine, rapid antibody production and protection from rabies [39]. Furthermore, In order to study the impact of MPL adjuvanted rabies vaccine on histopathology two organs; liver and kidney are used as they are commonly studied organs by other workers as well [40]. intravenous administrations of MPL produced inflammatory changes such as decreased platelets and WBCs and increased spleen weights at the two highest doses [41]. No treatment-related histopathological changes in a full panel of tissues and organs were observed and considered a no-observed adverse-effect-level [42]. An increasing dosage-response effect and higher group means were exhibited for hemoglobin and hematocrit of the test article groups relative to controls. On the contrary our data was disagreeing with [43] recording tat however, since the magnitude of change was small and not correlated with other biochemical or histopathologic effects, no meaningful toxicological significance was attached to this observation. As well as microscopic changes were observed in the eyes, heart, kidneys, liver, lung and spleen of rats from each of the MPL treated groups which were sacrificed at study termination [44]. The changes were generally characterized by minimal to mild infiltrations of mononuclear inflammatory cells and are probably related to the pharmacologic action of the test article [45]. Similar changes were observed in

rats which died or were sacrificed moribund [46]. In addition, either edema or hemorrhage was observed in the brain and spinal cord in the dead rats or were sacrificed moribund [47]. It was recoded that kidney and liverare affected MPL adjuvanted rabies vaccine which is evident in form of pathological changes such as necrosis of hematopoietic tissue, vacuolation of tubular cells, dilation of glomerular capillaries and degeneration of epithelial cell linings [48-49]. The treated liver with MPL adjuvanted rabies exhibited tissue vacuolization, pyknotic and distorted artery wall which has been in comparison with control shows normal arrangement of hepatocytes with nucleus, bile canaliculi and sinusoid. Liver of control. showing Normal histology of liver tissue with hepatic cells, nucleus and sinusoids while liver of infected fish is marked by vacuolization of the tissue, ruptured vein and hemorrhages [50-51]. Kidney treated by MPL adjuvanted rabies showed degenerated renal tubules, pyknotic, hemorrhages and glomerular degeneration as compared to its control with normal histology showing glomerulus, renal tubules and hematopoietic tissue [52]. The control kidney showed normal renal tubules, glomerulus and hematopoietic tissue while necrosis, tissue vacuolization, congested renal tubules and hemorrhages were evident in the kidney of treated with MPL adjuvanted rabies. Such changes have been reported by other workers but from fishes inhabiting water bodies lying in Southern India [53]. Similar to the present study, in other report hepatocytes showed marked cytoplasmic vacuolization and sinusoids, in most areas were distended and central veins appeared severely damaged due to marked swelling and degeneration of the endothelial lining cells [54]. Acute toxicity impacts of hexavalent chromium on kidney of Channa punctatus showed hypertrophy of epithelial cells of renal tubules with reduced lumens, atrophy of the renal tubules, glomerular contraction in the Bowman's capsules and necrosis of hematopoietic tissues [55]. The inter-renal cells of the head kidney exhibited distinct hypertrophy and vacuolization. Thus, these changes influences fish health and therefore the population of the fish in this zone. This will ultimately influence the major protein source available to the population as well as can endanger the species surviving there [56].Cytokines as indicators and regulators of the immune network play important roles in the immune and inflammatory responses [57] Th1- favored response is primarily characterized by secretion of IFN-γ by CD4+ cells, resulting in macrophage activation, B-cell differentiation to IgG1 synthesis, and support CTLs. In the present study IFN-γ and IL-6 were monitored aiming to evaluate the effect of rabies vaccine immunization adjuvanted with alum and MPL adjuvanted and non-adjuvanted vaccine on the cellular and mucosal immune response [58]. It was reported that vaccine protection correlates with the magnitude of the IFN-γ response induced by the vaccine resulting in an accelerated pulmonary recruitment of IFN-γ-producing T cells during disease. In agreement with recorded data, the IFN-γ recall response was associated with the appearance of IL-17 secretion [59]. Although in vivo studies have shown that alum potentially induces the production of IL-1β and IL-18 from DCs and the consequent expansion and differentiation of naïve CD4+ T cells into Th2 cells and promote the production of Abs. It has been experimentally shown that IL-4 and IL-13 are not necessary for alum to enhance Th2 responses but these cytokines strongly suppress Th1 responses, thus alum's role in regulating Th2 response may be mediated via Th1 suppression [60]. Although IL-4 has been shown to direct both CD4+ and CD8+ T cells to produce Th2 cytokines in vivo. [61] reported IL-4- dependent increased level of MHCII, CD86, CD83, IL- 1α, IL-1β, tumor necrosis factor (TNF), IL-4, and IL-6 in human PBMCs which later acquired a DC morphology IL-6 mainly produced by activated Th2 cells and mast cells, acted on B cells to induce proliferation and differentiation into immunoglobulin (Ig) producing cells. IL-6 is an important cytokine for the mucosal immune system, which distinguishes it from the systemic immune compartment [62]. IL-6 was promoted found for the generation of cytotoxic T cells from thymocytes. In thymocytes IL-6 induces the expression of high affinity IL-2 receptors. In contrast to murine IL-6 also acts on B-cells. It induces the proliferation of pre-activated B-cells and their differentiation. Murine IL-6 also stimulates the production and secretion of IgM and IgA by B- cells.Also, IL-6 controls the production and functions of eosinophils and basophiles [63].

