



IMMUNOHISTOCHEMICAL AND HISTOLOGICAL CHANGES IN THE SPLEEN INDUCED BY GOLD NANOPARTICLES AS ALTERNATIVE ADJUVANT AGAINST RIFT VALLY FEVER VIRUS

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ABSTRACT

Nanoparticles considered as promising tools because of its high applicability. Recently, nanoparticles are evaluated for their capability to increase immune responses as an adjuvant. In the present study different concentrations of spherical (S) and rod (R) gold nanoparticles (GNPs) were subcutaneously injected into rats as a vaccineadjuvant against rift valley fever virus (RVFV) to demonstrate the histopathological and immunohistochemical changes in their spleen tissues. Although aluminum (Alum) salt is renowned for its ability to induce cellular memory as well as generating long-term protective immunity. Our findings suggested that GNPs resulted in a greater total antigen-specific antibody response which is ideal for producing a vaccine capable of long-term protective immunity. Our results emphasized that nanorod appears to adhere more effectively than nanosphere, allowing for drug targeting to a specific type of cells.

KEYWORDS: Rift Valley fever virus, gold nanoparticles, adjuvants, rats.

INTRODUCTION

Rift Valley fever virus (RVFV) is a mosquito-borne zoonotic pathogen causing disease outbreaks in Africa and the Arabian Peninsula^[1]. A vaccine is a biological preparation that improves immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing microorganism and is often made from weakened or killed forms of the microbe, its toxins or one of its surface proteins. This agent stimulates the body's immune system to recognize the foreign agent, destroy it, and "remember" it so that the immune system can more easily recognize and destroy any of these microorganisms that may encounters later^[2]. Vaccines do not usually show good immunogenic properties similar to native microorganisms because of either chemical modifications or usage of none whole live microorganism^[3].

Adjuvants can be used for multiple purposes: to enhance immunogenicity, provide antigen-dosesparing, to accelerate and evoke the immune response and reduce the need for booster immunizations. They also increase the duration of protection and improve efficacy in poor responder populations^[4]. The optimally formulated adjuvant must be safe, stable before administration, readily biodegraded and eliminated, able to promote an antigen-specific immune response, inexpensive to

produce, and easy to use^[5]. Aluminum (Alum)salt is a dominant kind of adjuvants used, but it has limited capacity to generate the cellular immune response required for vaccines against cancers and viral diseases. It is desirable to develop new and efficient adjuvants with the aim of improving the cellular immune response against the antigen. As nanotechnology increasingly plays a significant role in vaccine development, the use of nanoparticles in vaccine formulations may allows improved antigen stability and immunogenicity, targeted delivery and slow release. Gold nanoparticles can be used as a new immune adjuvant to improve immune responses^[6]. The spleen is thought to be central in regulating the immune system and a metabolic asset that involved in the endocrine function^[7]. As the spleen is organized into the red pulp and white pulp, that are separated by the marginal zone (MZ)^[8]. The splenic red pulp contains macrophages that serve mostly to filter blood and recycle iron from aging red blood cells while the white pulp that is structurally similar to lymph node and contains T-cell and B-cell zones. The structural organization and multicellular composition of the spleen also permit monitoring of most of the blood in the red pulp and MZ. Diverse splenic populations not only trap and remove blood-borne antigens but also initiate innate and adaptive immune responses against pathogens. Additionally, the spleen is a site where immune

responses that are deleterious to the host can be regulated through several subsets of cells^[9]. Immunohistochemistry (IHC) refers to the process of detecting antigens (e.g. proteins) in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues^[10]. It is widely used in basic research to understand the distribution and localization of biomarkers and differentially expressed proteins in different parts of a biological tissue. Proliferating cell nuclear antigen (PCNA) plays an essential role in nucleic acid metabolism as a component of the replication and repair machinery. In this study, the ability of GNPs as an adjuvant to evoke the immune response against RVFV was investigated and the immunohistochemical and histopathological changes that induced in the rat splenic tissues were determined.

