Introduction

Complement-dependent sperm-immobilizing antibodies (SI-Abs) have frequently been detected in the sera of infertile women (Isojima et al., 1968, 1972, 1989; Kobayashi, 1990). Identification of the epitopes recognized by SI-Abs is important not only for understanding the mechanism of immunological infertility but also for developing a means for treatment of infertility due to SI-Abs. With this in mind, many monoclonal antibodies with sperm-immobilizing activity including human antibodies have previously been generated (Shigeta et al., 1980; Kurokchitchiev et al., 1986; Kameda et al., 1991; Komori et al., 1997). Analysis of these monoclonal antibodies as well as patients’ anti-sperm antibodies have shown that the carbohydrate moieties of sperm and seminal plasma are major epitopes of these antibodies (Koyama, 1991; Kurpisz and Alexander, 1995).

Previously, using peripheral B lymphocytes from an infertile woman, we established a stable human–mouse heterohybridoma, H6-3C4, producing a human monoclonal antibody with high titres of SI-Ab (Isojima et al., 1997). Analysis of these monoclonal antibodies as well as patients’ anti-sperm antibodies have shown that the carbohydrate moieties of sperm and seminal plasma are major epitopes of these antibodies (Koyama, 1991; Kurpisz and Alexander, 1995).

Recently, several research groups have suggested that MAB H6-3C4 recognized the male reproductive tract CD52 (mrtCD52) molecule (Dieckman et al., 1999; Kirchhoff and Schroter, 2001). Originally, CD52 was identified as an antigen for a monoclonal antibody called campath-1 that was produced using a human spleen cell antigen (Hale et al., 1990; Valentin et al., 1992). Human CD52 is expressed in virtually all lymphocytes, mature spermatoozaoa and seminal plasma (Hale et al., 1993). The sperm maturation associated CD52 is produced and secreted by epithelial cells of the cauda epididymis. Mature spermatooza acquire the molecules from the epididymal fluid secreted by the epithelium during their passage, though extra CD52 can be shed into seminal plasma (Kirchhoff, 1998; Kirchhoff and Schroter, 2001). The core protein of lymphocyte and male reproductive tract CD52 (mrtCD52) has a common small peptide comprising 12 amino acids encoded by a single copy gene located on chromosome 1 (Xia et al., 1991), and a glycosylphosphatidylinositol (GPI) anchor attached to Ser at the COOH-terminus (Xia et al., 1991; Schroter et al., 1999). The 12 amino acid peptide backbone carries a multi-branched N-linked carbohydrate attached to the Asn3 position, having distinct structural differences between lymphocytes and sperm (Treumann et al., 1995; Schroter et al., 1999). Male reproductive tract CD52 is characterized by extremely heterogeneous carbohydrate molecules compared to lymphocyte CD52. In this study, we analysed the epitopes of MAB H6-3C4 and compared them with those of two other sperm-immobilizing monoclonal antibodies, 1G12 and campath-1. In addition, we produced anti-CD52 core peptide antiserum in rabbits to examine its immunological and biological properties against sperm.

Materials and methods

Monoclonal antibodies

MAB H6-3C4, an IgM sperm-immobilizing human monoclonal antibody, was obtained from a human–mouse heterohybridoma that was generated using peripheral B cells of an infertile patient bearing SI-Ab (Isojima et al., 1987). 1G12, an IgM sperm-immobilizing mouse monoclonal antibody, was generated by immunization with human sperm membrane fraction as previously described (Komori et al., 1997). Campath-1, the monoclonal antibody (IgM)
reactive with human CD52, was purchased as clarified ascites from Calbiochem Ltd, Darmstadt, Germany. Generally, MAb H6-3C4 and 1G12 were used without dilution and campath-1 was used at a dilution of 1:500. A monoclonal antibody (4E12: IgM) that reacts to a zona pellucida antigen but does not relate to sperm antigen was used as the negative control.

**Antiserum to CD52 core peptide**

The 12 amino acid (GQNDTSQTSSPS) core peptide of CD52 was custom-synthesized (Chiron Mimotopes, Victoria, Australia) and conjugated to diphtheria toxoid (DT) as a carrier protein by the maleimide method (Lee et al., 1980). 100 μg of the conjugate was injected into a rabbit three times at 1 month intervals. The first injection was carried out with complete Freund’s adjuvant (Difco Laboratory, Detroit, MI) and the second and third injections were with incomplete Freund’s adjuvant. Blood was collected 1 month after the third injection.

