Modeling neutralization of Shiga 2 toxin by A- and B-subunit-specific human monoclonal antibodies

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Received: 16 November 2015 / Accepted: 17 March 2016 / Published online: 7 May 2016
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Abstract A mathematical model for Shiga 2 toxin neutralization by A- and B-subunit-specific human monoclonal antibodies initially delivered in the extracellular domain is presented, taking into account toxin and antibodies interaction in the extracellular domain, diffusion of toxin, antibodies, and their reaction products toward the cell, the receptor-mediated toxin and complex composed of toxin and antibody to A-subunit internalization from the extracellular into the intracellular medium and excretion of this complex back to the extracellular environment via recycling endosomal carriers. The retrograde transport of the intact toxin to the endoplasmic reticulum and its anterograde movement back to the vicinity of the plasma membrane with its subsequent exocytotic removal to the extracellular space via the secretory vesicle pathway is also taken into account. The model is composed of a set of coupled PDEs. A mathematical model based on a system of ODEs for Shiga 2 toxin neutralization by antibodies in the absence of cell is also studied. Both PDE and ODE systems are solved numerically. Numerical results are illustrated by figures and discussed.

Keywords Toxin · Antibody · Cell receptor · Microtubule transport · Molecular motors

1 Introduction

Shiga toxin 2 (Stx2) is one of two immunologically distinct Stx holotoxins, designated Shiga toxin 1 (Stx1) and Stx2, synthesized by some strains of *Escherichia coli* bacteria [1]. Infection of children with Stx2 is the leading cause of hemolytic uremic syndrome [1–6]. Stx2 internalization and its intracellular transport include a number of steps (see, e.g., [1, 2, 7–9], and refs. therein). The Stx2 molecule is assembled from an A-subunit monomer

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and a B-subunit pentamer. The B-subunit binds to the eukaryotic cell surface-specific receptor Gb₃, triggering internalization of the receptor-bound toxin molecule [1, 7, 8] and its intracellular transport, whereas the A-subunit mediates Stx2 toxic activity [2]. The internalized toxin molecule undergoes retrograde trafficking to the Golgi apparatus and the endoplasmic reticulum (ER) where it is enzymatically cleaved into the A- and B-subunits. The A-subunit is then translocated from the ER into the cytosol [9, 10] where it reaches the ribosomes. The A-subunit has RNA-glycosidase activity that removes a single adenine from 28S rRNA [1, 11, 12]. This adenine is on the loop of ribosomal RNA (rRNA) that is important for elongation factor binding. Lacking this factor disrupts the mRNA translation in the process of protein synthesis, resulting in cell death. From this point of view, the toxin concentration at the ER and the incoming intact toxin flux into the ER are quantities that can be used for estimation of the toxic impact of Stx2 on cell functioning and evaluation of the protective characteristics of the antibodies.

The comprehensive experimental study of a large number of human monoclonal antibodies (HuMAbs) [1, 2] shows that the most effective among them are 5H8 and 5C12, specific for B- and A-subunits, respectively. Moreover, work [2] reveals that B-subunit-specific HuMAb 5H8 blocks binding of the Stx2 molecule to the HeLa cell membrane receptors, Gb₃, in a dose-dependent manner. The doses of 5H8 from 250 to 16 $\mu\text{g/ml}$ completely inhibit 2.5 ng/ml Stx2 binding with cells. In contrast, the A-subunit-specific HuMAb 5C12 does not block binding of toxin with the cells at any dose but its particles bind to those Stx2 molecules that are already bound to Gb₃ [2] and form a complex (Stx2-Gb₃)-5C12. It has been shown [2, 13, 14] that a free HuMAb 5C12 molecule binds to a free Stx2 particle forming a complex Stx2-5C12, which also binds to the cell receptor Gb₃ and forms a complex (Stx2-5C12)-Gb₃. It has also been shown [2] that these two complexes, (Stx2-Gb₃)-5C12 and (Stx2-5C12)-Gb₃, possessing the same structure, are internalized into the cell and accumulate in the early endosome (EE). Following [2], we assume that these complexes are subsequently cleaved into receptor molecule Gb₃ and complex Stx2-5C12 and that this complex is excreted into the extracellular environment via the recycling endosomes (RE). Since complex Stx2-5C12 accumulates in the EE and then is removed via the RE from the body, this shows [2] that HuMAb 5C12 blocks the retrograde transport of this complex toward the Golgi network and ER and causes it to use an intracellular route distinct from that in which a free internalized toxin molecule moves. Blocking the retrograde transport prevents the complex Stx2-5C12 from entering the ER and then the cytosol where Stx2 exerts its cytotoxic effect. However, a clear understanding of the mechanism by which 5C12 neutralizes Stx2 *in vitro* awaits further studies [1]. It has been experimentally proven [1] that the most effective of HuMAbs used to neutralize RNA-glycosidase activity of Stx2 by blocking retrograde transport also neutralize Stx2 toxicity in a dose-dependent manner.

Although the HuMAbs 5C12 and 5H8, which effectively neutralize the cytotoxic effects of the Stx2 toxin in HeLa cells, are highly protective in both mice and gnotobiotic piglets, no specific protective measures or therapy against the Stx2 poisoning are at present available in humans [13, 15]. The development of new antibodies usually includes expensive experimental studies [15]. To reduce the cost of this experimental burden and facilitate exertions to increase the protective characteristics of antibodies, mathematical modeling can be applied.

Ribosome-inactivating Shiga 2 and the dimeric plant toxin ricin belong to the AB family, which is highly toxic to mammalian cells [9, 16]. A mathematical model for the toxin

ricin trafficking towards the ER and inhibition of its cytotoxic effect on the cell by using a monoclonal antibody (in fact, against the B subunit of the ricin molecule) is presented and studied numerically in [17]. This model includes formation of a toxin-antibody complex, diffusion of the toxin, antibody, and toxin-antibody complex in the extracellular space, toxin clearance flux, pinocytotic and receptor-mediated free toxin internalization, lysosomal toxin degradation and its exocytotic removal from the intracellular domain, free toxin diffusion in the intracellular medium and the directed (retrograde and anterograde) toxin transport along the microtubules of the cell skeleton. We stress that the toxin-antibody complex cannot bind to membrane receptors and thereby cannot be internalized into the cell.

