



When is allergen immunotherapy effective?

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ABSTRACT

Allergen immunotherapy is used to treat allergic symptoms such as rhinitis and itchy eyes in Japanese patients with cedar pollen allergy (JCPA). Administration of small amounts of pollen over several years may suppress severe allergic symptoms when these patients are later exposed to large amounts of pollen in the environment. Herein, we developed a simple mathematical model to identify conditions in which allergen immunotherapy is effective. We considered the dynamics of type 2 T helper cells (Th2) and regulatory T cells (Treg), both of which differentiate from naive T cells. Therapy was considered successful under the following three conditions: (1) Without therapy patients develop allergic symptoms upon exposure to environmental pollen, (2) with therapy patients do not develop symptoms upon exposure, and (3) patients do not develop allergic symptoms to the therapy itself. We defined scores for therapeutic success and identified ranges of parameters in which allergen immunotherapy is likely to be successful. Treg cells have a longer lifespan than Th2 cells, allowing accumulation over many years. In accordance, therapy with linear dose increases (rather than constant doses) reduced the risk of allergies to the therapy itself, and led to stronger accumulation of resistance to pollen exposure.

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1. Introduction

Allergies are undesirable reactions that are caused by hypersensitivity to harmless materials, such as wheat, mites, and tree pollen. From February to March, many people in Japan suffer from inflammation of the eyes and nose due to Japanese cedar pollen allergies. Runny noses and itchy eyes cause physical and mental suffering and degrade quality of life (Shiomori et al., 2007). To control allergic symptoms, otolaryngologists prescribe anti-allergic drugs according to the severity of symptoms (Yamada et al., 2014). However, anti-allergic drugs do not provide a permanent cure, and patients must visit the doctor every year.

Recently, allergen immunotherapy was introduced for the treatment of Japanese cedar pollen allergy (JCPA). During therapy, patients take an allergen (pollen extract) in multiple small dose for months, and subsequent allergic reactions to pollen-rich air are suppressed. This therapeutic approach was first documented in 1911 in a study of immunotherapy for allergy to gramineous plants using subcutaneous injections (Noon, 1911; Freeman, 1911), and is now known as subcutaneous immunotherapy (SCIT). More recently, sublingual immunotherapy (SLIT) was developed to improve safety. In this approach, patients take drops of pollen extract via the sublingual route (Moingeon and Mascarelli, 2012). The effects of SLIT for JCPA were subsequently investigated and the therapy

was shown to be effective for reducing symptoms (Okubo and Go-toh, 2009). Moreover, Pfaar et al. (2015) recently discussed mechanisms of allergen immunotherapy, and proposed corresponding therapeutic guidelines.

Allergy has been described in terms of the following chain of events: Firstly, antigen-presenting cells (APCs) receive antigens such as pollen and process these into peptides. APCs then present peptides to naive T cells (Th0 cells), which then differentiate into type 2 T helper cells (Th2 cells) in the presence of interleukin-4 (IL-4). Th2 cells then activate B cells by producing IL-4 and activated B cells produce IgE antibodies that bind receptors on the surfaces of mast cells and are cross-linked with each other via the antigen. Subsequently, mast cells degranulate and release molecules such as histamine, which induce inflammation and allergic symptoms.

Regulatory T cells (Treg cells) suppress exaggerated allergic responses (Sakaguchi et al., 2008). An experimental study suggested that after grass pollen immunotherapy, the number of Treg cells increased and play an important role in suppressing recruitment and activation of Th2 cells (Radulovic et al., 2008). Immunotherapy using cow milk protein as allergen also induces Treg cells in mice (Smaldini et al., 2015). Moreover, observations of natural immunotherapy indicate that exposure to farm environments may prevent allergy and induction of Treg cells (Luis et al., 2014), and maternal exposures have also been shown to be effective (Schaub et al., 2009). These examples have been explained using the hygiene hypothesis, which suggests that microbiome exposures

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prevent allergic symptoms and asthma, and has been investigated as a microbiome-based therapeutic approach (Liu, 2015).

Mechanisms of immune suppression by natural and allergen immunotherapy remain poorly understood, and several alternative theories have been proposed (Vignali et al., 2008). For example, Taylor et al. (2006) suggested that Treg cell cytokines such as IL-10, TGF- β , and various surface molecules play multiple roles in the suppression of immune responses. In addition, Wing et al. (2008) suggested that Treg cells suppress immune responses by preventing APCs from activating Th0 cells.

We considered two types of T cells: induced regulatory T cells (iTreg cells) and naturally occurring regulatory T cells (nTreg cells). Similar to Th2 cells, iTreg cells differentiate from Th0 cells in peripheral tissues. In contrast, nTreg cells are produced in the thymus via a different route. Accumulation of iTreg cells has been demonstrated in peripheral tissues, and corresponds with regulatory memories of localized areas of the human body (Burzyn et al., 2013).

Fishman and Segel (1996) developed the first mathematical model of allergen immunotherapy and emphasized the roles of cross regulation between Th1 and Th2 cells. The balance of Th1 and Th2 cells was also considered in models of melanoma therapy (Kogan et al., 2013), and for asthma development depending on quantities of air dust particles (Kim et al., 2013). Moreover, Gross et al. (2011) developed a mathematical model for allergen immunotherapy, and included Th1/Th2 balance and the effects of Treg cells. In this study, allergen immunotherapy was administered in two phases. In the first phase, patients began by taking small amounts of pollen and gradually increased doses to a certain point, and in the latter phase patients took constant doses of pollen (maintenance phase). These investigators concluded that the maintenance phase should be started as early as possible to facilitate growth of Treg cell populations and suppress Th2 cells.

In the present study, we investigated a simple dynamic model of Th2 and Treg cells. We adopted the number of Th2 cells as an index for the severity of allergic symptoms and expressed the dynamics of Th2 and Treg cell numbers using a system of ordinary differential equations. Subsequently, we defined explicit scores for successful therapy, and identified conditions in which therapy is likely to be successful. We conclude that, for this therapy to be effective, the longevity of Treg cells must be greater than that of Th2 cells so that Treg cells can accumulate and contribute to suppression of reactions to environmental pollen exposures. We also compared the efficacies of various treatment schedules.

2. Model

To examine suppressive effects of regulatory T cells in allergen immunotherapy, we used the model illustrated in Fig. 1. This model describes the stage in which naive T cells are differentiated into Th2s and Tregs. Following presentation of antigens such as pollen extracts to naive T cells (Th0) by antigen-presenting cells (APC), Th0 cells differentiate into regulatory T cells (Treg) or helper T cells (Th2) depending on the chemical environment (Luckheeram et al., 2012). Because Th2 cells trigger allergic reactions (Luckheeram et al., 2012), we assume that the magnitude of allergic symptoms is represented by Th2 cell numbers. We assumed that patients show allergic symptoms when the value of Th2 cell numbers (H) exceeds θ , which is the threshold for revealing symptoms. In contrast with Th2 cells, Treg cells suppress allergic responses by preventing activation of naive T cells and differentiation into Th2 cells (Wing et al., 2008).

We considered a therapy phase of two or three years, in which patients take drops of pollen extract, and a subsequent exposure phase, in which patients are exposed to large doses of pollen over

several months. For simplicity, we did not consider exposure to environmental pollen during the therapy phase.

We denoted Treg cell numbers as R and Th2 cell numbers as H , and calculated dynamics using the following differential equations:

$$\frac{dR}{dt} = b \cdot P(t) \cdot (1 - c) - d_r \cdot R, \quad (1a)$$

$$\frac{dH}{dt} = \frac{b \cdot P(t) \cdot c}{1 + m(R + n)} - d_h \cdot H. \quad (1b)$$

The pollen dose $P(t)$ was calculated as follows:

$$P(t) = \begin{cases} a & \text{for } t < T \text{ (during therapy phase)} \\ A & \text{for } t > T \text{ (during exposure phase)} \end{cases} \quad (2)$$

During a therapy phase of duration T , patients take small amounts of pollen every day, and the intensity of pollen dose is represented by a in Eq. (2). Subsequently, patients are exposed to large doses of environmental pollen (exposure phase) and the pollen doses in this phase are denoted by A , as in Eq. (2).

