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The monoclonal antibody 26 raised against tetanus toxoid also recognizes tetanus toxin and β_2-glycoprotein I – its binding properties *in vitro* and potential applications

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Abstract: A murine monoclonal IgG_1 antibody, marked as MAb26, specific for tetanus toxoid has been immunochemically characterized. By performing enzyme-linked immunosorbent assays (ELISAs) and western blot analyses, it was demonstrated that MAb26 reacted with tetanus toxoid, tetanus toxin and β_2-glycoprotein I (β_2GPI). According to the results, MAb26 recognized the sequential epitope on the tetanus heavy chain. The affinity constant, calculated from Scatchard plots of MAb26 binding to tetanus toxoid, was 1.145×10^8 M^{-1} and the measurement of the relative affinity of MAb26 by ELISA using thiocyanate elution showed a significantly higher affinity of MAb26 to the toxoid (p = = 0.0012) in comparison to the toxin. Additionally, the reactivity of MAb26 toward the toxoid forms increased when the tetanus toxin was detoxified using 8 mM and higher formaldehyde concentrations. The similarity of the tetanus toxoid to several sera proteins, either at the level of its conformation (IL-1α) or at the level of peptide sequences (β_2GPI, laminin) favors its role in autoimmunity by the mechanism of molecular mimicry. As the induction of an autoimmune disease is dependent on the breakdown of tolerance, which could be the result of an overt hyperstimulation, the control of the presence and concentration of self-reactive epitopes in vaccine preparations is a prerequisite. In this study, it was shown that MAb26 can: 1) discriminate between the tetanus toxin and different toxoid forms, which makes it a good candidate for antibody control during vaccine preparation; 2) due to its cross-reactivity with β_2GPI, it could provide information on the presence of a potentially dangerous sequential epitope expressed at the protein surface.

Keywords: tetanus toxoid; tetanus toxin; monoclonal antibodies; formaldehyde.

INTRODUCTION

Tetanus toxin (TTn) is an enormously potent neurotoxin[1] secreted by the anaerobic soil bacterium, *Clostridium tetani* and is comprised of two polypeptide

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chains, termed heavy (H, 100 kD) and light (L, 50 kD) chains, linked by a disulphide bond. These two chains are functionally distinct; the H chain is responsible for binding and cell entry, while the L chain is catalytically active and functions as a zinc-dependent endopeptidase. TTn traffics to the central nervous system by retrograde axonal transport followed by trans-synaptic spread into inhibitory interneurons,[2,3] where it specifically cleaves the synaptic vesicle protein synaptobrevin. Synaptobrevin cleavage by TTn blocks the release of inhibitory neurotransmitters leading to spastic paralysis. In concentration of approximately 1 ng/kg,[4] TTn induces death in non-vaccinated humans.

As TTn induces death before an adaptive immunity could be generated, active immunization[5] with tetanus vaccine (TTdV) is crucial for the prevention of death caused by tetanus. At present, protection is routinely induced through immunization with a TTn derivate, tetanus toxoid (TTd), obtained by chemical modification with formaldehyde,[6] which was first described more than 80 years ago. TTn inactivated by formaldehyde is devoid of toxicity but is still highly immunogenic with a stabilized native conformation.

The similarity between TTn and several sera proteins (β_2GPI, laminin) at the level of short peptide sequences favors its role in autoimmunity by the mechanism of molecular mimicry.[7,8] This fact implies that the application of TTd requires prior detailed characterization and precise quantification. The cross-reactivity of TTn, especially with β_2GPI drew our attention as it has been shown that β_2GPI is a major antigen in the antiphospholipid syndrome (APS).[9]

With the intent of examining further the specificity of anti-tetanus antibodies, an anti-tetanus monoclonal antibody (MAb), marked as MAb26, which recognizes the epitope located on both forms of tetanus: natural toxin and its chemical derivative, toxoid, was immunochemically characterized. In addition, MAb26 cross-reacted with β_2GPI and could be regarded as a convenient tool for an investigation related to the potential generation of some "surprising" and potentially hazardous anti-β_2GPI autoantibodies, possibly through the molecular mimicry mechanism. There is a general consensus that autoimmune diseases depend on genetic and environmental factors. As studies on experimental APS models proved that there is molecular mimicry between β_2GPI-related synthetic peptides and structures within bacteria and viruses, TTd, might be a cause for experimental APS.[10] Since the pathogenic potential of MAb26 *in vivo* has already been demonstrated,[11] this study was conducted with the aim of further analyzing the immunochemical characteristics of MAb26, with the emphasis on the analysis of the reactivity of MAb26 toward different tetanus antigenic determinants. The obtained results also indicate that formaldehyde modification potentiates the conformational expression of β_2GPI cross-reactivity.