In this article, we model the Stx2 extra- and-intracellular transport, taking into account the ability of the A- and B-subunit-specific HuMAbs to inhibit (or neutralize) the Stx2 enzymatic (intracellular) and cell binding (extracellular) activity, respectively. We define the Stx2 enzymatic activity as its ability to inhibit protein synthesis by inactivating ribosomes and thereby reducing the pool of ribosomes available for protein synthesis. As in [17], we assume that toxin and both antibodies are initially delivered in the extracellular medium where the Stx2 reacts with each antibody, forming complexes composed of toxin-antibody to A-subunit (TA_a) and toxin-antibody to B-subunit (TA_b). We also take into account binding of the antibody to A-subunit (A_a) to complex TA_b to form $A_a(TA_b)$ complex and binding of the antibody to B-subunit (A_b) to complex TA_a to form $A_b(TA_a)$ complex. We suppose that species T , A_a , A_b , TA_a , TA_b , $A_a(TA_b)$, $A_b(TA_a)$ diffuse in the extracellular medium and that T and TA_a can bind to the cell membrane receptor S_r , forming complexes TS_r and $S_r(TA_a)$. We also assume that antibody A_a can bind to TS_r , forming complex $A_a(TS_r)$ and that complexes TS_r , $S_r(TA_a)$, and $A_a(TS_r)$ can be internalized into the intracellular space. As mentioned above, complexes $S_r(TA_a)$ and $A_a(TS_r)$ are localized in the EE and are not transported to the ER for subsequent inactivation of ribosomes.

To model the intracellular transport of the free toxin (that evades formation of complexes with the A- and B-subunit-specific antibodies) toward the ER we follow papers [18, 19] and assume that all intact toxin molecules are divided into particles that are bound via motor proteins to the microtubules of the cell skeleton and those that are detached from them. We also assume that the detached particles undergo diffusion and divide the attached toxin molecules into outward going particles (that move anterogradely from the ER toward the vicinity of the cell membrane) driven by kinesin motor protein and inward going particles (that move retrogradely) driven by dynein motors.

We also studied a model for Shiga 2 toxin neutralization by A- and B-subunit-specific human monoclonal antibodies in the case where no cell is present. This model can be described by a system of coupled ODEs. To describe the models by systems of deterministic differential equations we assume that the number of molecules of all species involved in reactions is sufficiently large.

The paper is organized as follows. In Section 3, we construct the models. In Section 4, we discuss numerical results. Some remarks in Section 5 conclude the paper.

2 Notation

ρ	– the distance to the origin;
$S_m = \{\rho : \rho = \rho_m\}$	– the surface of the cell membrane of a spherical cell;
$\Omega_e = \{\rho : \rho \in (\rho_m, \rho_e)\}$	– the extracellular domain;

$S_e = \{\rho : \rho = \rho_e\}$	– the surface of the external sphere (external surface of Ω_e);
$S_n = \{\rho : \rho = \rho_n\}, \rho_n < \rho_m$	– the surface of the spherical envelope of the domain occupied by ER;
$\Omega_i = \{\rho : \rho \in (\rho_n, \rho_m)\}$	– the intracellular domain lying between the ER and cellular membrane;
T	– the Stx2 toxin in domains Ω_e and Ω_i ;
$A_a, A_b, C_a, C_b, C_{ab}$, and C_{ba}	– the antibody to A-subunit, antibody to B-subunit, and complexes $TA_a, TA_b, A_b(TA_a)$, and $A_a(TA_b)$, respectively;
r_0	– the concentration of receptors confined to the cell membrane;
$r_0\theta_1$	– the density of the toxin T-bound receptors $S_r = Gb_3, TS_r$;
$r_0\theta_2$	– the density of the antibody A_a -bound complexes $TS_r, A_a(TS_r)$;
$r_0\theta_3$	– the density of the complex C_a -bound receptors $S_r, S_r(TA_a)$;
r_d and r_k	– the number of sites on the surface of a cytoskeletal microtubule to which toxin molecule can bind via dynein and kinesin, respectively;
u_a and u_b	– the bulk concentration in Ω_e of anti-A and anti-B subunit antibodies, respectively;
$u_{C_a}, u_{C_b}, u_{C_{ab}}$, and $u_{C_{ba}}$	– the bulk concentration in Ω_e of the complexes C_a, C_b, C_{ab} , and C_{ba} , respectively;
u_T and u_T^i	– the bulk concentration of the intact toxin T in Ω_e and Ω_i , respectively;
u_{dT} and u_{kT}	– the bulk concentration in Ω_i of the intact toxin T particles bound to microtubule sites via dynein (TS_d) and kinesin (TS_k), respectively;
u_T^0, u_a^0, u_b^0	– the initial concentration of the toxin T and antibodies A_a and A_b ;
$\kappa_T, \kappa_a, \kappa_b, \kappa_{C_a}, \kappa_{C_b}, \kappa_{C_{ab}}$, and $\kappa_{C_{ba}}$	– the diffusivity of species T, $A_a, A_b, C_a, C_b, C_{ab}$, and C_{ba} in Ω_e , respectively;
κ_T^i	– the bulk diffusivity of the intact toxin in the intracellular domain Ω_i ;
k_a and k_{-a}, k_b and k_{-b}	– the rate constant of the forward and reverse reaction between the T and A_a, T and A_b , respectively;
k_{ab}, k_{ba} and k_{-ab}, k_{-ba}	– the rate constant of the forward and reverse reaction between the A_b and TA_a, A_a and TA_b , respectively;
k_1 and k_{-1}	– the toxin T binding to and detachment from the cell receptors rate constants;
k_2 and k_{-2}	– the antibody A_a binding to and detachment from the complex TS_r rate constants;
k_3 and k_{-3}	– the complex C_a binding to and detachment from the cell receptor S_r rate constants;

k_4, k_5 , and k_6	– the complexes TS_r , $C_{ar}=A_a(TS_r)$, and $C_{ra}=S_r(TA_a)$ internalization rate constants, respectively;
γ_m	– the constant of the T exocytotic flux at the cell membrane S_m ;
γ_n	– the T absorbtion rate constant by the ER through its surface S_n ;
p_d and p_k	– the bulk concentration of the cytoskeletal microtubule sites S_d and S_k to which T molecules may bind via dynein and kinesin, respectively;
k_d and k_{-d}	– the rate constant of T particles binding to and detachment from the microtubule site S_d to which they may bind via dynein;
k_k and k_{-k}	– the rate constant of T particles binding to and detachment from the microtubule site S_k to which they may bind via kinesin;
v_d and v_k	– the positive drift velocity of the microtubule-bound toxin particle TS_d and TS_k , respectively;
α_k	– the loading rate constant for particles of species T at the ER envelope;
α_d	– the fraction of the internalized toxin T flux coming to their free diffusion in Ω_i ;
$\partial_t = \partial/\partial t$, $\Delta = \rho^{-2} \frac{\partial}{\partial \rho} (\rho^2 \frac{\partial}{\partial \rho})$	– the Laplace operator.

