SEROLOGICAL DIAGNOSIS

Influence of cardiolipin antibodies on the binding of treponemal specific antibodies in the fluorescence treponemal antibody absorption test and the *Treponema pallidum* immobilisation test

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The aim of the present study was to investigate the biological role of cardiolipin antibodies during *Treponema pallidum* infection. Inhibition of the binding of treponemal specific antibodies at the early and late stages of infection by cardiolipin antibodies was shown in the fluorescence treponemal antibody absorption (FTA-ABS) test and *T. pallidum* immobilisation (TPI) test. Incubation of treponemes with cardiolipin antibodies followed by a second incubation with treponemal specific antibodies resulted in a reduction of the titres of the FTA-ABS test and the TPI test. The findings suggest that cardiolipin antibody production should be considered as a virulence mechanism of pathogenic treponemes with the purpose of evading the host defence mechanisms.

Introduction

The occurrence of cardiolipin antibodies was first described for syphilis [1] and later to a lesser degree for other infectious diseases caused by viruses, mycoplasms or protozoa [2]. The occurrence of antibodies against cardiolipin and the β 2-glycoprotein I as an antigenic cofactor has been described in the antiphospholipid syndrome (APS) [3, 4]. Syphilis cardiolipin IgG antibodies belong predominantly to the IgG1 and IgG3 subclasses and, in contrast to the APS cardiolipin antibodies, they are characterised by low avidity [5]. The biological role of cardiolipin antibodies in the course of syphilis has not yet been clearly elucidated. Following infection with *Treponema pallidum*, cardiolipin antibodies are detectable 1–2 weeks after the appearance of the primary chancre. The titres of cardiolipin antibodies depend on the activity of infection, and in contrast to treponemal specific antibodies which may persist for years or even for life, cardiolipin antibodies usually disappear after appropriate antibiotic treatment; therefore, these antibodies are of great diagnostic value. The Venereal Disease Research Laboratory (VDRL) test is used routinely to determine cardiolipin antibodies in the course of the treponemal infection [6].

The occurrence of cardiolipin antibodies is associated with the pathogenicity of treponemes; these antibodies are not detectable in healthy individuals colonised with saprophytic treponemes or in individuals immunised with killed *T. pallidum* [7].

For a long time it was assumed that the formation of cardiolipin antibodies during *T. pallidum* infection was primarily a result of the destruction of host cells and the release of cardiolipin from the membranes of mitochondria [8]. Recent data have shown that treponemes produce cardiolipin themselves [9, 10]. In 1995 Radolf and co-workers succeeded in isolating the outer membrane of *T. pallidum* and determining its chemical composition [10]. Only small amounts of cardiolipin were found in this compartment, unlike whole cell lysates where high proportions of cardiolipin were detected.

Cardiolipin antibodies do not seem to play a protective role as, despite their production in high titres, the treponemal infection continues. In 1993 Baker-Zander and co-workers [11] reported that cardiolipin antibodies enhanced phagocytosis of *T. pallidum* but failed to facilitate macrophage-mediated killing. They suggested that this mechanism could be utilised by *T. pallidum* to evade the extracellular immune response by residing within macrophages and causing persistent infection.

Another hypothesis for explaining the role of cardiolipin antibodies in the promotion of treponemal
infection includes the possibility that their target epitopes are in a close spatial relation to those of treponemal specific epitopes responsible for evoking antibodies with bactericidal capacity. So the binding of treponemal specific antibodies or the antibody-mediated antigen aggregation, which is a prerequisite for complement activation [12], could be impaired.

The purpose of the present study was to analyse a possible role of cardiolipin antibodies in the survival of treponemes in the infected host. Special emphasis was focused on the influence of cardiolipin antibodies on the binding of treponemal specific antibodies in the *T. pallidum* immobilisation (TPI) test and the fluorescence treponemal antibody absorption (FTA-ABS) test.

**Materials and methods**

**Animals**

Adult male ’Russian’ rabbits were obtained from Moellegaard Breeding Centre (Denmark), housed individually at 18–20°C and given antibiotic-free food and water. The rabbits were examined by the VDRL test to exclude an infection with *T. paraluis-cuniculi*.