In Eqs. (1a) and (1b), the first term of the right-hand side represents the supply of Treg or Th2 cells by differentiation from naive T cells. Pollen grains are given to the patient at rate $P(t)$, and stimulate mature T cell production at a rate that is proportional to the amount of pollen received $P(t)$, with proportionality coefficient b . Among newly differentiated T cells that are reactive to pollen antigens, fraction c ($0 < c < 1$) differentiate into helper T cells, and the remaining fraction $1 - c$ differentiate into regulatory T cells. In addition, Treg cells suppress differentiation into Th2 cells, as indicated by the denominator of the first term in the right-hand side of Eq. (2). The coefficient m is the strength of this effect, and we assume that nTreg cells are produced separately regardless of naive T cell differentiation, and their abundance is denoted by n .

The second term of Eq. (1) represents the decay of T cells. Specifically, d_r and d_h denote decay rates of Treg and Th2 cells, respectively. We assume that “memory Treg cells”, which live long life in local nonlymphoid tissue (Burzyn et al., 2013), have the role of suppressing immunoreaction. Therefore, we set the decay rates to satisfy $d_r < d_h$, allowing relative accumulation of Treg cells in the local tissue.

Eqs. (1) and (2) have the following ten parameters θ , a , A , b , c , d_h , d_r , m , n , and T , and each of these parameters influence the success of therapy. Accordingly, we examined the model using simulations with 3^{10} combinations of parameters in which each parameter takes three different levels (Table 1). For each set of parameters, we considered conditions with therapy and without therapy. In Fig. 2, we illustrate four cases that differed in the presence of symptoms, and in therapy and exposure phases. Fig. 2A shows a case of successful therapy and Fig. 2B shows a case in which the therapy failed to suppress symptoms of pollen exposure. In Fig. 2C, the patient showed no symptoms without therapy, and in Fig. 2D the patient experienced allergic symptoms during the therapy phase.

2.1. Therapeutic success scores

To determine conditions in which allergen immunotherapy is effective, we evaluated the influences of various parameters on therapeutic successes and defined corresponding scores that can be used to identify patients in whom therapy would be effective. Therapy was regarded as successful when (1) without therapy the patient developed allergic symptoms upon exposure to the environmental pollen, (2) with therapy the patient did not develop symptoms upon exposure, and (3) the patient did not develop allergic symptoms due to the therapy itself. Scores were then calculated as the sum of the three components, and are expressed in

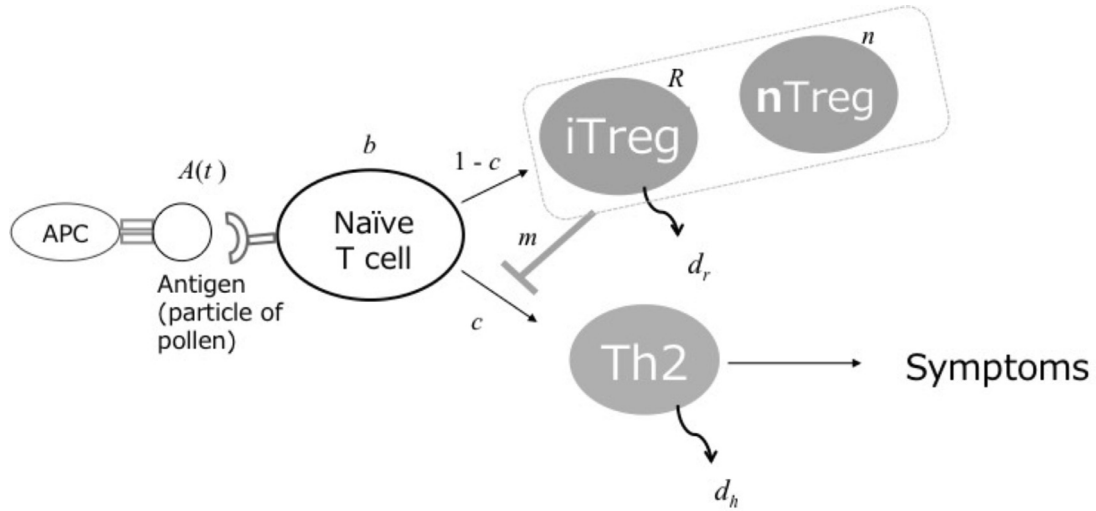


Fig. 1. Scheme of the model. An antigen-presenting cell presents $P(t)$ pollen particles to naïve T cells (Th0), which subsequently produce b differentiated T cells. Fraction c of Th0 cells differentiate into type-2 T helper cells (Th2 cells), and fraction $1 - c$ of these differentiate into regulatory T cells (Treg cells). Th2 cells and Treg cells decay at rates of d_h and d_r , respectively; Numbers of naturally occurring regulatory T cells (nTregs) are indicated by n .

Table 1

Values of the parameters. Three values are shown for each parameter, resulting in 3^{10} parameter combinations. We calculated $H(t)$ and $R(t)$ for each set of 3^{10} parameters and present units for parameters in which PU is the unit amount of pollen and CU is the unit number of cells.

	Symbol	Low	Intermediate	High	Unit
Threshold of having symptoms	θ	10	50	90	CU
Intensity of pollen dose as therapy	a	0.1	0.5	0.9	PU/day
Intensity of pollen exposure	A	10	50	90	PU/day
Numbers of differentiating naïve T cells per unit dose of pollen	b	10	100	1000	CU/PU
Rate of differentiation into Th2 cells	c	0.5	0.7	0.9	1
Decay rates of Th2 cells	d_h	0.5	1.0	1.5	1/day
Decay rate of Treg cells	d_r	0.001	0.01	0.1	1/day
Suppression effect of Treg cells	m	0.001	0.01	0.1	1/CU
Numbers of nTreg cells	n	2	20	200	CU
Length of therapy phase	T	100	500	900	day

terms of three peaks of $H(t)$ curves. Let $H_0(t)$ be the number of Th2 cells without therapy and $H_w(t)$ be the number of Th2 cells with therapy.

For example, $\max_{t>T} H_0(t)$ is the peak $H(t)$ without therapy. If this is larger than the threshold θ , the difference $(\max_{t>T} H_0(t) - \theta)$ indicates the severity of allergic symptoms that the patient experiences without therapy and $H(t)$ peaks that are smaller than θ indicate absence of detectable symptoms. Therefore, $(\max_{t>T} H_0(t) - \theta)$ should be positive for patients who have allergic symptoms.

The expression $\max_{t<T} H_w(t)$ is the $H(t)$ peak in the therapy and the expression $\max_{t>T} H_w(t)$ is the peak pollen exposure after the therapy. Therapy was regarded as failed if pollen administration itself caused allergic symptoms ($\max_{t<T} H_w(t) > \theta$) and if allergic symptoms to environmental pollen were not suppressed by the therapy ($\max_{t>T} H_w(t) > \theta$). For successful therapy, $\max_{t<T} H_w(t) < \theta$ and $\max_{t>T} H_w(t) < \theta$, and the differences between sides in these inequalities were regarded as margins of safety during or after therapy.

We introduced symbols for differences between peaks of $H(t)$ and the threshold θ , as follows:

$$S_0 \text{ (relative peak height without Therapy)} = \max_{t>T} H_0(t) - \theta, \quad (3a)$$

$$S_T \text{ (relative peak height in Therapy)} = \max_{t<T} H_w(t) - \theta, \quad (3b)$$

$$S_E \text{ (relative peak height in Exposure)} = \max_{t>T} H_w(t) - \theta. \quad (3c)$$

If these quantities are positive, allergic symptoms appear in respective situations, and magnitudes of symptoms are expected to increase with these quantities, as shown in Figs. 2A, B, and D. Therapy is successful if $S_0 > 0$, $S_T \leq 0$, and $S_E \leq 0$. Under these circumstances, a possible measure of therapeutic success is the severity of symptoms S_0 .

