Langerhans Cell Dynamics During Epicutaneous Immunotherapy Using Agent-Based Modeling

By: Andrew Hinton

Executive Summary

It is estimated that up to 15 million Americans have a potentially deadly food allergy. There are approximately 200,000 emergency room visits and nearly 10,000 hospitalizations each year from food allergies. In children under the age 18, food allergies have increased 18% from 1997 to 2007. When factors such as clinician visits, hospitalizations, and special foods are taken into account, it is estimated that the annual economic cost of food allergies is an astounding $24.8 billion.

Currently, there is no cure for food allergies and the only approved way to prevent possible life-threatening anaphylactic reactions are strict avoidance of the offending foods. Recently, epicutaneous immunotherapy (EPIT) clinical trials have shown great promise as a possible cure for food allergies. While the exact cellular mechanisms governing tolerance of a food allergen as a result of EPIT are unknown, this therapy has had success in desensitizing some patients. A key component of EPIT is the activation of Langerhans cells(LC) where their propensity to regulate tolerance in lieu of immunity to food allergens is hypothesized to greatly impact EPIT outcomes.

The motivation of this project is to identify and understand key model parameters that can be defined as measurable patient attributes. These patient attributes can then be used to initialize model optimization routines thereby maximizing patient-specific therapeutic success rates. The insight uncovered as part of this study is a direct result of simulated cellular interactions that are based on the most current research related to the theoretical behaviors of key immune cells.

The application of Agent-based modeling to immunology is well researched and has been successfully used to model the immune response to various immunological phenomena. In the case of the immune response to EPIT, cellular interactions can be programmed based on theoretical behaviors and occur in a spatially dependent manner. As such, a first of its kind 3-D agent-based model was developed in Net Logo to model, simulate, and analyze the complex behaviors of key immune cells during EPIT. This model was primarily focused on two response variables as functions of the 7 model input parameters: number of activated LC and Free Allergen. The model environment was scaled to 1/512 the size of a common skin patch used for the transdermal delivery of allergen.

The verified model was analyzed using statistical methods to understand the main and interaction effects of each of the seven input parameter on the key response variables after a dose of allergen. The analysis revealed the activation thresholds of IL-1β and TNF-α combined with the epidermis diffusion coefficient had the most significant impact on the number of LC activated. Free Allergen, the allergen not ingested by LC, was strongly influenced by the epidermis diffusion coefficient. Minimization of free allergen could be achieved by lower diffusion coefficients which are directly related to allergen delivery and the skin barrier.

With the exact mechanisms governing allergen tolerance during EPIT unidentified, this model demonstrates how agent-based modeling can be used to explicate the mysteries surrounding immunotolerance relative to EPIT. Future work will be focused on relating key model parameters to patient specific attributes in an effort to optimize the efficacy of EPIT. Although clinical trials are effective ways to test new therapies, computer simulation of this can be used by doctors and researchers to safely test countless EPIT dosing protocol tailored to each patient. Further research and development of this model and models of this kind could prove instrumental in curing potentially fatal allergies.

Introduction

With an estimated 15 million people suffering from food allergies, this seemingly asymptomatic condition can easily lead to anaphylaxis which, in severe cases, can result in death (Facts and Statistics - Food Allergy Research & Education. 2015). Currently, there is no cure for food allergies and meticulous avoidance is the only treatment. Astonishingly, EPIT was shown to be successful in 1921 by Vallery-Radot where allergen was placed onto scarified skin resulting in decreased allergy symptoms in patients allergic to horses (Senti, von Moos, & Kündig, 2014). EPIT is administered with a patch placed over the skin and serves as a safe and painless alternative to subcutaneous immunotherapy (Senti et al.). The epidermis, the outermost layer of skin, is uniquely suited for allergen administration because of its vast population of potent antigen presenting LC and its lack of vascularization (Senti, von Moos, & Kündig, 2011). Since the epidermis is made up of nonvascularized tissue, the risk of allergen cross-linking with IgE-coated mast cells, basophils and eosinophils are minimal thus reducing the risk of severe allergic reactions. As shown in (Senti, von Moos, & Kündig, 2011) EPIT has an excellent safety record.

