Med Mol Morphol (2012) ■■:■■–■■
DOI 10.1007/s00795-012-0587-2

AWARD REVIEW

Munekazu Naito · Hayato Terayama · Shuichi Hirai
Ning Qu · Livia Lustig · Masahiro Itoh

Experimental autoimmune orchitis as a model of immunological male infertility

Received: May 11, 2012 / Accepted: June 26, 2012

Abstract Clinically, 60–75% of male infertility cases are categorized as idiopathic spermatogenic disturbance. In previous studies of this condition, lymphocytic infiltration and immune deposits were present in several testis biopsy specimens, indicating that inflammatory or immunological factors contribute to the occurrence of the lesions. However, there is currently little evidence regarding immunological infertility in men. Previously, we established an immunological infertility model, experimental autoimmune orchitis (EAO), that can be induced in mice by two subcutaneous injections of viable syngeneic testicular germ cells without the use of any adjuvant. In this EAO model, lymphocytes surround the tubuli recti and then induce spermatogenic disturbance. In addition, after the active inflammation stage of this model, the seminiferous epithelium is damaged irreversibly, resembling the histopathology of human male idiopathic spermatogenic disturbance. In the majority of patients with testicular autoimmunity, there is a chronic and asymptomatic development of the inflammatory reaction. Therefore, this disease is very difficult to diagnose at the ongoing stage, and it is possible that the histopathology of idiopathic spermatogenic disturbance in the clinic is reported at the post-active inflammation stage of autoimmune orchitis. In this review, the histopathology of EAO before and after inflammation is discussed, comparing it with human orchitis.

Key words Testis · Blood–testis barrier · Autoimmunity · Orchitis · Male infertility

M. Naito (✉) · H. Terayama · S. Hirai · N. Qu · M. Itoh
Department of Anatomy, Tokyo Medical University, 6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, Japan
Tel. +81-3-3351-6141; Fax +81-3-3341-1137
e-mail: munekazu@tokyo-med.ac.jp

M. Naito · L. Lustig
Institute for Research in Reproduction, School of Medicine, University of Buenos Aires, Buenos Aires, Argentina

Introduction

Of all cases of infertility, 40–50 % occur in men.[1,2] Although the literature reports that there are many causes of male infertility, 60–75 % of cases are caused by idiopathic disturbance of spermatogenesis.[3] The histopathology of idiopathic spermatogenic disturbance is characterized by seminiferous tubules (STs) showing Sertoli cell-only syndrome or maturation arrest.[1] In a study of 68 Japanese patients with idiopathic male infertility, testicular biopsies showed that 14 patients had Sertoli cell-only syndrome and 32 patients had maturation arrest.[4] It was also noted that the degrees of spermatogenic disturbances varied widely in each ST, showing either the Sertoli cell-only feature or maturation arrest at the level of spermatogonia, spermatocytes, or round spermatids.[1] However, previous studies have reported that, in approximately 10 % of idiopathic male infertility cases, lymphocytic infiltration was present in several testis biopsy specimens (Table 1).[5–8] It was also noted that deposits of immunoglobulin G (IgG) and third components of complement (C3) could be detected in 20–70 % of cases of idiopathic spermatogenic disturbance (Table 1).[7,9–11] These results imply that some inflammatory or immunological factors are involved in the development of tissue damage; however, there is little documented evidence on immunological infertility in men.

Haploid cells do not appear in the seminiferous epithelium until puberty, when immune tolerance has already been established. Therefore, they contain various autoimmunogenic antigens, which are recognized as foreign by the self-immune system. The blood–testis barrier (BTB), formed by Sertoli cells, protects autoimmunogeneic spermatozoa from attack from the self-immune system.[12,13] However, if the BTB is functionally damaged, the autoantigens of haploid cells leak out beyond the BTB, leading to a continuous supply of autoantigens inducing testicular inflammation for a prolonged length of time. Indeed, it has been reported that damage of the BTB in one testis (following an infection, biopsy, torsion, or surgery in the scrotal area) induced orchitis in the contralateral testis.[6,14,15] Therefore, the testis

could be considered a critical target of autoimmune damage. Here we review the histopathology of experimental autoimmune orchitis (EAO), an immunological infertility model, and discuss it in comparison with human male infertility.