To combine three aspects of therapeutic success, we used a simple sum of the three quantities, each indicating one requirement as follows:

$$\text{score} = \alpha + \beta + \gamma. \quad (4)$$

The first component α is related to the symptoms experienced by a patient without therapy and is calculated from S_0 . We consider symptoms as not detectable if $S_0 \leq 0$, and define the following quantity as the first component of the score using $[x]_+ = x$ if $x \geq 0$, $[x]_+ = 0$ if $x < 0$:

$$\alpha = ([S_0]_+)^{0.1}, \quad (5a)$$

where S_0 is the relative peak height without therapy (Eq. (3a)). We adopted a power function of S_0 with a small positive power of 0.1 to avoid regarding runs with very large S_0 as successful cases when the other requirements were violated. Hence, using a small positive power, the impact of very large S_0 is mitigated and the score becomes high only when all three conditions are satisfied.

If $S_T > 0$ or $S_E > 0$ or both hold, the case is not successful. Because evaluations of these cases are desirable, we introduce penalty terms. Specifically, if $S_T > 0$, symptoms appear during therapy and are greater if S_T is larger. Hence, we introduce a

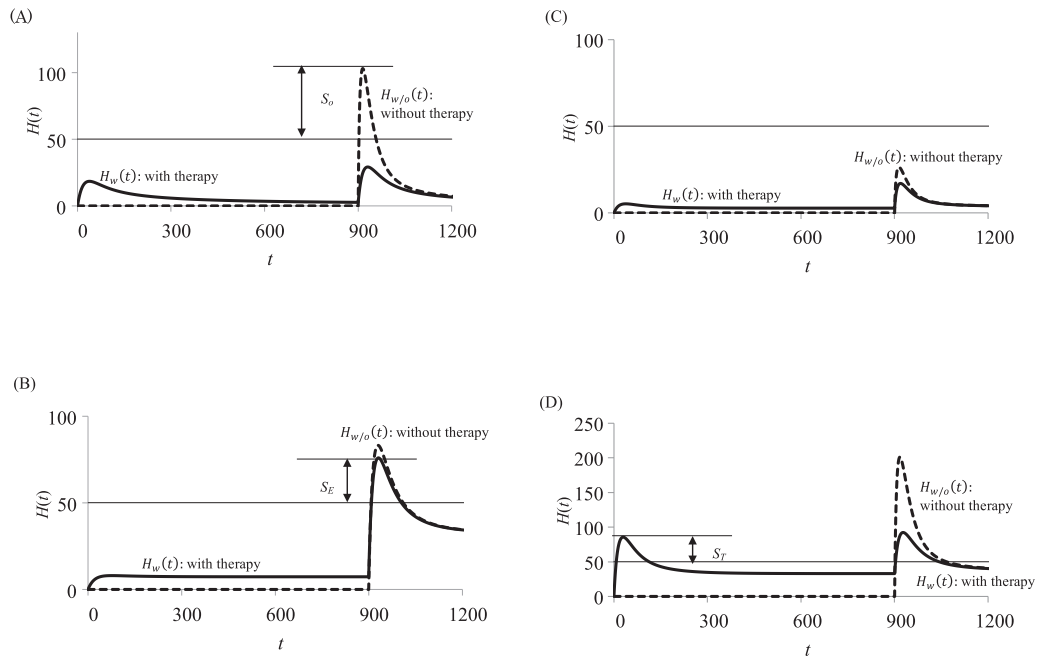


Fig. 2. Results of four therapeutic schedules. The vertical axis shows the number of Th2 cells $H(t)$, and the horizontal axis shows time t . Broken and solid lines represent without and with therapy, respectively. The phase for $t < T=900$ is the therapy phase, and the phase for $t > T=900$ is the environmental pollen exposure phase. (A) Therapy was successful ($\theta = 50$, $a = 0.5$, $A = 10.0$, $b = 10.0$, $c = 0.9$, $d_h = 0.05$, $d_r = 0.001$, $m = 0.1$, $n = 20.0$, $T = 900.0$). Without therapy, $H(t)$ exceeds the threshold for symptoms ($\theta = 50$) in the exposure phase. With therapy, $H(t)$ did not reach $\theta = 50$. We regard this condition as success of allergen immunotherapy. S_0 , S_T , and S_E were used to calculate scores (see Eqs. (3a), (3b), and (3c)). (B) Therapy fails to suppress symptoms in the exposure phase ($\theta = 50$, $a = 0.5$, $A = 10.0$, $b = 10.0$, $c = 0.9$, $d_h = 0.05$, $d_r = 0.01$, $m = 0.045$, $n = 200.0$, $T = 900.0$). Both without and with therapy, $H(t)$ exceeds the threshold $\theta = 50$ in the exposure phase. (C) Even without therapy, patients do not have symptoms ($\theta = 50$, $a = 0.5$, $A = 10.0$, $b = 10.0$, $c = 0.9$, $d_h = 0.05$, $d_r = 0.01$, $m = 0.45$, $n = 20$, $T = 900.0$). Therapy is unnecessary under these conditions. (D) In the therapy phase, $H(t)$ exceeds the threshold $\theta = 50$ and patients experience allergic symptoms ($\theta = 50$, $a = 2.0$, $A = 10.0$, $b = 10.0$, $c = 0.9$, $d_h = 0.05$, $d_r = 0.01$, $m = 0.045$, $n = 20$, $T = 900.0$).

penalty term of the following value:

$$\beta = -([S_T]_+)^{0.1}. \quad (5b)$$

Similarly, we introduced another penalty term when symptoms remained even under therapy ($S_E > 0$) using the following function:

$$\gamma = -([S_E]_+)^{0.1}. \quad (5c)$$

Eqs. (5b) and (5c) are second and the third components of the score given in Eq. (4).

In further computations, we examined combinations of parameters that are likely to be associated with successful therapy. Ten parameters were examined with three (high, intermediate, and low) values each, as listed in Table 1 (see also caption to the table). Scores for each run of 3^{10} parameter sets were calculated, and parameter sets that achieved scores in the top 10% of all runs were regarded as successful cases, although we do not have clear reason of why we choose 10% instead of other percentage for identifying runs achieving a high score.

3. Parameter sensitivity

Parameter sensitivity of the model was examined for each of the ten parameters using high, intermediate, and low values for the likelihood of successful therapy, and the influence of each parameter on therapeutic success was investigated. Our aim in the current study is to know the condition in which all of the three criteria of therapy success are satisfied ($S_0 > 0$, $S_T \leq 0$, and $S_E \leq 0$), using “score”. In addition, parameter sensitivity of S_0 , S_T and S_E are also important to know the effect of parameters on therapy results. First, we investigated parameter dependence of S_0 , S_T and S_E and then we discuss parameter sensitivity of each parameter using “score”.

We set parameter range in order to illustrate a range of the model's behavior as in Fig. 2; therapy success (Fig. 2A), having symptom after therapy (Fig. 2B), having no symptoms even without therapy (Fig. 2C) and having symptom in therapy (Fig. 2D). Since our main purpose of the current study is to develop the simplest mathematical model of allergen immunotherapy and to elucidate the logical basis of the system, rather than to develop realistic model, identifying the realistic parameter range should be done in future development of this paper.

In these computations, as shown in Table 1, $\theta = 10$, 50 and 90 were set for threshold of the number of Th2 cells for having symptoms. $a = 0.1$, 0.5 and 0.9 were set as the intensity of pollen intake in the therapy phase and we set $A = 10$, 50 and 90 (i.e., 100 times $a = 0.1$, 0.5 and 0.9) as the environmental pollen exposure in the exposure phase. Numbers of naïve T cells produced in response to a unit of pollen were set as $b = 10$, 100, and 1000 for low, intermediate, and high, respectively. The fraction of naïve T cells that differentiated into Th2 cells was denoted by c , and $c = 0.5$ indicates that half of all naïve T cells differentiate into Th2 or Treg cells. Subsequently, $c = 0.7$ and $c = 0.9$ were set as intermediate and high values of c . Mortality of Th2 cells was denoted by d_h and $d_h = 1.0$ for the intermediate value and $d_h = 0.5$ and 1.5 for low and high mortality rates, respectively. We assumed that the turnover of Treg cells is much slower than that of Th2 cells. Hence, d_r was considered much smaller than d_h and the three values were set to $d_r = 0.001$, 0.01, and 0.1. Correspondingly, the strength of suppression m was set to 0.001, 0.01, and 0.1, and numbers of nTreg cells n were set to 2, 20, and 200. If both m and n are large, all runs will indicate that cases have no symptoms even without therapy (Fig. 2C). However, if both m and n are small, cases will have strong symptoms during or after therapy (Fig. 2B and D), leading to failure of therapy. T is the duration of the therapy phase in days,

and was set to $T=900$ as the longest length (about two and a half years), and shorter phases were set to $T=100$ and 500 .

