Lymph node histopathological studies in a combined adjuvant–collagen induced arthritis model in albino rat *Rattus rattus*

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Abstract   There are at least 16 types of collagen. Antibodies, to collagens, in particular to types I and II have been reported in a number of autoimmune diseases. In this study, we used rat and bovine collagens to study the effects of anti-collagen antibodies on the lymphoid organs of rats His-tological examination of the lymph node sections showed that immunization with different collagen types combined with the adjuvant used in this study induced arthritis and immune reaction in lymph nodes. The degrees of the disease symptoms and immune reaction depended on the foreign-ness of the stimulating collagen and on whether it was accompanied with adjuvant. Anti-collagen antibodies led to a decrease in the thickness of the lymph node cortex and atrophy, with medullary hyperplasia, small granulomas appeared in the section. Immunizing with either bovine collagen or BSA has shown a remarkable distortion in architecture appeared, follicular hyperplasia and reactive inflammatory hyperplasia of the LN. Passive transfer of rat anti-rat collagen antibodies either accompanied with Freund's adjuvant or not with a lesser degree of severity, resulted in epithelioid cells forming granuloma surrounded by collar of lymphocytes, scattered apoptotic bodies, lympha-tic sinus ectasia accompanied with lymphoid follicles atrophy. The results of our study present a reliable model for collagen induced arthritis which is suitable for the investigation of the mechanism of disease induction and treatment approaches.

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Introduction

It is generally assumed that the main job of the immune system is to distinguish between what is self and non self. Once the distinction has been made, self is preserved and non self is destroyed. At the most general level, this is true, and human beings remain alive and healthy only because it is so. It has become clear; how-ever, that at the finer level of detail the distinction between self and other is not absolute (Rose and Mackay, 2006).

The immune system is made up of a network of cells, tissues, and organs that work together to protect the body against infectious organisms and other invaders. Through a series of events called the immune response, the immune system attacks organisms and substances that invade body systems and cause disease. The cells of the immune system are organized into specific structures called central lymphoid tissues which include bone marrow, thymus, and peripheral lymphoid tissues which include lymph nodes, spleen, and mucosa-associated lymphoid tissues (Schindler, 1991).

In both cell-mediated and humoral responses, the recognition of antigen by receptors present on the lymphocyte surface promotes changes in the morphology of the lymphocyte (Daugherty and Hansson, 2000). A humoral immune response takes place when the antigenic determinant is recognized by B-lymphocytes which differentiate to plasma cells upon their activation. B cells have the capacity to synthesize and secrete antibodies (immunoglobulin) that are specific for and complex with the antigenic determinant (Kathryn et al., 2007). Upon recognition of the antigen and induction of humoral immune response, germinal centers (GCs) are formed within LNs. Histologically; GCs describe microscopically distinguishable parts in lymphoid tissues. GCs appear pale in staining because most of their cells are large and their cytoplasm is abundant and pale in staining. It contains large lymphocytes, a large number of medium sized lymphocytes, lymphoblasts, plasma cells and macrophages. All these cells are anchored in a network from the dendrites of the follicular dendritic cells interacting with antigen-reactive B cells (Meyer-Hermann, 2002).

The GC reaction is a complex process that requires at least three cellular components: follicular dendritic cells (FDCs), antigen specific helper T lymphocytes, and B lymphocytes (Kelsoe, 1995, 2008). The GC forms a specialized micro-environment that participates in the proliferation and differentiation of B lymphocytes (Klaus et al., 1980; MacLennan and Gray, 1986; Tew et al., 1989). The major constituents of GCs are activated B lymphocytes and follicular dendritic cells (FDCs) with some macrophages and T-lymphocytes (Butcher et al., 1982).

Collagens are the most abundant proteins found in the animal kingdom. It is the major protein comprising the extracellular matrix (ECM). There are at least 30 different collagen genes dispersed through the human genome. These 30 genes express proteins that combine in a variety of ways to create over 20 different types of collagen fibrils. Types I, II and III are the most abundant and form fibrils of similar structure. Type IV collagen forms a two-dimensional reticulum and is a major component of the basal lamina. Collagens are mainly synthesized by fibroblasts with some contribution from epithelial cells (Layman et al., 1971; Paz Mercedes and Gallop, 2010; Killen and Striker, 1979).

Collagen is a primary component of the connective tissue located in the dermis, the tough inner layer of the skin. This kind of connective tissue is also found in mucous membranes, nerves, blood vessels, and organs. Collagen in these structures imparts strength, support, and a certain amount of elasticity. Collagen is also a component of a kind of connective tissue that surrounds organs. This connective tissue encases and protects delicate organs like the kidneys and spleen. Collagens also, are widely employed in the construction of artificial skin substitutes used in the management of severe burns (Liu et al., 2002). However, this treatment is controversial since many people develop allergic reactions to collagen (Askari et al., 2011; Tang et al., 2011).

Antibodies to native and denatured collagens, in particular to types I and II, have been reported in a number of diseases including ankylosing spondylitis, emphysema, gout, juvenile chronic arthritis, lepromatous leprosy, osteoarthritis, paget's disease, psoriatic arthritis, relapsing polychondritis, rheumatoid arthritis (RA), scleroderma, systemic lupus erythmatosus, and traumatic synovitis (Clague et al., 1981; Ebringer et al., 1981; Trentham, 1981; Stuart et al., 1983; Morgan et al., 1987; Choi et al., 1988; Charriere et al., 1988).

Collagen-induced arthritis (CIA) is an experimental auto-immune disease and the most widely used mouse model for human rheumatoid arthritis (RA) (Luross and Williams, 2001; Myers et al., 1997). CIA can be elicited in susceptible strains of rodents (rat and mouse) and non-human primates by immunization with type II collagen, the major constituent protein of articular cartilage. Following immunization, these animals develop an autoimmune polyarthritis that shares several clinical and histological features with RA. Susceptibility to CIA in rodents is linked to the class II molecules of the major histocompatibility complex (MHC). The immune response to collagen II are characterized by both the stimulation of collagen-specific T cells and the production of high titers of antibody specific for both the immunogenic collagen whether it is autologous or heterologous (Williams, 2007).