EXPERIMENTAL

Antigens

TTn was purified from fermentation cultures of *Clostridium tetani* and inactivated at a fixed concentration of 180 Lf/ml by adding formaldehyde to final concentrations of 0, 2, 4, 8, 16, 32, 64 and 128 mM. The mixture was adjusted to pH 7.4 and incubated at 35 °C for 4 weeks. The resulting "detoxified samples" were then dialyzed and filtered to remove the excess of inactivation reagents. After a final pH adjustment and filtration, the samples were aliquoted and used in the experiments. In the following text, when the formaldehyde concentration is not specifically indicated, the abbreviation TTd refers to the tetanus toxoid obtained using 128 mM formaldehyde.

β_2GPI was purified from normal human plasma using sequential precipitation with $(NH_4)_2SO_4$, 65 % saturation, from the supernatant obtained following perchloric acid addition to human plasma (final concentration 1.75 %) and chromatographic steps (Mono S column; ÄKTA Purifier, Pharmacia Amersham, Uppsala, Sweden). Both Ags were checked for purity by SDS-PAGE (Phast System, Pharmacia Amersham, Uppsala, Sweden) and identified by immunoblot, using either commercially available mouse anti-human β_2GPI IgG1 (IgG1; clone 5F7, ICN Biomedicals, Aurora, USA) or anti-TTd standard antibodies.

Production of MAbs specific for TTd

MAbs to TTd were produced by the hybridoma technology.[12] MAb26 was IgG_1 which was demonstrated by ELISA, using commercially available biotin-labeled MAbs specific for mouse IgG subclasses (ICN Biomedicals, Aurora, USA) for detection. MAb26 producing hybridoma was cultured in integra bottles (Integra CL 350, Integra Biosciences, Switzerland) and the MAb was affinity purified from the supernatant on a Protein A-Sepharose 6B (Pharmacia Amersham, Uppsala, Sweden) column according to the instructions of the manufacturer. The purity of MAb preparations were determined by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). The isolated MAb26 was used for $F(ab')_2$ and Fab' production according to a procedure previously described.[13] The resulting proteolytic digestion samples were dialyzed against 100 mM Tris-HCl buffer, pH 8.1, and intact Fc fragments and non-digested antibody were removed by Protein A-Sepharose chromatography. The $F(ab')_2$ preparation showed a single band at 100 kD and a doublet of bands at 25 kD in non-reducing and reducing SDS-PAGE, respectively. The Fab' preparation showed a single band at 50 kD and 25 kD in non-reducing and reducing SDS-PAGE, respectively. The obtained antigen-binding fragments, as well as intact MAbs were biotin labeled (–B) according to a previously described procedure.[14]

Reactivity of MAb26 with TTn and TTd

ELISA plates (MaxiSorp, Nunc) were covered (50 µl/well) with TTn or TTd (1µg ml^{-1} PBS) by overnight adsorption at 4 °C. A 1 % BSA/PBS solution was used for blocking non-specific binding for 2 h at room temperature). The saturation, as well as each subsequent ELISA step, was followed by washing with 0.05 % Tween 20/PBS (4×200 µl/well). MAb26 (0.50–10 µg/ml) was added to the plate and incubated for 1 h at room temperature. Biotin--labeled antibodies specific for mouse IgG (ICN) (50 µl/well) were incubated for 1 h at room temperature. The system extrAvidin-peroxidase/OPD was used for "visualization" of Ag–Ab interaction. The absorbance was read at 492/692 nm.

The measurement of the relative MAb26 affinity by ELISA using thiocyanate elution

The affinities of MAb26 to TTd and TTn were estimated by use of aqueous solutions containing different potassium thiocyanate (KSCN) concentrations.[15] Analysis of the in-

fluence of thiocyanate ion (SCN^-) on the specific MAb26 binding was performed with ELISA-based procedures. The protocol was similar to those performed for the reactivity determination of MAb26 with TTd and TTn. The single difference was an additional 1-hour incubation with aqueous KSCN solutions after the binding of MAb26 to the adsorbed Ags. Aqueous solutions containing increasing KSCN concentrations (0–6 M) were employed for MAb26 desorption. The SCN^- concentration ($[SCN^-]$) that induced a 50 % reduction of initial absorbance ($[SCN^-]_{50\ \%}$) was calculated for each sample upon linearization of the corresponding Ag–Ab dissociation profile (Binding (%) = $f([SCN^-])$). A preliminary experiment showed that KSCN in the concentrations used did not induce desorption of coated TTd or TTn. To evaluate the significance of the observed differences in MAb26 affinity toward TTd and TTn, dissociation profiles (Binding (%) = $f([SCN^-])$ were compared by a paired Student's *t*-test.