We chose these ranges of parameters (in Table 1) for the model to accommodate all four kinds of cases, as illustrated in Fig. 2A–D. For example, although c can be smaller than 0.5 within the range of $0 < c < 1$, we chose $c=0.5, 0.7$ and 0.9 . With c smaller than 0.5 , the cases having symptoms during or after therapy is less likely to appear and then we start with $c=0.5$.

3.1. Parameter sensitivity of relative peak height without therapy, in therapy and after therapy (S_0 , S_T , S_E)

We investigated the parameter sensitivity of three relative peak heights, S_0 , S_T and S_E . The results are explained in detail in Appendix A. Larger b and c and smaller d_h , m and n are more likely to achieve a peak of $H(t)$. These are situations in which the patients are likely to have symptoms.

3.2. Parameter sensitivity of the score

Next, we aim to consider these three conditions simultaneously, using “score”. Fig. 3 shows the influences of each parameter on the realization of successful therapy. In Fig. 3A, the effect of A is represented. A is fixed as one of the three values on the horizontal axis (high, intermediate, and low), and the vertical axis indicates the number of runs achieving scores in the top 10% among all combinations of the other seven parameters ($3^{10}=59,049$ runs).

(i) Threshold of having symptoms, θ

Among the three values, the largest (90; Fig. 3A) is most likely to be included in runs with top 10% high score. A high threshold of having symptoms avoids having symptoms in the therapy and exposure phases.

(ii) Intensity of pollen dose as therapy, a

The largest (0.9; Fig. 3B) was the most likely to be included in runs with top 10% high score. This suggests that larger amount of pollen dose suppresses the symptoms in each exposure phase more effectively. If the amount of dose is too small ($a=0.1$), the therapy is not enough to suppress symptoms. Note that the largest and the intermediate are almost the same value. This suggests that too large amount of dose may lead side effects of therapy, symptoms caused by therapy itself.

(iii) Intensity of pollen exposure, A

Among the three A values, the largest (90; Fig. 3C) was the most likely to be included in runs with scores in the top 10%. This suggests that therapy is appropriate when the patient is exposed to a large amount of environmental pollen. If A is smaller, fewer patients have allergic symptoms and the therapy is increasingly redundant.

(iv) Numbers of differentiating naive T cells per unit dose of pollen, b

The largest b value (1000) was the most likely to be included in the top 10% of runs (Fig. 3D). To accumulate sufficient numbers of Treg cells for successful therapy, large numbers of naive T cells need to be stimulated by APCs. In contrast, if b is too large, Th2 and Tregs are produced in response to small doses of pollen and cause allergic symptoms during therapy, leading to failure. We discuss this problem later.

(v) Rate of differentiation into Th2 cells, c

The smallest c value (0.5) appeared more frequently in the top 10% of runs than the other two values (Fig. 3E). If a smaller fraction of naive T cells differentiate into Th2 cells, symptoms become

less likely, although more Treg cells are produced and enhance the therapeutic effect.

(vi) Decay rates of Th2 cells, d_h

The largest d_h value (1.5) was more often included in the top 10% of cases than the other two values (Fig. 3F). As d_h increases, Th2 cells decay more rapidly and the therapy becomes more likely to succeed.

(vii) Decay rate of Treg cells, d_r

The smallest d_r value was more likely to be included in the high score group than the larger d_r value (Fig. 3G). Hence, the therapy was more likely to succeed if Treg cells had a longer lifespan, presumably reflecting accumulation of Treg cells and enhanced suppressive effects.

(viii) Suppression effect of Treg cells, m

The largest m value (0.1) was more likely to be included in the top 10% of cases than the smaller values (Fig. 3H). Hence, stronger suppression prevented differentiation into Th2 cells and blocked allergic symptoms more effectively.

(ix) Numbers of nTreg cells, n

The smallest n value (2.0) was more likely to be included among the top 10% of cases than the larger values (Fig. 3I). Patients with small numbers of nTreg cells had symptoms and needed therapy. However, when n was large, high nTreg cell numbers made patients insensitive to pollen, resulting in no allergic symptoms. On the other hand, during the earliest stages of therapy when few iTreg cells had been produced, nTreg cells were crucial to suppress symptoms during therapy. If numbers of nTreg cells were very small, allergic symptoms appeared during therapy. We will discuss this problem later.

(x) Length of therapy phase, T

The longest therapy phase T (900) was more likely to be included in the top 10% of cases than shorter phases (Fig. 3J). If a patient received longer therapy, more Treg cells were accumulated, making the therapy more likely to be successful.

3.3. Relative peak heights (S_0 , S_T and S_E) and score when parameters are randomly sampled

We performed parameter sensitivity analysis with random choice of parameters from uniform distribution. Relative peak heights (S_0 , S_T and S_E) and score are examined. Detailed results are explained in Appendix C. Parameter dependence of desirable parameter values is qualitatively the same as in the analysis reported in Section 3.2.

4. Alternative scoring for therapeutic success

In further computations, we examined several alternative methods for scoring the results. Specifically, we considered weighting of the three components (α , β , and γ), and various ways of measuring each component. In Appendix B, we explain these methods in more detail.

4.1. Alternative weighting of various components for therapeutic success

Although we changed the weights for α , β , and γ , only the relative values for these weights were consequential and the model remained the same if we multiplied all three weights by the same factor. Thus, we increased the weight for α by factors of 10 and