3 The models

In this section, we consider two models: (i) the first model is devoted to the Shiga 2 toxin transport to the ER and reduction of its toxic effect on the cell (or neutralization of Stx2) with introduction of antibodies to toxin A-and B-subunits; (ii) the second one describes neutralization of Shiga 2 toxin with introduction of antibodies to toxin A-and B-subunits in case of cell absence.

We first study model (i) and suppose that toxin and antibodies initially are delivered in the extracellular domain Ω_e where toxin T competitively reacts with antibodies A_a and A_b , forming $C_a=TA_a$ and $C_b=TA_b$ complexes. In turn, C_a and C_b can react with antibodies A_b and A_a to form complexes C_{ab} and C_{ba} , respectively. Toxin T, which evades reaction with antibodies, free antibody A_a , and free complex C_a move toward the cell membrane where toxin T can bind to cell receptor, $S_r=Gb_3$, forming complex TS_r , antibody A_a can bind to complex TS_r , forming a new complex $C_{ar}=A_a(TS_r)$ [2], and C_a can bind to free receptor S_r to form complex $C_{ra}=S_r(TA_a)$. Receptor-bound toxin TS_r , which evades formation of complex C_{ar} , is internalized and undergoes diffusion and retrograde transport to the Golgi apparatus and ER [16] whereas complexes C_{ra} and C_{ar} are localized in the EE and then cleaved into S_r and TA_a and subsequently complex TA_a is removed via recycling endosomes RE from the cell to the extracellular environment [2, 20]. We assume that one portion of the internalized intact toxin T is exocytosed from the intracellular domain Ω_i back to the Ω_e , whereas another its portion is transported to the ER where it is cleaved into the A-and B-subunits. Then the A-subunit is translocated [16] across the ER envelope into the cytosol where it inactivates ribosomes, inhibiting protein synthesis.

To describe the intact toxin T transport inside Ω_i we follow [18] and [17] and assume that one toxin portion undergoes diffusion whereas the other portion moves retrogradely or anterogradely (i.e., uses the dynein or kinesin-driven transport on the immobile microtubules towards the ER or back to the vicinity of the cell membrane and then out of the cell via the exocytotic pathway, respectively). We neglect details of the T molecules binding to motor protein particles and following [21–23] suppose that the diffusing toxin T molecule can bind to diffusing motor protein molecules (kinesin or dynein) to form a particle composed of toxin T bound to the motor protein. We also suppose that this new particle diffuses and can bind via the motor protein molecule to the skeletal microtubules and detach from them. We assume that the detached particle can split into the motor protein molecule and toxin T particle and that the latter can bind to a dynein or kinesin molecule. We also assume that microtubule-bound T-motor protein particle is transported along the microtubule at a permanent motor velocity and that the pool of motor proteins is large enough so that the variation of their concentrations can be neglected.

To construct equations in Ω_e for the mathematical model we suppose that the cell membrane receptors are distributed continuously and use the following reactions:

- (i) between the toxin $T=Stx2$ and anti-A subunit antibody A_a , toxin T and anti-B subunit antibody A_b , antibody A_b and complex TA_a , antibody A_a and complex TA_b in domain Ω_e ,

- (ii) between toxin T and cell receptor S_r , antibody A_a and complex TS_r , complex C_a and cell receptor S_r on the cell membrane S_m ,

- (iii) the cleavage reaction of complexes C_{ar} and C_{ra} into C_a and S_r in the intracellular domain Ω_i ,

Here T , A_a , A_b , C_a , C_b are the $Stx2$, anti-A subunit antibody, anti-B subunit antibody, complexes TA_a and TA_b , respectively, C_{ab} and C_{ba} are complexes formed of A_b bound to TA_a and of A_a bound to TA_b , respectively, in domain Ω_e , TS_r is the receptor-bound toxin, and C_{ar} and C_{ra} are complexes formed of A_a bound to TS_r and of TA_a bound to receptor S_r , respectively.

As mentioned above, the toxin, both antibodies, both complexes formed of the toxin and one of antibodies, and both complexes composed of toxin T and both antibodies are transported by the diffusion in domain Ω_e . In the intracellular domain Ω_i , toxin T moves toward the ER using diffusion and retrograde transport and by diffusion and via anterograde transport back to the vicinity of the plasma membrane. We also take into account the final stage of intact toxin exocytotic removal from the vicinity of the plasma membrane out of the cell. It is known [24] that the final stage of the exocytosis is based on fusion of the secretory vesicles with the plasma membrane. However, it is not currently clear [24] how these vesicles finally dock and fuse with the plasma membrane to release their contents (intact toxin) into the extracellular space. Therefore, for simplicity, we assume that the final stage of the exocytotic toxin excretion out of the cell is rapid and describe the toxin exocytotic flux by the expression $(\gamma_m u_T^i + v_k u_{kT})|_{\rho_m-0}$ where γ_m is a constant.

Following [17], we treat the cell as a sphere of radius ρ_m and the ER as the concentric sphere of radius $\rho_n \ll \rho_m$ (see Fig. 1). We assume that microtubules are directed radially from the center toward the cell membrane. Let r_d and r_k be the number of sites on the microtubule surface for toxin binding via dynein and kinesin molecules, respectively, and suppose that q is the number of microtubules of the cell skeleton. Then quantities $p_d = qr_d/V$, $p_k = qr_k/V$, $V = 4\pi/3(\rho_m^3 - \rho_n^3)$, represent the bulk concentrations of the microtubule sites for binding of toxin T molecules via dynein and kinesin, respectively. We assume that binding sites of the same and different microtubules do not compete for free toxin molecules. We neglect the lysosomal toxin degradation and assume that a portion of the diffusing toxin T and microtubule-bound toxin component TS_d , where S_d is the microtubule site for T to bind via dynein, penetrates into the ER via the outgoing fluxes through its envelope, $\gamma_n u_T^i|_{\rho=\rho_n+0}$ and $v_d u_{dT}|_{\rho=\rho_n+0}$, respectively.