CIA is an experimental auto-immune model that provides the opportunity to investigate the role of a defined auto-antigen in the disease induction and progression. Both T-cell and B-cell mediated immune responses have been implicated as having important roles in the pathogenesis of CIA (Stuart and Dixon, 1983; Holmdahl et al., 1985b). The induction of collagen arthritis as well as the induction of an antibody response toward type II collagen is intimately associated with the expression of certain class II MHC genes (Wooley et al., 1981; Holmdahl and Hansen, 1986a). These suggest that auto-antibodies related to type II collagen may play an important role in immunoregulation and, in this context, may be an example of dysregulation in the control of pathogenic autoimmunity. With this regard, it was possible to induce arthritis after immunization with autologous type II collagen (Trentham et al., 1977; Holmdahl et al., 1985a). Furthermore, both B cells and T cells from arthritis-susceptible strains can be activated with autologous type II collagen (Holmdahl et al., 1985a, b). In this work, we aimed to study the effect of anti-collagen immune response toward autologous and heterologous collagens on some lymphoid tissues in rats.

Materials and methods

Animals

Inbred Male and female albino rats of Charles River strain (*Rattus rattus*) obtained from the "animal house" of Asyut University, Egypt, were used just after suckling period (8 weeks old). Animals were kept in rat cages in the animal house at room temperature for about two weeks to reach their optimal conditions of weight and maturity. The humidity was adjusted and the animal house was made sure to be infection free. Food and drinking water were provided *ad libitum*. Animal care and use were inspected and approved by the department committee.

Antigens

Rats were sacrificed, and their tails were washed in distilled water and the skin was removed completely, and then the longitudinal tendons were extracted from rat tails. The extraction of rat tail collagen (Q collagen) and solution fabrication was run according to Habermehi (2005). For extraction of bovine collagen, the same method as for rat tail collagen extraction was applied but with using tendon of Achilles of bovine instead of the rat tail. A card with pertinent experimental information was fastened to the outside of the cage where the animals were housed.

Adjuvant preparation

Crude lipopolysaccharides (LPS) of *Pseudomonos* preparation was run according to Apicella (2008) and Al-Hendy et al. (1991). Exactly 8.5 ml of mineral oil, 1.5 ml Arlacel A, and 50 mg LPS were mixed thoroughly, the mixture was autoclaved for 15 min at 15 Lbs.

Antigen-adjuvant emulsion preparation

Sterile solutions of antigen were prepared by filtration through a Millipore filter. 10 ml of 10% potassium alum was added to 25 ml of 1% solution of antigen drop wise with stirring. The pH of the mixture was adjusted to be 6.5 by drop wise addition, with stirring, using 1 N NaOH and was let to stand for 30 min. The suspension was centrifuged at about 1000g, at about 5 °C for 10 min. The supernatant fluid was decanted and saved. More than 99% of the antigen was adsorbed to alum by estimating the amount of protein remaining in the supernatant fluid according to the method of Henry (1964). The precipitate was washed twice by gentle inversion in about 50 ml of saline and recovered each time by centrifugation. The washed precipitate was re-suspended in 25 ml of saline and stored at 4 °C. Of alum-precipitated antigen 10 ml was drawn into 10 ml syringe and added in aliquots of 1–1.5 ml to the mixture prepared in the 1st step (optimal emulsification is obtained using equal volumes of antigen solution and adjuvant (Fischel et al., 1952; Munoz 1964; Williams and Chase 1967). The mixture was emulsified thoroughly after each addition of the antigen by ejection of the emulsion drawn repeatedly into a 20 ml syringe with a 13 gauge needle. The emulsion was made just prior to use.

Preparation of anti-sera and sera collection

This study was designed to include two treatment conditions. The first treatment condition is based on immunization of rats with the antigen (collagen, or BSA) combined with adjuvant. The collagen induced arthritis is referred as CIA. The second treatment condition was based on passive transfer of rat sera including anti-collagen antibodies which is referred as antibody induced arthritis (AIA). The first treatment condition included about 18 mature albino rats their weight ranged from 80 to 120 g, were divided into three groups of six rats each. Group (A1), was immunized with one ml of the emulsion of the rat collagen combined with adjuvant. Group (A2), received an immunization with one ml of the emulsion of bovine collagen combined with adjuvant. Group (A3), was injected with one ml of the emulsion of bovine serum albumin (BSA)

combined with adjuvant, this group served as negative control group to collagen. The three groups were injected twice, two weeks apart. Sera were collected from each group after 5 days of the 2nd immunization and then stored at −20 °C.

During the first condition of the experiment; Animals were immunized twice; the first immunization was induced with one ml of emulsion as 0.1 ml of emulsion for each footpad, 0.1 ml for each side of the neck and 0.4 ml for intraperitoneal injection. The second shot was run with 0.7 ml of emulsion as 0.1 ml for each footpad, 0.1 ml for each side of the neck and 0.1 ml for intraperitoneal injection. In the second condition of the experiment; the dose (sera of first condition animals) was divided into first shot and second shot two weeks apart. The same doses as the first condition were applied. Four days later, the animals were immunized with one ml of adjuvant only.

In the second treatment conditions rats were divided into five groups, of 6 rats each. Group (B1) rats were injected with one ml of serum containing anti-rat's collagen Abs (obtained from the serum of group A1 in the first treatment condition). After two weeks, rats received 0.7 ml of serum as a boost dose without immunization with adjuvant. After four days of the 2nd antibody treatment, the rats were immunized with adjuvant only.

