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Artificial in vivo antigen presentation by the APCs and subsequent T-cell activation: a feasibility analysis

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Abstract Inappropriate antigen presentation by the antigen-presenting cells (APCs) is a cause of various diseases. One of the ways to combat these diseases is to immobilize the APCs near the infected tissue or a tissue which is susceptible to an antigen. The antigen is presented by the APCs present in the immobilized form on an implant and these upon binding to T_H-cells result in triggering of a cascade of events as part of the natural immune response leading to the destruction of the antigen. This system has been modeled as a dialysis bag containing immobilized receptors inside the bag and the ligand diffusing out of the bag. The simulations show that by using the implant, the concentration of the ligand that has diffused into the tissue matrix can be substantially reduced and by suitably choosing the coupler size, the T_H-cells can also effectively be activated.

Key words: T-cell; Immune response; Antigen; Implant; Dialysis; Receptor; Ligand; Simulation

1. Introduction

In the immune response of animals, the helper T-cells (T_H-cells) are activated by interactions with the antigen-presenting cells (APCs) through the formation of an immunological synapse. These APCs internalize the foreign body and present the peptides of the foreign bodies on their surfaces using major histocompatibility complexes (MHCs). The APCs can be of different types, and comprise primarily of macrophages and dendritic cells. The T_H-cells bind to these APCs and stimulate the B-cells, and/or T_H- and T_C-cells by mediating the response through a chain of mediators called cytokines. In the end, the foreign body is destroyed or it is neutralized. If the foreign body is a virus, which has infected a host cell, T_H-cells recognize it and initiate a cascade of events leading to the destruction of the infected cells [1].

However, in many diseases, T_H-cells fail to activate in response to antigen or are inappropriately activated. Many of such diseases occur due to inappropriate antigen presentation by the APCs either due to defective APCs or due to a low APC concentration in the plasma. These include genetic disorders [2,3], viral infections [4,5], and cancer [6,7]. One means to combat these diseases is to present MHC peptide in the affected region so that the T_H-cells can bind to these sites and stimulate the immune response. Another way is to immobilize the APCs (specifically primed against an antigen) on a polymer support and implant it in the vicinity of the concerned tissue. In this case, the antigen will bind to these APCs. This treatment strategy is therefore able to provide normal (and effective) APCs in adequate concentrations (or counts) in the region of the body that is vulnerable to a specific antigen. The T_H-cells present in the bloodstream identify these MHC peptides and bind to them, and thereafter the natural immune response of the body takes over and does the needful to effectively destroy the antigen source of the infection or the disease.

In case of antigens like virus or toxic compounds produced by bacteria, there is a competition between the binding to the APCs (and subsequent endocytosis) and their diffusion into the tissue. The diffusion of the antigen may be across the bloodstream or into the tissue matrix [8] depending on the specific location of the disease-causing antigen. Therefore, the implant must be designed such that the free antigen concentration is extremely small, at least until the natural immune response takes over. The local region where implant is present can be modeled as an immobilized receptor system (immobilized APCs involving receptor-mediated antigen binding, on a polymer matrix) in a dialysis bag (tissue) containing free ligand in the medium (antigen). In this paper, we have modeled the implant system to study the response and have attempted to estimate the size of the coupler (e.g. PEG–biotin) for immobilizing the receptors in order to achieve the objective. We also expect to study the feasibility of this idea and thus determine the constraints on the applicability of this technique. The analysis is believed to be helpful in the design of such implants for tackling a variety of diseases.

Previous works on T-cell activation and its modeling [9–15] have studied interactions between the T-cell receptors (TCRs) and the ligands in solution but this is the first study of its kind in which an immobilized receptor has been considered with respect to the physiological-like situations. Therefore, in the absence of any experimental observations available for the analysis, representative parameter values have been taken from T-cell–MHC peptide binding assuming that the interactions are similar. Even though these are two different systems, however, an analysis with representative parameter values can provide an insight into the process. Moreover, some of the assumptions in previous analysis [10], like ligand concentration being much more than the receptor concentration, are not necessarily valid under physiological conditions. In fact, the receptor concentration may be much more than the ligand

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concentration in case of immobilized systems like the implant we are studying in this paper.