V. CONCLUSION

According to the recorded data it can be concluded thatboth alum and MPL are promising adjuvants and of a promising immune enhancing potential based on the elevated immune mediators and antibodies levels despite the drawbacks detected in different tissues due to the pro-inflammatory reactions accompanied with significant effect on the cell cycle profile post exposure to Alum and MPL adsorbed and non-absorbed vaccine candidates

VI. RECOMMENDATION

Accordingly, it is recommended to find out the role of adjuvant concentration and related safety measures. Monitoring of the IgG subtypes developed post vaccination in addition to the antioxidant level that may have a role in the tissue toxicity.

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REFERENCE

[1] Balachandran, A., & Charlton, K. (1994). Experimental rabies infection of non-nervous tissues in skunks (Mephitis mephitis) and foxes (Vulpes vulpes). Veterinary pathology, 31(1), 93-102.
[2] Barnard, R. J. O., Elleder, D., & Young, J. A. T. Avian sarcoma and leukosis virus-receptor interactions: From classical genetics to novel insights into virus–cell membrane fusion. Virology, 344(1), 2006,25-29
[3] Boonham, N., Tomlinson, J., & Mumford, R. Microarrays for rapid identification of plant viruses 1. Annu. Rev. Phytopathol., 45, 2007,307-328.
[4] Callaway, E. M. Transneuronal circuit tracing with neurotropic viruses. Current opinion in neurobiology, 18(6), 2008, 617-623.
[5] Charlton, K. M., Casey, G. A., Wandeler, A. I., &Nadin-Davis, S. Early events in rabies virus infection of the central nervous system in skunks (Mephitis mephitis). Acta neuropathologica, 91(1), 1995,89-98.
[6] Charlton, K. M., Nadin-Davis, S., Casey, G. A., &Wandeler, A. I.The long incubation period in rabies: delayed progression of infection in muscle at the site of exposure. Acta neuropathologica, 94(1), 1997,73-77.
[7] Connolly, J. D., Hashemi- Nezhad, M., & Lyon, D. C. (2012). Parallel feedback pathways in visual cortex of cats revealed through a modified rabies virus. Journal of Comparative Neurology, 520(5),2012, 988-1004.
[8] Dietzschold, B., Schnell, M., &Koprowski, H. Pathogenesis of rabies. The World of Rhabdoviruses,2005, 45-56.
[9] Dietzschold, B., Li, J., Faber, M., & Schnell, M. Concepts in the pathogenesis of rabies. Future virology, 3(5), 2008, 481-490
[10] Dukes, J. P., King, D. P., &Alexandersen, S. Novel reverse transcription loop-mediated isothermal amplification for rapid detection of foot-and-mouth disease virus. Archives of virology, 151(6), 2006,1093-1106
[11] , A. R., Johnson, N., Freuling, C. M., Wakeley, P. R., Banyard, A. C., McElhinney, L. M., et al. Emerging technologies for the detection of rabies virus: challenges and hopes in the 21st century. PLoSNegl Trop Dis, 3(9),2009, e530.
[12] Gosztonyi, G., & Ludwig, H. Interactions of viral proteins with neurotransmitter receptors may protect or destroy neurons. In the Mechanisms of Neuronal Damage in Virus Infections of the Nervous System ,2001, (pp. 121-144). Springer Berlin Heidelberg.
[13] Gurrala, R., Dastjerdi, A., Johnson, N., Nunez-Garcia, J., Grierson, S., Steinbach, F., & Banks, M. .Development of a DNA microarray for simultaneous detection and genotyping of lyssaviruses. Virus research, 144(1), 2009, 202-208
[14] Etessami, R., Conzelmann, K. K., Fadai-Ghotbi, B., Natelson, B., Tsiang, H., &Ceccaldi, P. E. Spread and pathogenic characteristics of a G-deficient rabies virus recombinant: an in vitro and in vivo study. Journal of General Virology, 81(9), 2000, 2147-2153.