Group (B2) was treated as group B1 but without immunization with adjuvant. Group (B3) was injected twice, 1 week apart, with one ml of serum containing anti-bovine's collagen Abs (serum of group A2). Rats received 0.7 ml of serum as a boost dose then immunized with one ml of adjuvant four days later. Group (B4) Rats was injected as in group 3 but without immunization with adjuvant. Group (B5) was injected twice, 1 week apart; with serum of anti-bovine serum albumin (BSA) antibodies (obtained from group A3 in the 1st treatment condition) followed four days later by immunization with the adjuvant only.

At the end of the experiment the animals were fastened for 12 h prior to bleeding to reduce plasma lipid. Blood was collected from the jugular vein in the neck where a thick needle was inserted into the neck at the position of the vein; the blood was allowed to drip into clean tubes. The tube was kept on ice for five minutes to allow slow blood coagulation. The serum was collected, pooled together for each group and kept at −20 °C until use.

Histological preparations

The animals were narcotized, after the determined period of treatment, the animal under anesthesia was dissected, the cervical, auxiliary, brachial, inguinal, femoral and mesenteric lymph nodes, and spleen, were collected and prepared for histological examination. All the chosen lymph nodes are located in the lymph drainage route. The organs were washed in saline solution and run through the routine histological H&E technique of Drury and Wallington (1980).

Results

Animal's general health conditions and internal organs during dissection

Control group rats looked fine with normal vital activities after injection where there were no signs of illness or swelling and redness in the sites of injection. At the time of animal

dissection the lymph nodes, liver and spleen were of normal size and shape. The rats of other groups after injection either with emulsion containing antigen in the 1st condition or the passive transfer of anti collagen antibodies in the 2nd condition, were not looking well. They appeared weak and exhausted especially the groups that were immunized with either collagen or BSA (groups of the first condition). Once they were injected, swelling and redness of the injected joints started (Fig. 1a and b), and the movement of rats decreased, gradually swelling and redness increased and movement decreased until about five days. After five days the swelling was ameliorated and the movement was improved. The redness remained for about 15 days after injection. The inflammation of joint disappeared completely after about 38 or 40 days after injection. The groups that received a passive transfer with the serum containing antibodies (AIA) appeared better than the groups immunized with either collagen (CIA) or BSA. After about half an hour from injection, the joints swelled and became blue in color; the rats scarcely could move and appeared tired. After one week from the first injection, the inflammation of joints improved and completely disappeared after 10 days. In all groups, at the sites of injection (neck), there were granulomas (Fig. 1c); with a half ball shape pointing internally from the dermis. The lymph nodes generally were enlarged compared to control groups, but those of the groups immunized with collagen were less tangible than of the groups treated with passive transfer of serum containing antibodies (the second condition groups).

Histological examination of the lymph node section of the control group is shown in (Fig. 2), the cortex contained a num-
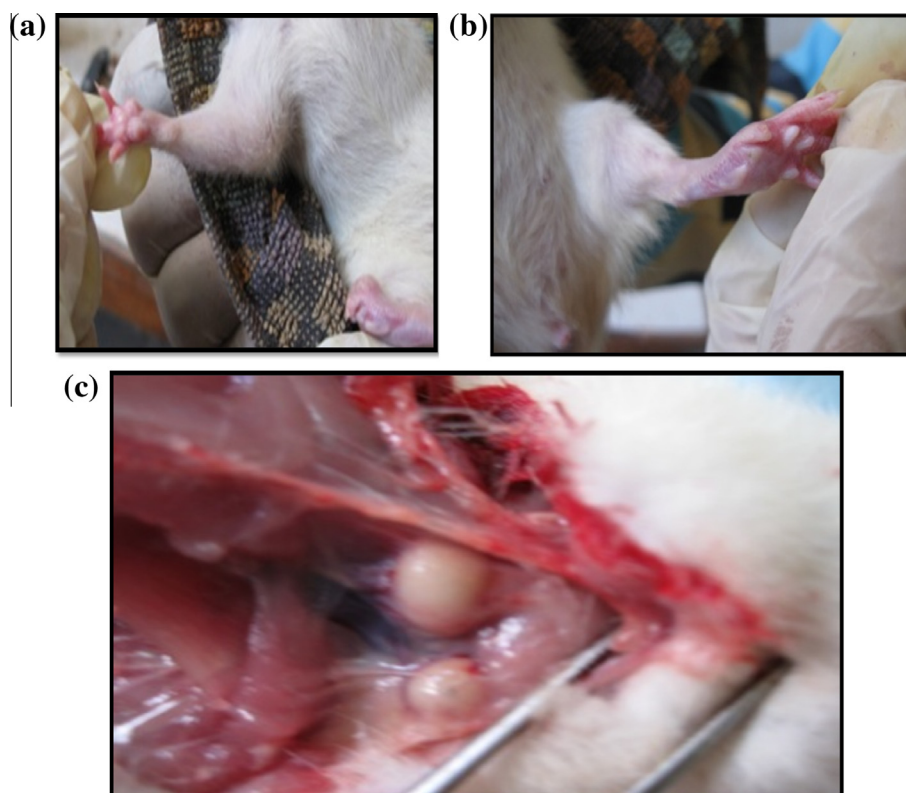

Figure 1 (a) A photograph showing the inflammation of fore limb. (b) A photograph showing the inflammation of hind limb. (c) A photograph showing the granulomas at the injection site (neck).
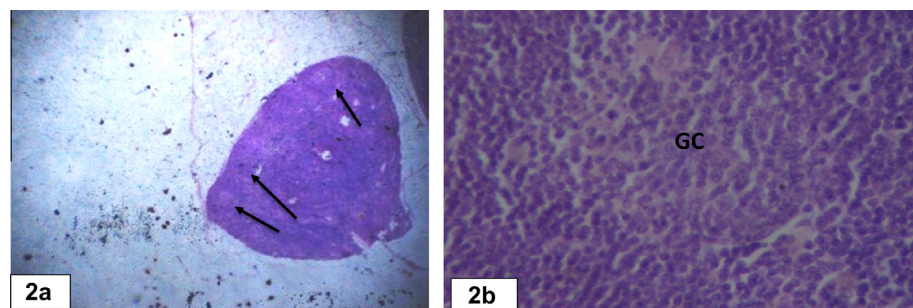

