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Expression of CD30 in Testis and Epididymis of Adult Mice

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CD30 is a member of tumor necrosis factor receptor (TNFR) superfamily and has pleiotropic functions including cell activation, proliferation, differentiation, and death, depending on cell types and stage of differentiation. Although CD30 expression has been described mainly in hematopoietic tissues, several types of nonhematopoietic tumors including embryonic carcinoma and germ-cell tumors express CD30. We examined CD30 distribution in the testis and epididymis from wild type and CD30-deficient mice. In the testis, spermatogonia, spermatocytes and Sertoli cells expressed CD30, but not in spermatids. Spermatogonia and spermatocytes near the basement membrane strongly reacted to anti-CD30. In the epididymis, CD30 expression was exclusively observed in luminal epithelia and some interstitial cells. Taken together, these results show a spatio-temporal regulation of CD30 expression in mouse testis and epididymis and suggest a possible role of CD30 in spermatogonia and spermatocytes.

CD30 antigen is a member of the tumor necrosis factor (TNF) receptor superfamily (Smith et al., 1990; Durkop et al., 1992), which was originally identified on Reed-Sternberg cells of Hodgkin's diseases (Schwab et al., 1982). CD30 was subsequently revealed to be expressed also in different non-Hodgkin's lymphomas (NHLs), as well as in several virally transformed T- and B-cell lines (Herbst et al., 1993; Gruss and Dower, 1995), and normal $CD45RO^+$ T cells after activation by a variety of T-cell stimuli (Ellis et al., 1993). Further studies have described that CD30 expressed on normal T and B lymphocytes following activation is involved in cell activation, differentiation and death (Gruss et al., 1994a; Gruss et al., 1994b; Telford et al., 1997; Opat and Gaston, 2000). Therefore, CD30 has been extensively studied for clinical application as a specific marker for diagnosis (Piris et al., 1990; Hodges et al., 1991; Paulli et al., 1995) or a target molecule for treatment (Barth et al., 2000; Levi et al., 2001; Willers et al., 2003) of lymphomas. CD30 has been also proposed as a type 2 helper T (Th2) cell marker (Romagnani et al., 1995; Annunziato et al., 1998) despite several debates (Bengtsson et al., 1995; Hamann et al., 1996). In consideration that polarized Th1/Th2 response is causally related with pathogenesis of diseases (Romagnani, 1996; Romagnani et al., 1997), identification and functional control of type 2 helper T (Th2) cells using CD30 antigen may provide important clues for successful therapies of those related diseases (Romagnani, 1995; Romagnani et al., 1995; D'Elios et al., 1997).

CD30 outside lymphatic system has been rarely found. To our knowledge, CD30 expressed by normal non-hematopoietic cells was documented solely on decidual endometrial stromal cells (Papadopoulos et al., 2001). Another types of non-hematopoietic cells expressing CD30 are several germ cell tumors including embryonal carcinomas (Pallesen and Hamilton-Dutoit, 1988; Latza et al., 1995), seminoma or mixed germ cell tumor (Pallesen and Hamilton-Dutoit, 1988), and mesenchymal tumors (Mechtersheimer and Moller, 1990). Furthermore, CD30 expression was down-regulated during stem cell differentiation of *in vitro* cultured embryonal carcinoma cells (Pera et al., 1998). All of these reports showing CD30 distribution in reproductive system raise a hypothesis that CD30 may play a role in germ cell differentiation. However, it remains unknown whether CD30 is expressed in developing germ cells or other somatic cells in reproductive system. Based on the fact that germ-cell tumors are the most common malignant neoplasms of the testis, we investigated a possibility of CD30 expression during spermatogenesis.

Spermatogenesis has been studied most extensively in the mammalian testis (Eddy and O'Brien, 1994; Fawcett, 2001; Jung et al., 2001). Mammalian spermatozoa are produced in coiled tubes called seminiferous tubules of the testes. These seminiferous tubules consist of two types of somatic cells: the myoid or smooth muscle-like cells and the sertoli cells, and five types of germ cells:

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spermatogonia, primary and secondary spermatocytes, spermatids, and spermatozoa. From each testis, spermatozoa pass into a coiled tube called the epididymis, which stores spermatozoa while they develop motility and fertilizing ability. Spermatogenesis in seminiferous tubules of adult testis is regulated by several hormones and cell-cell interactions, implying the importance of signals contributing to cell differentiation.

