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Immunohistochemical Analysis of F4/80-Positive Macrophages in the Interstitial Tissue of Mouse Testis

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Abstract

This research work focuses on the localization and distribution of F4/80-positive macrophages in interstitial tissue from the adult mouse testis with the intent of their involvement in spermatogenesis and testicular homeostasis. Herein, we have stained sections from five male C57BL/6 mice by immunohistochemistry and identified an extraordinary accumulation of F4/80-positive macrophages with irregular shapes displaying a dendritic-like appearance in the interstitial spaces. These cells form a network that allows them to interact with germ cells and Sertoli cells, playing an important role in sperm development and immune functions in this immune-privileged organ. Macrophages may also modulate the function of Leydig cells and thereby testosterone production, underlining their highly versatile roles in male reproductive health. Our findings highlight the possible role of F4/80+ macrophages as a therapeutic target in male infertility and further studies investigating the interaction of these macrophages with testicular cells under various pathological conditions may be required.

Introduction

The testis is one of the most important organs in the male reproductive system. It assumes a dual function involving spermatogenesis and the secretion of necessary hormones, especially testosterone, which highly contributes to the development of male secondary sexual characteristics, adding to many other physiological processes(1). The testis is an organ with a very organized structure, containing seminiferous tubules where the differentiation and maturation of germ cells occur, along with interstitial spaces with Leydig cells involved in testosterone synthesis. Complex architecture is of immense importance in maintaining male fertility and therefore overall reproductive health(2).The testis is a very complex organ that develops from seminiferous tubules and interstitial tissue. The seminiferous tubules, lined by stratified epithelium, represent the major sites of spermatogenesis and contain germ cells in various stages, including spermatogonia, spermatocytes, and spermatids, along with Sertoli cells, which support and nourish the developing spermatozoa(3).

The interstitial tissue is interspersed between the seminiferous tubules and consists of Leydig cells. These are generally known for the production of testosterone. Present within this area are also blood vessels, lymphatics, and immune cells such as macrophages, implicated in testicular microenvironment regulation. Generally speaking, the architecture of the testis is highly relevant to its reproductive function in supporting sperm production and hormonal activity(4)(Fig1).

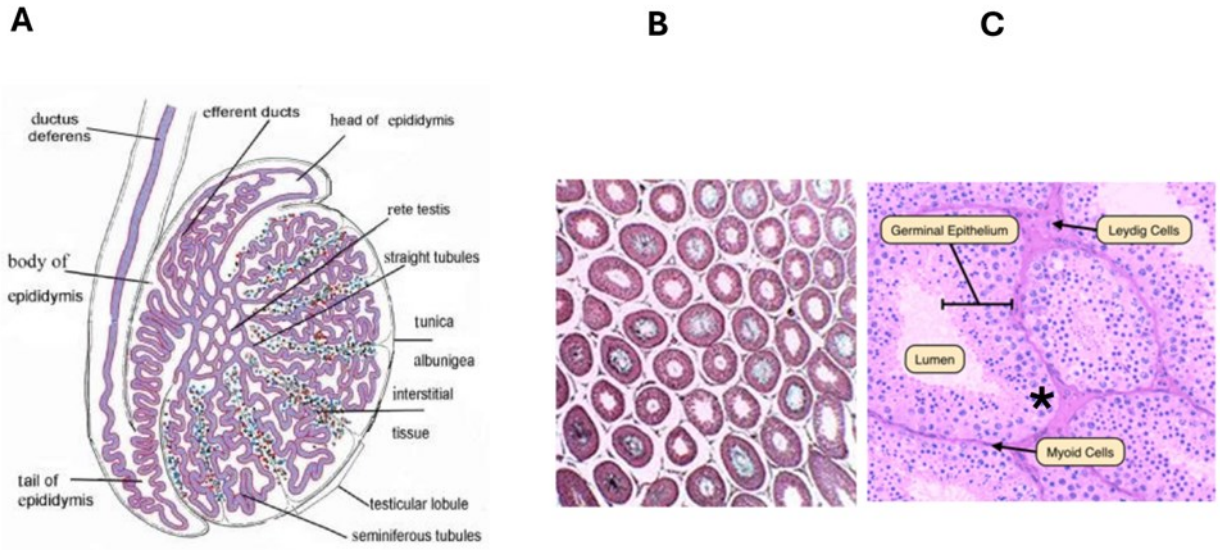

Figure 1, A: General overview of the histological organization of testis(5), B: Histological image of seminiferous tubules(6), C: High magnification of spermatogenic tubules and surrounding tissue(7). *: Interstitium