Overview of Model Component

Langerhans Cells

Langerhans cells are a type of professional antigen presenting dendritic cell that is found in the epidermis (Romani, Clausen, & Stoitzner, 2010) LC alert the immune system of invading antigen by sampling, processing, and presenting substantial amounts of antigen to T-Cells. After antigen has been processed, LC mature and migrate from the epidermis to regional lymph nodes via the afferent lymphatics. In (Shklovskaya et al., 2011), direct evidence was found that suggest Langerhans are committed to inducing peripheral tolerance. Peripheral tolerance is characterized by T-cell anergy and activation-induced cell death. The aim of EPIT is to induce tolerance by targeting the LC in the epidermis which presents antigen to T-cell in a way that does not allow or encourage an immune response.

Peanut Allergen

Peanuts, *Arachis hypogaea*, are groundnuts that belong to the Leguminosae family (Sharma & Bhatnagar, 2006). Peanut allergen belongs to a specific group of protein superfamilies' that includes cupin, prolamin, and the plant defense proteins (Zhuang & Dreskin, 2013). To date, there are 17 allergens associated with peanuts that are documented in the Allergen Nomenclature database that is managed by the World Health Organization (WHO). Of the known peanut allergens, 35-95% of patients allergic to peanuts are sensitized to Ara h 1 and 95% are sensitized to Ara h 2 (Yang Zhou et al., 2013). Ara h 1, a glycoprotein, is used in this model to represent the peanut extract used in the simulation.

Skin

The major layers of the skin are the epidermis, dermis, and hypodermis where the epidermis represents the outermost layer of skin and the hypodermis makes up the innermost. This model focuses on the epidermal and dermal layers of skin. The epidermis is further broken down into the Stratum Corneum, Stratum Lucidum, Stratum Granulosum, Stratum Spinosum, and the Stratum Basale. The stratum corneum, the apical layer, is commonly thought to acts as a barrier that prevents the entry of environmental antigens through the skin, however, the skin is a permeable and antigen and allergen can penetrate (Berard, Marty, Nicolas, 2003).

Cytokines

Cytokines or cell signaling molecules are used in this model to create the dynamic cytokine environment found during EPIT that leads to LC activation and migration. IL-1β is a multifunctional cytokine that is expressed by keratinocytes (KC) and LC in the epidermis and functions to initiate the immune response (Uchi, Terao, Koga, & Furue, 2000). TNF-α is produced by KC and LC in the epidermis and functions to induce adhesion molecules that encourage neutrophils and lymphocytes to migrate to the skin (Uchi, Terao, Koga, & Furue, 2000). Based on (Villablanca & Mora, 2008), LC need the help of both IL-1β and TNF-α in order to cross the epidermal basement membrane during migration to regional lymph nodes.

Model Design

The 3-D environment in (Figure 1) was modeled with the dimensions [40 x 40 x 90] (XYZ) patches in NetLogo. The brown layer represents the stratum corneum, the pink layer represents remaining epidermis and the layer below (not colored) represents the dermis. In the model, lymph vessels are represented as blue cylinders and are located lower in the dermis. A single model patch represents 20 μm in length, width and height equating to a volume of 8000 μm^3. The scaled dimensions of the virtual environment are [800 x 800 x 1800] μm. The Stratum Corneum was modeled 20 μm or 1 patch thick and the remainder of the epidermis was modeled 60 μm thick. The dermis was sized at 75 patches or 1500 μm / 1.5mm. The Langerhans Cells in this model are located in the stratum spinosum and was modeled using the reference density of 18,217 – 27,955 $\frac{LC}{mm^3}$ from (Bauer et al., 2001). When this density was scaled for the stratum spinosum model environment:

$$\text{Resulting Range} = \left(\frac{(0.06*0.8*0.8)mm^3}{1000\ mm^3} * \text{Reference Density}\right) = 700 - 1073\ \frac{LC}{\mu m^3}, \quad (1)$$