Reactivity of MAb26 with TTn adsorbed onto gangliosides

ELISA plates (PolySorp, Nunc) were covered (50 µl/well) with the appropriate ganglioside: GT_{1b} or GD_{1b} (10 µg/ml in methanol) by overnight evaporation at room temperature. A 1 % BSA/PBS solution was used for saturation for 2 h at room temperature. The saturation, as well as each subsequent ELISA step, was followed by washing with PBS (4×200 µl/well). TTn/1 % BSA (20 µg /ml) or 1 % BSA were added to the plate and incubated for 1 h at 37 °C. MAb26 (10 µg/ml) or TTd-specific mouse anti-serum (polyclonal anti-TTd Abs, 1:400) were added to the plate and incubated for 1 h at 37 °C. After washing, the biotin-labeled antibodies specific for mouse IgG (ICN) was added to the plate (50 µl/well). System extrAvidin-peroxidase/OPD was used for "visualization" of the Ag–Ab interaction. The absorbance was read at 492/692 nm.

SDS-PAGE and Western blot

TTn, TTd and β_2GPI were resolved by SDS-PAGE on 1-mm-thick 9 % separating gels, with 4 % stacking gels (Mini Protean II System, Bio-Rad, USA). The proteins were electrophoretically transferred to PVDF membranes (Millipore Corporation, Bedford, MA, USA) for 1 h at 4 °C and 1–1.5 mA/cm^2 (Multiphor II System, LKB, Sweden) in buffer containing 25 mM Tris (Pharmacia), 193 mM glycine (Pharmacia), and 15 % methanol (Fluka). To prevent non-specific antibody binding, the membranes were incubated with blocking buffer, 3 % BSA (Sigma) in PBS for 2 h. MAb26 was diluted in blocking buffer and the incubations were performed for 1 h at room temperature. The membranes were incubated with anti-mouse IgG-B (Sigma) diluted 1:5000, followed by streptavidin–phosphatase (Sigma) diluted 1:1000. The membranes were washed three times with 0.05 % Tween 20 (Sigma)/PBS and twice with PBS after the incubations. The antibody binding was visualized by exposure to 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) (Bio-Rad).

SDS-PAGE under reducing conditions was performed similarly to that described above with the following differences: 1) the proteins were resolved by electrophoresis on a gradient 4.0–15 % polyacrylamide gel (Pharmacia) and 2) the samples contained 5 % 2-mercaptoethanol.

Reactivity of MAb26 with β_2*GPI*

ELISA plates (MaxiSorp, Nunc) were covered (50 µl/well) with β_2GPI (10 µg/ml β_2GPI/PBS) by overnight adsorption at 4 °C. A 1 % BSA/PBS solution was used for saturation for 2 h at room temperature. The saturation, as well as each subsequent ELISA step, was followed by washing with 0.05 % Tween 20/PBS (4×200 µl/well). MAb26 (0.50–10 µg/ml) was added to the plate and incubated for 1 h at room temperature. The biotin-labeled antibodies specific for mouse IgG (ICN) (50 µl/well) were incubated for 1 h at room tem-

perature. The system extrAvidin-peroxidase/OPD was used for "visualization" of the Ag–Ab interaction. The absorbance was read at 492/692 nm.

Competitive inhibition ELISA: binding of MAb26 for β_2GPI in the presence of differently detoxified forms of TTn

The β_2GPI was bound to the MaxiSorp (Nunc) microtiter plates by overnight adsorption at 4 °C, at a concentration of 10 μg/ml. Detoxified samples (20 μg/ml), made by adding formaldehyde to final concentrations of 0, 2, 4, 8, 16, 32, 64 and 128 mM, were mixed with $F(ab')_2$ MAb26, 10 μg/ml, then pre-incubated for 1 h at 25 °C in a water bath and further incubated with β_2GPI coated on the microplate walls. ExtrAvidin-peroxidase/OPD was used as the detection system. The percentage binding (*BI*) was calculated after determination of the free MAb26 from the standard curve $A_{492/692} = f((F(ab')_2MAb26))$.

Double immunodiffusion

Double immunodiffusion[16] was performed for subclass determination and for the detection of the precipitating properties of MAb26.