We first construct the partial differential equation model (referred to as the PDE model). In what follows, we consider the case of spherical symmetry. Taking into account diffusion

Fig. 1 Toxin pathway to ER: 1 external surface of the extracellular domain Ω_e , 2 cell membrane, 3 spherical envelope of the ER, 4 receptor, 5 toxin, 6 antibody to A-subunit, 7 antibody to B-subunit, 8 receptor-toxin complex, 9 toxin-antibody to A-subunit complex, 10 toxin-antibody to B-subunit complex, 11 complex of toxin-antibody to A-subunit bound with antibody to B-subunit, 12 complex of toxin-antibody to B-subunit bound with antibody to A-subunit, 13 complex of receptor-toxin bound with antibody to A-subunit, 14 complex of toxin-antibody to A-subunit bound with receptor

of species, applying the mass action law to reactions (1), (2), and (3) and using the Langmuir kinetics with the Eley–Rideal reaction step (see term $k_2 u_a \theta_1$ in (4), (10), and (11)) we derive equations in the extracellular domain Ω_e for concentrations $u_T, u_a, u_b, u_{C_a}, u_{C_b}, u_{C_{ab}}, u_{C_{ba}}$ and fractions $\theta_1, \theta_2, \theta_3$ where $r_0 \theta_1, r_0 \theta_2$, and $r_0 \theta_3$ denote the density of complexes TS_r, C_{ar} , and C_{ra} , respectively:

$$\begin{cases} \partial_t u_a = -k_a u_T u_a + k_{-a} u_{C_a} - k_{ba} u_a u_{C_b} \\ \quad + k_{-ba} u_{C_{ba}} + \kappa_a \Delta u_a, & \rho \in (\rho_m, \rho_e), \quad t > 0, \\ \partial_\rho u_a|_{\rho_e=0} = 0, & t > 0, \\ \kappa_a \partial_\rho u_a|_{\rho_m+0} = r_0(k_2 u_a \theta_1 - k_{-2} \theta_2)|_{\rho_m+0}, & t > 0, \\ u_a(0, \rho) = u_a^0(\rho), & \rho \in (\rho_m, \rho_e), \end{cases} \quad (4)$$

$$\begin{cases} \partial_t u_b = -k_b u_T u_b + k_{-b} u_{C_b} - k_{ab} u_b u_{C_a} \\ \quad + k_{-ab} u_{C_{ab}} + \kappa_b \Delta u_b, & \rho \in (\rho_m, \rho_e), \quad t > 0, \\ \partial_\rho u_b|_{\rho_e=0} = \partial_\rho u_b|_{\rho_m+0} = 0, & t > 0, \\ u_b(0, \rho) = u_b^0(\rho), & \rho \in (\rho_m, \rho_e), \end{cases} \quad (5)$$

$$\begin{cases} \partial_t u_T = -k_a u_T u_a + k_{-a} u_{C_a} - k_b u_T u_b \\ \quad + k_{-b} u_{C_b} + \kappa_T \Delta u_T, & \rho \in (\rho_m, \rho_e), \quad t > 0, \\ \partial_\rho u_T|_{\rho_e=0} = 0, \\ \kappa_T \partial_\rho u_T|_{\rho_m+0} = r_0(k_1(1 - \theta_1 - \theta_2 - \theta_3)u_T - k_{-1}\theta_1)|_{\rho_m+0} \\ \quad - (v_k u_{kT} + \gamma_m u_T^i)|_{\rho_m-0}, & t > 0, \\ u_T(0, \rho) = u_T^0(\rho), & \rho \in (\rho_m, \rho_e), \end{cases} \quad (6)$$

$$\begin{cases} \partial_t u_{C_a} = k_a u_T u_a - k_{-a} u_{C_a} - k_{ab} u_b u_{C_a} \\ \quad + k_{-ab} u_{C_{ab}} + \kappa_{C_a} \Delta u_{C_a}, & \rho \in (\rho_m, \rho_e), \quad t > 0, \\ \partial_\rho u_{C_a}|_{\rho_e=0} = 0, \\ \kappa_{C_a} \partial_\rho u_{C_a}|_{\rho_m+0} = r_0 k_3 u_{C_a}(1 - \theta_1 - \theta_2 - \theta_3)|_{\rho_m+0} \\ \quad - r_0((k_{-3} + k_6)\theta_3 + k_5 \theta_2)|_{\rho_m+0}, & t > 0, \\ u_{C_a}(0, \rho) = 0, & \rho \in (\rho_m, \rho_e), \end{cases} \quad (7)$$

$$\begin{cases} \partial_t u_{C_b} = k_b u_T u_b - k_{-b} u_{C_b} - k_{ba} u_a u_{C_b} \\ \quad + k_{-ba} u_{C_{ba}} + \kappa_{C_b} \Delta u_{C_b}, & \rho \in (\rho_m, \rho_e), \quad t > 0, \\ \partial_\rho u_{C_b}|_{\rho_e=0} = 0, & t > 0, \\ \partial_\rho u_{C_b}|_{\rho_m+0} = 0, & t > 0, \\ u_{C_b}(0, \rho) = 0, & \rho \in (\rho_m, \rho_e), \end{cases} \quad (8)$$

$$\begin{cases} \partial_t u_{C_{ab}} = k_{ab} u_{C_a} u_b - k_{-ab} u_{C_{ab}} + \kappa_{C_{ab}} \Delta u_{C_{ab}}, & \rho \in (\rho_m, \rho_e), \quad t > 0, \\ \partial_\rho u_{C_{ab}}|_{\rho_e=0} = 0, & t > 0, \\ \partial_\rho u_{C_{ab}}|_{\rho_m+0} = 0, & t > 0, \\ u_{C_{ab}}(0, \rho) = 0, & \rho \in (\rho_m, \rho_e), \end{cases} \quad (9)$$