**Bacteria**

*T. pallidum* subsp. *pallidum* (Nichols strain) was maintained by intratesticular passage in rabbits without the use of cortisone acetate. Treponemal suspensions were prepared from the testes of rabbits 6–8 days after intratesticular infection; organisms were either extracted into sterile phosphate-buffered saline (PBS) or basal medium consisting of 16.7 ml of an aqueous solution of sodium thioglycolate 1.5%, 3.6 ml of glutathione 1.23%, 3.6 ml of D,L-cysteine 1.26%, 1.76 ml of sodium pyruvate 1%, 6.46 ml of sodium bicarbonate 1.26%, 16.7 ml of 0.077 M phosphate buffer (pH 7.2) and 12.5 ml of heat-inactivated normal human normal serum according to Berlinghoff [13].

Suspensions were centrifuged twice for 10 min at 500 g to remove gross testicular debris. The number of motile treponemes in the supernate was counted by dark-field microscopy and adjusted with PBS to (3–5) × 10⁴ treponemes/ml for passage. For the TPI test the treponemal suspension was adjusted to 2 × 10⁶/ml of basal medium.

*T. pallidum* rabbit immune sera

To yield TPI-reactive rabbit sera, animals infected intratesticularly were bled 4 weeks (early sera) or 6 months (late sera) after infection. Early sera were precipitated with VDRL antigen (Behring, Germany) according to the method described by Baker-Zander et al. [11] to remove cardiolipin antibodies. Late sera were also examined in the VDRL test and, in case of a positive result, precipitated with VDRL antigen.

**Syphilis patient sera**

A total of 50 FTA-ABS and TPI test reactive serum samples from patients at early stages of infection (<2 years after infection, 19S IgM-FTA-ABS test positive) and at late stages (>2 years after infection, 19S IgM-FTA-ABS-test negative, latent infection) were used. To remove cardiolipin antibodies, sera were precipitated with VDRL antigen.

**Isolation of cardiolipin antibodies from rabbits**

Five rabbits were immunised with VDRL antigen–antibody complexes as described by Baker-Zander et al. [11]. The immunisation yielded sera with VDRL titres of 256–1024, cardiolipin-ELISA-IgG titres of 6400–25600, and ELISA-IgM titres of 400–800. Cardiolipin immune sera were also fractionated according to Baker-Zander et al. [11]. The IgM-enriched fraction was adjusted to a VDRL titre of 128 and the IgG fraction to 16 for use in the TPI and the FTA-ABS tests.

**Isolation of cardiolipin antibodies from sera of syphilis patients**

A volume of 0.5 ml of VDRL antigen was mixed with 0.5 ml of sterile sodium chloride 0.9% solution (NaCl 0.9%) and centrifuged for 10 min at 10000 g. The sediment was washed three times with NaCl 0.9%. The last sediment was resuspended in the starting volume of NaCl 0.9% and mixed with 2 ml of VDRL reactive syphilis serum pool. The mixture was shaken for 1 h at room temperature; the precipitates were then centrifuged for 10 min at 10000 g and washed three times with NaCl 0.9%. The final sediment was resuspended into 1 N acetic acid and stirred for 1 h at room temperature. After centrifugation for 10 min at 10000 g the antibody-containing supernate was removed, neutralised with 1 N Tris(hydroxymethyl)-aminomethane and dialysed against PBS. The reactivity in the VDRL test was determined and adjusted with PBS to a titre of 16.

**FTA-ABS test and 19S IgM-FTA-ABS test**

The FTA-ABS test was performed according to the method described by Hunter [14], the 19S IgM-FTA-ABS test according to Müller [15].

**VDRL test**

Cardiolipin antibodies were determined with the VDRL carbon antigen (bioMérieux, France) according to manufacturer’s instructions.

**Avidity assay**

A modified FTA test was used to determine the avidity of treponemal specific IgG in rabbit sera. Two-fold serial dilutions of serum samples (10 μl) were placed on to two *T. pallidum*-coated slides, one with dilutions
from 64 to 4096 and the other with dilutions from 8 to 512. After incubation in a moist chamber for 30 min at 37°C, the slide with the higher dilutions was washed twice (5 min each) in PBS and the slide with the lower dilutions for 5 min with PBS containing 8 M urea and then for 5 min with PBS. Both slides were rinsed with distilled water, air-dried and treated with anti-rabbit IgG-fluorescein isothiocyanate (FITC) conjugate (1 in 40; SIFIN, Germany). A titre ratio of ≥ 32 was considered as low avidity, and a ratio of ≤ 4 was considered as high avidity.