Examining expression of CD30 in adult mouse testis and epididymis, we here report that expression of CD30 is developmentally regulated in tissue and cell specific-manners during spermatogenesis in testis and epididymis of adult mice.

Materials and Methods

Mice

Male C57BL/6 mice (10 weeks old; Samtako Inc., Osan, Korea) and CD30-deficient mice (Amakawa et al., 1996) (a generous gift from Dr. Tak W. Mak, Ontario Cancer Institute, Toronto, Ontario, Canada) were housed under controlled 14 h light and 10 h darkness, and fed a standard diet of pellets and water ad libitum.

Antibodies

Antibodies to mouse CD30 and fluorescein isothiocyanate (FITC)-conjugated anti-hamster IgG and isotype control were purchased from BD Pharmingen (San Diego, CA. USA).

Tissue preparation

At 12 weeks of age, animals were decapitated and the testis and epididymis were removed immediately and mixed with Tissue-Tek ® O.C.T. compound (Miles Inc., USA), then frozen in liquid nitrogen, and stored at -80°C until use.

Staining and immunofluorescence microscopy

The distribution of CD30 in mature mouse testes and epididymis was determined by indirect immnunofluorescence microscopy of frozen sections. Serial sections (6 μl thick) cut with a cryostat microtome were thaw-mounted on albumin-coated glass slides. The mounted sections were dried in air for 2 h and fixed with acetone at -20°C for 5 min as described by Graus et al. (Graus et al., 1984). Sections were washed twice with phosphate-buffered saline (PBS) for 10 min and then incubated with 5% bovine serum albumin (BSA) in PBS for 15 min at room temperature. After washing with PBS twice, they were incubated with monoclonal antibody diluted in PBS containing 5% BSA overnight at 4°C. They were washed with cold PBS four times, and then incubated with FITC-conjugated goat anti-hamster IgG antibody diluted in PBS to 1:100 for 3 h. After washing with PBS five times, the sections were sealed with a coverslip. To identify nuclei I μl/ml of a DNA-specific fluorescein dye (Hoechst 33342) was added. All fluorescent samples were observed with a Carl Zeiss Axiovert 25 microscope equipped for epifluorescence and photographed on a 35 mm film (Presto 400, Kodak Film). A control staining was performed according to identical procedure except using a isotype control (hamster IgG) instead of primary antibody.

Reverse transcription-PCR (RT-PCR)

Frozen tissue samples were ground in liquid nitrogen. Then total RNAs were extracted using the acid-phenol method of Chomczynski and Sacchi (1987). Semi-quantitative RT-PCR was used to assess CD30 mRNA leuel in tissues, based on the relative expression of 2 mRNAs: CD30 and GAPDH. Primers used were CD30 sense, 5'-CAACCCTGGCTGAGTTACTC-3', and anti-sense, 5'-AGCGGCAGGTTCTTCAGGTA-3', leading to a 942-bp PCR product and GAPDH sense, 5'-TGA AGG TCG GAG TCA ACG GAT TTG GT-3' and antisense, 5'-CAT GTG GGC CAT GAG GTC CAC CAC-3', leading to a 983-bp PCR product.

Total RNA was reverse transcribed and PCR was carried out using RT-PCR PreMix (Bioneer Co., Korea). The reaction mixture contained 1 μg total RNA, 20 U M-MLV reverse transcriptase, 10 U RNasin, 10 mM Tris-Cl (pH 8.3), 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM dNTP, 10 pmole of each primer, and 1 U Taq polymerase. The reverse transcription and PCR amplification was simultaneously performed in one batch tube. The reverse transcription was carried out for 60 min at 42°C, and the mixture was heated to 94°C for 3 min to terminate the reaction. PCR was performed for 35 cycles of denaturation at 94°C for 45 sec, annealing at 55°C for 90 sec, and extension at 72°C for 90 sec. A final extension step was followed at 72°C for 5 min.