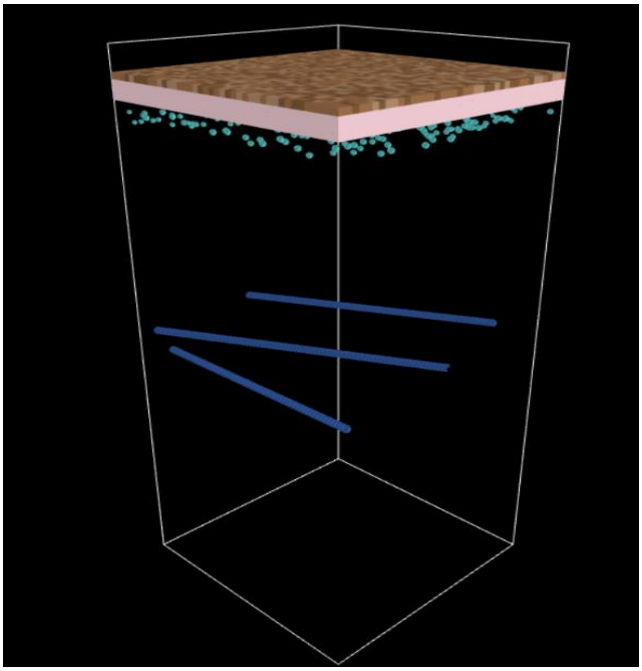

Figure 1. Simplified 3D Environment of Integument.

DBV technologies currently manufacture the VIASKIN Peanut Patch U.S. which is commonly used during EPIT. A review of the VIASKIN peanut patch patent 7,635,488 B2 revealed the dimensions of the patch is similar to the FINN chamber patch test device. Evaluation of the specifications for the FINN chamber revealed the patch has a contact surface of 50 mm^2. The surface area of the Stratum Corneum of this model is 0.64 mm^2. The modeled dose is based on 0.0004 μm dose of Ara h 1 protein which is $\cong \frac{1}{512}$ of the equivalent dose expected on a FINN chamber patch. The model assumes the protein is distributed evenly across the skin. The number of protein molecules is estimated in (Table 1). Protein molecules diffuse from the patch above to the patch below where flux is based on the allergen concentration gradient. For simplicity, lateral diffusion was not considered in this work but will be considered in future work. Penetration through the epidermal basement membrane by peanut protein is

also not permitted in this model. The cytokines expressed by the KC (not modeled) were however allowed to diffuse in all direction.

Table 1. Distribution and Estimation of Number of Protein Molecules per Simulated Dose

Protein	MW (kDa)	Dose (µg)	Protein Per NetLogo Patch	Estimated Total Proteins
Ara h 1	64	0.0004	1897	3.03×10^6

Model Parameters and Agent Behaviors

The model parameters used in this simulation controls diffusion coefficients, antigen uptake, dose intervals and activation thresholds of LC. The parameters, their value ranges, and description are listed in (Table 2).

Table 2. Model Parameters

Parameter	Value Range	Description
EpidermisDiffusionCoefficient(EDC)	0 - 1	Controls the percentage of Protein diffusing through the epidermis
LC_AntigenUptake	0 to 1	Controls the percentage of antigen taken up by each LC at each Time Step
IL1BDiffusionRate	0 to 1	Controls the percentage of IL1B that can diffuse at each time step
TNFaDiffusionRate	0 to 1	Controls the percentage of TNF-a that ca diffuse at each time step
LC_IL_ActivationThreshold	0 to 10000	Sets the number of IL1B protein received by the Langerhans Cell
LC_TNFa_ActivationThreshold	0 to 1000	Sets the number of TNF-a protein received by the Langerhans Cell
LangerhansCellPopulationThreshold	0 to 1073	Regeneration rate for LC ensures LC maintain a steady state amount after others have migrated
DoseInterval_Days (DI)	0 to 60	Control the amount of days between doses.