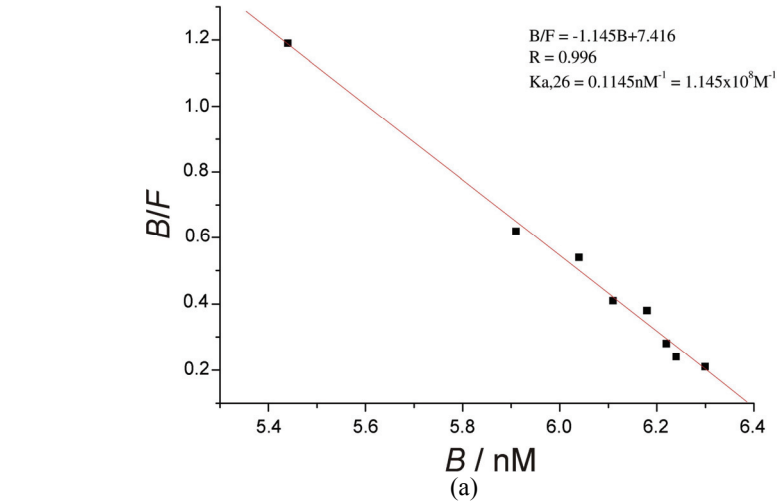
RESULTS AND DISCUSSION

Preliminary tests, performed with supernatants of hybridoma 26, indicated that MAb26 reacts with TTd, TTn and β_2GPI. The hybridoma 26, secreting MAb26 of the IgG1 subclass (pI 7.4), was further subcloned, propagated in Integra bottles and characterized. Although this characterization included the conventional approach to study MAbs, the focus was on the investigation of the unusual binding features of MAb26, the localization of its partial epitope and potential applications.

Binding properties of MAb26

The reactivity of the isolated MAb26 toward TTd and TTn was tested using direct ELISA and this MAb displayed strong reactivity toward tetanus antigenic determinants. The reactivity pattern undoubtedly demonstrated anti-TTd binding specificity with a K_a value of 1.145×10^8 M^{-1}, determined for isolated TTd (150 kD) in competitive ELISA (Fig. 1a). The reactivity toward TTd was higher in comparison to the reactivity toward TTn, which was confirmed by analysis of the influence of KSCN on MAb26 binding to TTd and TTn (Fig. 1b). Significant differences between the elution profiles of MAb26 from TTn and TTd following incubation with KSCN in various concentration were revealed by paired Student's *t*-test ($p = 0.0012$), indicating a large difference in the affinity of MAb26 toward these antigens. The calculated $[SCN^-]_{50\ \%}$ values confirmed the higher affinity of MAb26 toward TTd compared to TTn. Parallel binding curves of MAb26 and its $F(ab')_2$ and Fab' fragments for TTd or TTn revealed the same binding pattern, which excluded the necessity of bivalent binding of MAb26 (Fig. 2), which is in accordance with the high affinity of MAb26 toward these Ags.

The MAb26 was also reacted with β_2GPI. It was not possible to calculate the affinity of MAb26 toward β_2GPI by the standard Scatchard method. The first reason could be its low affinity toward β_2GPI and the second, probably more im-



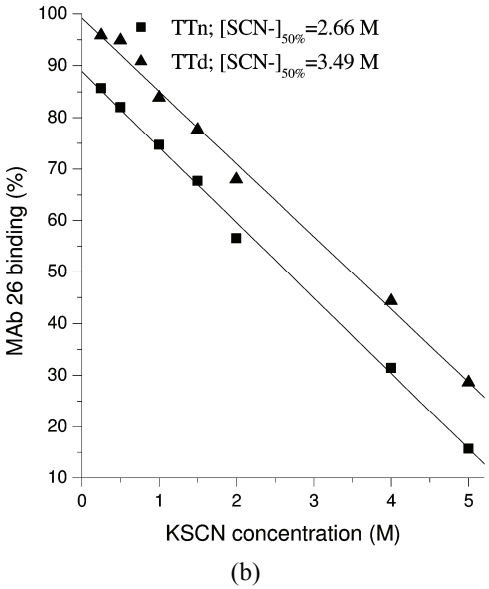


Fig. 1. a) Scatchard plot ($B/F = f(B)$) of the binding of MAb26 to TTd measured by ELISA at 25 °C, B – concentration of MAb26 bound to TTd, F – concentration of free MAb26, and b) measurement of the relative affinity of MAb26 toward TTn and TTd by ELISA using thiocyanate elution. The corresponding $[SCN^-]_{50\,\%}$ values are indicated within the graph.

portant, is the requirement of its bivalent binding.[17] The necessity to use a high concentration of β_2GPI (10 μg/ml) for the detection of MAb26 binding implies a low affinity of MAb26 toward β_2GPI (Fig. 3a). Bivalent binding of MAb26 to β_2GPI could explain the non-linear dependence of MAb26 binding to β_2GPI following its incubation with solutions containing different concentration of KSCN and the relatively high value of $[SCN^-]_{50\,\%}$ (Fig. 3b). Finally, as was shown in a previous study,[11] the absence of Fab' binding to β_2GPI adsorbed in a concentration which allows the detection of MAb26, but the presence of binding

of intact MAb26 and its F(ab')$_2$ to β_2GPI, confirmed the requirement of bivalent binding of MAb26 to β_2GPI.