**Sperm immobilization test (SIT)**

Human semen was collected from healthy donors under informed consent. Each semen specimen was mixed with 10 ml of BWW medium (Biggers et al., 1971) containing 0.3% BSA (Sigma, St Louis, MI) and centrifuged at 600 g for 5 min. The sperm pellet was washed with BWW medium twice and incubated in 5% CO2 in air for 3 h. Swim-up sperm were collected and concentrated to the required concentration as detailed for the individual experiments below.

Complement-dependent sperm immobilization tests were carried out as described previously (Isojima and Koyama, 1979). Briefly, 10 μl of diluted antiserum, 1 μl of sperm suspension (40×10^6/ml) and 2 μl of active or inactive complement (Low-Tox guinea pig complement; Cedarlane, Ontario, Canada) were mixed in each well of a microplate, (Greiner, Germany), incubated at 32°C for 1 h, and motile sperm were counted under an inverted microscope (×200 magnification). SI-Ab activity (SI_{30} unit) was estimated from a regression curve obtained by plotting sperm immobilization activity against serial dilutions of antiserum (Koyama et al., 1988). Three assays were performed with spermatozoa from different donors. SI_{30} unit was represented as mean ± SD.

**Immunofluorescent staining of spermatozoa**

Swim-up sperm prepared as described above were washed with phosphate-buffered saline (PBS), pH 7.2, and an aliquot of sperm suspension (concentration of 2×10^6/ml) was placed on a glass slide. The specimen was fixed with 10% neutral formalin for 10 min, and incubated with MAb H6-3C4, 1G12, campath-1 or rabbit antiserum to the CD52 core peptide for 60 min. As a negative control, the rabbit serum immunized with DT was used. After washing with PBS for 10 min, 200-fold diluted fluorescein-isothiocyanate (FITC)-labelled goat anti-human IgM, anti-mouse IgM, anti-rat IgM or anti-rabbit IgG antibodies (ICN/Cappel, Aurora, OH) was applied and incubated for 30 min. The specimen was mounted in a mounting medium (Vector Laboratories, Burlingame, CA, USA) for observation under a UV microscope (TE300; Nikon, Tokyo, Japan).

**Preparation of human sperm extract**

Sperm pellet was suspended in a mixture of Milli-Q water:chloroform:methanol (3:4:8 volume ratio) and centrifuged at 9200 g for 5 min. The supernatant was evaporated to dryness by vacuum centrifugation and stored at −80°C until use. This preparation was used as crude mrtCD52.

**Western blot analysis**

For detection of antigens reactive to the monoclonal antibodies, sperm extract was subjected to SDS–PAGE (Laemmli, 1970) through 5–20% polyacrylamide gel. The separated proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane. The blots were blocked with PBS containing 5% bovine serum albumin and incubated with primary antibodies, MAB H6-3C4 (supernatant), 1G12 (supernatant), campath-1 (ascites at 1:500 dilution) and the antiserum to CD52 core peptide (1:200 dilution), for 20 h at 4°C. The blots were washed with PBS three times and incubated with a secondary antibody (1:1000 dilution) which was horseradish peroxidase-conjugated goat anti-human IgM for MAb H6-3C4, anti-mouse IgM for 1G12, anti-rat IgM for campath-1 or anti-rabbit IgG for antiserum against the synthetic peptide (ICN/ Cappel, Aurora, OH) for 1 h at room temperature. The blots were washed as above, and colour was developed with 4-chloro-1-naphthol and 0.01% H2O2 in PBS containing 10% methanol. In some experiments, a biotin-avidin system (Vector Laboratories) was applied to enhance the detection of the antigenic components. High resolution two-dimensional PAGE was carried out according to O’Farrell (O’Farrell, 1975) with a slight modification. In brief, the sperm extract was dissolved in a sample solution containing 6 mol/l urea, 2% Nonident P-40, 0.05 mol/l 2-mercaptoethanol, and 5% of a pH-gradient reagent (pH 2–4 or pH 3–10) (Serva, Heidelberg, Germany). Isoelectric focusing for the first dimension was carried out at 150 V overnight, and SDS–PAGE for the second dimension was carried out at 20 mA for 90 min. Calibration kits for pl determination (Amersham Bioscience, Tokyo, Japan) and Precision protein standards (Bio-Rad, Laboratories, Hercules, CA) were used for pH and Mr markers respectively.