As a control to exclude non-specific damage to treponemes by 8 M urea, treponeme-coated slides were exposed to 8 M urea for 5 min, then washed twice with PBS, rinsed with distilled water and air-dried. The quantitative FTA test with treponemal specific rabbit sera should not show a reduction of titre, nor should it reveal a loss of intensity of fluorescence of the urea-pretreated slides when compared with the untreated slides.

Determination of the avidity of human treponemal specific IgG was performed as described above after absorption of the sera with T. phagedenis ultrasonicate (Behring, Germany). A FITC-labelled anti-human IgG (1 in 40; SIFIN, Germany) was used as conjugate.

The avidity of cardiolipin-specific IgG from rabbits was determined in a modified cardiolipin ELISA according to Rupin et al. [17]. Briefly, microtitration plates (Greiner, Germany) were coated with VDRL antigen (Behring, Germany) 50 µl/well diluted 1 in 25 in ethanol. The ethanol was allowed to evaporate overnight. After blocking for 2 h with horse serum 10% v/v in PBS, 100 µl of serum samples (serial dilutions beginning with 1 in 100) in duplicate were incubated for 2 h at room temperature. The wells were washed three times (5 min each) with PBS containing 8 M urea or, as a control, with PBS only. The whole plate was washed twice with PBS and then treated with horseradish peroxidase conjugated anti-rabbit IgG (1 in 8000; Southern Biotechnology Associates, USA) for 1 h at room temperature. The wells were washed three times with PBS without urea, followed by the substrate reaction with tetramethylbenzidine and H2O2 in citrate buffer (pH 5.0). The substrate reaction was stopped by the addition of 50 µl of 2 M H2SO4. The plates were read immediately on a spectrophotometer (Anthos) at 450 nm. According to Hedman and Seppälä [18] the avidity of IgG was evaluated as high if ≥ 70% or medium if ≥ 50 < 70% of antibodies remained bound after urea treatment. If < 30% of antibodies remained, their avidity was considered low. Non-specific damage to the antigen caused by urea treatment was excluded by the inclusion of appropriate controls.

Determination of the influence of rabbit VDRL antibodies on the binding of human treponemal specific antibodies in the IgG FTA-ABS test and the 19S IgM FTA-ABS test

In the first step, 10 µl of rabbit VDRL antibodies – VDRL hyperimmune serum (titre 256), IgG (titre 16) or IgM fraction (titre 128) – or rabbit normal serum were placed on to treponeme-coated slides and incubated for 30 min at 37°C in a moist chamber. The slides were washed twice with PBS, rinsed with distilled water and air-dried. In the second step, T. phagedenis ultrasonicate absorbed syphilis patient sera (serial dilutions, starting with 1 in 40) or 19S IgM fractions (serial dilutions, starting with 1 in 10) were applied to the pretreated slides and incubated for 30 min at 37°C. The slides were washed with PBS, followed by the conjugate incubation with FITC-labelled anti-human IgG or IgM (both 1 in 40; SIFIN, Germany) for 30 min. The reduction of titres on the slides pretreated with VDRL antibodies was determined with a fluorescence microscope in comparison with the controls pretreated with normal serum.

TPI test

The TPI test was conducted and evaluated according to the method described by Nelson and Mayer [19]. The 50% immobilisation titres of T. pallidum rabbit sera and syphilis patient sera were calculated by means of semi-logarithmic graphics. VDRL hyperimmune sera were tested in the TPI test in the presence of an excess of complement and lysozyme and in the absence of treponemal specific antibodies to exclude a membrane-destabilising activity.

Simultaneous incubation of T. pallidum with VDRL antibodies and treponemical antibodies

A volume of 50 µl of T. pallidum immune serum (final dilution from 1 in 10 to 1 in 20480) from rabbit or human patients was mixed with 50 µl of VDRL antibodies (whole serum, IgG-enriched fraction, IgM-enriched fraction, isolated cardiolipin antibodies from syphilis patient sera) or rabbit normal serum, 200 µl of T. pallidum suspension (1 × 10⁶ treponemes/ml) in basal medium and 250 µl of active complement (guinea-pig serum, Harlan) or 250 µl of heat-inactivated complement (control), and incubated in an oxygen-reduced atmosphere (N2 95%, CO2 5%) for up to 18 h at 36°C.
**Sequential incubation of T. pallidum with VDRL antibodies and treponemical antibodies**

A volume of 400 μl of treponemal suspension in basal medium was mixed with 100 μl of VDRL hyper-immune serum or rabbit normal serum and 500 μl of heat-inactivated complement. The mixture was incubated in an oxygen-reduced atmosphere for 8 h at 36°C. Treponemes were then centrifuged at 10,000 g for 5 min, washed three times in basal medium, and their mobility was tested by dark-field microscopy. Pretreated treponemes (200 μl volumes) were mixed with 50 μl of treponemal specific rabbit serum and 250 μl of active complement in the test tubes, or heat-inactivated complement in the control tubes and incubated in an oxygen-reduced atmosphere for 12 h at 36°C.