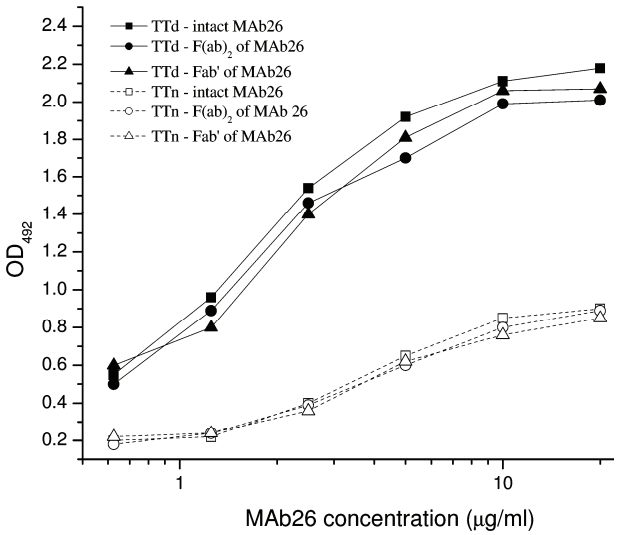


Fig. 2. Binding curves of MAb26 and its F(ab')$_2$ and Fab' fragments to TTd and TTn.

Partial characterization of the MAb26 binding epitope

Western blot revealed that MAb26 recognized the band of 150 kD in TTd/ /TTn and cross-reacted with β_2GPI (Fig. 4a). These results suggest that the epitope recognized by MAb26 was most probably sequential and not dependent on the native structure of TTd/TTn or β_2GPI. Further Western blot experiments performed after SDS-PAGE obtained under reducing conditions revealed that MAb26 binding epitope most probably lies on the tetanus heavy chain (Fig. 4b).

The reactivity of MAb26 toward different detoxified samples, made by adding formaldehyde to final concentrations of 0, 2, 4, 8, 16, 32, 64 and 128 mM, was tested by ELISA. It could be seen (Fig. 5) that the reactivity of MAb26 increased when the Ags were used in which the TTn had been detoxified using 8 mM and higher formaldehyde concentrations. A similar MAb26 binding pattern was revealed by Western blot (Fig. 6b) performed after SDS-PAGE obtained under reducing conditions with detoxified samples (Fig. 6a).

The results of MAb26 binding to TTn adsorbed directly onto a microtiter plate or a microtiter plate pre-coated with gangliosides supported the hypothesis that the epitope recognized by MAb26 is located on the H chain of the TTn/TTd molecule. The gangliosides (GD_{1b} and GT_{1d}) represent natural receptors for TTn and the ganglioside binding site is located on the C-terminal domain of the H chain of TTn. It was shown (Fig. 7) that adsorption of TTn to gangliosides almost completely inhibited its recognition by MAb26, most probably due to steric hindrance.[18]