**Amino acid sequence analysis of the antigen reacting to MAb H6-3C4**

MAb H6-3C4 from human–mouse heterohybridoma cultivated in protein-free medium (PFHM-II; Invitrogen, Tokyo, Japan) was purified by HiTrap™ IgM Purification HP (Amersham Bioscience). Sperm extract (equivalent to 2.5×10^7 spermatozoa in 100 μl of PBS) was incubated with the purified MAB H6-3C4 (20 μg) overnight at 4°C and centrifuged at 9200 g for 10 min. The pellet was dissolved in a sample buffer for SDS–PAGE (Laemmli, 1970), electrophoresed in 5–20% of gradient polyacrylamide gel at 20 mA for 60 min. Proteins were transferred onto a PVDF membrane under a constant current of 160 mA for 1 h, and stained with Coomassie brilliant blue (CBB). The region reactive to MAB H6-3C4 was excised from the membrane. The amino acid sequence was determined by an automated gas-phase protein sequence system based on Edman degradation (Procise 491 cLC; Applied Biosystems, Tokyo, Japan).

**Removal of N-linked and O-linked carbohydrates**

To remove N-linked carbohydrate chains, sperm extract was treated with 10 units/ml N-glycosidase F (Calbiochem, Darmstadt, Germany) in 0.1% SDS, 0.05 mol/l 2-mercaptoethanol in 20 mmol/l sodium phosphate buffer (pH 7.2) at 37°C for 20 h. The reaction mixture was separated by SDS–PAGE or two-dimensional PAGE. To remove O-linked carbohydrate chains, chemical two-step deglycosylation was performed according to Duk et al. (1997). Briefly, N-glycosidase-treated sperm extract was incubated in 0.025 mol/l sulfuric acid for

**Figure 1. Hypothetical structure of mrtCD52.** The mrtCD52 molecule is composed of a 12 amino acid peptide, N-linked and O-linked carbohydrates, and a glycosylphosphatidylinositol (GPI) anchor portion inserted in the plasma membrane. This structure is based on Schroter et al. (1999) and Yeung et al. (2001). The amino acid sequence used for producing the polyclonal antibody is shown in capitals in the box. Carbohydrate chains are shown by ovals. This model includes the potential sites for O-linked carbohydrates.
1 h at 80°C for desialylation (first step), dialysed against Milli-Q water and lyophilized. The lyophilized material was treated with 0.055 mol/l NaOH for 16 h at 40°C to hydrolyse O-Ser/Thr (second step).

Lectin binding assay
Sperm extract was separated by SDS–PAGE and transferred onto a PVDF membrane as described above. Excess protein binding sites on the membrane were blocked with 3% BSA in PBS for 15 min at room temperature. Biotinylated Peanut Agglutinin (PNA) or Wheat Germ Agglutinin (WGA) (Vector Laboratories) was applied to the blot membrane at 10 mg/ml and incubated for 20 h at 4°C. After washing three times with PBS, the membranes were treated with horseradish peroxidase-coupled avidin D (Vector Laboratories) in PBS at room temperature. After washing with PBS, colour was developed with 0.5 mg/ml of 4-chloro-1-naphthol and 0.01% H₂O₂ in PBS containing 10% methanol.

Results
Antiserum to the CD52 core peptide
An antiserum reactive to the 12 amino acid core peptide of the CD52 molecule was produced by immunization with a synthetic core peptide (Figure 1) conjugated with DT. In Western blot analysis, the polyclonal antibody reacted with the conjugate of Mr 60–70K (Figure 2a, lane 1) and with sperm components of Mr 17–23K (Figure 2a, lane 2). The ability of the antibody to react with sperm extract was lost after absorption with human sperm (Figure 2a, lane 3), indicating that the synthetic peptide could induce antibodies reactive to the naturally occurring CD52 on sperm. The antiserum exhibited sperm agglutination and complement-dependent sperm immobilizing (SI) activity with the SI50 unit of 81.1 ± 10.6 (n = 3). Undiluted antiserum completely blocked sperm motility. The anti-DT antiserum did not show SI activity. This indicates that the peptide portion of CD52 could induce SI-Ab.