**Statistical analysis**

Data are presented as mean and SD. Comparisons were made by Student's t test. Differences were considered as significant when p < 0.05.

**Results**

**Avidity**

As early as 14 days after experimental infection of rabbits with *T. pallidum*, treponemal specific antibody titres of 512–2048 were detected in the FTA test. Only a slight increase in antibody titre was found during the subsequent course of infection. However, maturation of avidity of treponemal specific IgG was noted. Until 7–8 weeks after infection, antibodies were characterised by low avidity, expressed as a titre ratio of ≥ 32 assessed after urea treatment. It was not until 4–6 months after infection that highly avid antibodies became predominant (Fig. 1). No maturation of avidity of human treponemal specific antibodies was seen as described above for rabbit sera. Even though the average IgG titre ratio in the group of early sera was 4–8 and higher when compared to the group of late sera with IgG titre ratios of 1–2, not a single serum with low-avidity treponemal IgG (i.e., a ratio of ≥ 32) could be identified. Maturation of cardiolipin IgG after experimental infection of rabbits with *T. pallidum* did not occur, as shown by the VDRL ELISA. Independent of the stage of infection (from 2 weeks to 3 months after intratesticular infection) > 70% of bound antibodies were released by urea treatment. This result was considered as evidence of the predominant existence of low-avidity antibodies. The same result was shown with IgM VDRL antibodies. Even cardiolipin antibodies induced by intravenous immunisation of rabbits with VDRL immune complexes were characterised by a low strength of binding (only 10–20% of antibodies remained bound after urea treatment).

**Inhibition of treponemal specific antibodies in the FTA-ABS test**

VDRL hyperimmune sera as well as isolated cardiolipin IgG exerted an inhibitory effect on human treponemal specific antibodies in the IgG-FTA-ABS test and the 19S IgM-FTA-ABS test. In the IgG-FTA-ABS test performed with 10 syphilitic sera with titres in the range 1280–5120, cardiolipin antibody pretreatment (whole serum and IgG fraction) led to an average four-fold titre reduction (Fig. 2). In the 19S IgM-FTA-ABS test five syphilitic sera with titres in the range 80–640 were tested. Pretreatment of treponemes with cardiolipin antibodies (whole serum and cardiolipin IgG) caused an eight-fold decrease in titres (Fig. 3). Cardiolipin IgM failed to produce an inhibitory effect on the treponemal specific antibodies in the IgG-FTA-ABS test as well as in the 19S IgM-FTA-ABS test.

![Fig. 1. Maturation of avidity of treponemal specific IgG antibodies after intratesticular infection of rabbits with *T. pallidum* tested in the FTA test with urea (titre ratio ≥ 32 low avidity; titre ratio ≤ 4 high avidity). Data are presented as medians of five serum samples taken weekly.](image)

![Fig. 2. Inhibitory effect of rabbit VDRL antibody treatment of treponemes on human treponemal-specific IgG antibodies in the FTA-ABS test (NRS, normal rabbit serum; VDRL-HIS, VDRL hyperimmune serum; VDRL-IgG, IgG-enriched fraction of VDRL-HIS; VDRL-IgM, IgM-enriched fraction of VDRL-HIS). Data are presented as medians of a single representative experiment done in triplicate out of 10 experiments performed.](image)
Inhibition of treponemal specific antibodies in the TPI test

Simultaneous incubation with human syphilitic sera and cardiolipin antibodies. Cardiolipin antibodies, active complement and lysozyme did not influence the survival and mobility of treponemes over a period of 18 h.

In the TPI test, human treponemal specific antibodies at early stages of infection (IgM positive) with low 50% immobilisation titres were inhibited by the rabbit VDRL hyperimmune serum and the isolated VDRL IgG and IgM fractions (Fig. 4). As shown in Fig. 5 at later stages of infection (IgM negative) only the whole cardiolipin hyperimmune serum and the IgG fraction were effective, but not the IgM fraction.