$$\begin{cases} \partial_t u_{C_{ba}} = k_{ba} u_{C_b} u_a - k_{-ba} u_{C_{ba}} + \kappa_{C_{ba}} \Delta u_{C_{ba}}, & \rho \in (\rho_m, \rho_e), \quad t > 0, \\ \partial_\rho u_{C_{ba}}|_{\rho_e=0} = 0, & t > 0, \\ \partial_\rho u_{C_{ba}}|_{\rho_m+0} = 0, & t > 0, \\ u_{C_{ba}}(0, \rho) = 0, & \rho \in (\rho_m, \rho_e), \end{cases} \quad (10)$$

$$\begin{cases} \partial_t \theta_1 = k_1(1 - \theta_1 - \theta_2 - \theta_3)u_T - (k_{-1} + k_2 u_a + k_4)\theta_1 + k_{-2}\theta_2, \\ \quad \rho = \rho_m, \quad t > 0, \\ \theta_1|_{t=0} = 0, & \rho = \rho_m, \end{cases} \quad (11)$$

$$\begin{cases} \partial_t \theta_2 = k_2 \theta_1 u_a - (k_{-2} + k_5)\theta_2, & \rho = \rho_m, \quad t > 0, \\ \theta_2|_{t=0} = 0, & \rho = \rho_m, \end{cases} \quad (12)$$

$$\begin{cases} \partial_t \theta_3 = k_3 u_{C_a}(1 - \theta_1 - \theta_2 - \theta_3) - (k_{-3} + k_6)\theta_3, & \rho = \rho_m, \quad t > 0, \\ \theta_3|_{t=0} = 0, & \rho = \rho_m. \end{cases} \quad (13)$$

Here, $k_4 r_0 \theta_1$, $k_5 r_0 \theta_2$, and $k_6 r_0 \theta_3$ are the outgoing fluxes across the cell membrane of complexes TS_r , Car , and C_{ra} , respectively, and $(v_k u_{kT} + \gamma_m u_T^i)|_{\rho_m-0}$ is the exocytotic intact toxin flux. The algebraic terms in the first equations of systems (4)–(10) describe the variation rate of respective species conditioned by chemical reactions, while the last ones are caused by diffusion.

To derive equations in the intracellular domain Ω_i , we use the following reactions between the intact toxin T and microtubule binding sites S_d and S_k :

Here, TS_d and TS_k are microtubule-bound toxin T via dynein and kinesin, respectively. To construct boundary conditions for the equations determined in domain Ω_i , we assume that the α_d portion of the internalized toxin flux $r_0 k_4 \theta_1$ comes to the diffusion in Ω_i of the intact toxin T and that the remaining portion $1 - \alpha_d$ moves retrogradely. We also assume that the $\alpha_k u_T^i|_{\rho=\rho_n+0}$ concentration of the intact toxin near the ER envelope with a constant α_k is transported anterogradely toward the vicinity of the cell membrane. Applying the mass action law, and the Langmuir mechanism to reactions (14), and taking into account diffusion of the intact toxin T and the boundary conditions on the ER envelope and cell membrane, we derive equations for the bulk concentrations u_T^i , u_{dT} , and u_{kT} in Ω_i of T, TS_d , and TS_k ,

$$\begin{cases} \partial_t u_T^i = -k_d u_T^i (p_d - u_{dT}) + k_{-d} u_{dT} - k_k u_T^i (p_k - u_{kT}) \\ \quad + k_{-k} u_{kT} + \kappa_T^i \Delta u_T^i, \quad \rho \in (\rho_n, \rho_m), \quad t > 0, \\ \kappa_T \partial_\rho u_T^i|_{\rho_m-0} = \alpha_d r_0 k_4 \theta_1|_{\rho_m+0} - \gamma_m u_T^i|_{\rho_m-0}, \quad t > 0, \\ \kappa_T \partial_\rho u_T^i|_{\rho_n+0} = (\gamma_n + \alpha_k) u_T^i|_{\rho_n+0}, \quad t > 0, \\ u_T^i|_{t=0} = 0, \quad \rho \in [\rho_n, \rho_m], \end{cases} \quad (15)$$

$$\begin{cases} \partial_t u_{dT} = k_d u_T^i (p_d - u_{dT}) - k_{-d} u_{dT} \\ \quad + v_d \partial_\rho u_{dT} + 2v_d u_{dT}/\rho, \quad \rho \in (\rho_n, \rho_m), \quad t > 0, \\ u_{dT}|_{\rho_m-0} = (1 - \alpha_d)(r_0 k_4 \theta_1)/v_d|_{\rho_m+0}, \quad t \geq 0, \\ u_{dT}|_{t=0} = 0, \quad \rho \in [\rho_n, \rho_m], \end{cases} \quad (16)$$

$$\begin{cases} \partial_t u_{kT} = k_k u_T^i (p_k - u_{kT}) - k_{-k} u_{kT} \\ \quad - v_k \partial_\rho u_{kT} - 2v_k u_{kT}/\rho, \quad \rho \in (\rho_n, \rho_m), \quad t > 0, \\ u_{kT}|_{\rho_n+0} = \alpha_k u_T^i/v_k|_{\rho_n+0}, \quad t \geq 0, \\ u_{kT}|_{t=0} = 0, \quad \rho \in [\rho_n, \rho_m]. \end{cases} \quad (17)$$

Equations (4)–(13) and (15)–(17) compose the PDE model. Note that, due to the Langmuir kinetics, the transition terms that model binding of toxin particles to the skeletal microtubules in (15)–(17) are nonlinear.