Amino acid sequence of the cognate antigen of MAb H6-3C4
MAb H6-3C4 reacted to Mr 17–25K components in sperm extract (Figure 2b, lane 1). It gave similar results to the anti-CD52 peptide antiserum as described above. For amino acid sequence analysis sperm extract was incubated with MAb H6-3C4, the immuno-complexes formed were separated by SDS–PAGE, transferred onto a PVDF membrane and stained with CBB. The region of Mr 17–25K (Figure 2b lane 2) was excised into three fractions. CBB staining detected heavy and light chains derived from MAb H6-3C4 IgM. No other proteins were detected at the region reactive to MAb H6-3C4, suggesting that the amount of proteins recognized by MAb H6-3C4 was below the level of detection by the method used. Amino acid sequence analysis was carried out using the three fractions. The highest-yield amino acids (aa) in each cycle of the upper fraction were as follows: 1st aa: G (1.98 pmol), 2nd aa: Q (2.10 pmol); 3rd aa: not detected; 4th aa: D (1.35 pmol); 5th aa: T (0.89 pmol); 6th aa: S (0.88 pmol); 7th aa: Q (1.73 pmol); 8th aa: T (0.58 pmol); 9th aa: S (0.64 pmol); 10th aa: S (0.77 pmol); 11th: P (0.74 pmol); 12th aa: not detected. This resulted in the determination of a sequence, GQXDTSQTSSPX, where the two unidentified residues are indicated by ‘X’, one probably due to glycosylation and the other due to the COOH-terminal position, in all three samples. These amino acids,
except for the two unidentified residues, were identical with the core peptide sequence of the CD52 molecule on human lymphocytes in which the amino acid sequence has been shown to be GQNDTSQTSSPS. Therefore, the unidentified positions 3 and 12 are probably N and S. The yields of ‘T’ and ‘S’ at positions 5, 6, 8, 9 and 10 were less than those of other amino acids in the sequence, suggesting that these residues were partially O-glycosylated.

**Characterization of monoclonal antibodies**

We analysed the reactivity of the anti-CD52 peptide antibody and three monoclonal antibodies, MAb H6-3C4, 1G12 and campath-1, with sperm. The immunofluorescent staining showed that all the antibodies tested reacted to the whole surface of the sperm (Figure 3). No immunofluorescence was detected in the negative control.

To characterize the epitopes of the antibodies, sperm extracts were subjected to high resolution two-dimensional PAGE with the first dimension in a pH 3–10 range and then Western blot analysis was conducted using the four antibodies described above. MAb H6-3C4 and 1G12 yielded staining patterns similar to those with campath-1 and anti-CD52 peptide antibody, reacting with the extremely acidic components (pH <3.5) with Mr 17–25K. When a pH range of 2–4 for the first dimension and a biotin-avidin system for immunological detection were used for more precise analysis, MAb H6-3C4 and 1G12 showed a similar polymorphic pattern.

**Figure 4.** Two-dimensional PAGE and Western blot analysis of sperm extract with monoclonal anti-CD52 peptide antibodies. When a pH range of 3–10 (wide range) was used for the first dimension, MAb H6-3C4 (a), 1G12 (b), campath-1 (c), and anti-peptide antibody (d) reacted to the extremely acidic region (pH <3.5) of Mr 17–25K. When a pH range of 2–4 for the first dimension and a biotin-avidin system for immunological detection were used for more precise analysis, MAb H6-3C4 (e), 1G12 (f) campath-1 (g) and anti-peptide antibody (h) showed a similar polymorphic pattern.

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We conducted a similar analysis in a pH range of 2–4 for the first dimension of the two-dimensional PAGE and then a biotin-avidin system to enhance immunological detection. With all the antibodies used, positive stainings appeared in the region of Mr 25–35K as well as 17–25K, both of which showed heterogeneous patterns comprising at least six different pH components (Figure 4e–h). The similarity of the staining patterns confirmed that MAb H6-3C4 and 1G12 recognize the CD52 molecule as is the case for campath-1 and the anti-CD52 peptide antiserum. The anti-CD52 peptide antiserum reacted strongly to the regions of lower Mr and higher pH, suggesting that the components of higher Mr contain more carbohydrates than the components of lower Mr, which interfered with the binding of the anti-core peptide antibody. No positive reaction was detected with 4 E12 or anti-DT rabbit antiserum.