Each tick of the NetLogo model represents an hour and so the movement and diffusion rate of LC were adjusted to ensure movement and distances traveled closely replicate actual cellular motility. The feedback system shown in (Figure 2) depicts the positive feedback loop required for activation of LC via cell-specific cytokines. The reinforcing cytokine loop follows the progression listed below:

1) Allergen comes in contact with Keratinocyte.
2) Keratinocytes secrete IL-1β
3) Langerhans secrete IL-1β that interacts with KC
4) KC then secrete TNF-α to LC where this process repeats as long as there is allergen present in the epidermis. When the two signals have been received the activation and migration of LC from epidermis may occur.

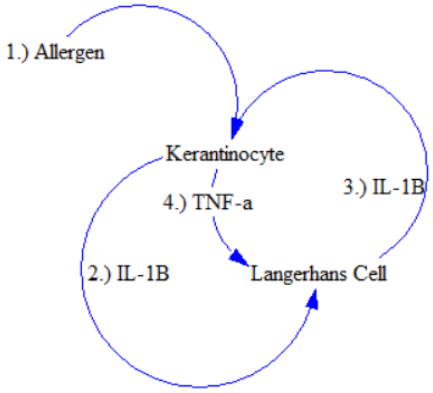

Figure 2. Reinforcing Cytokine Loop.

LC in this model process antigen, receive signals from IL-1β, TNF-α and activate based on the aggregation of cytokine signals and migrate to the nearest lymph vessel upon activation. Patch Variables act as keratinocytes and secrete cytokines if allergen is detected on the patch. Allergen is uniformly distributed throughout the patch variables that make up the stratum corneum. Once the dose interval has been detected the stratum corneum has allergen applied to the patches and on subsequent ticks the allergen diffuses toward the dermis.

Model Verification and Validation

Model Verification

Model verification was done by verifying that the output of the response variables equaled the expected values for the corresponding minimum and maximum values of the input parameters. Each verification test was done independently due to the complex interaction between input parameters on response variable output. Minimum, maximum, and expected values for model verification are shown in Table 3.

Table 3. Model Verification Expected Values

			Expected Value	
	Min	Max	Activated LC(min)	Activated LC(max)
IL1BDiffusionRate	0	1	0	>0
DoseInterval_Days	0	12	0	increase corresponding to dose interval
LC_TNFa_ActivationThreshold	0.1	4500	>0	0
LC_AntigenUptake	0	1	0	>0
EpidermisDiffusionCoefficient	0	1	0	>0
LC_IL_ActivationThreshold	0.1	4500	>0	>0
TNFaDiffusionRate	0	1	0	>0

Model Validation

The model was designed to generate insight based on known theoretical behaviors where little experimental data exist for validation purpose. Face validity was used to validate this model given its strong dependence on theoretical behaviors. The insight gained as part of this model can be used to generate novel experimental hypotheses of actual cellular dynamics. Experimental hypotheses generated as part of this model will provide the basis of future experiments that will generate data in an iterative process that can be used to refine and validate the model until the model represents the actual system.

Results

The goal of this model was to expound LC dynamics during EPIT. As such, one variable of interest is the number of activated LC and their subsequent migration to regional lymph nodes during EPIT. To better understand how the model parameters influence the number of activated LC, a two level 2^{7-} full factorial experiment was used to screen the 7 model input parameters. The parameters of the experiment are shown in table 4.

Table 4. Full Factorial Experiment Parameters

Response Variable: Activated LC Total LC Activated (3 Reps) n=384

Factor		Level
Factor 1	IL1BDiffusionRate	[0.1, 1]
Factor 2	DoseInterval_Days	[1, 10]
Factor 3	LC_TNFa_ActivationThreshold	[1000, 5500]
Factor 4	LC_AntigenUptake	[0.1, 1]
Factor 5	EpidermisDiffusionCoefficient	[0.1, 1]
Factor 6	LC_IL_ActivationThreshold	[1000, 5500]
Factor 7	TNFaDiffusionRate	[0.1, 1]

Based on an analysis of the response data shown in (Figures 3 and 4) from the full factorial experiment, the EpidermisDiffusionCoefficient, LC_TNFa_ActivationThreshold, and the LC_IL_ActivationThreshold were identified as the most significant key model parameters.