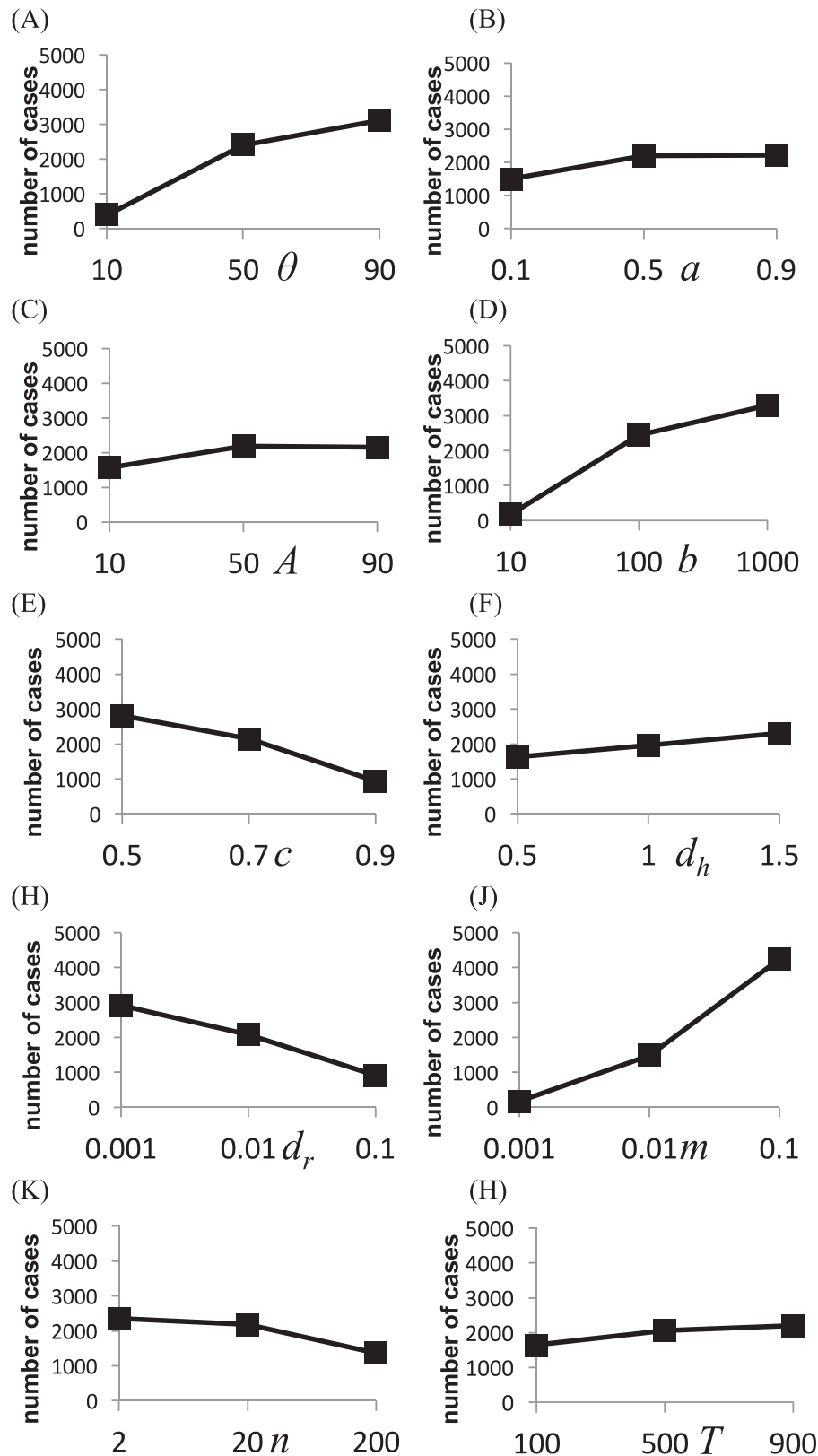


Fig. 3. Analysis of the influence of each parameter value. The score given by Eqs. (4), (5a), (5b), and (5c) was adopted and the model is presented for 3^{10} parameter sets (Table 1). The horizontal axis shows three levels for each parameter and the vertical axis shows the number of cases achieving scores in the top 10% with the focal parameters specified in the horizontal axis. (a) Threshold of having symptoms, θ ; (b) Intensity of pollen dose as therapy, a ; (c) Intensity of pollen exposure, A ; (d) Numbers of differentiating naive T cells per unit dose of pollen, b ; (e) Rate of differentiation into Th2 cells, c ; (f) Decay rates of Th2 cells, d_h ; (g) Decay rate of Treg cells, d_r ; (h) Suppression effect of Treg cells, m ; (i) Number of nTreg cells, n ; (j) Length of Therapy phase, T . Values that show large numbers of cases are likely to be associated with therapeutic success.

100 without changing those for β and γ . Using the score with modified weights, successful therapy was most likely for parameter values that were different from those obtained with the standard score from equal weights for all three components, as shown in Eq. (4). However, scores with enhanced weights for α were high for different parameter values. Specifically, larger c , smaller d_h , and smaller m were frequently included in the top 10% of successful cases when we adopted larger weights for α . These observations indicate that more naïve T cells differentiate into Th2 cells, that Th2 cells have a longer life span, and that the weaker suppressive effects of Treg cells increase the likelihood of allergic symptoms. Because a large α represents symptom severity without therapy, this result is quite plausible. In contrast, the results remained the same as those for the standard case even when weights for β or γ only were increased, and the recommended parameter values were the same as the original score. Hence, greater weight for one component over the other components corresponds with greater importance.

4.2. Variations of scoring methods

We examined various alternative functional forms for the three components of scores α , β , and γ , using the functions S_0 , S_T , and S_E , respectively. The original method for calculating these three components (Eqs. (4), (5a), (5b), and (5c)) only considers the value of $H(t)$ when it is greater than the threshold. Hence, these criteria do not distinguish cases with $H(t)$ values that are slightly smaller than the threshold, and in these cases, the peak is much lower than the threshold. This is justified because no symptoms appear if $H(t)$ is below the threshold θ . Accordingly, we considered the following three variations, which are explained in more detail in Appendix B.

Variation 1: We considered contributions of the $H(t)$ peak when it was lower or higher than the threshold. Using this score, we considered a penalty for choosing patients with no symptoms without therapy, and gave a larger score to those with larger margins of symptoms following therapy.

Variation 2: We only considered positive parts of each component of variation 1. In comparisons these with the standard score, this variation differed in β and γ , and a positive score for a larger margin was considered instead of a penalty for failure to satisfy the corresponding conditions for successful therapy.

Variation 3: We considered the functions of power at 0.5 instead of at 0.1. Accordingly, we allowed one component of the score to be very much larger than the other components.

For each method, we changed weights for α , β , and γ , as in Section 4.1.

Formulas for these variables and the results of analyses using these variations are explained in Appendix B. Generally speaking, these variations provided almost the same results as the standard scoring method (Eqs. (4), (5a), (5b), and (5c)). However, in some parameters, the desirable level for successful therapy changes with weights of α , β , and γ (Table 2). Variations 1 and 2 consider $H(t)$ values below the threshold θ and assume that numbers of Th2 cells can be measured irrespective of the presence of symptoms.

If a very large weight is given to one component and the power is not very small, other conditions for therapeutic success may be violated in high-score cases (Variation 3). See Appendix D for details.

5. Temporal patterns of administration

In Eq. (2), we consider cases in which the patient receives pollen at a constant daily rate during the therapy phase. Below, we consider various patterns of daily pollen intake as specified by

different $P(t)$ ($t < T$) values, and examine the performance of therapy with other fixed parameters. Specifically, we studied the effects of (1) intervals between dates of pollen administration and the amount of pollen per dose, (2) gradual changes of pollen doses throughout the therapy, and (3) the total amount administered. To evaluate the performance of these temporal patterns of administration, we focused on peaks of $H(t)$ in the therapy phase ($\max_{t < T} H_w(t)$) and in the exposure phase ($\max_{t > T} H_w(t)$).

To fit the length of phase with symptoms into about 90 days, which is the length of a typical pollen season (Horiguchi et al., 2008), we set $d_h = 0.01$ and $m = 0.045$. For other parameters, we fixed parameters A , b , c , d_h , d_r , m , n , and T at 50.0, 10.0, 0.5, 0.01, 0.001, 0.045, 200.0, and 900.0, respectively.

5.1. Intervals and amounts of pollen doses

First, we varied the length of dose intervals and the amount of pollen in one dose, fixing total amount of pollen constant (Appendix E). We compare three methods with different length of intervals. Giving smaller amount of pollen at shorter dose intervals is the best in terms of avoiding symptoms in therapy.

5.2. Constant, increasing, and decreasing doses

Next, we examined variations of temporal pattern of administration. Details are explained in Appendix E.

- (i) *Gradual changes in daily doses over time (constant, increasing, or decreasing)*

We examined three patterns of daily doses; constant, gradually increasing, and gradually decreasing with time. Among these, gradual increase was the most likely to suppress symptoms in therapy.

- (ii) *Total doses of treatment schedules*

Next, for each of three patterns in (i), the total amount of pollen throughout therapy was varied and dynamics of the number of Th2 cells were calculated. The results were similar among three patterns: if the total amount of pollen was too small, the therapy was inefficient, and if it was too large, the therapy caused symptoms in the therapy phase.

- (iii) *Therapeutic effects under conditions of symptom control*

Considering the result in (ii), we applied another criterion in which the risk of having symptoms in therapy is fixed and we compared therapy effect. We choose representative for each of three patterns and examined maximum A (the amount of environmental pollen) to which the therapy can suppress symptoms after the therapy. As a result, gradually increasing dose can cope with the largest A . If we control the risk of having symptoms, increasing dose with time is the most effective method among three patterns of administration.

5.3. Conventional method of administration with induction and maintenance phases

In current medical practice, the term induction phase refers to the period in which increasing amounts of pollen extract are administered (Horiguchi et al., 2008). Accordingly, we examined dosing schedules during the induction phase in which the amounts of doses are gradually increased as $P(t) = st$, and in the maintenance phase, in which doses remained constant until the end of therapy. The total length of therapy T was fixed at 900, and the length of the induction phase T_i was varied. We set k as the sizes of maintenance doses.