2. Modeling and mathematical analysis

The modeled system has been considered as a dialysis bag in a dialysis chamber. The dialysis bag contains immobilized receptors and free ligand, which can either bind to the receptors or diffuse out of the dialysis bag. The valency of ligand, which corresponds to the maximum number of bonds a ligand can form with receptors, has been taken as four for our analysis. A valency of four has been chosen to demonstrate multivalency, though the trends observed for other valencies of the ligands are similar. The tetravalent ligand means that for effective antigen binding to the APCs, it is desirable to have tetramerically bound receptor–ligand complex. Therefore, we concentrate only on those receptors which have the capability to result in tetravalent binding. Since the receptor distribution is random, we can consider Poisson distribution. The immobilization surface can be divided into spheres each of volume V_s such that the radius of the sphere is described as access radius, r [16]. Access radius can be considered as the distance from the center, which a receptor can traverse and bind to ligand molecule. Based on Poisson distribution, probability of k receptors in a sphere,

$$P(k) = (R_{tot}^{imm} V_s N_a)^k / k! \cdot \exp(-R_{tot}^{imm} V_s N_a) \quad (1)$$

where R_{tot}^{imm} is the total concentration of the immobilized receptor and N_a is Avogadro's number. Since we are concerned about spheres with four or more receptors, the total receptor concentration for tetravalent binding is given as

$$R_{tot}^{tetra} = R_{tot}^{imm}[1-\Sigma((R_{tot}^{imm} V_s N_a)^i / i! \cdot \exp(-R_{tot}^{imm} V_s N_a)] \quad (2)$$

where summation is for $i = 0$, 1, 2 and 3. For the analysis of receptor–ligand binding, we have used the following notation:

L = total ligand concentration in the dialysis bag (mol l^{-1})
L_F = free ligand concentration inside the dialysis bag (mol l^{-1})
α = permeability of the bag material (h^{-1})
L_{out} = ligand concentration outside the bag (mol l^{-1})
L_{bound} = total bound ligand concentration (mol l^{-1})
K_X = cross-linking constant (l mol^{-1})
K_D = dissociation constant (mol l^{-1})
R_0 = free receptor concentration (mol l^{-1})
$L_i = L_i R$ complex with i sites bound (mol l^{-1})
v = valency of ligand (dimensionless)

The receptor–ligand binding may be considered to occur as shown in Fig. 1. Since all the receptors immobilized and present on the polymer matrix see a common ligand concentration equal to the bulk concentration inside the dialysis bag, irrespective of their presence in any particular sphere, we have

$$R_{tot}^{tetra} = R_0\{1 + v[L_F]/K_D \cdot (1 + K_X R_0)^{v-1}\} \quad (3)$$

and

$$L_i = {}^vC_i [L_F][R_0]^i / K_D \cdot K_X^{i-1} \quad (4)$$

These equations have been obtained by considering that rate of receptor–ligand binding is high compared to any other process occurring in the system. Therefore, the binding can be assumed to be at quasi-equilibrium. L_i in Eq. 4 corresponds to the amount of ligand with i sites bound to receptors in spheres with four or more receptors, and R_0 is the concentration of unbound receptor in the same spheres. These relations have previously been obtained for general receptor binding by multivalent ligands and have been applied to the release of histamine from basophils [9]. This model has frequently been used to describe various other situations like receptor clustering induced by incubation of T-cells with MHC peptide oligomers [10], a response which requires receptor cross-linking [11], IgE–Fχ receptor clustering [12], dissociation of insulin and nerve growth factor from their cross-linked receptors [13], viral attachment to cell surface receptors [14], and dimeric MHC peptide complexes binding to CD8$^+$ T-cells [15]. However, we would like to point out that under in vivo conditions, the interactions might be different due to the reasons mentioned earlier. Thus from Eq. 3, we get

$$[L_F] = 1/v \cdot (R_{tot}^{tetra}/R_0 - 1) \cdot K_D / (1 + K_X R_0)^{v-1} \quad (5)$$

From Eq. 4, we can determine the total amount of ligands bound to the receptors present in spheres with at least four receptors, L_{bound}^{tetra}.