Aside from its role in reproduction, the testis is an immunologically privileged site. This privilege serves as protection against autoimmune responses that could be directed against the developing sperm cells, which are foreign entities to the surface antigens presented on them. The balance of immune tolerance versus response within the testis is a delicate balance of equilibrium maintained by many members of the immune system, including macrophages. These cells comprise one of the most important immune cells, having a wide range of functions generally known, involving phagocytosis, antigen presentation, and the regulation of the inflammatory response(8).

Due to the fact that testicular macrophages are involved in the processes of spermatogenesis and testicular homeostasis, quite active studies have been conducted on them. Among their functions are the removal of apoptotic germ cells, the regulation of Sertoli cell functions, and the modulation of the local immune response. Their presence establishes an appropriate environment for the differentiation of spermatogonia into mature spermatozoa. Further, macrophages may affect Leydig cell function to modulate testosterone production and overall testicular function(9).

Identification and characterization of testicular macrophages have significantly improved through the application of specific markers, such as the F4/80 antibody, which stains only mature macrophages. Immunohistochemistry with the F4/80 antibody allows more detailed determination of the localization and distribution patterns of macrophages within the testicular microenvironment. Such knowledge is relevant for explaining the functional roles played by macrophages in spermatogenesis and their interaction with other testicular cell types (10).

Although their importance in the testis is duly recognized, there is a lack of extensive studies related to the localization and distribution pattern of macrophages. This seriously pertains to a knowledge gap in the role played by macrophages and therefore keeps from fully understanding testicular physiology and male fertility. Here-(11), we report the pattern of localization and distribution of macrophages in the

adult mouse testis using an F4/80 antibody-immunohistochemistry approach in a systematic manner. Characterization of the spatial distribution of these immune cells could provide clues about their functional roles in spermatogenesis and their interactions with germ cells and Sertoli cells(12).

Such research would therefore have a far-reaching consequence in terms of interactions between the immune system and reproductive health in such a way as would enable the identification of new therapeutic targets for male infertility. Study of the role of macrophages in maintaining testicular homeostasis and responding to pathological conditions may provide novel approaches for improving male reproductive health and fertility.

Methods and Materials

Animal:

To this end, the present study utilizes five male C57BL/6 mice in an investigation on the localization and distribution patterns adopted by macrophages within the adult testis. C57BL/6 mice are among the most commonly employed inbred strains due to the well-characterized nature of their immune system; their genetic background has been shown to possess very predictable characteristics.

The mice were obtained from an accredited animal facility and maintained in normal conditions in a temperature- and humidity-controlled environment at 22 ± 2°C with a 12-hour day-night cycle. They had ad libitum access to standard rodent chow and filtered water throughout the study period. Animals were acclimatized for at least one week in advance of initiating experiments to minimize stress and ensure well-being.

All experimental procedures were carried out in accordance with institutional guidelines for the care and use of laboratory animals and were approved by the relevant ethical review board. Every attempt was made to minimize animal suffering and distress, yet to adhere to the principles of 3Rs: Replacement, Reduction, and Refinement in animal research.

The mice were then sacrificed at the end of the study by isoflurane anesthesia followed by cervical dislocation to humanely end the experiment. The testes were then harvested for immunohistochemistry to determine macrophage localization and distribution patterns using the F4/80 antibody.

Immunohistochemical analysis

Tissue Processing

Immediately following euthanasia, the testes were carefully excised and fixed in 4% PFA in phosphate-buffered saline for 24 hours at 4°C. The tissues, following fixation, were washed in PBS and dehydrated in a gradient of ethanol (70%, 80%, 90%, 100%). This was followed by clearing in xylene. The testes were then embedded in paraffin wax, and 5 µm thick sections were cut using a microtome. The sections were then mounted onto glass slides and stored at room temperature until further processing.