MATERIALS AND METHODS

Preparation of adjuvants

Sphere and rod gold nanoparticles adjuvants

Sphere and rod gold nanoparticles were purchased as 2 Nm from Nano Tech. company.

Aluminum phosphate (Alum) adjuvant

Aluminum phosphate (Alum) was prepared in 40 ml normal saline according to^[11].

Animals

Male albino rats; were supplied from Theodore Bilharz Research Institute, Giza, Cairo. Body weights of rats ranged from 75-80 gm. that were housed in metallic cages. All rats were housed in VACCERA and kept under good conditions of light and temperature, allowed free access tap water and dry feed diet. Animals were kept under observation for about one week before the onset of the experiment for adaptation. Care and cleaning were important for maintaining the animals in a normal healthy state. Animals used in the present experiment were divided randomly into seven groups (ten rats in each group)

Group (1): served as the negative control group.

Other sex groups were immunized subcutaneously by 0.5 ml RVFV/rat as:

Group (2): RVF vaccine only

Group (3): RVF vaccine conjugated with alum phosphate

Group (4): RVF vaccine conjugated with 20 Nm SGNP

Group (5): RVF vaccine conjugated with 40 Nm SGNP

Group (6): RVF vaccine conjugated with 20 Nm RGNP

Group (7): RVF vaccine conjugated with 40 Nm RGNP

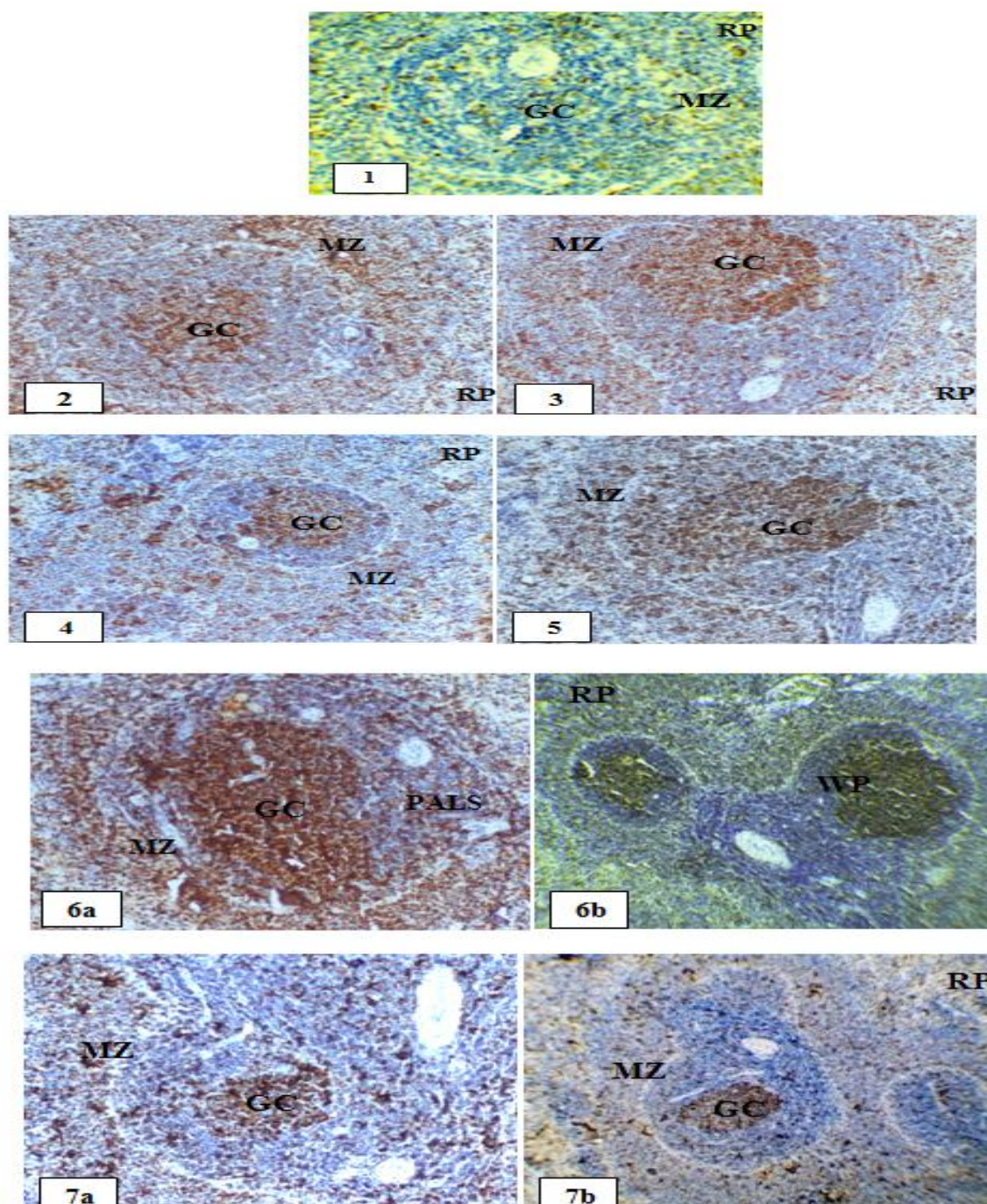
Immunohistochemical and histological Studies