Experimental autoimmune orchitis (EAO)

EAO is a model of chronic inflammation of the testis, which leads to male infertility.[16–20] EAO has been induced in several animals including monkeys, guinea pigs, rabbits, rats, and mice, and is characterized by T-cell-dependent lymphocytic inflammation and damage to the STs, that is, the shedding and apoptosis of germ cells.[16–26] Classically, murine EAO is induced by immunization with testicular homogenate (TH) plus complete Freund's adjuvant (CFA) plus *Bordetella* pertussigens (BP); it has been considered that treatment with these two adjuvants is needed to enhance the immune responses, resulting in the breakdown of testicular immune privilege.[15,27] Previously, we established another EAO model induced (with a very high incidence) by two subcutaneous injections of viable syngeneic testicular germ cells (TGCs) in both A/J and C3H/He mice, without the use of any adjuvant.[18,19] Histopathological examinations of both TH^+ CFA^+ BP-induced EAO and TGC-induced EAO revealed that the inflammatory responses progress differently in each model.[20] Namely, in TH^+ CFA^+ BP-induced EAO, lymphocytes preferentially infiltrate the peripheral testis away from the tubuli recti (TR) with spermatogenic disturbance. However, in TGC-induced EAO, lymphocytes surround the TR before spermatogenic disturbance is induced (Fig. 1).[28–30]

Table 1. Previous reports regarding immunological factors in testicular biopsy specimens from idiopathic male infertility

Incidence	Reference
Lymphocytic inflammation	
8.1% (13/160)	Hofmann and Kuwert (1979)[5]
4.8% (8/166)	Suominen and Söderström (1982)[6]
13.6% (22/159)	Hatakeyama (1984)[7]
9% (9/100)	Jahnukainen et al. (1995)[8]
Deposits of IgG or C3	
20% (10/50)	De-Casseye et al. (1980) [9]
70% (21/30)	Salomon et al.[10]
70% (14/20)	Hatakeyama (1984)[7]
40% (21/52)	Lehmann et al. (1987)[11]

IgG, immunoglobulin G; C3, third components of complement

Early stage of EAO

Even under normal conditions, the TR is an immunologically special site. In normal mice, the TR and rete testis (RT) lumen are permeable to anti-sperm antibodies and exogenously administered horseradish peroxidase; however, the ST lumen is impermeable to these substances.[31–33] Moreover, the occasional diffusion of lymphocytes and macrophages into the TR and RT has been reported in many animal species under normal conditions.[34] Indeed, we also found that these cells were occasionally present in the TR and RT of mice.[35,36] In the TR lumen, these lymphocytes were categorized as T lymphocytes and were localized close to spermatozoa under an electron microscope. These findings suggest that T lymphocytes or macrophages may be able to gain access to the autoantigens of spermatozoa inside the TR under normal conditions. Following further electron microscopy observations, it was found that the basal lamina of the TR had a wavy, multilayered structure; however, at the ST and RT, it had a nearly flat, single-layered appearence.[37] In addition, at the TR, it was noted