Ten microliters of each RT-PCR product were loaded on a 1% agarose gel and run for 0.5 hr at 120 V. The image of the ethidium bromide (0.1 μg/ml)-stained gel was digitalized by a video image analyzer (Vilber Lourmat, France).

Results

Immunofluorescence staining of testis

When the testes were assessed for their CD30 expression by immunofluorescence, as shown in Fig. 1, CD30 was predominantly distributed in spermatogonia, spermatocytes, interstitial cells, Leydig cells and Sertoli cells, whereas spermatids were negative (Fig. 1A). Antibody to CD30 stained whole cytoplasm of these cells, but not the nucleus (Fig. 1A). Images of nuclear staining (Fig. 1B) and Nomarski differential interference contrast microscopy

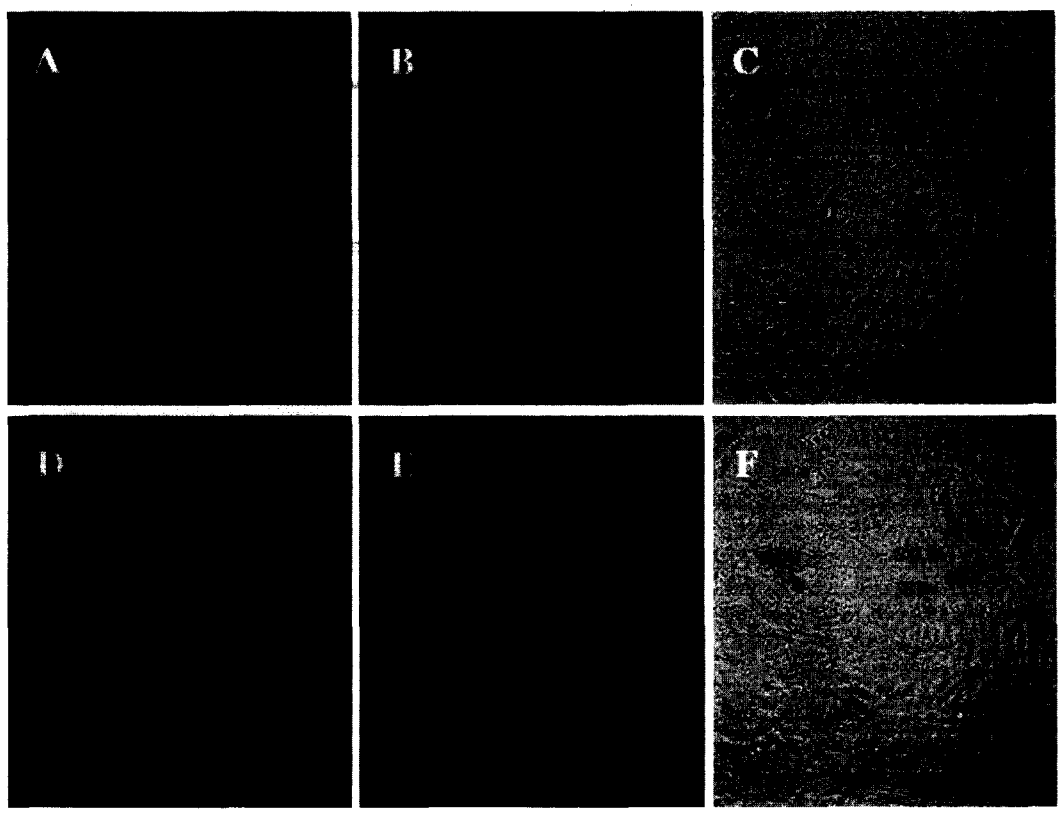

Fig. 1. Localization of CD30 detected by indirect immunofluorescence microscopy in the mouse testis. Serial sections from wild-type (A-C) and $CD30^{-/-}$ (D-F) mice were immunostained with anti-CD30 and then FITC-labeled goat anti-hamster IgG antibody (A, D), and then stained with Hoechst 33342 for DNA (B, E). Images with Nomarski optics (C, F) show normal morphology of seminiferous tubules in the mouse testis. bm, basement membrane; ps, pachytene spermatocytes; s, spermatids; sc, Sertoli cells; sg, spermatogonia. Scale bars=200 μm.