Figure 2 Photomicrographs of lymph node section of control group: (a) low microscopic power showing the normal lymph node architecture with primary and secondary lymphoid follicles (arrows) below the capsule in the cortex, the medulla appear in the center (4×), (b) higher magnification of a secondary lymphoid follicle containing germinal center (GC) (pale staining area) (40×) (H & E).
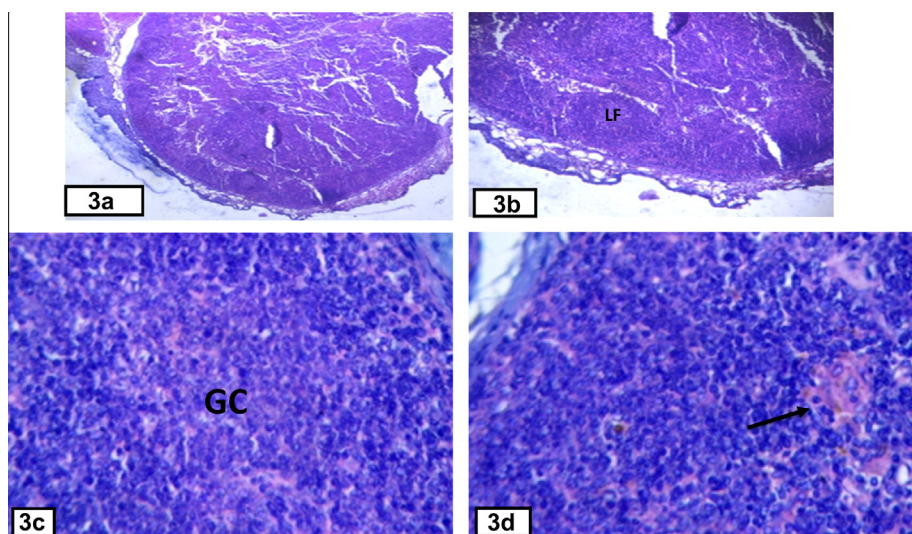
Figure 3 Photomicrograph of lymph node section of group A1 (1st condition of the experiment): (a) showing the decrease in the thickness of the cortex (lymph node depletion or atrophy) with hyperplasia (4×). (b) Showing lymphoid follicle (LF) containing germinal center (GC) (10×). (c) Higher magnification showing the germinal center (GC) (40×) and (d) High magnification showing small granulomas in the lymph node section (arrow) (40×) (H & E).
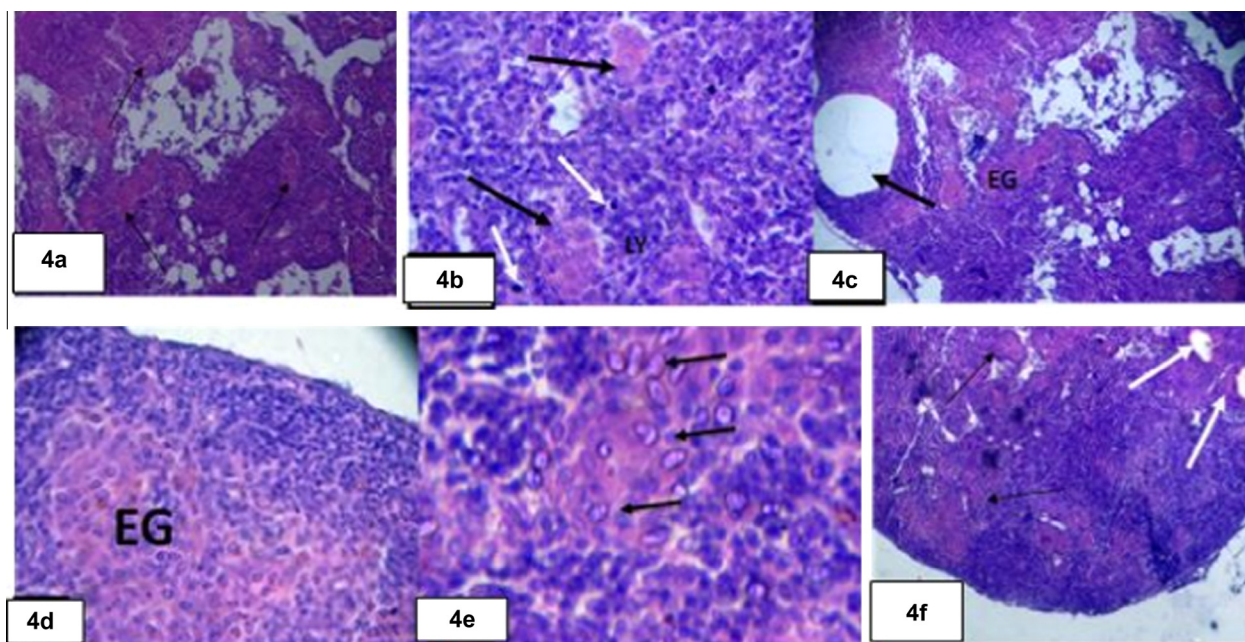

Figure 4 Photomicrograph of lymph node section of group B1 (second condition of the experiment). (a) Showing scattered epithelioid granuloma (arrow): subcapsular or cortical and medullary (10×). (b) Showing high power of epithelioid cells (black arrows) forming granuloma surrounded by collar of lymphocytes (LY), scattered apoptotic bodies (white arrows) (40×). (c) Showing lymphatic sinus ectasia (arrow): epithelioid granuloma (EG) accompanied with lymphoid follicle atrophy (cortical atrophy) (10×); (d and e) Showing epithelioid granuloma in a higher magnification power observing the epithelioid cells (arrows) (40×); (f) Showing lymphatic sinus ectasia (white arrows); epithelioid granuloma (black arrows) accompanied with lymphoid follicles atrophy) (10×) (H & E).

ber of lymphatic follicles, some of them are active and well developed with light central zones or germinal centers (Fig. 2a and b), the others are poorly developed and take intensive stain without germinal centers (Fig. 2a).