Fresh specimens of spleen were taken from the control and treated groups. The specimens were fixed in 10% neutral buffer formol for the histological and immunohistochemical 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 the method of^[12] and PCNA stain for immunohistochemical studies.

RESULTS

Immunohistochemical changes in rat splenic tissue

Immune reaction of splenic rat tissue was observed for non adjuvanated (Vac) and adjuvanated groups (Alum and R&SGNPs) after 60 days of treatment using PCNA. Mean while a negative immune reaction in the rat splenic tissue was recorded in the control group (Fig. 1). The rat splenic tissue of non adjuvanated (vac) group showed moderate immune reaction (++ve) (Fig. 2), while adense positive immune reaction (+++ve) was observed in the vac+alum group and the lymphocytes were showed in the red pulp (Fig. 3). The 20 &40 SGNP groups showed a dense immune reaction (+++ve) in the white pulp that slightly extend in the red pulp (Fig. 4&5). Rat splenic tissue of 20 RGNP group showed heavy dense immune reaction (++++ve) accompanied with many lymphocytes scattered in the red pulp (Fig. 6a). Also there was connected white pulps appered in this treated group (Fig. 6b). However a dense immune reaction (+++ve) was appeared in both of white and red pulps of the 40 nM RGNP group (Fig. 7a&b).