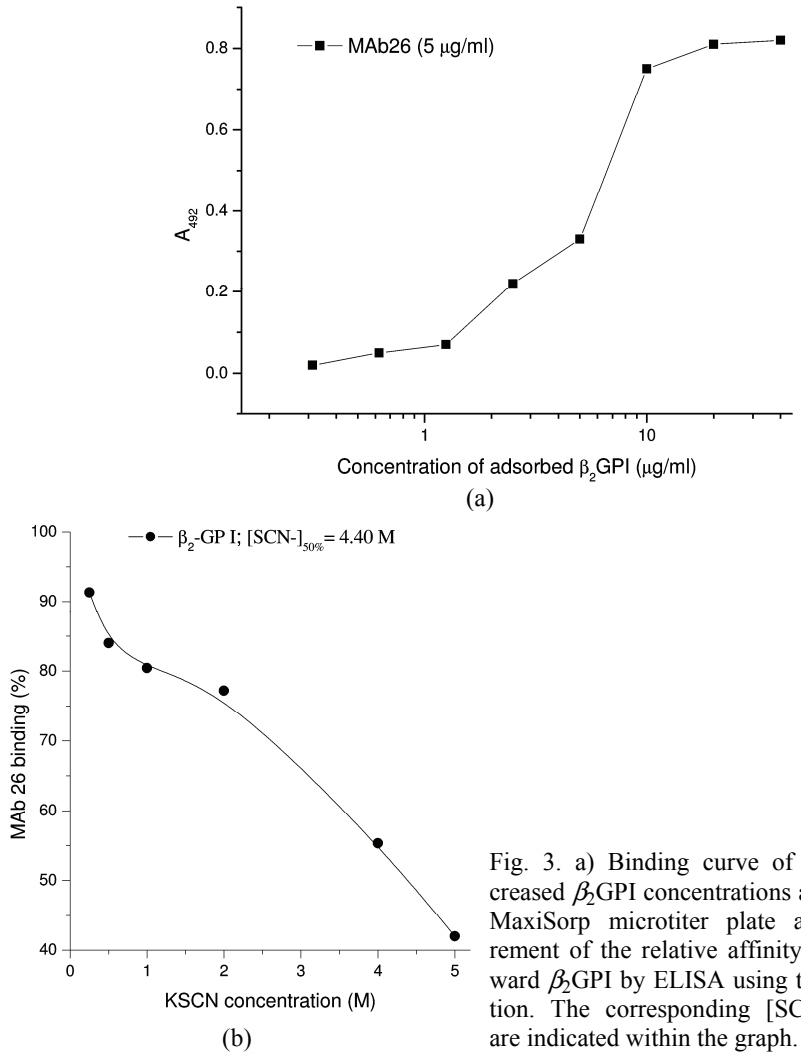


Fig. 3. a) Binding curve of MAb26 to increased β_2GPI concentrations adsorbed onto a MaxiSorp microtiter plate and b) measurement of the relative affinity of MAb26 toward β_2GPI by ELISA using thiocyanate elution. The corresponding $[SCN^-]_{50\,\%}$ values are indicated within the graph.

The observed differences in reactivity of MAb26 toward the different toxoid forms might be the result of the different extent of chemical modification and this fact could eventually be used for discrimination between TTn and the toxoid forms or to confirm the efficient transformation of TTn to toxoid forms.

Cross-reactivity of MAb26 with β_2*GPI*

By using "detoxified TTd samples" as inhibitors of MAb26–β_2GPI binding, an attempt was made to evaluate whether the formaldehyde concentration used in the detoxification process could have any impact on the structure of the β_2GPI-like epitope on the TTd molecule. It is evident that the β_2GPI-like epitope was pre-

sent in the TTn molecule and was potentiated through conformational changes induced by formaldehyde treatment. A 50 % inhibition of binding F(ab')$_2$ MAb26 to β_2GPI was obtained in the presence of as little as 4 mM formaldehyde in the TTn detoxification process (Fig. 8). This result could favor the possibility that the native conformation is stabilized by the formaldehyde-induced cross-links.

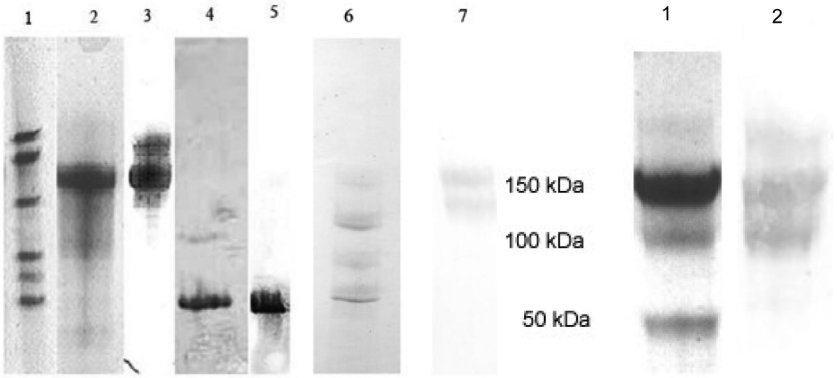


Fig. 4. a) SDS-PAGE of TTd (lane 2), β_2GPI (lane 4) and TTn (lane 6) on a 9 % polyacrylamide gel and Western blot analysis of MAb26 TTd (lane 3), β_2GPI (lane 5) and TTn (lane 7). The molecular weight marker kit (lane 1) consisted of myosin (212 kD), α-macroglobulin (170 kD), β-galactosidase (116 kD), transferrin (76 kD) and glutamic dehydrogenase (53 kD), (b) SDS-PAGE of TTd under reducing conditions and Western blot analysis.

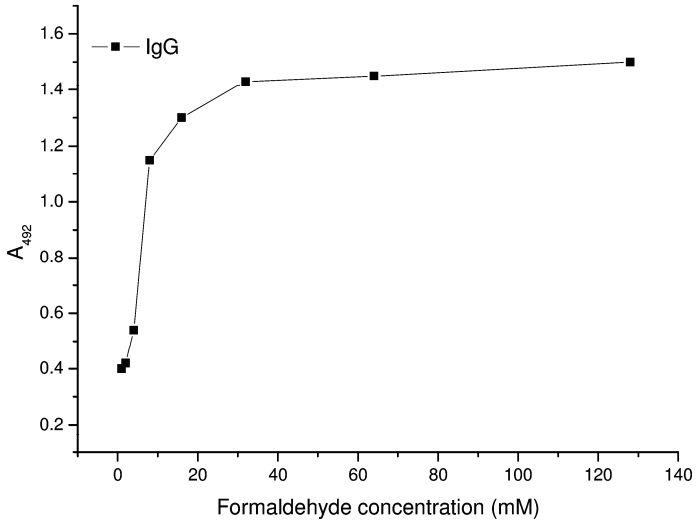
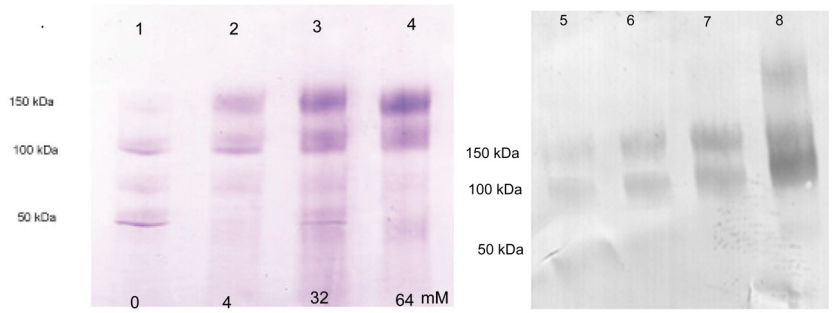