(Fig. 1C) show typical architecture of the seminiferous tubules and interstitial cells (Fig. 1C). In contrast, no CD30 expression was detected in $CD30^{-/-}$ mice (Fig. 1D), while they showed normal figure in nuclear staining (Fig. 1E) and Nomarski differential interference contrast microscopy (Fig. 1F).

Immunofluorescence staining of epididymis

When epididymis was examined, CD30 expression was found in luminal epithelia and interstium, whereas spermatozoa were negative (Fig. 2A). Images of nuclear staining and Nomarski differential interference contrast microscopy show typical architecture of the cauda epididymis constructed of epithelial cells, interstitial cells and spermatozoa (Fig. 2B and 2C). In $CD30^{-/-}$ mice as controls, no CD30 expression was detected (Fig. 2D) and nuclear staining (Fig. 2E) and Nomarski differential interference contrast microscopy (Fig. 2F) were normal.

Table 1 summarizes the immunohistochemical data on the differential localization of CD30 in testis and epididymis.

Negative control staining

We carried out negative control staining to confirm the specific reactivity of anti-CD30 antibody. Using isotype control (hamster IgG) for primary antibody, no significant positive staining in testis (Fig. 3A) and epididymis (Fig. 3D) were shown. Images by nuclear staining and Nomarski differential interference contrast microscopy show typical architecture of the seminiferous tubules (Fig. 3B and C) and epididymis (Fig. 3E and F). These results together indicate that CD30 reactivity shown above is not an artifact.

RT-PCR

In order to confirm the CD30 expression at RNA level in the testis and epididymis, we performed RT-PCR using frozen tissues and compared to $CD30^{-/-}$ mice. As shown in Fig. 4, CD30 RNA transcripts were detected in both the testis and epididymis, but not in $CD30^{-/-}$ mice.

Discussion

The results presented in the current study are, to our knowledge, the first immunohistochemical data showing the expression pattern of CD30 in the testis and epididymis of adult mice. The expression pattern was determined at a protein level by immunofluorescence