- (i) *Varying T_i with fixed maintenance doses (k)*

Table 2

Desirable levels of parameters changing with weights of α , β , and γ for each scoring method. Three values (large, intermediate, and low) were set for each of ten parameters and scores were calculated for all 3^{10} combinations. We then determined which of the three levels are most frequently included in successful parameter sets with scores in the top 10%. Scores were calculated as $score = w_\alpha \alpha + w_\beta \beta + w_\gamma \gamma$. We considered the scoring methods standard and variations 1, 2, and 3. Definitions of α , β , and γ differ among these methods (Eqs. (A.1), (A.2) and (A.3)) and w_α , w_β , and w_γ were varied at 1, 10, and 100 to examine the influence of increasing weights of single components in comparisons of the cases as follows:

(i) $(w_\alpha, w_\beta, w_\gamma) = (1, 1, 1)$, $(10, 1, 1)$, and $(100, 1, 1)$,

(ii) $(w_\alpha, w_\beta, w_\gamma) = (1, 1, 1)$, $(1, 10, 1)$, and $(1, 100, 1)$, and (iii) $(w_\alpha, w_\beta, w_\gamma) = (1, 1, 1)$, $(1, 1, 10)$, and $(1, 1, 100)$. For each table, the leftmost column shows the values of w_α , w_β , or w_γ and other columns show the parameter values that were the most successful of the three alternatives. Large, intermediate and small values are shown in brackets, but only for parameters in which the most successful level was changed by increases in the weights of components α , β , or γ .

(A) As a standard method, we calculated components of the score (α , β and γ) using Eqs. (4) and (5a), (5b), and (5c), and considered different weights of α , β , and γ .

Increasing the weight of α (w_α) influenced the results of c , d_h , and m .

(B) In variation 1, we calculated α , β , and γ using Eq. (A.1). In addition to the standard method, we evaluated $H(t)$ values that were below the threshold. (i) Increased w_α also changed the results of c , d_h , and m , whereas (ii) increasing w_β changed the results of b and n and (iii) increasing w_γ changed the result of A .

(C) In variation 2, we calculated α , β , and γ using Eq. (A.2). In this method, only the degree of success of each component was evaluated, and the results were similar to those of variation 1. Specifically, (i) increasing w_α changed the results of c , d_h , and m , (ii) increasing w_β changed the results of b and n , and (iii) increased w_γ changed the results of A .

(D) In variation 3, we calculated α , β , and γ using Eq. (A.3). The effects of extremely large components on the score were stronger than in the standard method. Specifically, increasing w_α changed the results of c , d_h , and m .

(A) standard			
w_α	c	d_h	m
1	0.5 (small)	1.5 (large)	0.1 (large)
10	0.9 (large)	1.0 (intermediate)	0.001 (small)
100	0.9 (large)	0.5 (small)	0.001 (small)
(B) variation 1			
(i)			
w_α	c	d_h	m
1	0.5 (small)	1.5 (large)	0.1 (large)
10	0.9 (large)	1.5 (large)	0.001 (small)
100	0.9 (large)	0.5 (small)	0.001 (small)
(ii)			
w_β	a	b	n
1	0.9 (large)	1000 (large)	2 (small)
10	0.5 (intermediate)	100 (intermediate)	2 (small)
100	0.1 (small)	10 (small)	200 (large)
(iii)			
w_γ	A	b	n
1	50 (intermediate)	1000 (large)	2 (small)
10	50 (intermediate)	1000 (large)	2 (small)
100	10 (small)	10 (small)	200 (large)
(C) variation 2			
(i)			
w_α	c	d_h	m
1	0.5 (small)	1.5 (large)	0.1 (large)
10	0.9 (large)	0.5 (small)	0.001 (small)
100	0.9 (large)	0.5 (small)	0.001 (small)
(ii)			
w_β	a	b	n
1	0.9 (large)	1000 (large)	2 (small)
10	0.1 (small)	100 (intermediate)	2 (small)
100	0.1 (small)	10 (small)	200 (large)
(iii)			
w_γ	A	b	n
1	50 (intermediate)	1000 (large)	2 (small)
10	10 (small)	10 (small)	200 (large)
100	10 (small)	10 (small)	200 (large)
(D) variation 3			
(i)			
w_α	c	d_h	m
1	0.7 (intermediate)	1.5 (large)	0.01 (intermediate)
10	0.9 (large)	0.5 (small)	0.001 (small)
100	0.9 (large)	0.5 (small)	0.001 (small)
(ii)			
w_β	a		
1	0.9 (large)		
10	0.1 (small)		
100	0.1 (small)		

Initially, we varied the length of the induction phase T_i and changed the rate of increase s . The daily dose in the maintenance phase was fixed at $k = 20$ (Fig. 4A). In Fig. 4, (A) the therapy schedule and (B) dynamics of the number of Th2 cells ($H(t)$), and (C) dynamics of the number of iTreg cells ($R(t)$) are shown. ($H(t)$) Thus, we have $s = k/T_i$. Dose increases during the entire therapy phase are shown as bold lines for $T_i = 900$, and those for the half therapy phase $T_i = 450$ are shown as broken lines. Dashed-dotted lines show changes for the induction phase of $T_i = 150$ and dotted lines show that for no induction phase $T_i = 0$. These computations show that $H(t)$ peaks during therapy are decreased with longer induction phases (Fig. 4B). However, longer induction phases led to larger $H(t)$ peaks after therapy, reflecting reduced numbers of iTreg cells in the exposure phase (Fig. 4C).

(ii) Varying T_i with fixed total doses

Under conditions of fixed total doses with varying lengths of the induction phase T_i , daily doses in the maintenance phase changed with T_i (Fig. 4D). Computations were performed for $T_i = 900$ (bold lines), $T_i = 450$ (broken lines), $T_i = 150$ (dashed-dotted lines) and $T_i = 0$ (dotted lines), and longer induction phases were associated with smaller $H(t)$ peaks (Fig. 4E). However, the peak $H(t)$ value in the exposure phase varied less between dosing schedules than the case of (i). This is because almost the same amount of iTreg accumulates until the exposure to environmental pollen (Fig. 4F).

For both (i) and (ii), increasing doses throughout the therapy phase were associated with low risks of symptoms during therapy. These circumstances differ from conventional treatments with induction and maintenance phases. However, continued increases in doses may be unrealistic because very large doses near the end of the therapy would likely cause additional complications of digestion and in the formation of regulatory T cells.

Under the conditions of (ii), $H(t)$ peaks after therapy were barely affected by changes in T_i , compared with changes in $H(t)$ peaks during therapy. These results reflect the choice of parameters. In particular, a small d_r value was required for successful therapy, as indicated in the previous section. However, this implies that Treg cells are long lived, that almost all Treg cells accumulate, and that the total dose directly affects the number of Treg cells (Fig. 4C and F).

6. Discussion

In this paper, we developed a very simple dynamic model of allergen immunotherapy to determine conditions in which therapy is most likely to be successful. To investigate relationships between treatments and each parameter, we defined scores for therapeutic success and calculated these for 3^8 sets of parameters, corresponding to a variety of physical traits and environmental conditions. According to the standard score (Eqs. (4) (5a), (5b), and (5c)), we concluded that therapy would be more effective to patients with more differentiating naive T cells b , larger fraction of differentiating into Th2 cells c , smaller Treg decay rates d_r , large decay rates of Th2 cells d_h , stronger suppressive effects of Treg cells m , and fewer of nTreg cells n . Although the range of parameters (Table 1) was chosen to cover all plausible values, some of these were difficult to identify, warranting further quantitative studies.

We performed computations using several differing scoring methods and found that the most successful value for each parameter depended on the method. Hence, the qualities of participants likely depended on the evaluation method and should be carefully chosen according to intended purpose. For example, if we prioritize the evasion of allergic reactions to the therapy, the component β in the score needs to be strongly weighted, whereas increased weight

for the component α is required for treatments of patients with severe symptoms. Hence, patient groups for these two therapies will likely include subjects with differing conditions.

The present computations indicate the need to accommodate multiple parameters simultaneously. Specifically, participants with severe allergic symptoms and very large α values may show reactions to the therapy, leading to larger than threshold $H(t)$ peaks during therapy. In Section 4.1, the weight of α was set at 10 and 100 and cases with $H(t)$ peaks that exceeded the threshold after therapy were included among those with scores in the top 10% (see Appendix B). This scenario represents a failure to choose appropriate participants and warrants moderate weighting of individual components.

In this paper, we investigated contributions of each parameter to the likelihood of therapeutic success and identified associated sets of parameters. Hence, the present computations will enable prospective selection of patients for whom therapy is likely to be successful.