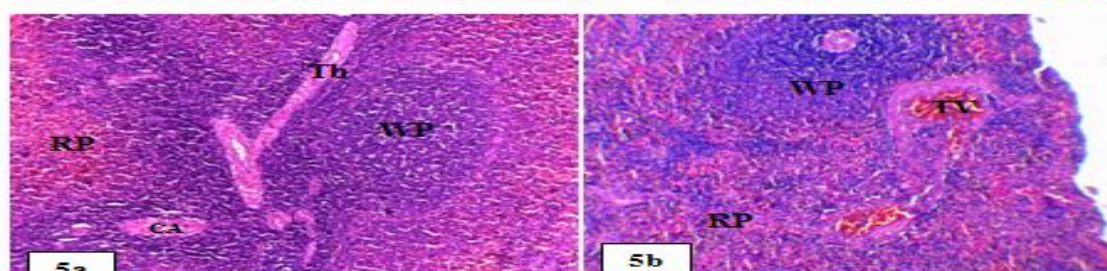
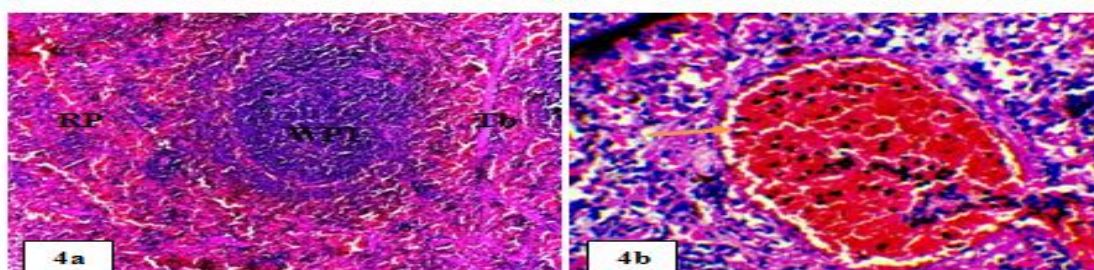
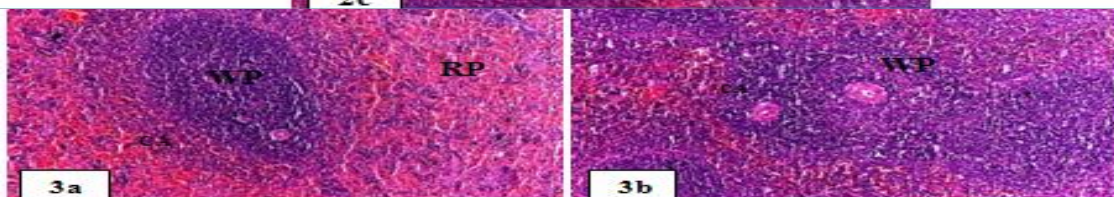
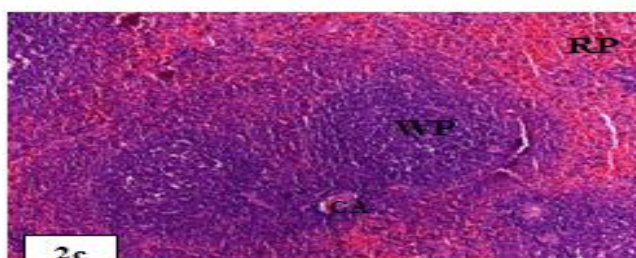
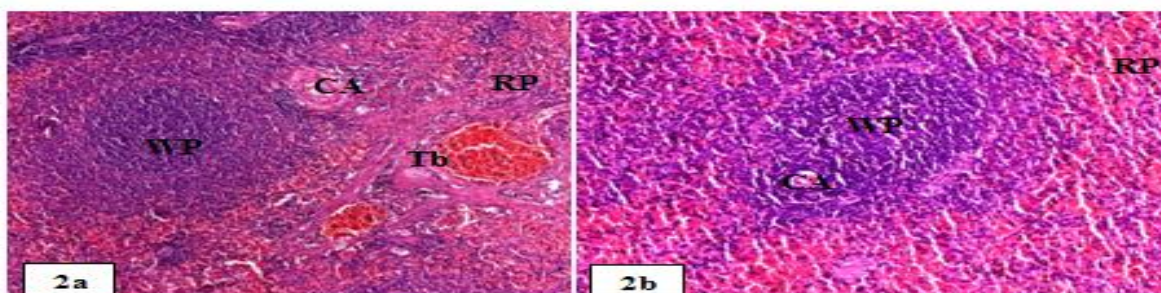
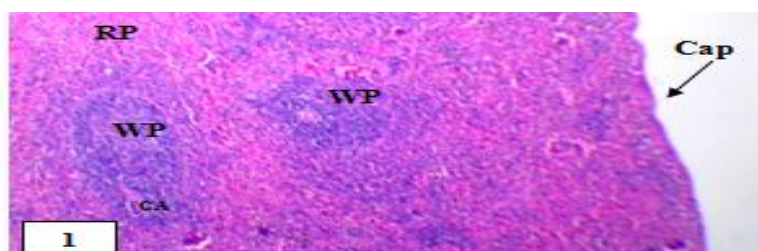
Histological changes in rat splenic tissue