Immunohistochemistry Procedure

The immunohistochemistry procedure was performed according to our previous publication(13), which is briefly described as follows: Deparaffinization and Rehydration: The paraffin-embedded tissue sections were deparaffinized by incubating them in xylene for 2 x 5 minutes, followed by rehydration through a graded series of ethanol (100%, 90%, 80%, and 70%), and finally rinsed in distilled water. Epitope Unmasking: The sections were heated in a microwave oven using a citrate buffer at pH 6.0 for antigen retrieval. Slides were

heated for 20 minutes at 95°C, then cooled to room temperature. Sections, following cooling, were washed in PBS. Blocking: Sections were incubated with 5% bovine serum albumin (BSA) in PBS for 1 hour at room temperature with the purpose of blocking non-specific binding. This blocking step was necessary to reduce the background that was present. Primary antibody incubation: The sections were incubated with the primary antibody F4/80 overnight at 4°C at a 1:100 dilution (serotec, Oxford, UK) for the specific labeling of macrophages. Thereafter, the sections were incubated and washed three times with PBS to remove the unbound antibodies. Incubation with Secondary Antibody: Following washes, the sections were treated for 1 hour at room temperature with a biotinylated secondary antibody , which was selected to bind the primary antibody for eventual detection. Staining: The sections were then immersed in an ABC solution according to the manufacturer's instructions . Subsequently, the sections were developed using 3,3'-diaminobenzidine as a chromogen that yielded a brown precipitate at the site of antibody binding. The reaction was followed closely, and stopped by quenching the slides in distilled water when desired intensity was reached. Mounting: The sections were then dehydrated through a graded series of ethanol, cleared in xylene, and mounted with a permanent mounting medium. The slides were then set aside to air dry overnight and were being observed under the light microscope. Microscopy and Analysis: Stained sections analyzed by light microscopy and images captured at various magnifications. Localization and distribution of F4/80-positive macrophages within testicular tissue was assessed both qhjknlnimualitatively and quantitatively. Thereafter, the sections were developed using DAB as a chromogen, yielding a brown-colored precipitate at the site of antibody binding. The reaction was followed by closely monitoring and stopping it at an appropriate intensity by rinsing the slides in distilled water. Mounting: Lastly, the sections were dehydrated through a graded series of ethanol, cleared in xylene, and mounted with a permanent mounting medium. Slides were then allowed to air dry completely, followed by examination under a light microscope. Microscopy and analysis: The light microscopic sections were observed, and photography was done in different magnification orders. Qualitative and quantitative assessment of the localization and distribution of F4/80+ macrophages was done within the testicular tissue.

Result

Histological observations indicated that the interstitium surrounding the rete testis and the adjacent interstitial tissue became increasingly distinct throughout development. Initially, the interstitial spaces contained a sparse population of interstitial cells. Immunohistochemical analysis revealed the presence of F4/80-positive cells within the testicular interstitium. These cells exhibited irregular shapes and were characterized by an arborized, dendritic-like morphology, suggesting a dynamic role in the testicular microenvironment. Furthermore, there was a significant accumulation of irregular or elongated F4/80-positive cells in the central region of the interstitium. These cells formed a complex mesh-like structure in conjunction with adjacent macrophages, indicating a potential functional relationship that may be crucial for tissue organization and immune response within the testis. This intricate network of interstitial cells likely plays an essential role in supporting spermatogenesis and maintaining the overall homeostasis of the testicular environment (Fig2).

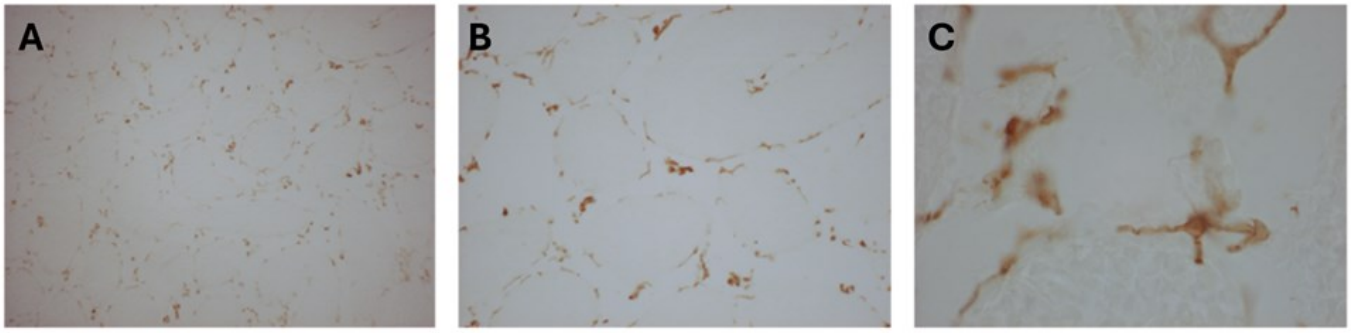

Figure 2, Immunohistopositive macrophages cell that localized by F4/80 Antibody. Magnification A: X5, B: X20 and C;