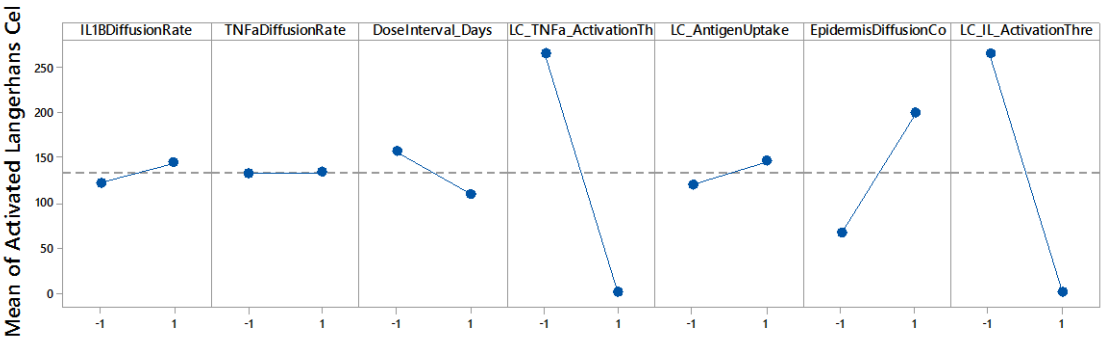

Figure 3. Main Effects Plot of Activated LC.

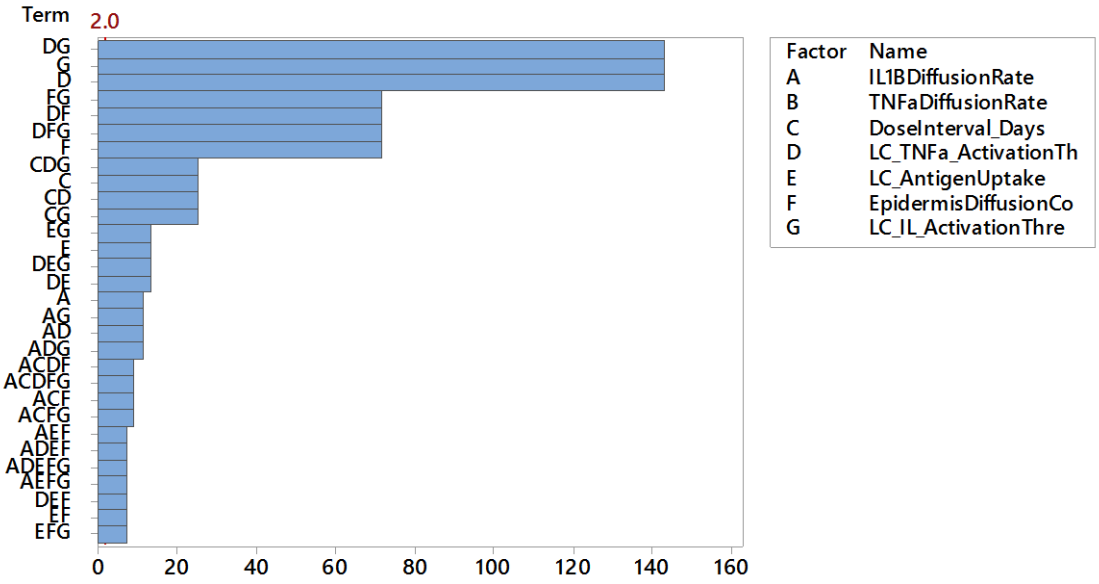

Figure 4. Pareto Chart of the Standardized Effects

With the number of factors reduced from 7 to 3, a central composite inscribed response surface design (Table 5) was used to gain further insight into how these model parameters affected the number of activated LC. The 5 level 20 run response surface design was analyzed with stepwise response surface regression where the resulting model (Table 6) narrowed down the significant parameters to LC_TNFa_ActivationThreshold and the LC_IL_ActivationThreshold.