The group immunized with rat collagen mixed with adjuvant (group A1) is shown in Fig. 3. The LN in the rats in these groups (CIA) showed a decrease in the thickness of the cortex, LN depletion and atrophy, with medullary hyperplasia (Fig. 3a), lymphoid follicle with a pale staining area (germinal center) (Fig. 3b and c) and small granulomas (Fig. 3d).

The histological examination of the LN section obtained after the end of the second phase of the experiment of the
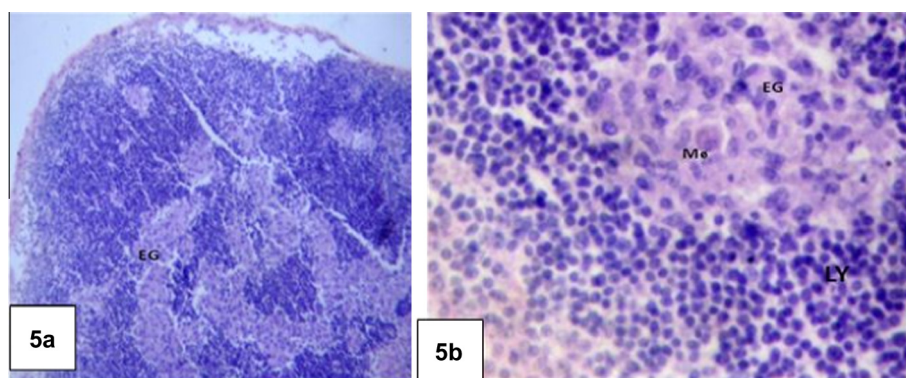
Figure 5 Photomicrograph of lymph node section of group B2 (2nd condition of the experiment). (a) Showing scattered epithelioid granuloma (EG), subcapsular or cortical or medullary accompanied with lymphoid follicle atrophy (10×). (b) Showing high power of granuloma; macrophages with ill defined borders and abundant cytoplasm surrounded by collar lymphocytes (LY) (40×) (H & E).
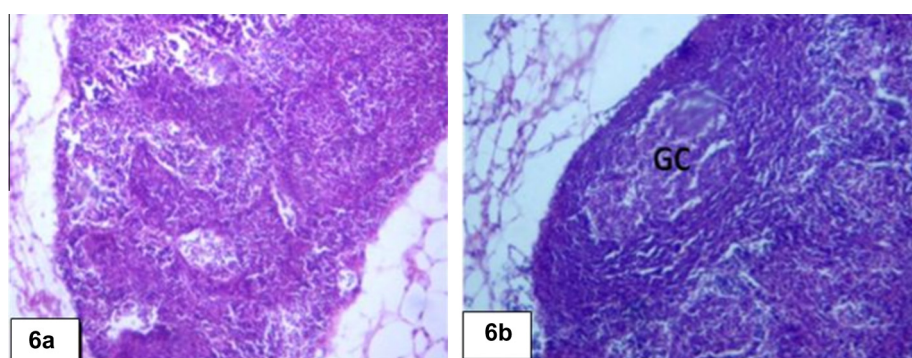

Figure 6 Photomicrograph of lymph node section of group A2 (1st condition of the experiment). (a) Showing distortion in architecture of LN (10×). (b) Showing follicular hyperplasia (10×) (H & E).

group passively received a serum containing anti-rat collagen antibodies accompanied with adjuvant (group B1) is shown in Fig. 4. The section appeared filled with masses of histiocyte cells (macrophages). The masses extend to medulla and distributed throughout the whole section, epithelioid granulomas (Fig. 4a–f), high magnification power of these masses observed (Fig. 6b, d and e) the epithelioid cells (cell with ill-defined border, abundant cytoplasm and vesicular nucleus) forming granuloma surrounded by collar of lymphocytes, scattered apoptotic bodies, lymphatic sinus ectasia accompanied with lymphoid follicles atrophy (cortical atrophy) appeared in the tissue section (Fig. 4a,c and f).

The group treated with rat serum containing anti-rat collagen alone (group B2) is shown in Fig. 5a and b, the section appeared filled with epithelioid granulomas (foreign body granuloma) which appear in higher microscopic power as Epithelioid cells (cell with ill defined border, abundant cytoplasm and vesicular nucleus) surrounded by collar of lymphocytes.

The group immunized with bovine collagen mixed with adjuvant (group A2) is shown in Fig. 6, The LN in the rats in this groups showed distortion in architecture appeared (Fig. 6a), follicular hyperplasia and reactive inflammatory hyperplasia of the LN (Fig. 6b).

The group treated with serum of bovine collagen immunized group accompanied with adjuvant (group B3) is shown in Fig. 7. Follicular hyperplasia (Fig. 7a), tangible body mac-

rophages and scattered apoptotic bodies inside the germinal center (sign of hyperplasia) (Fig. 7b), and abnormal proliferation of plasma cells (Fig. 7c).

The group injected with serum containing anti-bovine collagen antibodies without adjuvant (group B4) is shown in Fig. 8. Loss of normal architecture (Fig. 8a), scattered granulomas throughout the section (Fig. 8a and b), tangible body macrophages appeared in the section (Fig. 8c) and follicular hyperplasia (Fig. 8c and d).

The group injected with rat anti-BSA antibodies accompanied with adjuvant (group B5) The group immunized with BSA mixed with adjuvant (group A3) is shown in Fig. 9. The LN in the rats in this group showed a distortion in architecture and an abnormal excess in the frame work of the LN (Fig. 9a and b), with a follicular hyperplasia.