Herein we show that the strength of symptom suppression in the pollen exposure phase depends on the total dose. To prevent symptoms during therapy, gradual dose increases are effective, and enable symptom free administration of greater total amounts of pollen than constant doses. These assertions are consistent with current use of induction and maintenance phases (Horiguchi et al., 2008) and indicate the significance of the induction phase.

In a previous study by Gross et al., shorter induction phases improved therapeutic success because longer maintenance phases are good for increasing numbers of Treg cells, thus facilitating therapeutic success (Gross et al., 2011). In contrast, our analyses (Section 5.3) showed that differences in therapeutic success were related to the lifespan of Treg cells. Accordingly, in our models we assumed that the lifespan of regulatory T cells is much longer than that of Th2 cells, because immunotherapy was most effective under these conditions. However, no reliable measurements of regulatory T cell longevity have been published to date. In the model by Gross et al., small doses at the beginning of the induction phase failed to produce sufficient numbers of Treg cells to suppress symptoms after therapy. They assumed the same decay constant for all the cell types including Th2s and Tregs, and therefore Treg accumulation was not observed in the induction phase when patients start from a little amount of pollen that is not enough to produce Treg cells which can be maintained until pollen exposure. In contrast, Treg cells accumulated and suppressed allergic symptoms in our model. Moreover, increasing doses over long time periods may be effective for establishing sufficient numbers of regulatory T cells by the time of pollen exposure, and for avoiding allergic symptoms during therapy.

In the present model, small d_r values were important for therapeutic success. Previous studies of Treg cell longevity revealed that gamma chain cytokines from other cells prevent apoptosis and maintain Foxp3 and CD25 expression, which are important for Treg functions (Pandiyan and Lenardo, 2008). However, exact value of lifetime of Treg cells are not available and we consider that lifetime of iTreg cells are estimated by considering memory iTreg cells (Burzyn et al., 2013). Floess et al. (2007) reported that iTregs are unstable compared to nTregs, due to incomplete demethylation. Though decay rate of iTreg cells we set might be smaller than the actual decay rate, our study demonstrate that the therapy can be successful when decay of iTreg cells is very slow. This suggestion will be useful to explore new target of immunotherapy. For example, another recent study showed that treatments with the immunosuppressive drug rapamycin can enhance the half-lives of Treg cells (Singh et al., 2014). Rapamycin is commonly used in patients with organ transplants, and its effects may lead to smaller d_r values and facilitate success of allergen immunotherapy. Previously, Kanamori et al. (2016) suggested that demethylation of spe-

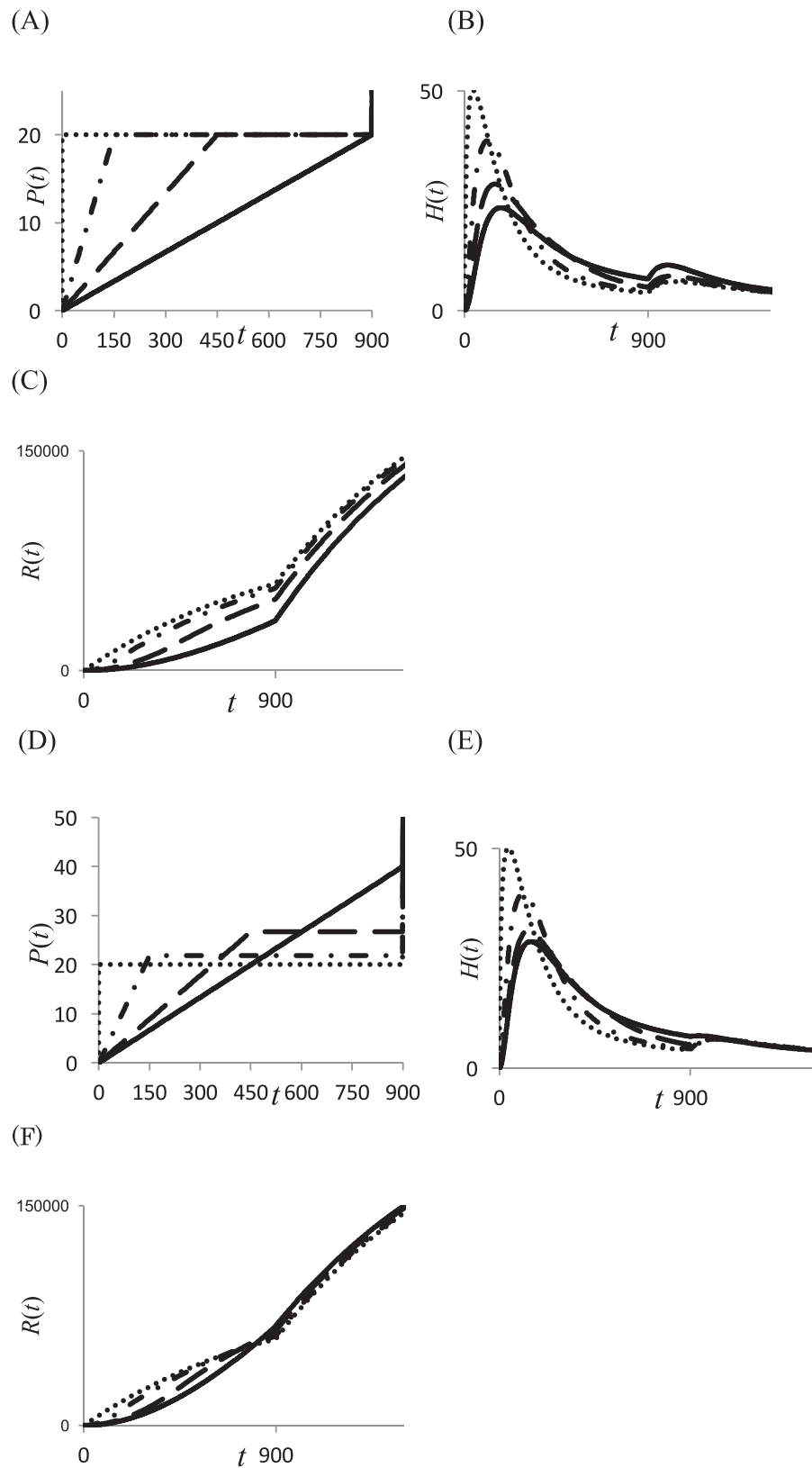


Fig. 4. Variation of conventional method of pollen administration. We changed the length of the induction phase T_i . The left graphs show therapy schedules; Vertical axes show $P(t)$ and horizontal axes shows time t . The right graphs show numbers of Th2 cells with time; Vertical axes shows $H(t)$ and horizontal axes shows time t . The length of therapy period was $T=900$ for all methods. In (A) and (B), bold lines show cases of $T_i=900$, broken lines show cases of $T_i=450$, broken and dotted lines show cases of $T_i=150$, and dotted lines show cases of $T_i=0$. (A) The constant dose in the maintenance phase was fixed at $k=20$ and T_i values were 0, 150, 450, and 900. (B) Constant total dose between methods with T_i values of 0, 150, 450, and 900.