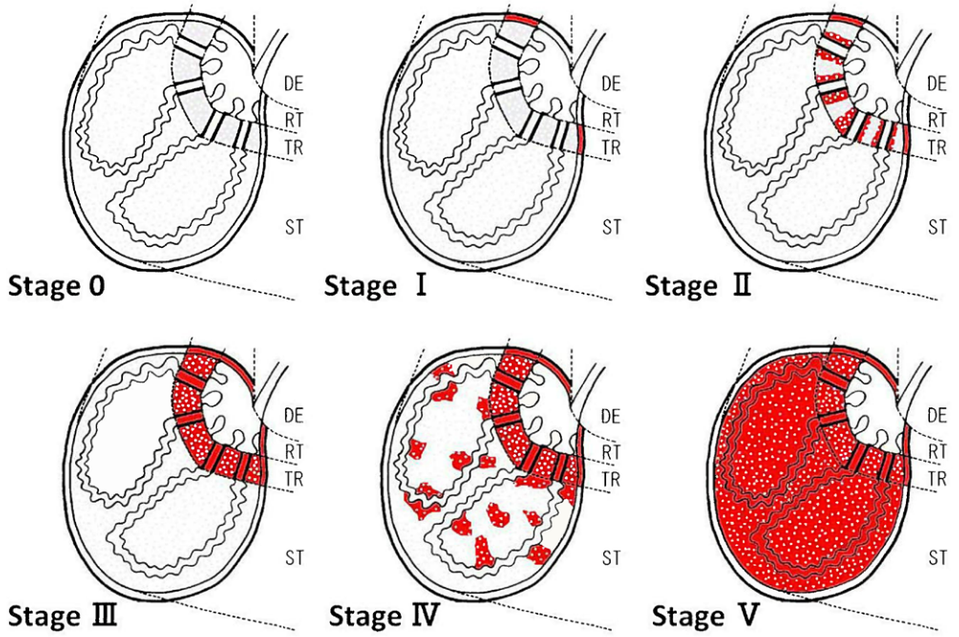

Fig. 1. Mode of inflammatory cell infiltration in the testes of mice injected with syngeneic testicular germ cells without adjuvant. *Red areas* indicate inflammatory cell infiltration. Histological findings are as follows: *stage 0*, no inflammation; *stage I*, focal inflammation in the tunica albuginea; *stage II*, focal inflammation adjacent to the tubuli recti (*TR*); *stage III*, inflammation surrounding the TR; *stage IV*, inflammation spreading around the seminiferous tubules (*ST*); *stage V*, widespread inflammation involving the TR and STs. *DE*, ductuli efferentes; *RT*, rete testis; *ST*, seminiferous tubule; *TR*, tubuli recti

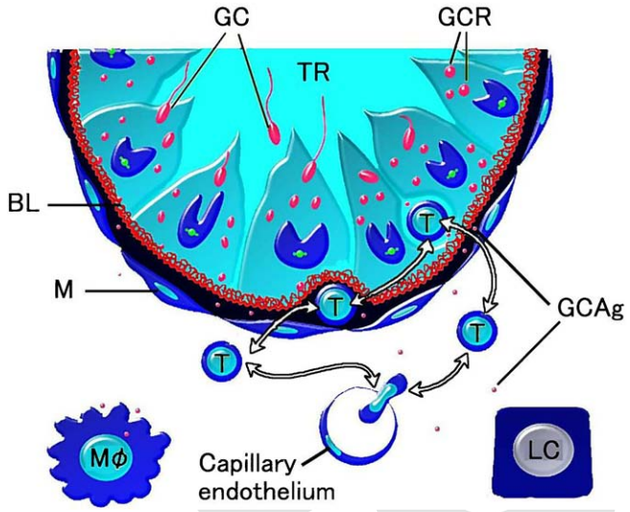

Fig. 2. Hypothesized presentation of the microenvironment at the tubuli recti (*TR*). The TR epithelial cells (i.e., modified Sertoli cells) actively phagocytose germ cell remnants (*GCR*s) and degenerated germ cells (*GC*s). Then, under normal conditions some germ cell autoantigens (*GCAg*s) are excreted from the wavy, multilayered basal lamina (*BL*) to the outside of the tubule. T lymphocytes (*T*s) can migrate into the TR epithelium from an adjacent capillary and gain access to the excreted GCAgs. *LC*, Leydig cell; *M*, myoid layer; *Mϕ*, macrophage; *TR*, tubuli recti; *GCR*, germ cell remnant; *GC*, germ cell; *GCAg*, germ cell autoantigen; *BL*, basal lamina; *T*, T lymphocyte

that wide gaps exist between the epithelial layer, basal lamina, and myoid cell layer. These observations suggest that the TR epithelial cells have a wide surface area at their base and, therefore, can effectively excrete or absorb various molecules between the lumen and interstitium. In TGC-induced EAO, the first infiltrating lymphocytes that surround the TR were found to be of both T- and B-cell lineage.[38] Moreover, even before damage to the seminiferous epithelium, these lymphocytes caused degeneration of the TR epithelium.[38] These findings indicate that TR epithelial cells are the first targets of autoreactive T and B lymphocytes in TGC-induced EAO. In general, the phagocytosis of TGC remnants within the TR epithelial cells (i.e., modified Sertoli cells) is regarded as a normal mechanism for the elimination of unwanted spermatozoa.[34,39,40] From these results, we hypothesize that under normal conditions, TR epithelial cells actively phagocytose germ cell remnants, as well as some degenerated germ cells, and then excrete certain germ cell autoantigens from the wavy, multilayered basal lamina to the outside of the tubule (Fig. 2). Therefore, few lymphocytes could migrate into the TR epithelium from an adjacent capillary with access to the excreted germ cell autoantigens, and if there is further autoreactive lymphocyte migration, autoimmune orchitis might be initiated, beginning from the TR to the peripheral ST and resulting in spermatogenic disturbance. In men, we speculate that modified Sertoli cells lining the TR capture and phagocytose degenerating spermatozoa and spermatids with a similar mechanism to that observed in mice. In the majority of patients, chronically torpid inflammatory reactions in the testes remain asymptomatic.[39] Therefore, it is difficult to diagnose the early stage of autoimmune orchitis from testicular biopsies obtained from patients, and no data regarding the early stage of autoimmune orchitis have been reported in men.