In case (ii) (i.e., in the absence of cells) we are interested only in the ability to neutralize toxin by both types of antibodies and model this process by a coupled system of ODEs (referred to as the ODE model) that directly follows from the first equations of systems (4)–(10) provided that all species initially are distributed uniformly:

$$\begin{cases} u'_a = -k_a u_T u_a + k_{-a} u_{C_a} - k_{ba} u_a u_{C_b} + k_{-ba} u_{C_{ba}}, \\ u_a(0) = u_a^0, \end{cases} \quad (18)$$

$$\begin{cases} u'_b = -k_b u_T u_b + k_{-b} u_{C_b} - k_{ab} u_b u_{C_a} + k_{-ab} u_{C_{ab}}, \\ u_b(0) = u_b^0, \end{cases} \quad (19)$$

$$\begin{cases} u'_T = -k_a u_T u_a + k_{-a} u_{C_a} - k_b u_T u_b + k_{-b} u_{C_b}, \\ u_T(0) = u_T^0, \end{cases} \quad (20)$$

$$\begin{cases} u'_{C_a} = k_a u_T u_a - k_{-a} u_{C_a} - k_{ab} u_b u_{C_a} + k_{-ab} u_{C_{ab}}, \\ u_{C_a}(0) = 0, \end{cases} \quad (21)$$

$$\begin{cases} u'_{C_b} = k_b u_T u_b - k_{-b} u_{C_b} - k_{ba} u_a u_{C_b} + k_{-ba} u_{C_{ba}}, \\ u_{C_b}(0) = 0, \end{cases} \quad (22)$$

$$\begin{cases} u'_{C_{ab}} = k_{ab} u_{C_a} u_b - k_{-ab} u_{C_{ab}}, \\ u_{C_{ab}}(0) = 0, \end{cases} \quad (23)$$

$$\begin{cases} u'_{C_{ba}} = k_{ba} u_{C_b} u_a - k_{-ba} u_{C_{ba}}, \\ u_{C_{ba}}(0) = 0. \end{cases} \quad (24)$$

System (18)–(24) possesses three mass conservation laws

$$\begin{cases} u_T + u_{C_a} + u_{C_b} + u_{C_{ab}} + u_{C_{ba}} = u_T^0, \\ u_a + u_{C_a} + u_{C_{ab}} + u_{C_{ba}} = u_a^0, \\ u_b + u_{C_b} + u_{C_{ab}} + u_{C_{ba}} = u_b^0. \end{cases} \quad (25)$$

In what follows, we are interested in the evaluation of the parameters:

- (i) toxin concentration at the ER envelope, $\psi(t) = (u_T^i + u_{dT})|_{\rho=\rho_n}$, conditioned by diffusion and retrograde transport in the PDE model,
- (ii) toxin flux into the ER, $\phi(t) = (\gamma_n u_T^i + v_d u_{dT})|_{\rho=\rho_n}$, determined by the PDE model,
- (iii) toxin concentration, $u_T(t)$, determined by the ODE model.

By substituting variables

$$\begin{cases} \rho = l\bar{\rho}, t = \tau_*\bar{t}, r_0 = lu_*\bar{r}_0, r_d = lu_*\bar{r}_d, r_k = lu_*\bar{r}_k, \\ f = \bar{f}u_*, f = u_T, u_a, u_b, u_{C_a}, u_{C_b}, u_{C_{ab}}, u_{C_{ba}}, u_T^i, u_{dT}, u_{kT}, p_d, p_k, \\ f = \bar{f}/(\tau_*u_*), f = k_a, k_b, k_{ab}, k_{ba}, k_d, k_k, \\ f = \bar{f}/\tau_*, f = k_{-1}, k_{-2}, k_{-3}, k_4, k_5, k_6, k_{-a}, k_{-b}, k_{-ab}, k_{-ba}, k_{-d}, k_{-k}, \\ f = \bar{f}l/\tau_*, f = \gamma_n, v_k, v_d, \gamma_m, \alpha_k, \\ f = \bar{f}l^2/\tau_*, f = \kappa_T, \kappa_a, \kappa_b, \kappa_{C_a}, \kappa_{C_b}, \kappa_{C_{ab}}, \kappa_{C_{ba}}, \kappa_T^i \end{cases} \quad (26)$$

into (4)–(13), (15)–(24) we can deduce the same systems, but now in the non-dimensional form expressed by the dimensionless (overscored) quantities. Therefore, for simplicity in what follows, we omit the bar and treat (4)–(13) and (15)–(24) as the non-dimensional ones.

4 Numerical results

We solve (18)–(24) by solver ode45 (see MATLAB ODE solvers [25, 26]). To solve (4)–(10) and (15) of the PDE model we used the implicit finite difference scheme [27]. Equations (11)–(13) were solved by the explicit difference scheme while (16) and (17) were written on the characteristic lines and solved by the Euler method. Results of simulations are presented in Figs. 2, 3, 4, 5, 6, 7, 8 and 9. Selection of the parameter values given in Table 1 was motivated by the values available in the literature. To model the various regimes possible outside and inside the eukaryotic cell, we use an extended range of these parameters. All results are presented in a non-dimensional form using the reference values $u_* = 6.02 \cdot 10^{13} \text{ cm}^{-3}$, $l = 10^{-2} \text{ cm}$, and $\tau_* = 1 \text{ s}^{-1}$ as a scale of concentration, length and time, respectively, that are typical for intracellular transport processes [28]. In all legends, we use values of non-dimensional parameters (bar omitted for simplicity). Values of parameters

Fig. 2 Dependence of the toxin flux $\phi(t)$ into the ER on the variation of the reaction rate constant $k_a = k_b$: 0.013 (1), 0.0065 (2), 0.0026 (3), 0.0013 (4) calculated for $k_1 = 0.0125$ and different values of the toxin binding rate constant k_1 : 0.0025 (5), 0.00625 (6) obtained at $k_a = k_b = 0.013$ in case $u_T^0 = 0.05$, $u_a^0 = u_b^0 = 0.5$ and $\kappa_T^e = \kappa_T^i = 10^{-4}$

that are not shown in legends correspond to non-dimensional ones determined by using values from Table 1. In the case where diffusivity of all species in the extracellular domain are equal we use κ_T^e for short.

Numerical results corresponding to the PDE model are presented in Figs. 2–8 and demonstrate a nonmonotone behavior of functions $\psi(t)$ and $\phi(t)$ in time. Figure 9 corresponds to ODE model.

Figure 2 illustrates the influence of the reaction rate constant $k_a = k_b$ and toxin binding to the cell receptor constant k_1 on the behavior of the toxin flux $\phi(t) = (\gamma_n u_T + v_d u_{dT})|_{\rho=\rho_n}$ into the ER at fixed values of the other parameters. This figure demonstrates the natural $\phi(t)$ growth as k_a decreases or k_1 increases.