Fig. 5. Binding of MAb26 to different detoxified samples (made by adding formaldehyde to final concentrations of 0, 2, 4, 8, 16, 32, 64 and 128 mM) immobilized directly onto a UV-irradiated MaxiSorp plate. All the samples were assayed in triplicate and the mean ±*SD* are presented.



(a) (b)

Fig. 6. SDS-PAGE of detoxified samples (with 0, 4, 8, 32 and 64 mM formaldehyde) under reducing conditions on a gradient 4–15 % PAG (a) and Western blot analysis of former samples 1–4 with MAb26 as 5–8, respectively (b).

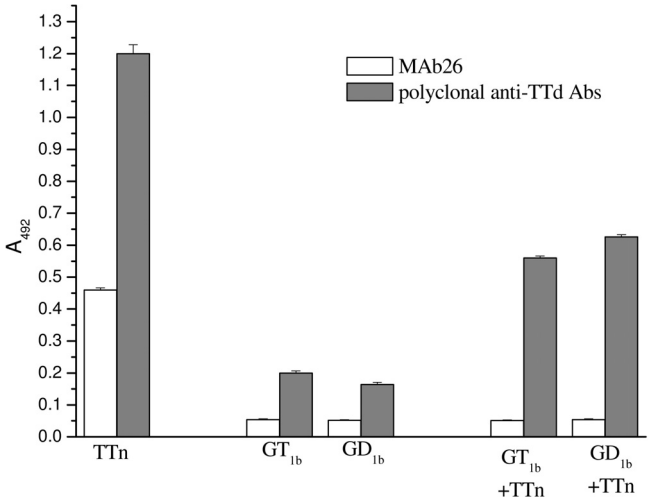


Fig. 7. Binding of MAb26 to TTn, gangliosides and TTn + gangliosides. Polyclonal anti-TTd anti-serum was used as the positive control.

MAb26 is cross-reactive with β_2GPI and might be employed as a convenient tool for investigations related to the potential generation of anti-β_2GPI autoantibodies, possibly by the molecular mimicry mechanism. The theory of molecular mimicry, one of the mechanisms thought to be responsible for the association of autoimmunity with infections, involves the display of epitopes resembling the host determinants by the infectious agent. The epitope of a pathogen may induce an immune response that breaks down self-tolerance by cross-reactivity, thus inducing an auto-immune response leading to disease. The involvement of molecular mimicry and immunization with TTd in the induction of APS was demonstrated in experimental animal models.[19–21] In a previous study,[11] experimental APS in BALB/c mice (which are not lupus-prone, *i.e.* the mice were not geneti-

cally predisposed to autoimmune diseases) was induced after they had been passively infused with MAb26.

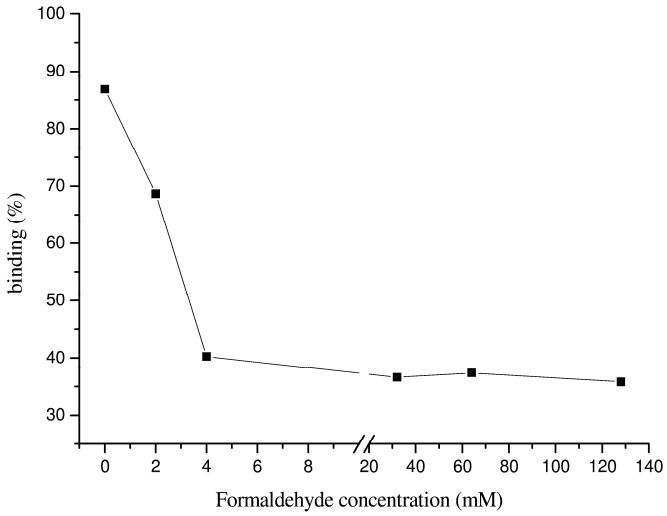


Fig. 8. Inhibition of MAb26 binding to β_2GPI in the presence of differently detoxified forms of TTn.