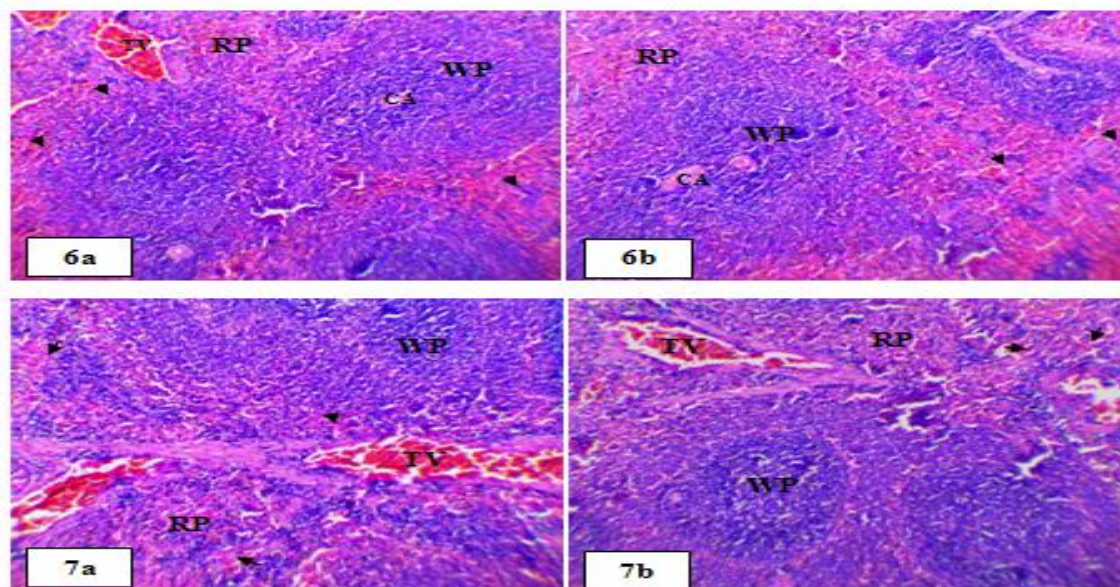
Hematoxylin and eosin stain was used for studying the splenic tissues of rats in different groups. As compared to the negative control group showed distinct red pulps, capsule surrounds the splenic tissue, spleen follicles, and clear white pulps with central arteries inside it (Fig 1). Splenic tissue of rats of the non adjuvanted group (Vac) showed a highly enlarged and thickened trabeculae which contain highly dilated and congested trabecular veins, highly thickened wall of central arteries with narrow lumen. Highly increasing and proliferation of lymphocytes comparing to negative control group were noticed in the white pulps which connected with each other while the blood sinuses in the red pulps were

highly dilated and congested (Figs. 2a, b, c). The Alum adjuvanted group showed highly thickened walls of the central arteries with narrow lumens while some white pulps showed increased proliferation of lymphocytes inside them (Figs. 3a&b). Gold adjuvanted groups showed highly disturbed arrangements of lymphocytes around the central arteries. Highly congested blood sinuses in the red pulps accompanied with branched white pulps and highly increased proliferation of lymphocytes. Additionally, some lymphocytes invaded the red pulps. Splenic tissue of rats of the 20&40SGNP adjuvanted group showed the central arteries with highly thickened walls and narrow lumens with increased lymphocytes in the white pulp. Congested

pools of blood sinuses and congested trabecular vein which contains numerous hemosiderin granules and hemolyzed blood cells were also noticed in the red pulps (Figs. 4a&b, Figs. 7a&b). Splenic rat tissue of the 20&40 Nm RGNP adjuvanted group showed elongated, corrugated, dilated and congested trabecular veins with thickened trabeculae. Connected white pulps with highly

increased proliferation of lymphocytes were also observed. In the red pulps, congested pools of blood sinuses and highly thickened walls of the central arteries with highly narrowed lumens in the white pulps were noticed (Figs. 5a&b, Figs. 6a&b). In addition, numerous lymphocytes invade the red pulp in 40 RGNP group was recorded.