Fig. 3 Time dependence of the toxin flux $\phi(t)$ determined for the initial toxin and antibody concentrations $u_T^0 = 0.05$ and $u_a^0 = u_b^0 = 0.5$ on different values of the antibody A_a and complex C_a binding rate constants k_2 and k_3 in case $\kappa_T^e = \kappa_T^i = 10^{-4}$

Fig. 4 Influence of the variation of the reaction rate constant k_a : 0.013 (1), 0.0065 (2), 0.0026 (3), 0.0013 (4) on the behavior of the toxin flux $\phi(t)$ determined for the initial concentrations $u_T^0 = 0.05$, $u_a^0 = 0.5$, $u_b^0 = 0$ and the toxin diffusivity $\kappa_T^e = \kappa_T^i = 10^{-4}$

Figure 3 demonstrates the effect of variation of the antibody A_a binding to TS_r complex and $C_a=TA_a$ binding to S_r constants k_2 and k_3 , respectively, on the toxin flux $\phi(t)$. We observe that the growth of k_2 and k_3 decreases values of $\phi(t)$ and that flux $\phi(t)$ is more sensitive to variation of k_2 than to variation of k_3 . Our calculations also show that the growth of k_2 and k_3 increases θ_2 and θ_3 , respectively, and hence decreases the concentration of free receptors S_r for binding of Stx2 molecules.

The influence of the reaction between toxin T and antibody A_a rate constant k_a and reaction between T and antibody A_b rate constant k_b on the toxin flux $\phi(t)$ is illustrated by Figs. 4 and 5, respectively, in case where only A-subunit-specific (Fig. 4) or B-subunit-specific (Fig. 5) antibody is used. In both cases, the growth of k_a and k_b naturally decreases flux $\phi(t)$.

Fig. 5 Effect of the reaction rate constant k_b : 0.013 (1), 0.0065 (2), 0.0026 (3), 0.0013 (4) on the toxin flux $\phi(t)$ calculated for the initial concentrations $u_T^0 = 0.05$, $u_a^0 = 0$, $u_b^0 = 0.5$ and toxin diffusivity $\kappa_T^e = \kappa_T^i = 10^{-4}$

Fig. 6 Time profiles of the toxin concentration ψ for different values of the initial concentrations of the antibodies, u_a^0 and u_b^0 (Figs. 6a and b), and the drift velocities $v_d = v_k$: 0.001 (solid line), 0.01 (dashed line) (Fig. 6a) obtained for the initial toxin concentration $u_T^0 = 0.05$ and diffusivities $\kappa_T^e = 10^{-1}$, $\kappa_T^i = 10^{-4}$

Plots in Fig. 7 illustrate the effect of variation of the initial concentration (dose) of the A- and B-subunit-specific antibodies, u_a^0 and u_b^0 (Figs. 6a and b, respectively) and the influence of the directed (retrograde and anterograde) transport velocity $v_d = v_k$ (Fig. 6a) on the behavior of the toxin concentration, $\psi(t) = (u_T + u_{dT})|_{\rho=\rho_n}$, at the ER envelope. Figure 6a shows that this concentration decreases as $v_d = v_k$ decreases. Moreover, this figure demonstrates a sudden growth of $\psi(t)$ for $v_d = v_k = 0.001$ at $t = 80$ s. This effect of the microtubule-assisted velocity $v_d = v_k$ on the behavior of toxin concentration $\psi(t)$ can be easily explained. Indeed, since domain Ω_i is initially free of toxin particles there will be a time delay before toxin particles arrive at the ER. If no detachments of toxin particles from the microtubules occur, the attached particle will travel from the cell toward the ER in time $(\rho_m - \rho_n)/v_d$. For $v_d = 0.001$ and 0.01 , this value is 80 and 8 s. Toxin concentration at the ER envelope, $\psi(t)$, also decreases as either u_a^0 or u_b^0 increases, i.e., demonstrates the dose-dependent Stx2 neutralization. Moreover, our calculations show that

$$\max_t \psi |_{(u_a^0=k, u_b^0=0)} / \max_t \psi |_{(u_a^0=0, u_b^0=k)} = 0.299, 0.3018, 0.3126, 0.322, 0.3354$$

Fig. 7 Time profiles of the toxin flux $\phi(t)$ calculated for different values of the internalization rate constant k_4 , the initial concentrations $u_T^0 = 0.05$, $u_a^0 = u_b^0 = 0.5$, and diffusivities $\kappa_T^e = 10^{-1}$, $\kappa_T^i = 10^{-4}$

Fig. 8 Dependence of time profiles of the toxin flux $\phi(t)$ determined at $u_T^0 = 0.05$ and $\kappa_T^e = 10^{-1}$ on different values of the toxin diffusivity κ_T^i : 0.1 (solid line), 0.001 (dashed line) and initial antibody concentrations u_a^0 and u_b^0

at $k = 0.8, 0.75, 0.6, 0.5, 0.4$, respectively, at fixed values of kinetic parameters. This shows that in case of application of the same dose of u_a^0 or u_b^0 the A-subunit-specific antibody is more effective (about three times) in the u_T reduction.

Figure 7 illustrates the dependence of the toxin flux $\phi(t)$ on the toxin internalization rate constant k_4 and shows that $\phi(t)$ grows as this constant increases. Our calculations reveal (results not shown) that the influence of the internalization rate constants k_5 and k_6 on the behavior of $\phi(t)$ at the same values of the other kinetic parameters is practically inappreciable.

The influence of the initial concentrations of A- and B-subunit-specific antibodies, u_a^0 and u_b^0 , respectively, and the intact toxin diffusivity, κ_T^i , inside the cell on the toxin flux $\phi(t)$ is plotted on Fig. 8. This figure demonstrates the increase of $\phi(t)$ as κ_T^i increases or u_a^0 and u_b^0 decrease.