$$\begin{aligned}[L_{bound}^{tetra}] = \Sigma L_i &= \Sigma^v C_i (K_X)^{i-1} [L_F]/K_D \cdot [R_0]^i \\ &= [L_F]/(K_D K_X) \cdot \Sigma^v C_i (K_X R_0)^i \\ &= [L_F]/(K_D K_X)[(1 + K_X R_0)^v - 1] \\ &= (1/vK_X) \cdot (R_{tot}^{tetra}/R_0 - 1) \cdot [(1 + K_X R_0) - 1/ \\ &\quad (1 + K_X R_0)^{v-1}] \end{aligned} \quad (6)$$

obtained by substituting for $[L_F]$ using Eq. 5. For dialysis through a dialysis bag, the flux is proportional to the concentration gradient or

$$d[L]/dt = -\alpha \cdot ([L_F] - [L_{out}]) \quad (7)$$

L_{out} can now be obtained by carrying out a mass balance over ligand. Taking the volumes of the dialysis bag (or the microenvironment around the implant) as V_{in} and of the dialysis chamber (or the tissue) as V_{out},

$$V_{in}[L_{tot}] = V_{in}([L_F] + [L_{bound}]) + V_{out}[L_{out}] \quad (8)$$

Since we intend to maximize the tetramerically bound receptor–ligand complex, it is important to select the access radius such that $[R_{tot}^{tetra}]$ is very large compared to the other possibilities of spheres with one, two or three receptors. Therefore, in order to obtain $[R_{tot}^{tetra}]$ equal to at least, say, 90% of $[R_{tot}^{imm}]$, we must choose the access radius suitably using Eq. 2. As a consequence of this design constraint, the amount of ligand bound to the receptors present in spheres with less than four receptors will be very small compared to those bound by the receptors present in spheres with at least four receptors, i.e. $[L_{bound}] \approx [L_{bound}^{tetra}]$. Hence,

$$[L_{out}] = ([L_{tot}] - [L_F] - [L_{bound}^{tetra}]) \cdot (V_{in}/V_{out}) \quad (9)$$

Therefore, using Eqs. 7 and 9, we have

$$\begin{aligned} d[L]/dt = &-\alpha\{1/v \cdot (R_{tot}^{tetra}/R_0 - 1) \cdot K_D/(1 + K_X R_0)^{v-1} - (V_{in}/V_{out}) \cdot \\ &([L_{tot}] - 1/v \cdot (R_{tot}^{tetra}/R_0 - 1) \cdot K_D/(1 + K_X R_0)^{v-1} - 1/(vK_X) \cdot \\ &(R_{tot}^{tetra}/R_0 - 1) \cdot [(1 + K_X R_0) - 1/(1 + K_X R_0)^{v-1}])\} \end{aligned} \quad (10)$$

Since $[L] = [L_F] + [L_{bound}]$, we have that $d[L]/dt = d[L_F]/dt + d[L_{bound}]/dt = (d[L_F]/dR_0 + d[L_{bound}]/dR_0) \cdot dR_0/dt$, or Eq. 10 becomes

$$\begin{aligned} dR_0/dt = &-\alpha\{1/v \cdot (R_{tot}^{tetra}/R_0 - 1) \cdot K_D/(1 + K_X R_0)^{v-1} - (V_{in}/V_{out}) \cdot \\ &([L_{tot}] - 1/v \cdot (R_{tot}^{tetra}/R_0 - 1) \cdot K_D/(1 + K_X R_0)^{v-1} - 1/(vK_X) \cdot \\ &(R_{tot}^{tetra}/R_0 - 1) \cdot [(1 + K_X R_0) - 1/(1 + K_X R_0)^{v-1}])\}/\{K_D/v \cdot \\ &[(-R_{tot}^{tetra}/R_0^2)/(1 + K_X R_0)^{v-1} + (R_{tot}^{tetra}/R_0 - 1)(1-v)K_X/ \\ &(1 + K_X R_0)^v] + (1/vK_X) \cdot [(-R_{tot}^{tetra}/R_0^2) \cdot \{(1 + K_X R_0) - 1/ \\ &(1 + K_X R_0)^{v-1}\} + (R_{tot}^{tetra}/R_0 - 1)\{K_X - (1-v)K_X/(1 + K_X R_0)^v\}]\} \end{aligned} \quad (11)$$