Shown in Fig. 10 are obliteration of subcapsular sinuses, follicular hyperplasia and increase in the cortical area (Fig. 10a), partial distortion of the architecture of the L.N (Fig. 10b), nuclear clearing due to intra-nuclear inclusions and apoptotic bodies that may have appeared (Fig. 10c).

Discussion

Immunologic hypersensitivity to collagen, the major structural component of the connective tissue, could explain both the systemic nature and chronicity of the inflammation occurring in
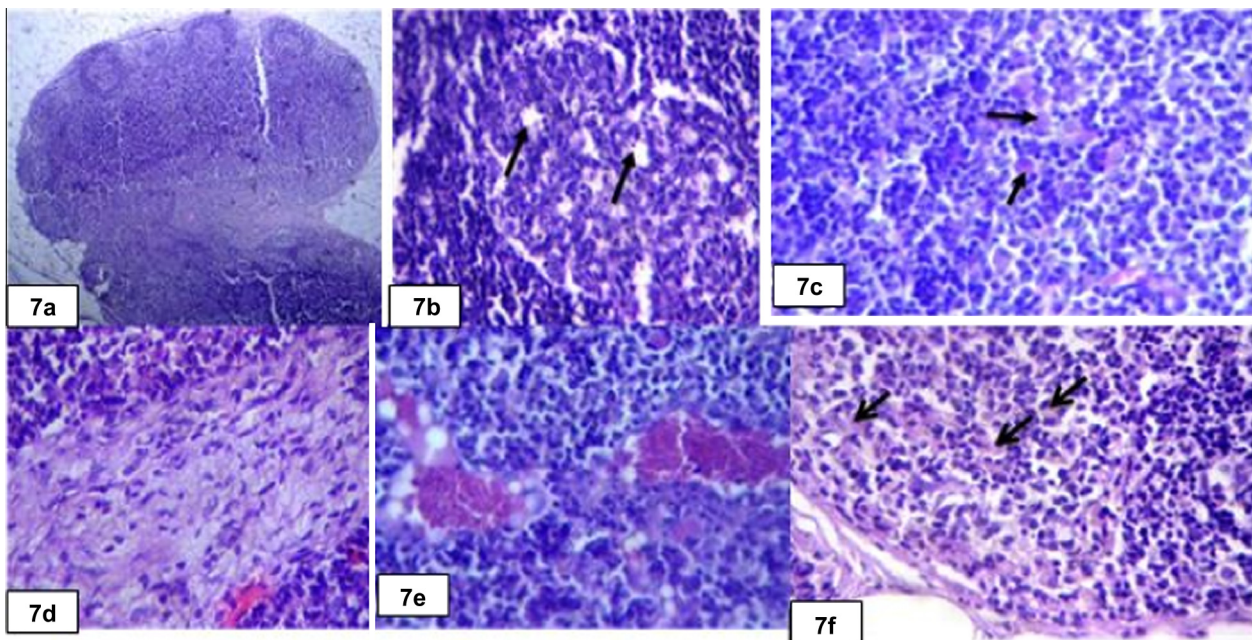
Figure 7 Photomicrograph of lymph node section of graph B3 (2nd condition of the experiment): (a) Showing Tangible body macrophages (arrows) and scattered apoptotic bodies inside the germinal center (40×). (c) Showing abnormal proliferation of plasma cells (arrows) (40×). (d) Showing increase thickness of the subcapsular trabeculae in LN (40×). (e) Showing dilation of the sinusoids with blood congestion (40×). (f) Showing aggregation of plasma cells (arrows) (40×) (H & E).
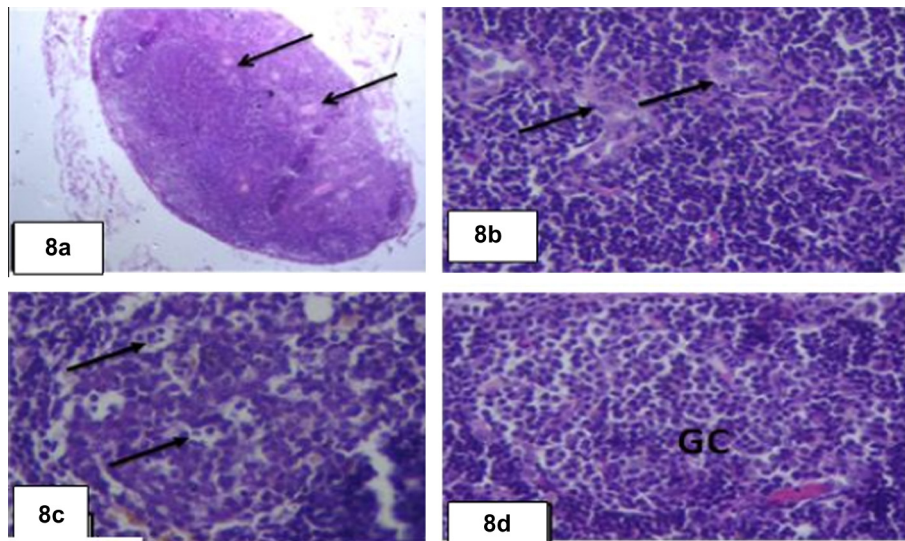

Figure 8 Photomicrograph of lymph node section of group B4 (2nd condition of the experiment): (a) Low microscopic power showing distortion in architecture and scattered granulomas throughout the section (arrows) (4×). (b) Higher magnification power of granuloma (srrows) (40×). (c) Showing Tangible body macrophages (arrows) (40×) and (d) showing hyperplasia of the germinal center (40×) (H & E).

rheumatoid arthritis. Different demonstrations of anti- bodies to collagen in sera from patients with rheumatoid arthritis support this premise (Steffen and Timpl, 1963; Steffen, 1970; Michaeli and Fudenberg, 1974; Cracchiolo et. al., 1975; Andri-opoulos et al., 1975, 1976). Also consistent with this hypothesis is the distribution of collagen in structurally distinct types in various tissues. For example, types I and III collagens are found in the skin and parenchyma of several organs, whereas type II exists in the cartilage (Seraffini-Fracassini and Smith, 1974).