[15] Hooper, D. C. The role of immune responses in the pathogenesis of rabies. Journal of neurovirology, 11(1), 2005,88-92
[16] Hughes, G. J., Smith, J. S., Hanlon, C. A., &Rupprecht, C. E. Evaluation of a TaqMan PCR assay to detect rabies virus RNA: influence of sequence variation and application to quantification of viral loads. Journal of clinical microbiology, 42(1), 2004, 299-306
[17] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry.72(1-2),2005, 248-254,
[18] Mosmann T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. Journal of lmmunological Methods, 65 (1-2),2003, 55-63, 198.
[19] Kuo HM, Chang LS, Lin YL, Lu HF, Yang JS.et al. Morin inhibits the growth of human leukemia HL-60 cells via cell cycle arrest and induction of apoptosis through mitochondria dependent pathway. Anticancer Res 27(1A), 2007,395- 406,.
[20] Jack, P. J., Amos-Ritchie, R. N., Reverter, A., Palacios, G., Quan, P. L., Jabado, O.et al. B. Microarray-based detection of viruses causing vesicular or vesicular-like lesions in livestock animals. Veterinary microbiology, 133(1), 2009,145-153.
[21] Jackson, A. C.. Rabies pathogenesis. Journal of neurovirology, 8(4), , 2002 267-269.
[22] Jackson, A. C. Rabies: new insights into pathogenesis and treatment. Current opinion in neurology, 19(3), 2006, 267-270.
[23] Jackson, A. C., Ye, H., Phelan, C. C., Ridaura-Sanz, C., Zheng, Q., Li, Z., et al. Extraneural organ involvement in human rabies. Laboratory investigation; a journal of technical methods and pathology, 79(8), 1999, 945-951.
[24] Jackson, A. C., Phelan, C. C., & Rossiter, J. P.. Infection of Bergmann glia in the cerebellum of a skunk experimentally infected with street rabies virus. Canadian Journal of Veterinary Research, 64(4), 2000,226.
[25] Jacob, Y., Badrane, H., Ceccaldi, P. E., &Tordo, N. Cytoplasmic dynein LC8 interacts with lyssavirus phosphoprotein. Journal of virology, 74(21), 2000,10217-10222.
[26] Piza, A. S. D. T., Santos, J. L. F., Chaves, L. B., & Zanetti, C. R. An ELISA suitable for the detection of rabies virus antibodies in serum samples from human vaccinated with either cell-culture vaccine or suckling-mouse-brain vaccine. Revista do Instituto de Medicina Tropical de São Paulo, 41(1), 2002, 39-43.
[27] Cameron, C. M., Barnard, B. J. H., Erasmus, E., & Botha, W. J. S. Antibody response in cattle to oil emulsion rabies and ephemeral fever vaccines, 54, 1987, 157-158,
[28] Wilde, H., &Lumlertdacha, B. Rabies Research in Resource- Poor Countries. Advances in Imaging and Electron Physics, 79, 2011,449-455.
[29] Yousaf, M. Z., Qasim, M., Zia, S., Ashfaq, U. A., & Khan, S. Rabies molecular virology, diagnosis, prevention and treatment. Virology journal, 9(1), 2012, 50.
[30] Zaytseva, N. V., Montagna, R. A., Lee, E. M., &Baeumner, A. J. Multi-analyte single-membrane biosensor for the serotype-specific detection of Dengue virus. Analytical and bioanalytical chemistry, 380(1), 2004, 46-53.
[31] Baylor, N. W., Egan, W., & Richman, P. Aluminum salts in vaccines—US perspective. Vaccine, 20, 2002, S18-S23
[32] Lindblad, E. B. Aluminium adjuvants—in retrospect and prospect. Vaccine, 22(27), 2004 ,3658-3668.
[33] Gherardi, R. K., Aouizerate, J., Cadusseau, J., Yara, S., &Authier, F. J. Aluminum adjuvants of vaccines injected into the muscle: Normal fate, pathology and associated disease. Morphologie, 100(329), 2016, 85-94.