Analysis of carbohydrates of mrtCD52
A lectin binding assay was used to detect N-linked and O-linked carbohydrate chains in the mrtCD52. Sperm extract was treated with N-glycosidase F and subjected to electrophoresis. To detect N- and O-linked carbohydrates, WGA lectin and PNA lectin were used which bind to Galβ1–4GlcNAc and Galβ1–3GalNAc respectively. The sperm extract showed N-linked carbohydrate-positive spots at Mr 17–35K (Figure 5a), but these spots disappeared after treatment of the sperm extract with N-glycosidase F (Figure 5b). The components at Mr 17–35K also reacted with PNA lectin indicating the presence of O-linked carbohydrates (Figure 5c). Mr 27–35K components (Figure 5a and c) are probably dimeric forms of Mr 17–25K. After treatment with N-glycosidase F the PNA reactivity shifted to a region of Mr 13–22K (Figure 5d). This reactivity disappeared after a mild alkaline treatment which removed O-linked carbohydrates (Figure 5e). Anti-core peptide polyclonal antibody showed a positive band of Mr 13K on the membrane prepared by the same treatment as lane e, indicating that the molecule corresponds to the core peptide and GPI anchor of mrtCD52 (Figure 5f). The disappearance of PNA reactivity by a mild alkaline treatment was not due to peptide lysis by this treatment. The staining pattern with PNA lectin in high-resolution two-dimensional PAGE (Figure 5g) was quite similar to those with MAb H6-3C4, 1G12 and campath-1 as shown in Figure 4e–g. These results suggested that the mrtCD52 contained O- as well as N-linked carbohydrates.

**Figure 6.** Effect of deglycosylation treatments on mrtCD52 epitopes for MAb H6-3C4, 1G12 and campath-1. The CD52 epitope for MAb H6-3C4 was lost after removal of N-linked carbohydrate (a). 1G12 reacted to six different pH components after removal of N-linked carbohydrate (b), and a single spot of Mr 12K at pH 3.5 after further removal of O-linked carbohydrate (d). Campath-1 reacted to three different pH components after removal of N-linked carbohydrate (c), and the same single spot as observed with 1G12 after further removal of O-linked carbohydrate (e).

Reaction of antibodies after removal of carbohydrates
The reaction of MAb H6-3C4 disappeared after sperm extract was treated with N-glycosidase F, indicating that the epitope for MAb H6-3C4 is present in the N-linked carbohydrate moiety of CD52 (Figure 6a). In contrast, 1G12 and campath-1 showed positive reactions after N-glycosidase F treatment, with a shift of staining to lower Mr regions (Figure 6b and c) and persisting pH heterogeneity. 1G12 reacted with six spots at different pH (Figure 6b), while campath-1 reacted with three different spots (Figure 6c). This indicates that the epitope for 1G12 is not identical to that of campath-1. Further mild alkaline treatment to remove O-linked carbohydrates resulted in a decrease in the number of spots reacting to 1G12 and campath-1 reacting to only one spot at Mr 13K and pH 3.5 (Figure 6d and e). These results indicated that 1G12 bound to an epitope in the GPI anchor and/or peptide portion.

Discussion
We have previously shown, using glycerolipids purified from various tissues, that MAb H6-3C4 reacts with N-acetyllactosamine [Galβ1→4GlcNAc] with and without terminal N-acetyllactosaminic acid and fucose (Tsuji et al., 1988). Mass spectrometric analysis showed that the polylactosamine structure [Galβ1→4GlcNAc]n is present in N-linked carbohydrates of mrtCD52 (Schroter et al., 1999). In the present study, we found that the reactivity of sperm extract with MAb H6-3C4 completely disappeared after removal of the N-linked carbohydrate by treatment with N-glycosidase F. Taken together, these results indicated that MAb H6-3C4 recognizes the...
[Galβ1→3GlcNAc]n structure in the N-linked carbohydrate of mrtCD52. Although the unit of [Galβ1→4GlcNAc] is found in various organs, a combination of an extreme unit repetition and specific carbohydrate branches in the male genitals may provide a unique epitope for MAb H6-3C4.

1G12 is a mouse monoclonal antibody having strong sperm-immobilizing activity but its epitope has not been determined. In the present study, 1G12 was shown to recognize a structure formed by the GPI anchor and/or peptide portion (Figure 6d). The epitope for campath-1 has been reported to include the COOH-terminal three amino acid sequence on the core peptide and GPI anchor portion (Hale, 1995; Teuermann et al., 1995). The epitopes for these two monoclonal antibodies were similar but distinct (Figure 6b and c). It suggests that the GPI anchor portion of the mrtCD52 contains heterogeneous immunogenicity. 1G12 has been demonstrated to cross-react with lymphocytes (Komori et al., 1997). The binding site for 1G12 is probably a structure formed by the GPI anchor and/or the peptide portion of mrtCD52 also present in lymphocyte CD52. Two-dimensional PAGE and Western blot analysis showed that 1G12 recognized three higher Mr spots before removal of the O-linked carbohydrate. However, Campath-1 (Figure 6b and c), whose epitope does not recognize carbohydrates (Valentin et al., 1992), did not. The O-linked carbohydrate may enhance the binding affinity of the antigen–antibody reaction by its hydrophilic property. Alternatively, the lack of campath-1 reactivity with the three higher Mr spots may be due to a masking of the campath-1 epitope by the O-linked carbohydrates.