It is not uncommon for vaccine sites to hurt after vaccination. After all, the injection site of a vaccine is a site where an aggressive immune inflammatory response is taking place and, consequently, the region can be swollen, red and painful. Our results indicated that the rats take five days to recover from the side effects of vaccination. It was expected for joints to swell and suffer inflammation due the anti-collagen inflammation which indicate the success of proposed objectives of this study (Fehrenbacher and M. R., 2012). The reason we mixed collagen of rat or bovine and BSA with CFA is that collagen
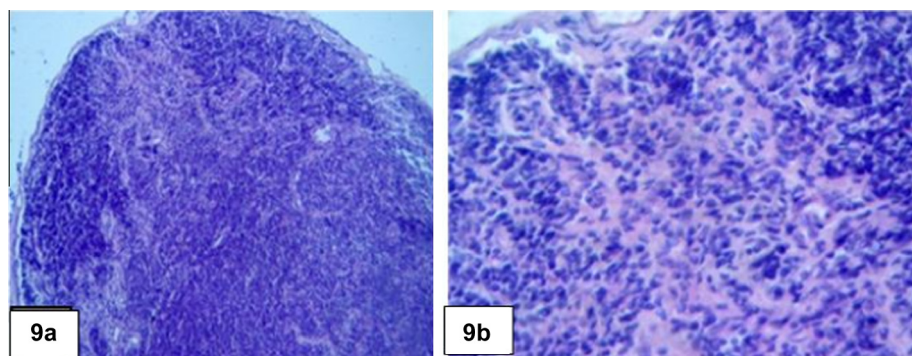
Figure 9 Photomicrograph of lymph node section of group A3 (1st condition of the experiment). (a) Showing the excessive frame work of the lymph node (10×). (b) Showing the increase in the normal frame work of lymph node (collagen like substance) in a higher power (40×) (H & E).
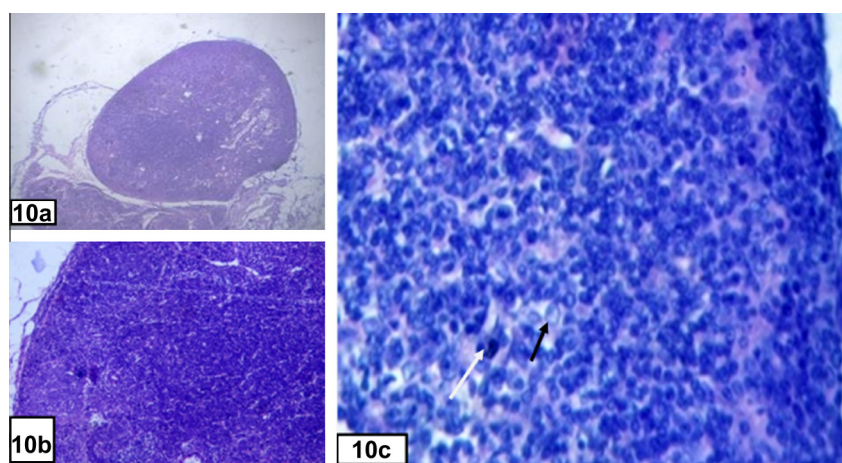

Figure 10 Photomicrograph of lymph node section of group B5 (2nd condition of the experiment): (a) showing obliteration of subcapsular sinuses, follicular hyperplasia and increase in the cortical area (4×), (b) showing partial distortion of the architecture of the LN (10×) and (c) showing nuclear due to intra-nuclear inclusions (black arrows), apoptotic bodies appeared (white arrows) (40×) (H & E).

by itself does not induce anti –collagen immune response unless it is mixed with CFA (Trentham et al., 1977).

Adjuvant is a substance injected along with an antigen that is intended to enhance the humoral and/or cell-mediated immune response to the antigen. Adjuvant may have up to five mechanisms of action: the ''depot'' effect, an antigen presentation effect, an antigen distribution or targeting effect, an immune activation/modulation effect, and a CTL effect (Cox and Coulter, 1997). Adjuvant generally permits the use of a smaller antigen dose and may modulate the immune response to the antigen. Adjuvant protects the antigen from both dilution and rapid degradation and elimination by the host. By localizing and slowly releasing intact antigen, the adjuvant permits a slow, prolonged exposure of the immune system cells to a low level of antigen. This prolonged exposure results in continued stimulation of antibody producing cells, leading to production of high levels of antibody by the host (Stills, 2005).

Lipopolysaccharides (LPS) used in this work acts as an endotoxin, induces a strong response from normal animal immune systems. It acts as the prototypical endotoxin because it binds the CD14/TLR4/MD2 receptor complex, which promotes the secretion of pro-inflammatory cytokines in many cell types. Activation of different TLRs leads to different cellular

and cytokine responses, which alters both the humoral and cellular immune responses (Heldwein and Fenton, 2002; Nigou et al., 2002; Means et al., 1999).

Lymph nodes function as filters of tissues and tissue fluids and are sites of origin and production of lymphocytes for normal physiological functions. As part of this normal function, they react to both endogenous and exogenous substances with a variety of specific morphological and functional responses. Lesions can be both proliferative and non-proliferative, and can be treatment-related or not. The histological evaluation of lymph nodes is necessary in order to understand the immunotoxic effects of chemicals with the resulting data providing an important component of animal risk assessment (Elmore, 2006a).

The group immunized with rat collagen mixed with adjuvant (group A1) has shown a decrease in the thickness of the cortex, lymph node depletion and atrophy, with medullary hyperplasia, lymphoid follicle has a pale staining area (germinal center) in the section, small granulomas appeared in the section. Lymphocyte hyperplasia can involve both the B-cell-rich follicles and the T-cell-rich paracortex and can be indicative of a humeral or cell-mediated response, respectively.