[34] Lapointe, R., Thumbi, D. K., &Lucarotti, C. J. Recent advances in our knowledge of baculovirus molecular biology and its relevance for the registration of baculovirus-based products for insect pest population control. Integrated Pest Management and Pest Control, 2012, 481-522

[35] Khader SA, Bell GK, Pearl JE, Fountain JJ, Rangel-Moreno J, Cilley GE, Shen F, Eaton SM, Gaffen SL, Swain SL, Locksley RM, Haynes L, Randall TD, Cooper AM Nat Immunol. 8(4),2007, 369-77.

[36] Theerasurakarn and ubol. Apoptosis induction in brain during the fixed strain of rabies virus infection correlates with onset and severity of illness. J. Neurol., 4, 2015, 407-14

[37] Franchi, L., &Núñez, G. The Nlrp3 inflammasome is critical for aluminium hydroxide- mediated IL- 1β secretion but dispensable for adjuvant activity. European journal of immunology, 38(8), 2008,2085-2089.

[38] Shaw, C. A., & Petrik, M. S. Aluminum hydroxide injections lead to motor deficits and motor neuron degeneration. Journal of inorganic biochemistry, 103(11), 2009, 1555-1562.

[39] Chang, M. F., Shi, Y., Nail, S. L., HogenEsch, H., Adams, S. B., White, J. L., & Hem, S. L.. Degree of antigen adsorption in the vaccine or interstitial fluid and its effect on the antibody response in rabbits. Vaccine, 19(20), 2001,2884-2889.

[40] Eisenbarth, S. C., Colegio, O. R., O'Connor, W., Sutterwala, F. S., &Flavell, R. A. Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. Nature, 453(7198), 2008, 1122-1126

[41] Li, H., Willingham, S. B., Ting, J. P. Y., & Re, F. Cutting edge: inflammasome activation by alum and alum's adjuvant effect are mediated by NLRP3. The Journal of Immunology, 181(1),2008. 17-21.

[42] Little, S. F., Ivins, B. E., Webster, W. M., Norris, S. L. W., & Andrews, G. P. Effect of aluminum hydroxide adjuvant and formaldehyde in the formulation of rPA anthrax vaccine. Vaccine, 25(15), 2007,2771-2777.

[43] Brady, R. C., Treanor, J. J., Atmar, R. L., Keitel, W. A., Edelman, R., Chen, W. H., et al. Safety and immunogenicity of a subvirion inactivated influenza A/H5N1 vaccine with or without aluminum hydroxide among healthy elderly adults. Vaccine, 27(37), 2009, 5091-5095

[44] Kass, P. H., Spangler, W. L., Hendrick, M. J., McGill, L. D., Esplin, D. G., Lester, S., et al. Multicenter case-control study of risk factors associated with development of vaccine-associated sarcomas in cats. Journal of the American Veterinary Medical Association, 223(9), 2008, 1283-1292.

[45] Hammad, H., Plantinga, M., Deswarte, K., Pouliot, P., Willart, M. A., Kool, M., et al. Inflammatory dendritic cells—not basophils—are necessary and sufficient for induction of Th2 immunity to inhaled house dust mite allergen. Journal of Experimental Medicine, 207(10), 2010,2097-2111.

[46] Takaki, A., Yasunaka, T., & Yagi, T. Molecular mechanisms to control post-transplantation hepatitis B recurrence. International journal of molecular sciences, 16(8), 2015,17494-17513.

[47] Jefferson, T., Rudin, M., & Di Pietrantonj, C. Adverse events after immunisation with aluminium-containing DTP vaccines: systematic review of the evidence. The Lancet infectious diseases, 4(2), 2004, 84-90.