Table 2. Comparison between idiopathic male infertility and the post-active inflammation stage of murine experimental autoimmune orchitis (EAO)

Parameter (incidence)	Idiopathic male infertility[a]	Post-active-inflammation stage of murine EAO
Lyphocytic inflammation	4.8–13.6%	Resolution
Sertoli only syndrome	20%	100%
Deposits of IgG and C3	20–70%	100%
Hypertrophied basal lamina	84%	100%

IgG, immunoglobulin G; C3, third components of complement
[a] Data of idiopathic male infertility in men are from previous studies[5–11]

Post-active inflammation stage of EAO

There have been many reports regarding the active inflammation stage of EAO; however, the post-active inflammation stage of EAO is not well understood.[16,17,20,27,29] In TH^+ CFA^+ BP-induced EAO, autoimmunity was induced against antigens of haploid germ cells, spermatogonia, Sertoli cells, Leydig cells, and the basal lamina of the ST. It also caused necrosis of the ST or Sertoli cell-only syndrome features at the post-active inflammation stage.[16,24,42,43] However, autoimmunity in TGC-induced EAO was induced only against antigens of haploid cells.[19,44] At the post-active inflammation stage (~1 year after the first immunization) of TGC-induced EAO, the seminiferous epithelium persistently presented maturation arrest and Sertoli cell-only syndrome, and all the EAO mice were infertile.[45] The degree of spermatogenic disturbance at the post-active inflammation stage varied widely in each ST, showing either features of Sertoli cell-only syndrome or maturation arrest at the level of spermatogonia, spermatocytes, or round spermatids. It was also noted that there were deposits of IgG and C3 on the thickened basement membrane of STs at the post-active inflammation stage of EAO.[45] It has been reported previously that the thickened basement membrane of the STs and the presence of immune deposits are significantly related to spermatogenic disturbance in infertile men.[9–11,46] These results indicate that persistent damage to the seminiferous epithelium caused by TGC-induced EAO after active inflammation may resemble the histopathology of idiopathic spermatogenic disturbance in man (Table 2). In the testicular biopsies of male patients with infertility, only some areas of the testis can be observed; however, in the EAO mouse model, we can analyze the whole testis at the same time. If we could examine the whole testis of men with idiopathic infertility, post-active inflammation in the testes could be observed more frequently than that reported previously. Recently, we demonstrated an increase in the intratesticular mRNA expression levels of both Fas and Bax during the