DISCUSSION

In the present study, GNPs were evaluated with various shapes and concentrations as an alternative adjuvant against RVFV. In the current work, the splenic rat tissues of Vac, Alum and S&RGNP groups showed many immunohistochemical changes compared to negative control group. Immune reaction in the spleen tissue using PCNA showed moderate immune reaction in Vac group, dense immune reaction in Alum and SGNPs groups and heavy dense immune reaction in RGNPs groups. This is due to the elevation of cytokines in all treated groups as^[13] who confirmed that the spleen develops and produces mature immune cells that are capable of identifying and destroying pathogens. As immune reaction in SGNP groups (20&40 nM) was observed in white pulp, it was appeared in both white and red pulps in the case of RGNP groups (20&40 nM). This may be due to that, the nanorods targeted to tissues as fast as nanospheres as proved by^[14] who confirmed that nanorod appears to adhere more effectively than nanosphere, allowing for drug targeting to a specific type of cells. It was observed that, Alum. sphere and rod GNP groups showed dense immune reaction compared to negative control and non adjuvanted group, these results are in agreement with^[15] who mentioned that adjuvants reduce the amount of antigen, accelerate the immune response and increase cross-protection. In the current study the splenic tissues of GNPs showed connected white pulps with increased proliferation of lymphocytes that come in agreement with^[6], who proved that, the treatment with NPs on splenic rat tissues linked to lymphocyte proliferation. As the exposure dose of gold was increased, the B- and T-lymphocyte proliferation stimulated was increased after 60 days. This is due to the using of gold nanorods that deliver an innate immune activator and stimulate antiviral responses of the host innate immune system as suggested by^{[16],[17]} demonstrated that the distribution of GNP is size-dependent, the smallest particles showing the most

widespread organ distribution including blood, heart, lungs, liver, spleen, kidney, thymus, brain, and reproductive organs.^[18] concluded that, small GNPs (25-nm GNPs) have a wider organ distribution than that of larger gold nanoparticles, with the liver and spleen being the dominant target organs.

The present study revealed that, the splenic rat tissues of Vac, Alum and GNP-treated groups showed many histopathological changes as compared to the control. These changes include increased number of connected white pulps with increased proliferation of lymphocytes and highly thickened wall of central arteries with narrow lumen. This is in agreement with^[19] who reported that the spleen from 8 to 37 nm GNP-treated mice showed various degrees of abnormality, as a significant aberration of white pulp of the spleens. However the white pulp normally consists of aggregates of lymphoid tissue and is responsible for the immunological function of the spleen, it showed a diffused splenic nodules in the experimental group. These abnormalities in the spleen may have been a consequence of direct contact with the invading GNPs, that may be transported through blood veins or through diffusion into the spleen.^[20] concluded that, spleen treated with Alum phosphate after 24 hr, 72 hr, 1 week, 4 weeks and 8 weeks showed histopathological changes as lymphocytic depletion associated with appearance of multiple megakaryocytes.^[21] study observed a dense necrosis of lymphocytes in the white pulp in association with congestion of the capillary sinuses in the red pulp. While the study of^[22] noticed that an intense congestion of red pulps and widened sinusoids.

CONCLUSION

This study showed that GNP can be used as an adjuvant against RVFV can induce an immune response after one dose. GNP showed immunohistochemical and histopathological changes.

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