Eq. 11 can be solved numerically to determine R_0 under a specific set of conditions and thereafter concentrations of free and various bound forms, and also of the ligand present outside the dialysis bag, can be calculated. However, for the purpose of the design of our implant, we have analyzed the concentrations of tetravalent receptor–ligand complex, and the ligand present outside the dialysis bag, which corresponds to the antigen that has diffused into the tissue. The initial condition for solving Eq. 11 is that the total ligand concentration in the dialysis bag at $t = 0$ equals the total ligand concentration when the ligand was added, i.e. $([L_F] + [L_{bound}])|_{t=0} = L_{tot}$.

3. Simulations

All the simulations were carried out using Mathematica 4 (Wolfram Research). Simulations were carried out for a fixed

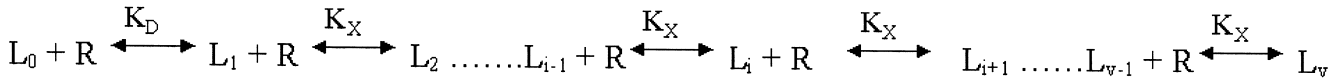

Fig. 1. Kinetic scheme showing various stages in receptor–ligand binding. The ligand used here has a valency v. L_i denotes the receptor–ligand complex with i ligand sites bound to the receptors.

receptor concentration and different ligand concentrations in the system. The time variations in the antigen concentration in the tissue, which corresponds to the ligand outside the dialysis bag, were obtained and analyzed. This was done in the presence and the absence of the implant to understand the constraints on the implant. In addition, the concentration of the immobilized receptor, which exists in tetramerically bound receptor–ligand complex form, was also determined. The amount of this complex may be expected to dictate the T-cell activation by the body itself. The parameter values used for the simulations are as shown in Table 1. The values taken were the typical values available in literature for different T-cell clones [10] and dialysis systems [17]. As mentioned earlier, it has been assumed, for the sake of analysis, that T-cell binding to MHC peptide is similar to the antigen binding to the APC surface receptors. It was assumed that the implant contains 0.1 μM receptor concentration as high concentrations can be achieved by immobilization, and even higher than what has been taken may be achievable. The ligand concentrations were taken to cover a wide range from 1 nM to 1 μM for comparison between the response with and without the implant (Fig. 2). However, for the determination of the tetravalently bound receptor concentration, the ligand concentration was taken as 10 nM (Fig. 3).

4. Results and discussion

The first step towards the designing of the implant involves the determination of the coupler length and as discussed previously, coupler length can be chosen such that the majority of receptors have the ability to form tetravalent binding with the ligand, as desired for the T-cell activation process. For a receptor concentration of 0.1 μM, which has been taken for all simulations, Eq. 2 gives that the coupler length should be at least 300 nm. This access radius is obtained by first determining the V_s such that R_{tot}^{tetra} is $0.9R_{tot}^{imm}$, for a given value of R_{tot}^{imm}. With this coupler length, more than 90% of the receptors are expected to be able to bind tetravalently. The coupler length can be controlled by using different means like streptavidin–biotin–PEG chain used for immobilization of the receptor to the polymer base.

The comparison of diffused ligand concentration for different initial ligand concentrations in the dialysis bag reveals that presence of the implant results in a drastic drop in $[L_{out}]$ even after long intervals of a few hours. As seen in Fig. 2a,b, after 1 h, the presence of implant leads to a diffused ligand concentration nearly an order of magnitude lower than what it is in the absence of the implant. However, when the ligand concentration is extremely high and is about an order of magnitude higher than the immobilized receptor concentration, we do not observe any significant difference between the diffused ligand concentrations in the presence or absence of the implant (Fig. 2c).