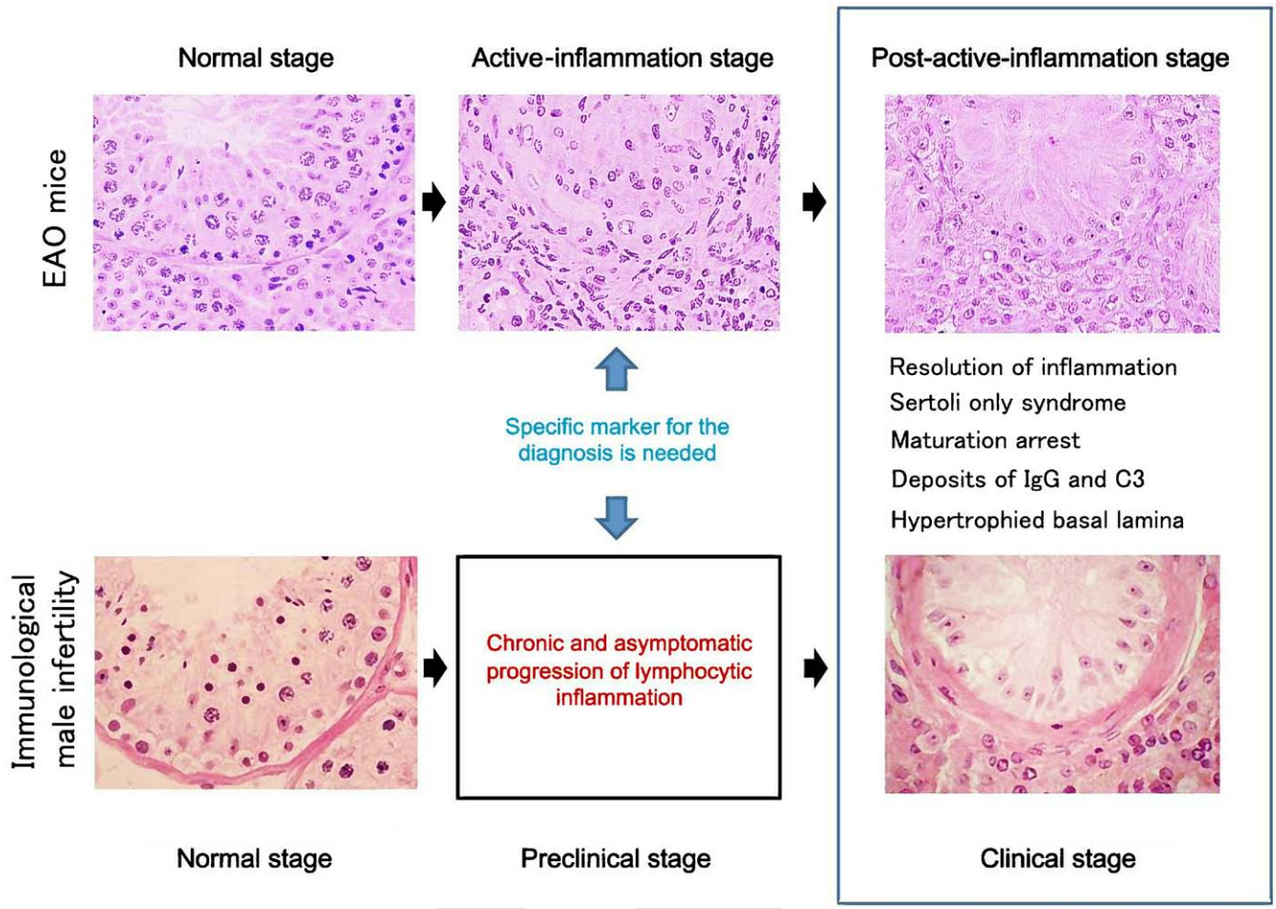

Fig. 3. Inflammatory process of immunological male infertility and murine EAO. *EAO*, experimental autoimmune orchitis; *IgG*, immunoglobulin G; *C3*, third components of complement

active inflammation stage of EAO, although these mRNA expressions decreased dramatically during the post-active inflammation stage.[47] In contrast, there was no significant change in the intratesticular mRNA expression levels of either Fas-L or Bcl-2 during the active inflammation stage, but extreme increases in the levels occurred during the post-active inflammation stage.[47] In a study on testicular biopsy specimens from male patients with Sertoli cell-only syndrome and maturation arrest, it was demonstrated that the mRNA expression of Fas-L was increased, but that the mRNA expression of Fas and caspase 3 did not change significantly compared with controls.[48] We predict that these clinical conditions may be similar to our experimental data in the post-active inflammation stage of TGC-induced EAO.

Conclusion

Most patients first suspect they may be infertile when they start trying to conceive a child. There is the possibility that the testicular histopathology of idiopathic male infertility is similar to the post-active inflammation stage of autoimmune orchitis (Fig. 3). If it is possible to find the early stage of autoimmune orchitis, we could treat it before spermatogenic disturbance results. The development of a specific marker for immunological infertility is needed for its diagnosis. The detection of autoantigens in TGC-induced EAO may contribute to the discovery of this specific marker in man.

Acknowledgments The authors thank Ms. Miyuki Kitaoka, Mr. Shinichi Kawata, Ms. Akiko Abo, and Ms. Yuki Ogawa for excellent secretarial and technical assistance.

Dr. Munekazu Naito, of the Department of Anatomy, Tokyo Medical University, Tokyo, is the winner of the Japanese Society for Medical Molecular Morphology Award for Promoting Young Researchers in 2011. Dr. Naito is recognized for his contribution in the experimental autoimmune orchitis as a model of immunological male infertility and its application in the field of medical molecular morphology.

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