Previously, Diekman et al. (2000) suggested the presence of O-linked carbohydrates in mrtCD52. This kind of oligosaccharide seems to be completely absent on the lymphocyte (Teuermann et al., 1995). We demonstrated the possible presence of the carbohydrates by the lectin binding assay on mrtCD52 in the present study. The amino acid sequence analysis showed that the yields of serine and threonine at amino acid positions 6, 8, 9 and 10 were less than other amino acids. The result supported the proposal that all potential sites were partially glycosylated by O-linked carbohydrates as shown in the hypothetical structure of mrtCD52 depicted in Figure 1. Although further experiments are necessary, human mrtCD52 seems to contain several O-linked carbohydrates different from the lymphocyte CD52. The rat counterpart of mrtCD52 has also been shown to contain extensive O-linked carbohydrate chains (Derr et al., 2001). The antiserum produced by immunization with the synthetic peptide of the CD52 core protein reacted to sperm and caused sperm agglutination and complement-dependent sperm immobilization. Collectively, the different portions of the CD52 molecule, including carbohydrate moieties, core peptide and GPI anchor, could induce the antibodies that interfere with sperm motility.

The biological function of CD52 in mature sperm is not well understood. The glycosylated molecules including N-linked and O-linked carbohydrates make the CD52 molecule highly negatively charged. This study showed that not only N- but also O-linked carbohydrate contribute to the heterogeneous negative charge of mrtCD52 (Figure 6). Such a structure may prevent lymphocytes and sperm from auto-agglutination and non-specific adherence to tissues as previously proposed (Kirchhoff and Hale, 1996; Kirchhoff and Schrotter, 2001). Considering monoclonal antibodies targeted to mrtCD52 exhibit extremely strong sperm-immobilizing activity with complement, it is an attractive hypothesis that CD52 possesses the function to suppress complement activity. Campath-1 recognizing lymphocyte CD52 has also been shown to induce complement-dependent cell lysis (Xia et al., 1993). The female genital tract is subject to frequent infection with various pathogens including sexually transmitted bacteria and viruses. However, its antibody-producing ability is not as high as mucosal tissues (Wu et al., 2000). Innate immunological systems such as complement are thought to mainly serve as a host defence mechanism. Functionally active complement exists in the female genital tract (Price et al., 1979) and follicular fluid (Perricone et al., 1992). Recently, complement regulatory proteins such as C1-INH, CD55, CD46 and CD59 were found on the surface of spermatozoa (Jiang and Pillai, 1998), and CD55 and CD59 were shown to be GPI anchor proteins (Kirchhoff and Hale, 1996). Collectively, it is speculated that GPI anchoring and complement-regulatory proteins seem to play a role in protecting sperm from complement attack during transportation to the fertilization site.

The mechanism by which mrtCD52 induces antibodies in the female genital tract is not known. Normally the N-linked carbohydrate of the CD52 molecule on sperm is not recognized by immunocompetent cells as an antigen, as shown by the low frequency of detection of SI-Abs in infertile women. Local pathogenic conditions such as genital infections may contribute to enhance the immunological responses. Spermatozoa falling into the abdominal cavity would be easily recognized by macrophages as a foreign antigen. Alternatively, it is possible that some molecular change of mrtCD52 caused by genetic alterations may enhance immunological responses in the female genital tract. In addition, several immuno-suppressive factors have been reported in seminal plasma (Skibinski et al., 1992; Kelly, 1995; Sakin-Kaindl et al., 2001). The deficiency of these factors in seminal plasma may enhance the production of sperm antibodies in women. Although the mrtCD52 is not the only aetiological sperm antigen for infertility, the knowledge about its physiological function and molecular structure is helpful for understanding the mechanism of production of anti-sperm antibodies and for developing means for treatment of infertility.

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Epitope analysis of sperm-immobilizing antibodies