We also studied the influence of the directed transport velocity on the toxin flux into the ER. Our calculations reveal (results not shown) that for $\kappa_T^i \in [0.001; 0.1]$ the influence of

Fig. 9 Effect of variation of the initial concentration of the antibody u_a^0 in case $u_b^0 = 0$ (Fig. 9a) and parameters $k_a = k_b = 0.013$ in case $u_a^0 = 0.5, u_b^0 = 0$ (Fig. 9b) on the toxin concentration $u_T(t)$ determined by the ODE model for $u_T^0 = 0.25$

Table 1 Parameters of simulations (mv – model value)

Parameter	Values	Ref.
k_a	$1.3 \cdot 10^{-15}, 2.6 \cdot 10^{-16} \text{ cm}^3 \text{ s}^{-1}$	[14],mv
k_b	$1.3 \cdot 10^{-15}, 2.6 \cdot 10^{-16} \text{ cm}^3 \text{ s}^{-1}$	[14], mv
k_{-a}, k_{-b}	$1.1 \cdot 10^{-4}, 10^{-7}, \text{ s}^{-1}$	[14], mv
k_{ab}, k_{ba}	$0.65 \cdot 10^{-15}, 1.3 \cdot 10^{-16} \text{ cm}^3 \text{ s}^{-1}$	[14], mv
k_{-ab}, k_{-ba}	10^{-7} s^{-1}	mv
k_4, k_5, k_6	$3.3 \cdot 10^{-5} \text{ s}^{-1}$	[29, 30]
k_d, k_k	$0.21 \cdot 10^{-17} \text{ cm}^3 \text{ s}^{-1}$	mv
k_{-d}, k_{-k}	$1.25 \cdot 10^{-4} \text{ s}^{-1}$	mv
k_1, k_2, k_3	$1.02 \cdot 10^{-16} \text{ cm}^3 \text{ s}^{-1}$	[31], mv
k_{-1}, k_{-2}, k_{-3}	$3.41 \cdot 10^{-3}, 5.2 \cdot 10^{-4} \text{ s}^{-1}$	mv
ρ_n	$2 \cdot 10^{-4} \text{ cm}$	[28]
ρ_m	10^{-3} cm	[28]
ρ_e	$1.8 \cdot 10^{-3} \text{ cm}$	[28]
γ_n	$5 \cdot 10^{-3} \text{ cm s}^{-1}$	mv
γ_m	$10^{-5} \text{ cm s}^{-1}$	mv
u_a^0, u_b^0	$3.01 \cdot 10^{13} \text{ cm}^{-3}$	mv, [28]
u_T^0	$0.05 \cdot 10^{13} \text{ cm}^{-3}$	mv
r_0	$1.26 \cdot 10^9 \text{ cm}^{-2}$	[28]
p_d, p_k	$6.02 \cdot 10^{10} \text{ cm}^{-3}$	mv
v_d, v_k	$10^{-5} \text{ cm s}^{-1}$	mv
α_k	$10^{-5} \text{ cm s}^{-1}$	mv
α_d	0.99 cm s^{-1}	mv
$\kappa_T, \kappa_a, \kappa_b, \kappa_{C_a}, \kappa_{C_b}, \kappa_{C_{ab}}, \kappa_{C_{ba}}, \kappa_T^i$	$10^{-5} \text{ cm}^2 \text{ s}^{-1}$	[28]

the motor transport velocity $v_d = v_k \in [0.001; 0.01]$ on the behavior of $\phi(t)$ is practically inappreciable.

Plots in Fig. 9 illustrate reduction of Shtx2 concentration, u_T , by increasing the initial concentration (Fig. 9a) of the A-subunit-specific antibody and by increasing the forward reaction rate constant k_a (Fig. 9b) in the case of cell absence. Figure 9a shows that in case of large antibody concentration ($u_a^0 > u_T^0$) all toxin is utilized during a fixed time depending on values of the kinetic constants and that concentration $u_T(t)$ tends to the steady-state value $u_T^0 - u_a^0$ as time increases if $u_a^0 < u_T^0$. This result also follows from mass conservation laws (25). All curves in Fig. 9b tend to zero as time grows because $u_a^0 > u_T^0$.

5 Concluding remarks

A unique mechanism of intracellular toxin neutralization by an antibody against the A subunit of Stx2 has been discovered in [2]. Both Stx2 A- and B-subunit-specific HuMAbs, 5C12 and 5H8, respectively, neutralize Stx2 via distinct mechanisms. The HuMAb 5H8 inhibits cytotoxicity by preventing binding of the unbound toxin to the cell receptors but is ineffective against the cell-bound toxin [2]. In contrast, the A-subunit-specific 5C12 binds to the cell-bound toxin and effectively neutralizes its ability to inhibit protein synthesis by redirecting its transport within the cell and thereby prevents toxin-mediated cell death [2].

In this paper, based on the results of experimental study [1, 2], we proposed and studied numerically a deterministic mathematical model (PDE model) for neutralization of Stx2 cytotoxic effect on the cell with introduction of Stx2 A- and B-subunit-specific antibodies. A model based on the ODEs for Stx2 neutralization in the case of cell absence is also studied. Both models enable extensive studies to increase the protective potential of antibodies before proceeding with targeted experimental studies. Both antibodies and toxin (Stx2) initially are delivered extracellularly. The model includes: toxin and antibodies competitive interaction in the extracellular domain, diffusion of toxin, antibodies, and their reaction products toward the cell, the receptor-mediated toxin and complex composed of toxin and antibody to A-subunit internalization from the extracellular into the intracellular domain and excretion of this complex back to the extracellular environment via the recycling endosomal carriers, the retrograde transport of the intact toxin to the endoplasmic reticulum and its anterograde movement back to the vicinity of the plasma membrane with its subsequent exocytotic removal back to the extracellular medium via the secretory vesicle pathway.

The main parameters we studied were the toxin flux $\phi(t)$ into the ER through its envelope and toxin concentration at the ER, $\psi(t) = (u_t^i + v_d u_{dT})|_{\rho=\rho_n}$. We presented numerical simulations showing how the kinetic and toxin transport (both tubule-mediated and by diffusion) parameters and initial concentrations (doses) of both antibodies influence behavior of functions $\psi(t)$ and $\phi(t)$.

To conclude, this paper we emphasize some shortcomings of our model. Our models are based on the assumption that all kinetic parameters are deterministic permanent quantities and concentrations of all species involved in reactions are sufficiently large so that process can be described by deterministic differential equations. Actually, some intracellular kinetic parameters (e.g., drift velocity of motor proteins v_d and v_k) can fluctuate (become stochastic) and concentrations of all species involved in reactions can become very small, so that fluctuations of values of these quantities can become appreciable. Thus, it would be interesting to refine our model and determine the influence of these fluctuations on the protective potential of antibodies.

Compliance with Ethical Standards

Competing Interests The authors declare that they have no competing interests.

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