The analysis of the tetravalently bound receptor concentration shows that more than 12% of the immobilized receptors exist in the tetravalently bound form (Fig. 3). These bound

Table 1
Parameter values used for the simulations for modeling the APC–antigen binding in the vicinity of a tissue

Parameter	Value
K_X	5×10^{7} M^{-1}
K_D	1.7×10^{-6} M
V_{in}/V_{out}	0.1
v	4
α	1.5 h^{-1}

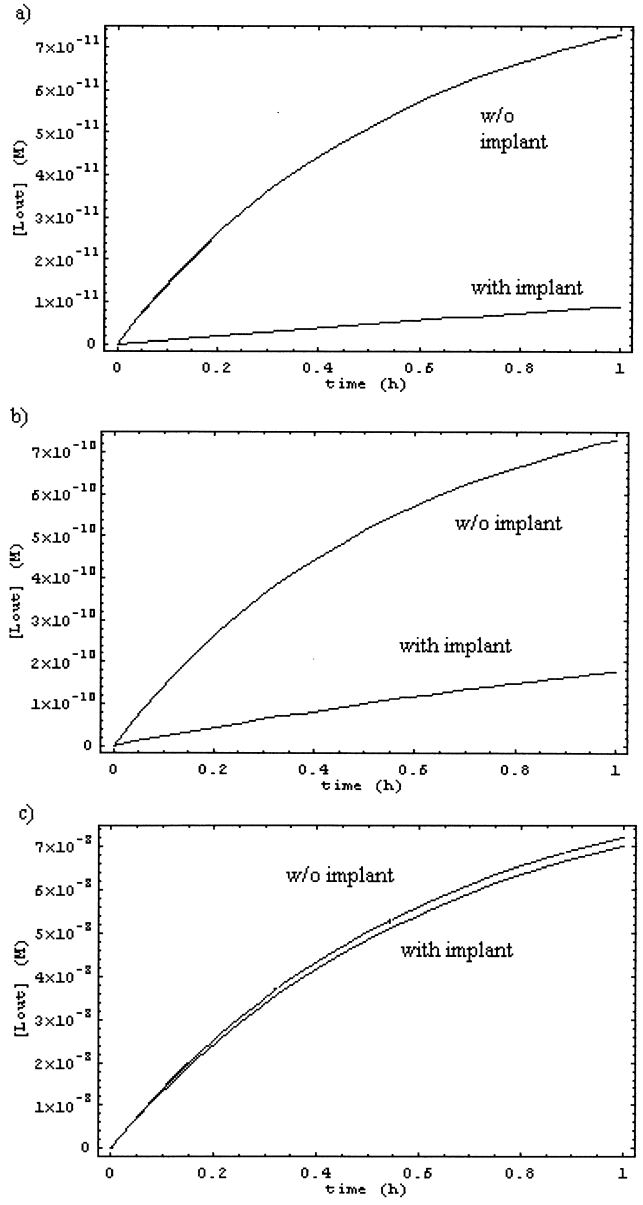

Fig. 2. Comparison of time variation of diffused ligand concentrations outside the dialysis bag in the presence and absence of immobilized receptor (or implant). Different ligand concentrations have been studied: (a) 1 nM, (b) 10 nM, and (c) 1 μM.

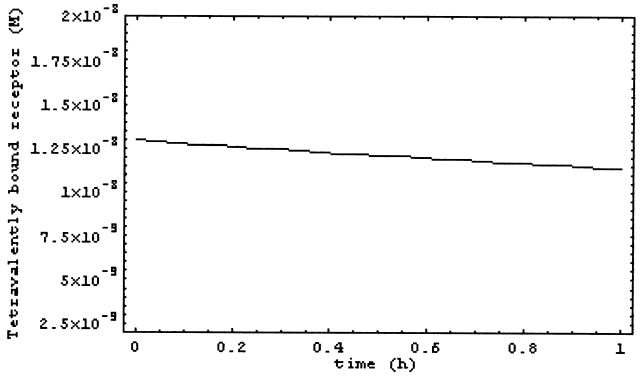

Fig. 3. Variation in concentration of tetramerically bound receptor with time at a ligand concentration of 10 nM and parameter values as shown in Table 1.