Lymphoid hyperplasia is generally a reactive or immune response and is not considered to be a preneoplastic lesion in the lymph node. Stimulated (reactive) follicles, also called secondary follicles, the case in group (A1), are usually larger than the unstimulated primary follicles and will have a paler staining germinal center with large lymphoblasts and increased numbers of apoptotic lymphocytes and tangible body macrophages. The mantle zone surrounding the germinal center is composed of small to medium-sized darker staining B lymphocytes. Hyperplastic follicles are identified by an increase in the number and size of follicles and conversion to secondary follicles. Hyperplasia of the paracortex is characterized by an increase in the cell density and, depending on the degree of hyperplasia, an increase in the paracortical area (Elmore, 2006a). The decrease in the LN cortex is due to medullary hyperplasia.

With the inflammation of Lymph nodes, it is accepted to see a granuloma in LN. Lymphoid necrosis may either be focal, multifocal or diffuse within a lymph node and there can be differences in the presence and severity of lymphoid necrosis between lymph nodes in the same animal, depending on the inciting factor and the effectiveness of the immune response (Sainte-Marie et al., 1982).

The groups A2 and A3 have shown a remarkable distortion in architecture appeared in the section, follicular hyperplasia and reactive inflammatory hyperplasia of the LN which is attributed to the foreignness of bovine collagen and BSA. This led to more potent responses in the lymphoid tissue. The enlarged germinal centers with B cell hyperplasia, are due to the proliferation of blasts of B cell origin in the center (Van den Broeck et al., 2006).

Autoantibody production is a characteristic of auto-immune diseases such as rheumatoid arthritis. Correspondingly, in mouse models of autoimmune arthritis, it is well established that Abs plays an essential role in disease development. In collagen-induced arthritis (CIA), high titers of IgG against collagen type II (CII) are essential for disease onset and transfer of the IgG to naive mice induces arthritis (Wooley et al., 1981; Nandakumar et al., 2003). In addition, mice lacking activating Fc gama receptors for IgG are protected from CIA, while absence of the inhibitory Fc gama RIIb results in augmented CIA (Diaz de Stahl et al., 2002; Kleinau et al., 2000). Thus, Fc gama RIIb is a negative regulator of B cells dampening the signaling strength of the B-cell receptor (BCR) and reducing Ab secretion, particularly in autoreactive B cells (Venkatesh et al., 2009).

The passive transfer of serum containing anti-rat collagen antibodies to induce AIA, accompanied with adjuvant resulted in masses of histiocyte cells that extend to medulla and distributed throughout the whole section, epithelioid granulomas, epithelioid cells with ill-defined border, abundant cytoplasm and vesicular nucleus forming granuloma surrounded by collar of lymphocytes, scattered apoptotic bodies, lymphatic sinus ectasia accompanied with lymphoid follicle atrophy. Lymphatic sinus ectasia can involve both the medullary and subcapsular sinuses. Diffuse sinus ectasia is typically associated with lymphoid atrophy. This lesion can be found in the mesenteric and mediastinal lymph nodes of aging mice (Elmore, 2006a). This lesion is characterized by the presence of dilated or cystic sinuses lined by lymph endothelium and filled with pale eosinophilic/amphophilic material that has a delicate lacy appearance and a few lymphocytes, plasma cells and macrophages mixed with the lymph.

The passive transfer of rat serum containing anti-rat collagen alone (group B2) has shown less severity where the section appeared filled with epithelioid granulomas, thus reflects the supportive role of adjuvant as indicated everywhere in the literature (Williams et al., 1997), but it does not eliminate the role of passively transferred Anti collagen II antibodies in inducing antibody induced arthritis (AIA).

Follicular hyperplasia, tangible body macrophages, scattered apoptotic bodies inside the germinal center, and abnormal proliferation of plasma cells were recorded for the group treated with anti BSA serum (group B5). Plasma cells are usually increased in number in response to antigenic stimulation that requires antibody production (Elmore, 2006a). Therefore B cell hyperplasia can occur simultaneously with plasma cell hyperplasia. Marked plasma cell hyperplasia, or plasmacytosis, is a common finding in rodents. The medullary cords normally contain plasma cells and their precursors as the dominant cell types and these cords are the primary sites of plasma cell hyperplasia. In cases of marked plasma cell hyperplasia, the node can be greatly enlarged, composed almost entirely of plasma cells, exhibit partial effacement of normal nodal architecture, and can be difficult to differentiate from neoplasia. Findings that support hyperplasia are a lack of cortical and capsular infiltration, typical plasma cells and metastases (Haley et. al., 2005).

Macrophage hyperplasia usually results from proliferation of resident sinusoidal macrophages but can also be seen as aggregates of macrophages within any region of the lymph node. Macrophage aggregates can be peripherally located around the paracortex or within the cortical, paracortical and medullary regions. Specific patterns (intrasinusoidal, cortical, paracortical, medullary) of macrophage hyperplasia in the same node within a dose group would be consistent with a treatment-related effect (Bouwens et al., 1986). Thus the results in this work came out with effective lymph node reactivity.

The different treatments used in this work either to induce CIA or AIA, resulted in reactive lymph nodes with different degrees depending on the foreignness of stimulator and whether it is accompanied with or without adjuvant which may be very effective in inducing the symptoms. The inflammation of lymph nodes clearly indicates a systemic inflammation where the selected lymph nodes are not adjacent to the injection sites.

Conclusion

In this work we established our own model for induction of anti-collagen arthritis (CIA) in rats with focus on histological changes of lymph node. The hallmark of this model is the reliable onset of robust inflammation. Antibody induced arthritis (AIA) is faster than antigen induced arthritis, but there is a possibility to be localized to the site of injection and its efficiency may be limited to the idiotypic determinants. While we recommend the antigen induced model in this work, we raise the warning sign of arthritic blood transfusion to healthy ones.

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