Table 5. Central Composite Design

Factors:	3	Replicates:	1
Base runs:	20	Total runs:	20
Base blocks:	1	Total blocks:	1

Two-level factorial: Full factorial

Cube points:	8
Center points in cube:	6
Axial points:	6
Center points in axial:	0

α: 1.68179

Table 6. ANOVA Table

Source	DF	Adj SS	Adj MS	P-Value
Model	4	340816	85204	0.000
Linear	2	211452	105726	0.000
LC_TNFa_ActivationThreshold	1	51873	51873	0.001
LC_IL_ActivationThreshold	1	159579	159579	0.000
Square	1	69506	69506	0.000
LC_IL_ActivationThreshold*LC_IL_ActivationThreshold	1	69506	69506	0.000
2-Way Interaction	1	59858	59858	0.001
LC_TNFa_ActivationThreshold*LC_IL_ActivationThreshold	1	59858	59858	0.001

Finally, a full factorial review of the contour plot (Figure 5) and the response surface (Figure 6) revealed minimum values of the parameters were associated with higher levels of activated LC.

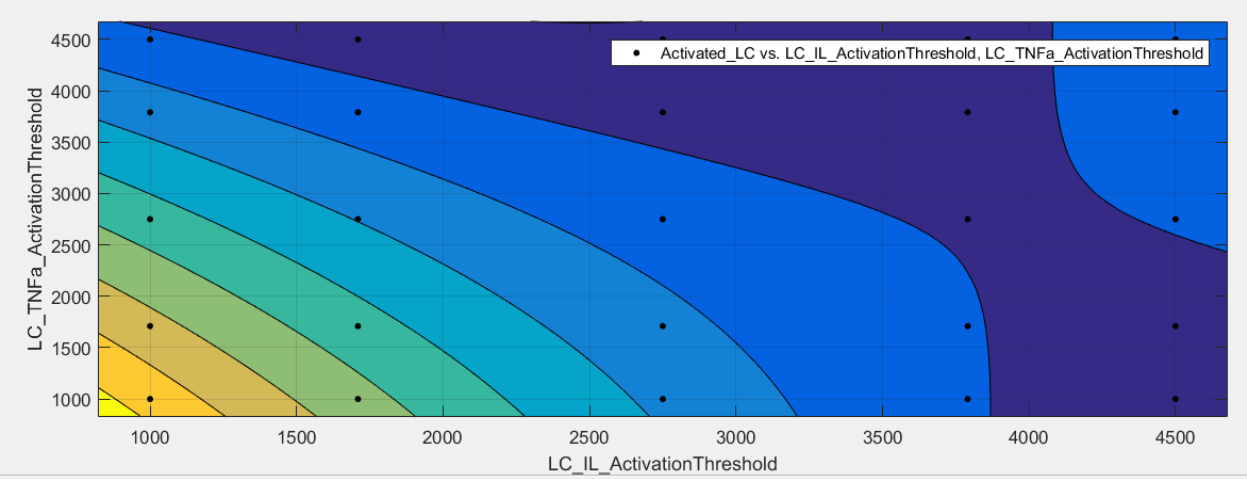

Figure 5. Contour Plot of Activated LC

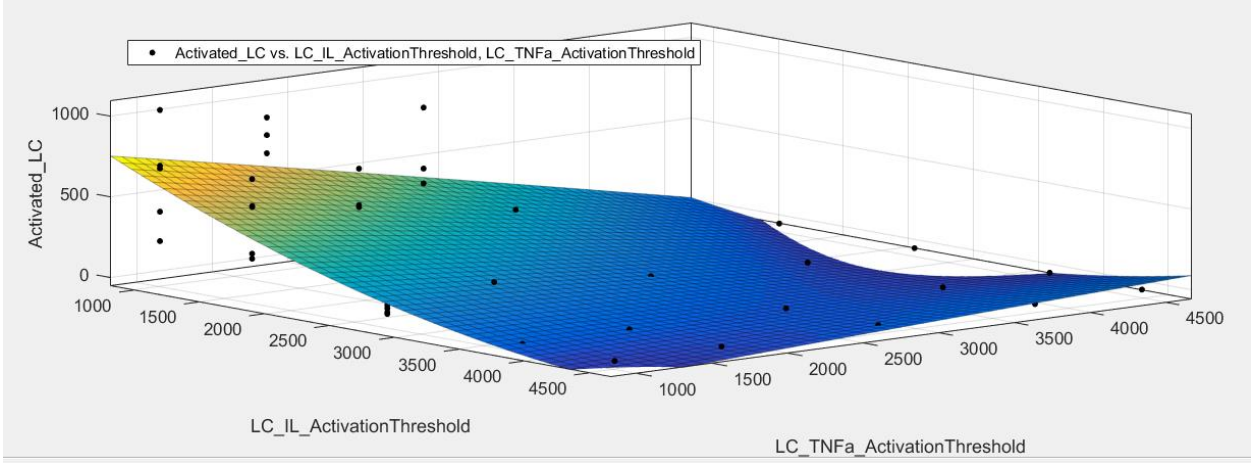

Figure 6. Response Surface Activated LC

A similar analysis of the model parameter that significantly affect the free Allergen levels following EPIT revealed the EpidermisDiffusionCoefficient as a significant contributor to the Free Allergen Levels. The relationship between the EpidermisDiffusionCoefficient and free allergen (Figure 7) suggest higher