receptors play an important role in the T-cell activation in order to destroy the antigen as the APCs are able to tightly bind the antigen and after internalization, they can be suitably presented on the surface. The T-cells forming TCR synapse release cytokines into the bloodstream and thereby result in activation of the immune response. Once the T-cells have been activated, the mediators can activate different components of the immune system like T_H-cells, B-cells and T_C-cells and along with various agents like macrophages, the antigen can be destroyed by the body itself. The activation of the immune response also ensures that the antigen is 'stored in memory' so that if the body encounters the same antigen again, it can be neutralized or destroyed immediately. Even in case of a high ligand concentration, though we do not observe any significant reduction in diffused ligand concentration, the amount of tetravalently bound receptor is as high as 15–20% of the immobilized receptor concentration. Again this results in T-cell activation and the cascade of events comprising immune body's immune response. Based on the fraction of the tetramerically bound receptors, suitable immobilized receptor concentrations as well as the coupler length may be taken to avoid any dose–response-related negative effect, if any (as the amount of antigenic peptide sequence displayed by the APC correlates directly with the tetramerically bound ligand).

Based on these results, we find that the presence of the polymer implant with immobilized APCs can effectively control the spread of antigen and its passage into the tissues. The implant, however, cannot be expected to replace the immune system completely but plays an important role during the initial stages which is the most crucial period for combating an infection. From the analysis of the model and considering the Poisson distribution of receptors on polymer surface, we can design an effective implant with desired properties. In this case, we studied a tetravalent ligand but there are a variety of ligands in nature and using similar analysis, a suitable implant can be designed. Similarly, to mimic the physiological scenario, appropriate APC loading on the polymer implant may also be determined and used. The presence of implant being able to reduce the antigen concentration in the tissue is also a major advantage. This becomes even more important in cases where the antigen concentration keeps increasing. Such situations are very common like bacteria or virus or a toxin produced by a bacteria. In such situations, the APCs reduce the concentration of these antigens immediately and therefore, in spite of growth of these disease-causing agents, the concentration is still maintained low. In addition, before these agents reach a dangerous limit, the body's immune response has already been activated to take the necessary action. It should be noted that the model does not take into account the internalization of the bound antigen. However, internalization of the antigen ensures that the concentration of the free receptors available for binding the antigen is higher than what has been predicted from the analysis. Therefore, the system performance is expected to be better than that obtained from the modeling.

The presence of a high concentration of APCs in immobilized form provides an excellent means to activate the immune response in those subjects who are susceptible to diseases due to failure of or inappropriate antigen presentation by the APCs. These implants can also be used in people who are already suffering from some disease due to the same reasons. An important point, which is brought to light from this analysis, is that these implants are highly effective when the antigen concentration is very low, i.e. either such implants can be used as vaccination or can be used for the treatment when the infection is at early stages of its development. But by suitable modifications in the system, this problem can also be tackled easily. In order to make the implant highly effective even at a high antigen concentration, we can either increase the immobilization density or increase the value of K_X or decrease the equilibrium dissociation constant, K_D. These changes will significantly improve the efficacy of the designed implant for a wide range of antigen concentrations.

5. Summary

In this modeling analysis, we have modeled the artificial antigen presentation as a dialysis system containing an immobilized receptor system and free ligand in the dialysis bag. The change in ligand concentration diffusing out of the dialysis bag has been determined for various ligand concentrations for a fixed receptor concentration. The diffused ligand corresponds to the antigen that may diffuse into a tissue and the smaller its concentration, the better it is. From the analysis, we find that for ligand concentrations which are lower than the receptor concentration, the diffused ligand concentration is nearly an order of magnitude lower than what it may be in the absence of any implant. In addition, the amount of tetramerically bound receptor is significant. This tightly bound ligand can be easily internalized by the APCs and thus presented on their surface as MHC peptide. A significant amount of these MHC peptides means an effective T-cell activation. However, at a very high antigen concentration, we observe that the implant with specific features may not be suitable. However, it may be modified by changing either the receptor density or by increasing binding affinity to the ligand. The analysis considers that the random distribution of receptors on a polymer base can be described by Poisson distribution and using this, the implant with desired properties can be designed. This kind of implant may be used for vaccination purposes as well as treatment of various diseases.

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