Highly avid treponemal specific antibodies (IgG-FTA titre ratio 4, 6 months after infection) were influenced by the VDRL hyperimmune serum and the IgG fraction, but not by the IgM fraction. The 50% immobilisation titres dropped from 9099 SD 141 in the control with addition of rabbit normal serum to 476 SD 583 (p < 0.005) in the presence of VDRL whole serum, and to 537 SD 440 (p < 0.005) in the presence of VDRL IgG. The immobilisation titre in the presence of VDRL IgM did not differ from the control.

Sequential incubation with rabbit treponemal specific serum and cardiolipin antibodies. The inhibitory effect of cardiolipin antibodies on the immobilisation by treponemical antibodies and complement was also demonstrated by a sequential incubation, but was weaker than with the simultaneous incubation. Pre-treatment of treponemes with VDRL rabbit hyperimmune serum (with rabbit normal serum in the control) for 8 h followed by the removal of non-bound antibodies by repeated washing of the treponemes in basal medium and incubation for 12 h with rabbit
treponemicidal antibodies and complement, resulted in a decreased immobilisation of treponemes from 78.4 SD 11% in the controls to 41.4 SD 8% (p < 0.001).

Not only cardiolipin antibodies obtained by immunisation of rabbits with VDRL immune complexes exerted an inhibitory effect on the immobilisation of *T. pallidum*. A similar result was obtained with isolated cardiolipin antibodies which were induced in human patients during *T. pallidum* infection. The influence of cardiolipin antibodies isolated from a serum pool of VDRL reactive syphilis patients on the course of immobilisation by human treponemal specific antibodies is shown in Fig. 6. The cardiolipin antibodies, which belonged predominantly to the IgG class, caused a delay of immobilisation as a function depending on their concentration.

**Discussion**

The predominant localisation and biological function of the cardiolipin antigen in different compartments of *T. pallidum* is still not clear. Radolf *et al.* [10] showed a paucity of cardiolipin in the outer membrane of pathogenic treponemes. They used a reliable method for the isolation of the treponemal outer membrane based upon isopyknic density gradient ultracentrifugation of treponemes following plasmolysis in sucrose 20%. The lipid constituents extracted from the outer membrane were identified by two-dimensional thin-layer chromatography. With the exception of cardiolipin, which was barely detectable in the outer membranes, the relative proportions of phospholipids and glycolipids in whole cells and outer-membrane fractions were similar. These results corresponded with the weak immunofluorescence of even high titred (VDRL titre ≥ 256) cardiolipin hyperimmune sera which was observed in the FTA-test. These findings were also reported by Baker-Zander *et al.* [11]. However, the ability of cardiolipin antibodies to opsonise *T. pallidum*, which is discussed by Baker-Zander as well as the binding of cardiolipin antibodies observed in the first step of the sequential TPI test, might suggest the presence of target structures for cardiolipin antibodies on the surface of treponemes. The results of the present study show that cardiolipin antibodies are not able to cause destruction of the outer membrane in the presence of complement under *in vitro* conditions. The TPI test performed with cardiolipin antibodies, active complement and an excess of lysozyme (5 µg/ml) did not show a loss of mobility of treponemes after 18 h. There are two possible explanations for the lack of bactericidal activity of cardiolipin antibodies in the TPI test, as described below.

1. The cardiolipin antigen concentration in the outer membrane and the antigen mobility is too low and, therefore, antibody-mediated antigen aggregation as a prerequisite for complement activation is impaired.

2. The molecular configuration and the insertion of the complement membrane attack complex C5b-9 activated by antibodies directed against outer-membrane proteins (OMPs) are distinct in contrast to the cardiolipin antibody activated C5b-9 complex. The latter possibly either fails to insert or inserts abortively, or does not insert at the same location in contrast to the former, i.e., OMP antibody-mediated C5b-9 complex. Different molecular configurations of the C5b-9 complex were reported recently by Joiner *et al.* [20] with gonococcal antibodies directed against gonococcal protein I. The authors demonstrated that monoclonal antibodies directed against closely associated surface-exposed epitopes on the gonococcal protein I differed markedly in their bactericidal

![Graph](https://via.placeholder.com/150)

**Fig. 6.** Influence of cardiolipin antibodies (VDRL titre 16), isolated from human syphilitic serum, on the course of immobilisation of *T. pallidum* by human treponemal specific antibodies. Data are presented as means and SD of a single representative experiment done in triplicate out of four experiments performed. VDRL antibodies added: ■, none (control); □, 10 µl; ■, 25 µl; ◊, 50 µl.
activity, despite leading to the deposition of nearly equivalent numbers of C9 molecules per micro-organism.