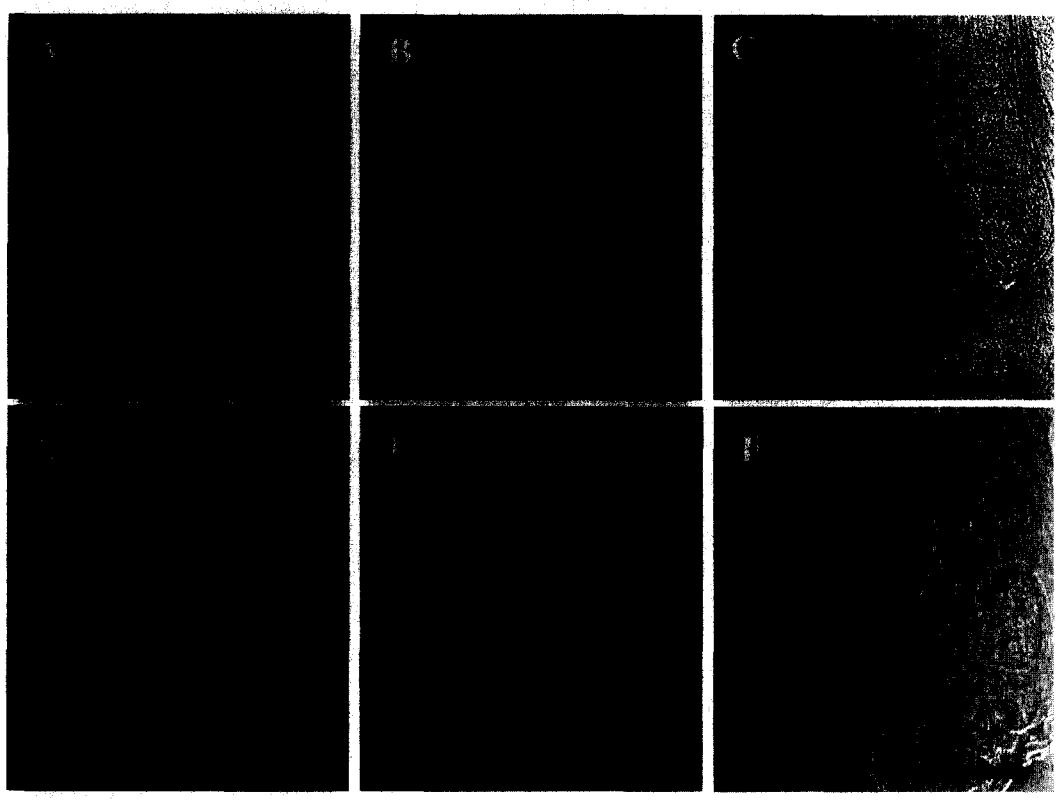

Fig. 2. Localization of CD30 detected by indirect immunofluorescence microscopy in the mouse epididymis. Serial sections from wild-type (A-C) and $CD30^{-/-}$ (D-F) mice were immunostained with anti-CD30 and then FITC-labeled goat anti-hamster IgG antibody (A, D), and then stained with Hoechst 33342 for DNA (B, E). Images with Nomarski optics (C, F) show normal morphology of the mouse epididymis. bm, basement membrane; e, epithelial layer; i, interstitial cells; sz, spermatozoa. Scale bars=200 μm.

with anit-CD30 antibody and also RNA level by RT-PCR analysis. In the testis, CD30 was detected in spermatogonia, spermatocytes, whereas spermatids were CD30 negative. These results indicate that CD30 may act as a signal molecule for further differentiation of spermatogonia and spermatocytes.

Sertoli cells are involved in the control of RNA and DNA synthesis of germ cells (Rivarola et al., 1985) and

Table 1. Distribution of CD30 in the testis and epididymis

Organs	Cell types	Degree of expression*
Seminiferous tubules	Spermatogonia	++
	Spermatocytes	++
	Spermatids	–
	Sertoli cells	++
Interstitium	Leydig cells	++
Epididymis	Epithelial cells	+
	Interstitial cells	+
	Spermatozoa	–

*CD30 expression was determined by indirect immunofloursence and representative scores from one experiment of three similar repeats are shown. ++, intense; +, moderate; –, negative

spermatogonial divisions and signaling to the developing sperm (Meehan et al., 2000; Meng et al., 2000). However, to date, whether and how the testicular CD30 from Sertoli cells is associated with spermatogenesis awaits to be defined. CD30 was abundantly distributed in Sertoli cells of seminiferous tubules (Fig. 2A), suggesting that CD30 may transduce any signals required for mitosis, meiosis or spermiogenesis of germ cells during spermatogenesis. Anti-CD30 monoclonal antibody stained the entire cytoplasm of germ cells, but not the nucleus, indicating that it may play some additional roles in the cytoplasm.

It has been generally accepted that spermatozoa are passed into epididymis for developing motility and fertilizing ability. In the epididymis, CD30 was not detected in spermatozoa, suggesting that CD30 may play any roles in maturation of germ cells to obtain fertilizing ability. Concomitantly, $CD30^{-/-}$ mice do not show any defects in fertility. So far, CD30 deficiency has been known to cause partial defects in immunological functions such as thymic negative selection (Amakawa et al., 1996) and deletion of autoreactive cytotoxic T cells (Kurts et al., 1999). In addition, control of germ cell apoptosis is possibly suggested as an alternative functional role of