As the induction of autoimmune disease is dependent on the breakdown of tolerance, which could be the result of overt hyperstimulation, control of the presence and concentration of self-similar epitopes in vaccine preparations is a prerequisite. This favors MAb26 as a valuable tool in the preparation process of a tetanus toxoid vaccine.

Precipitating properties of MAb26

In addition to the characterization of MAb26, it was shown that this MAb precipitated TTd and TTn in solution and also exhibited good precipitating properties in gels, as demonstrated by double immunodiffusion (Figs. 9a and 9b).

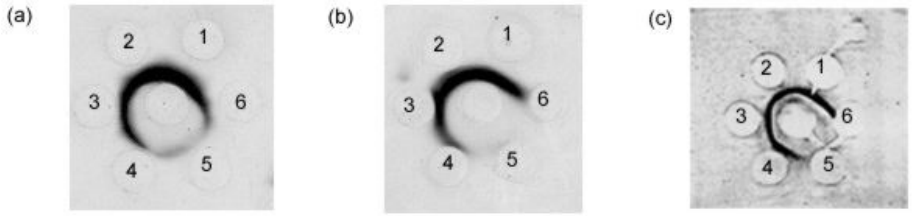


Fig. 9. Double immunodiffusion of MAb26 against TTd, TTn and β_2GPI performed in a 1.0 % agarose gel. TTd (a), TTn (b) and β_2GPI (c) were placed in the central wells at a concentration of 1.0 mg/ml. The concentrations of MAb26 that were placed in the peripheral wells were 2-fold dilutions starting with 0.50 (well 1), 0.25 (well 2), 0.125 (well 3), 0.0625 (well 4), 0.03125 (well 5) and ending with 0.015 mg/ml (well 6).

Although MAb26 could not precipitate β_2GPI in solution which is in accordance with data from the literature for anti-β_2GPI Abs, MAb26 exhibited good precipitating properties in gels (Fig. 9c).

CONCLUSIONS

According to the presented results, it can be concluded that the characterized MAb26 could be employed as a control antibody during vaccine preparation. Its different affinities toward tetanus toxin and toxoid might be used for following-up the detoxification process, which is indispensable for vaccine production. On the other hand, the cross reactivity with β_2GPI could expand the information of the presence of a potentially dangerous sequential epitope expressed at the protein surface after detoxification.

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ИЗВОД

МОНОКЛОНСКО АНТИТЕЛО 26 НАПРАВЉЕНО НА ТЕТАНУС ТОКСОИДУ РЕАГУЈЕ И СА ТЕТАНУС ТОКСИНОМ И β_2-ГЛИКОПРОТЕИНОМ I – КАРАКТЕРИСТИКЕ ВЕЗИВАЊА *IN VITRO* И МОГУЋА ПРИМЕНА

АЛЕКСАНДРА Б. ИНИЋ-КАНАДА, МАРИЈАНА М. СТОЈАНОВИЋ, ИРЕНА П. ЖИВКОВИЋ, ВЛАДИМИР Ж. ПЕТРУШИЋ и ЉИЉАНА ДИМИТРИЈЕВИЋ

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Овај рад описује имунохемијску карактеризацију мишјег IgG1 моноклонског антитела означеног као MAт26. Ензимским имуносорбентним тестом (ELISA) и Western blot анализом је показано да MAт26 реагује са тетанус токсоидом, тетанус токсином и β_2-гликопротеином I (β_2GPI). Према нашим резултатима, MAт26 препознаје секвенциони епитоп на тешком ланцу молекула тетануса. Константа афинитета MAт26 за тетанус токсоид, израчуната на основу Скачардовог дијаграма, је $1{,}145\times10^8$ M^{-1}. На основу елуције тиоцијанатом, коришћене за одређивање релативног афинитета MAт26 за тетанус токсин и тетанус токсоид, поступком базираним на ELISA-и, показан је знатно већи (p = 0,0012) афинитет MAт26 ка токсоидној форми. Такође, реактивност MAт26 ка токсоидној форми расла је са порастом концентрације формалдехида, почевши од 8 mM, коришћеног у процесу детоксификације. Сличност тетанус токсоида са различитим серумским протеинима на нивоу конформације и/или пептидних секвенција (β_2GPI, ламинин) указује на његову потенцијалну улогу у индукцији аутоимуности механизмом молекулске мимикрије. Будући да настанак аутоимунске болести подразумева нарушавање толеранције, на пример, прекомерном стимулацијом имунског система, контрола присуства и концентрације себи сличних епитопа се намеће као неопходна. У овом раду је показано да: 1) MAт26 може да прави разлику између тетанус токсина и различитих токсоидних форми што га чини потенцијално добрим антителом које би се користило у контроли током производње вакцина; 2) захваљујући унакрсној реактивности са β_2GPI, MAт26 може да пружи информације о присуству потенцијално опасних епитопа на површини протеина.

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