[48] Kool, M., Soullié, T., van Nimwegen, M., Willart, M. A., Muskens, F., Jung, S et al. N. Alum adjuvant boosts adaptive immunity by inducing uric acid and activating inflammatory dendritic cells. Journal of Experimental Medicine, 205(4), 2008,869-882

[49] Joffre, O. P., Segura, E., Savina, A., & Amigorena, S. Cross-presentation by dendritic cells. Nature Reviews Immunology, 12(8), 200, 557-569.

[50] Johnston, C. T., Wang, S. L., & Hem, S. L. Measuring the surface area of aluminum hydroxide adjuvant. Journal of pharmaceutical sciences, 91(7), 2002, 1702-1706.

[51] Johnston, R. J., Poholek, A. C., DiToro, D., Yusuf, I., Eto, D., Barnett, B., et al. (2009). Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. Science, 325(5943), 1006-1010.

[52] Hjorth, M., Axelsson, S., Rydén, A., Faresjö, M., Ludvigsson, J., & Casas, R. GAD-alum treatment induces GAD 65-specific CD4+ CD25 high FOXP3+ cells in type 1 diabetic patients. Clinical immunology, 138(1), 2011, 117-126.

[53] Miret, J., Nascimento, E., Sampaio, W., França, J. C., Fujiwara, R. T., Vale, A., et al.. Evaluation of an immunochemotherapeutic protocol constituted of N-methyl meglumine antimoniate (Glucantime®) and the recombinant Leish-110f®+ MPL-SE® vaccine to treat canine visceral leishmaniasis. Vaccine, 26(12), 2008,1585-1594.

[54] Trigo, J., Abbehusen, M., Netto, E. M., Nakatani, M., Pedral-Sampaio, G., de Jesus, R. S., et al. Treatment of canine visceral leishmaniasis by the vaccine Leish-111f+ MPL-SE. Vaccine, 28(19), 2010, 3333-3340

[55] Caldas, A., Favali, C., Aquino, D., Vinhas, V., Van Weyenbergh, J., Brodskyn, C., et al. Balance of IL-10 and interferon-γ plasma levels in human visceral leishmaniasis: implications in the pathogenesis. BMC infectious diseases, 5(1), 2005,113.

[56] Giannini, S. L., Hanon, E., Moris, P., Van Mechelen, M., Morel, S., Dessy, F., et al. Enhanced humoral and memory B cellular immunity using HPV16/18 L1 VLP vaccine formulated with the MPL/aluminium salt combination (AS04) compared to aluminium salt only. Vaccine, 24(33), ,2006 5937-5949.

[57] Garçon, N., & Van Mechelen, M. Recent clinical experience with vaccines using MPL-and QS-21-containing adjuvant systems. Expert review of vaccines, 10(4), 2011,471-486

[58] Kannanganat, S., Ibegbu, C., Chennareddi, L., Robinson, H. L., & Amara, R. R. Multiple-cytokine-producing antiviral CD4 T cells are functionally superior to single-cytokine-producing cells. Journal of virology, 81(16), 2007,8468-8476.

[59] Thakur, A., Pedersen, L. E., &Jungersen, G. Immune markers and correlates of protection for vaccine induced immune responses. Vaccine, 30(33), 2012, 4907-4920.

[60] Sun, P., Schwenk, R., White, K., Stoute, J. A., Cohen, J., Ballou, W. R., et al. Protective immunity induced with malaria vaccine, RTS, S, is linked to Plasmodium falciparum circumsporozoite protein-specific CD4+ and CD8+ T cells producing IFN-γ. The Journal of Immunology, 171(12),2003, 6961-6967.

[61] Evans, J. T., Cluff, C. W., Johnson, D. A., Lacy, M. J., Persing, D. H., & Baldridge, J. R. Enhancement of antigen-specific immunity via the TLR4 ligands MPL™ adjuvant and Ribi. 529. Expert review of vaccines, 2(2), 2003, 219-229

[62] Johnson, L. L., & Sayles, P. C. Deficient humoral responses underlie susceptibility to Toxoplasma gondii in CD4-deficient mice. Infection and immunity, 70(1), 2002, 185-191.

[63] Lourenço, E. V., Bernardes, E. S., Silva, N. M., Mineo, J. R., Panunto-Castelo, A., & Roque-Barreira, M. C. Immunization with MIC1 and MIC4 induces protective immunity against Toxoplasma gondii. Microbes and infection, 8(5), 2006,1244-1251.

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