Discussion

The localization and distribution patterns of F4/80 -positive macrophages in the adult mouse testis, as well as their possible functions in spermatogenesis and testicular homeostasis, have been significantly clarified by the current investigation(14). Our data indicate that, the interstitium surrounding the rete testis and neighboring interstitial tissue becomes progressively more well defined, associated with the number of F4/80-positive macrophages. These observations stress the importance of the role that macrophages play in the testicular microenvironment and their active participation in the maintenance of male reproductive health.

F4/80-positive cells with an irregular shape and arborized, dendritic-like morphology would indicate that these macrophages are active participants rather than passive residents within the testis in the maintenance of testicular function. This irregular and dendritic shape may provide them with greater capacity to interact with several testicular cell types, including germ cells and Sertoli cells. This is a very important interaction, because Sertoli cells provide important support and nourishment for developing spermatozoa, while macrophages are able to modulate Sertoli cell function and contribute to the clearance of apoptotic germ cells (15). Our findings indicate the profound accumulation of mostly irregular or elongated F4/80-positive macrophages in the area of the interstitium, establishing a complex network with neighboring macrophages.

Thus, the cells participate in a critical way in the organization of testicular architecture, promote communication between immune and germ cells, and, importantly, modulate local immune responses. Such a structured network may be required for maintaining the delicate balance between immune tolerance and immune response in an organ like the testis, which is considered to be an immune-privileged organ(16). Moreover, spermatogenesis would not have succeeded to such an extent without the supportive role from macrophages.

Previous studies have suggested that these macrophages take part in the phagocytosis of apoptotic germ cells, preventing the buildup of cellular debris that might interfere with spermatogenesis. The action of macrophages in removing dead or dying cells provides a good environment for the differentiation of spermatogonia into mature spermatozoa (17). Moreover, their influence on Leydig cells, responsible for testosterone production, suggests that macrophages might have an indirect consequence on hormonal regulation important in spermatogenesis and general reproductive health(18). The approach using the antibody F4/80 in immunohistochemistry has given us the possibility to visualize and characterize the distribution of

macrophages within the testicular microenvironment.

Our results contribute to the understanding of the pattern of macrophage localization, but further investigations are needed in order to explain in depth the functional role of these cells during spermatogenesis. Future studies need to address the interaction between macrophages with the germ cells and Sertoli cells, as well as the manner in which these cells would respond to pathophysiological conditions that may eventually culminate in defective male fertility. Current evidence has identified the F4/80+ macrophages as playing decisive roles in the testis, contributing multifunctionally to spermatogenesis and testicular homeostasis. Further study of the reciprocal interactions between macrophages and other testicular cell types may yield important insights into male reproductive health and fertility. Such information could potentially enable new strategies for improving male fertility using novel therapeutics, particularly those infertilities that are resulting from immune dysregulation.

Conclusion

The present study addresses the localization and functions of F4/80-positive macrophages in the adult mouse testis, a finding that highlights their critical role during spermatogenesis and testicular homeostasis. These findings suggest that these macrophages are active participants in the testicular microenvironment, appearing as irregular, dendritic shapes that allow better interaction with germ and Sertoli cells. Their presence is warranted by the need for clearance of apoptotic germ cells and regulation of Sertoli cell function, which contribute to the structural organization and immune balance within the testis. Moreover, macrophages may modulate Leydig cells and testosterone production further illustrating their multifaceted role in male reproduction. Although this study is important, more studies are required to understand the roles of macrophages during spermatogenesis and their response to various conditions that affect fertility. The study highlights the importance of F4/80-positive macrophages in male fertility and suggests novel pathways for therapeutic targeting in male infertility.

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