Assuming that there are few cardiolipin epitopes in the outer membrane, it appears likely that the inhibitory effect against treponemal specific antibodies can be attributed to a high avidity of cardiolipin antibodies. However, this hypothesis could not be confirmed. In the cardiolipin avidity ELISA with 8 M urea, the avidity was very low during the whole course of infection, whereas the avidity of the rabbit treponemal specific antibodies, as assayed in the FTA-test, increased. The human treponemal specific antibodies showed a high avidity even in serum samples taken from patients at the primary stage of infection. The avidity of TPI reactive antibodies could not be tested, because the common methods for the determination of the avidity are not applicable to the TPI-test. Some differences in the inhibitory activity of cardiolipin antibodies were observed in the FTA test and the TPI test. In the FTA-IgG test as well as in the FTA-IgM test, only the whole cardiolipin serum and the cardiolipin IgG fraction influenced the FTA titre of sera taken from patients at the early (19S IgM-FTA-ABS test positive) and the late stage (19S IgM-FTA-ABS test negative) of infection. However, in the TPI test in addition to cardiolipin hyperimmune serum and the cardiolipin IgG fraction, cardiolipin IgM produced a decrease in the 50% immobilisation titre at the early stage of infection. At later stages only the cardiolipin hyperimmune serum and isolated IgG were reactive in the inhibition of TPI antibodies. The different influence of cardiolipin IgM antibodies on the binding of treponemal specific antibodies in the TPI test and the FTA test appears to be the result of a different antigen presentation in the two systems. The TPI test is performed with viable treponemes, presenting poorly immunogenic rare OMP (Tromp) [21] in contrast to the FTA test, where treponemes are acetone-fixed and the outer membrane is partially damaged, resulting in the presentation of a high number of highly immunogenic [21] subsurface antigens. It seems possible that the average avidity of TPI reactive antibodies at the early stage of infection is much lower than the average avidity of FTA reactive antibodies. The incubation of freshly extracted viable treponemes with cardiolipin antibodies for 8 h, followed by repeated washing to remove unbound antibodies and the addition of treponemal specific serum and complement, produced a qualitatively similar effect to the simultaneous incubation, suggesting that cardiolipin antibodies bind to the intact outer membrane and impair the aggregation of the Tromp molecules. This would result in a lack of complement activation due to antibody binding. The restricted mobility of OMPs was demonstrated by Bourell et al. [22] who suggested that the antigens were stabilised by interactions between their periplasmatic domains and components of the protoplasmic cylinder. Furthermore, we propose that cardiolipin antibody complexes might inhibit the aggregation and cross linking of the OMP. Thus, the outer membrane is preserved in the presence of cardiolipin antibodies and the killing of treponemes is delayed. However, complete protection of treponemes from treponemical antibodies by cardiolipin antibodies did not occur. According to Lewinski et al. [23] the TPI test proceeds in a ‘two hit’ mechanism. In the first step, antibodies directed against the OMP antigens activate the complement membrane attack complex C5b-9 and the latter perforates the outer membrane. Then, endoflagella antibodies enter the periplasmic space and immobilise the treponemes. We conclude that, under the influence of cardiolipin antibodies, the entry of endoflagella antibodies into the periplasmic space is delayed; thus, the immobilisation rate of treponemes in the presence of cardiolipin antibodies is lower when compared to the cardiolipin antibody-free system.

The inhibition of the treponemal specific antibodies by cardiolipin antibodies as shown in the TPI test and the FTA test could be a pathogenic factor in the course of syphilis. We suggest that the results of the TPI test could reflect the situation in vivo during the first weeks of infection, when cardiolipin antibody titres are high and a maturation of avidity of treponemal specific antibodies has not yet occurred. During the further course of infection with the increase of titre and avidity of treponemal specific antibodies, the influence of cardiolipin antibodies would be diminished and finally negligible. It is conceivable that the loss of functional activity of outer-membrane antibodies at the beginning of infection partly accounts for the progression of syphilis [24]. Therefore, the presentation of the cardiolipin antigen should be considered as a possible virulence factor of pathogenic treponemes, mediating the obstruction of host defence mechanisms.

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References

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