permeability leads to exponentially higher levels of Allergen not processed by LC in the system.

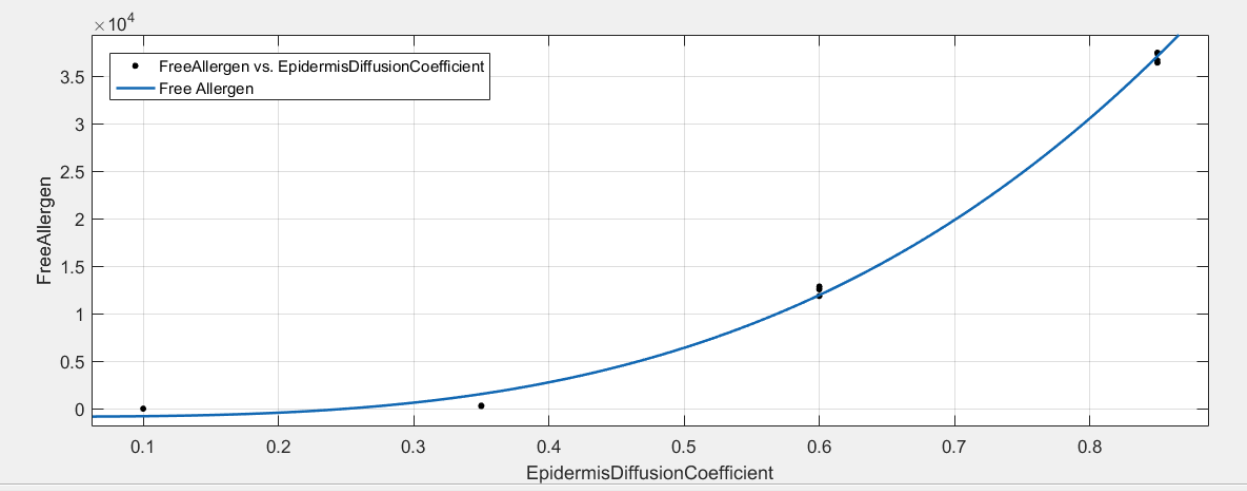

Figure 7. Free Allergen Response to the EpidermisDiffusionCoefficient Model Parameter

Conclusion

This model demonstrates the versatility of agent-based modeling in the immunology field. With customized treatment plans, patient treatment can be tailored to their current immune state and optimized to ensure the best result. The results of this model give rise to a patient specific factors of EPIT that could be further investigated in order to improve the efficacy of EPIT. Future work includes the refinement of model parameters in order to represent measurable quantities, possible laboratory validation of theses parameters and ultimately leading to validation of the model using clinical data with refined parameters. Additionally, future work will also include the addition of skin resident T regulatory cells, the potential activation of dermal dendritic cells and a heterogeneous peanut protein profile.

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