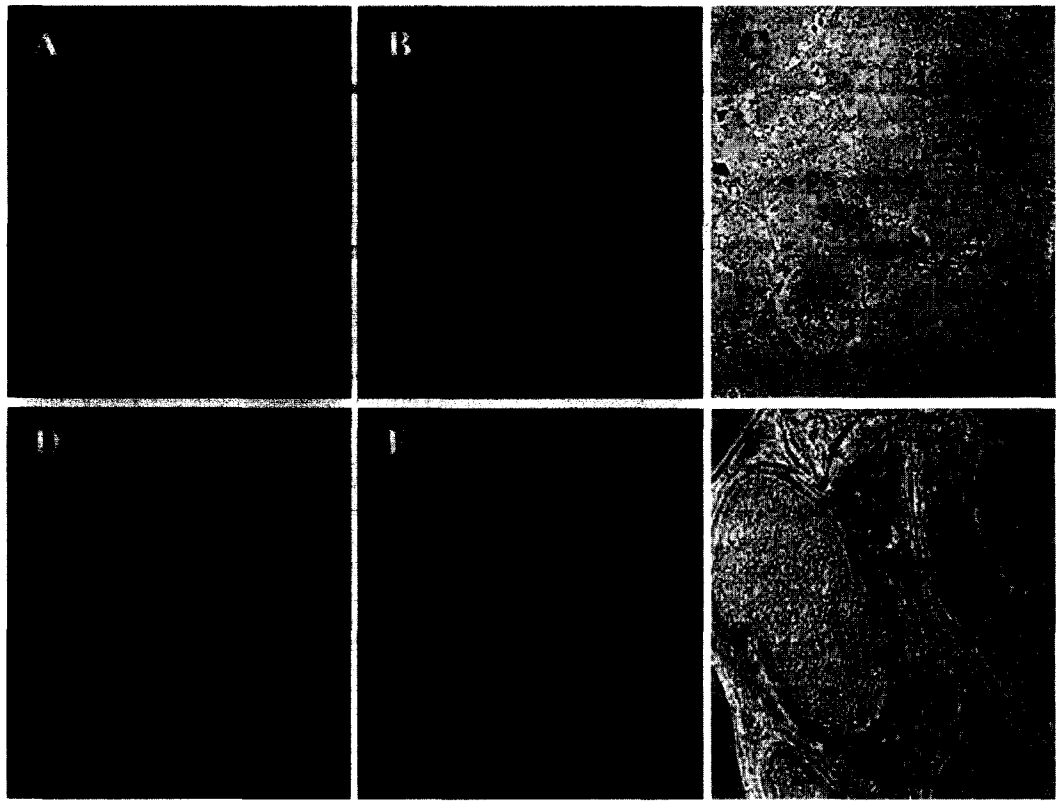

Fig. 3. Negative controls, consisting of the omission of the primary antibody, of seminiferous tubules in testis (A-C) and epididymis (D-F). Sections were stained with secondary antibody alone (A, D), and then with Hoechst 33342 for DNA (B, E). Images of Nomarski optics show normal morphology of the seminiferous tubules (C) and epididymis (F). bm, basement membrane; ps, pachytene spermatocytes; sc, sertoli cells; sg, spermatogonia; sz, spermatozoa; e, epithelial layer; i, interstitial cells. Scale bars=200 μm.

CD30, as CD30 signal leads to cell death (Gruss et al., 1994a). Fas, another member of TNFR family, mediates death signal of germ cells initiated by sertoli-germ cell interaction after toxicant exposure (Boekelheide et al., 1998). This issue needs to be addressed in combination with other signals responsible for germ cell survival. We are currently extending our study to the expression and function of CD30 in the ovary, which will hopefully provide further insight into the functional role of CD30 signals in germ cells.

In this study, it was concluded that the expression of CD30 in the testis and epididymis is spatio-temporally regulated during spermatogenesis, although the biological functions of CD30 in germ cells were not clarified.

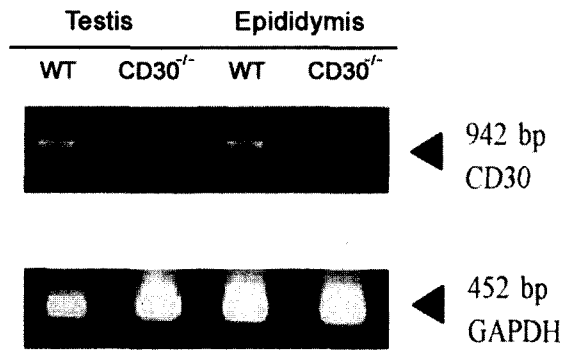

Fig. 4. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of CD30 expression in mouse testis and epididymis. Total RNA was prepared from frozen tissues from wild-type (WT) and $CD30^{-/-}$ mice. RT-PCR was performed as described at Materials and Methods.

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