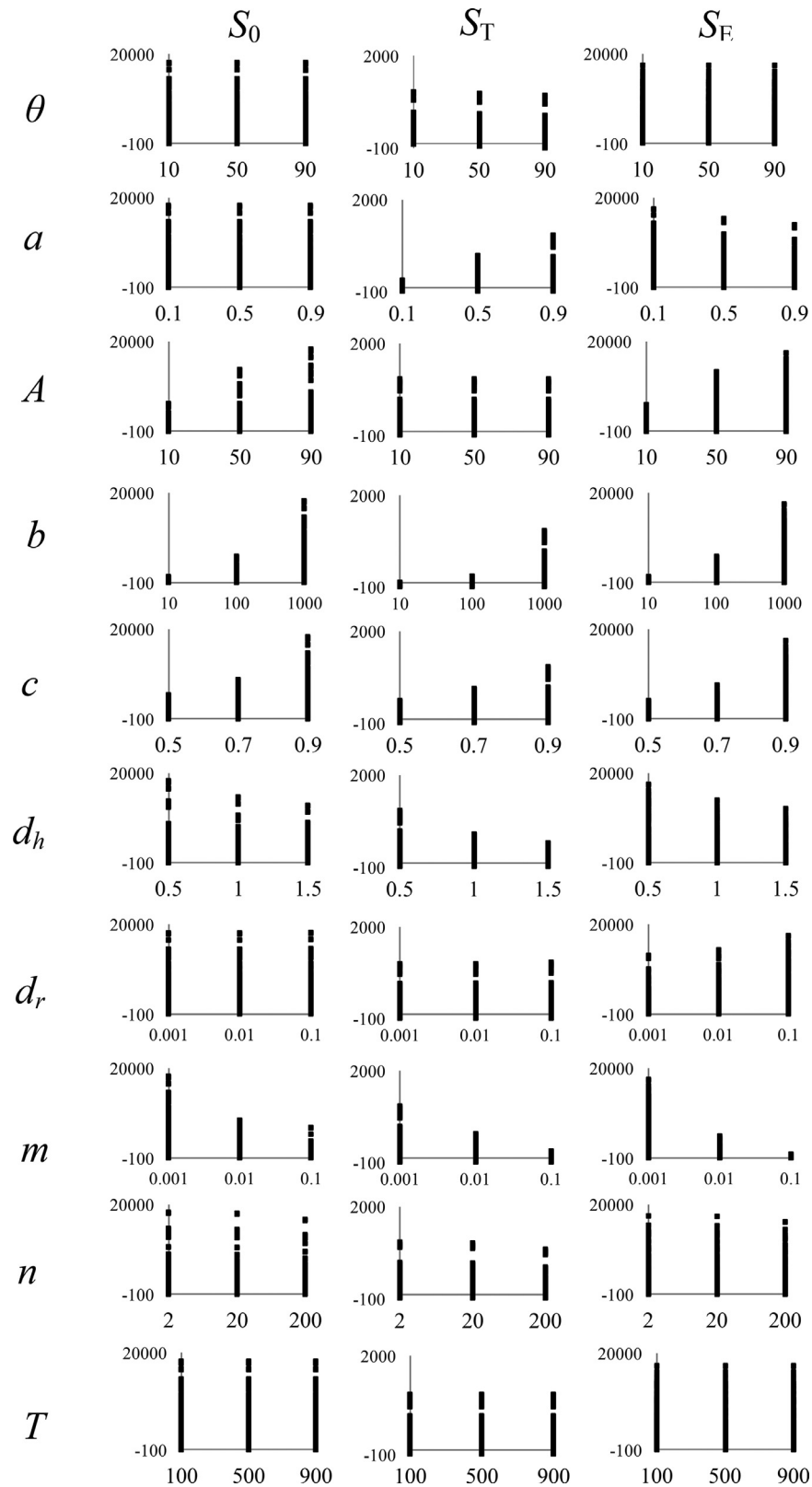


Fig. 5. Parameter sensitivity of relative peak height of $H(t)$. As shown in Table 1, three levels are set to each parameter and dynamics of $H(t)$ are calculated for $3^{10} = 59,049$ parameter sets. The value of the relative peaks without, in and after therapy (S_0 , S_T and S_E) are plotted in the graphs against three levels of parameters. Each row corresponds to each of ten parameters and S_0 , S_T and S_E are plotted on the graphs in the left, middle and right column, respectively.

cific DNA regions in Treg is important for stabilization and induction of iTregs, and listed candidate drug targets for upregulation and stabilization of iTregs.

For simplicity of the present model, we considered single therapy exposure phases. However, patients usually experience several exposure phases over several years, warranting further development of a more realistic model in which therapy and exposure phases are multiple. Our model also fails to describe patients who

are cured completely, because Tregs eventually run out after therapy. Further extensions of our model will be made to accommodate this shortcoming.

Although we included only two types of T cells, previous studies suggest other factors that play roles in the development of allergic symptoms. In particular, [Otsuka and Kabashima \(2015\)](#) showed that basophils facilitate differentiation of naïve T cells into Th2 cells, and [Stein et al. \(2016\)](#) showed

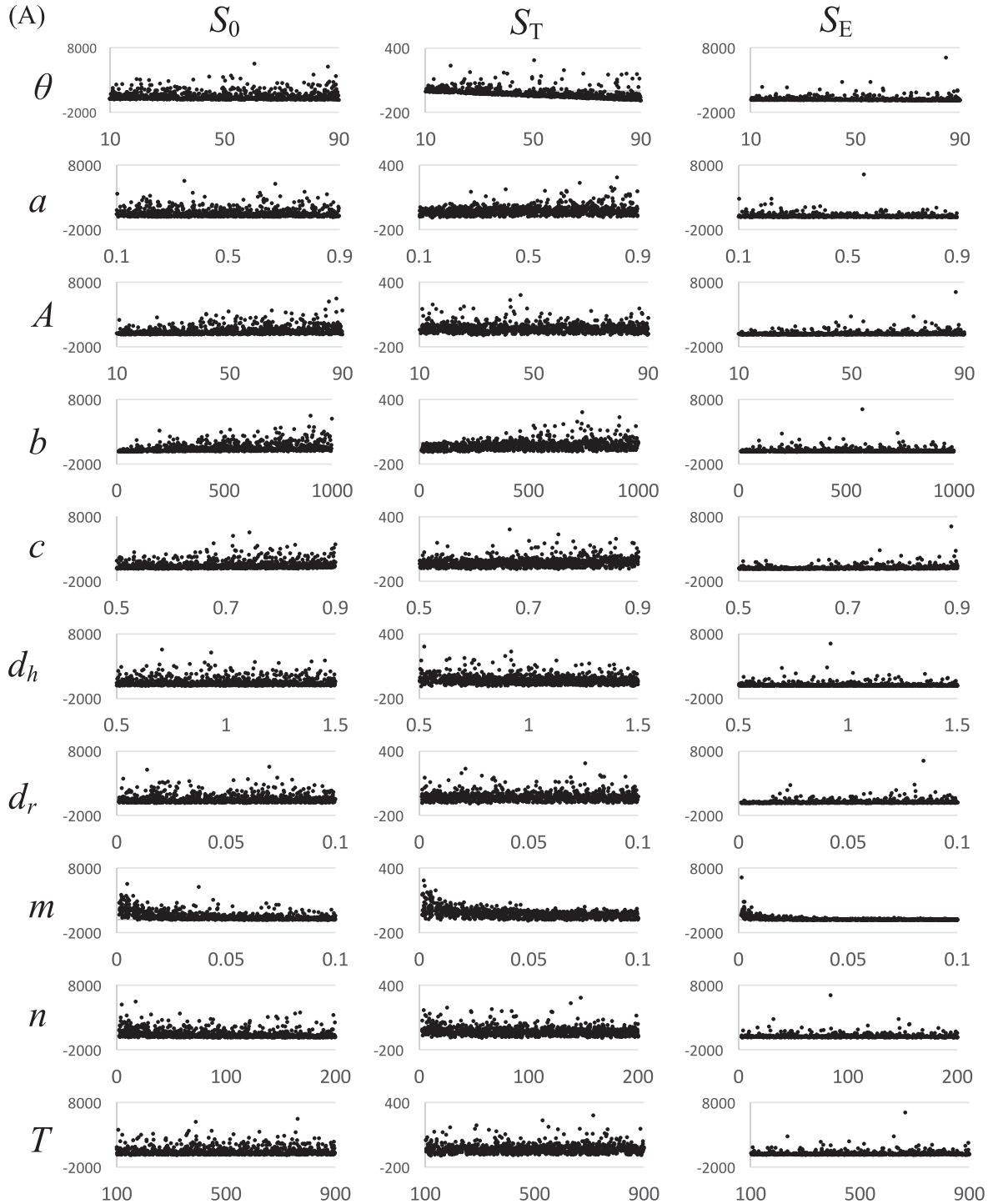


Fig. 6. Distributions of relative peak height (S_0 , S_T and S_E) and score calculated deriving parameters from uniform distribution. Deriving 1000 parameter sets from uniform distribution, we calculated $H(t)$ and plotted relative peak height (S_0 , S_T and S_E) and score. In (A), each row corresponds to each of 10 parameters and S_0 , S_T and S_E are plotted in the left, middle and right column, respectively. In (B), distribution of score given by Eqs. (4), (5a), (5b), and (5c) are plotted. Black dots correspond to the cases which achieved top 10% high score and gray dots are 90%, the rest of the cases.

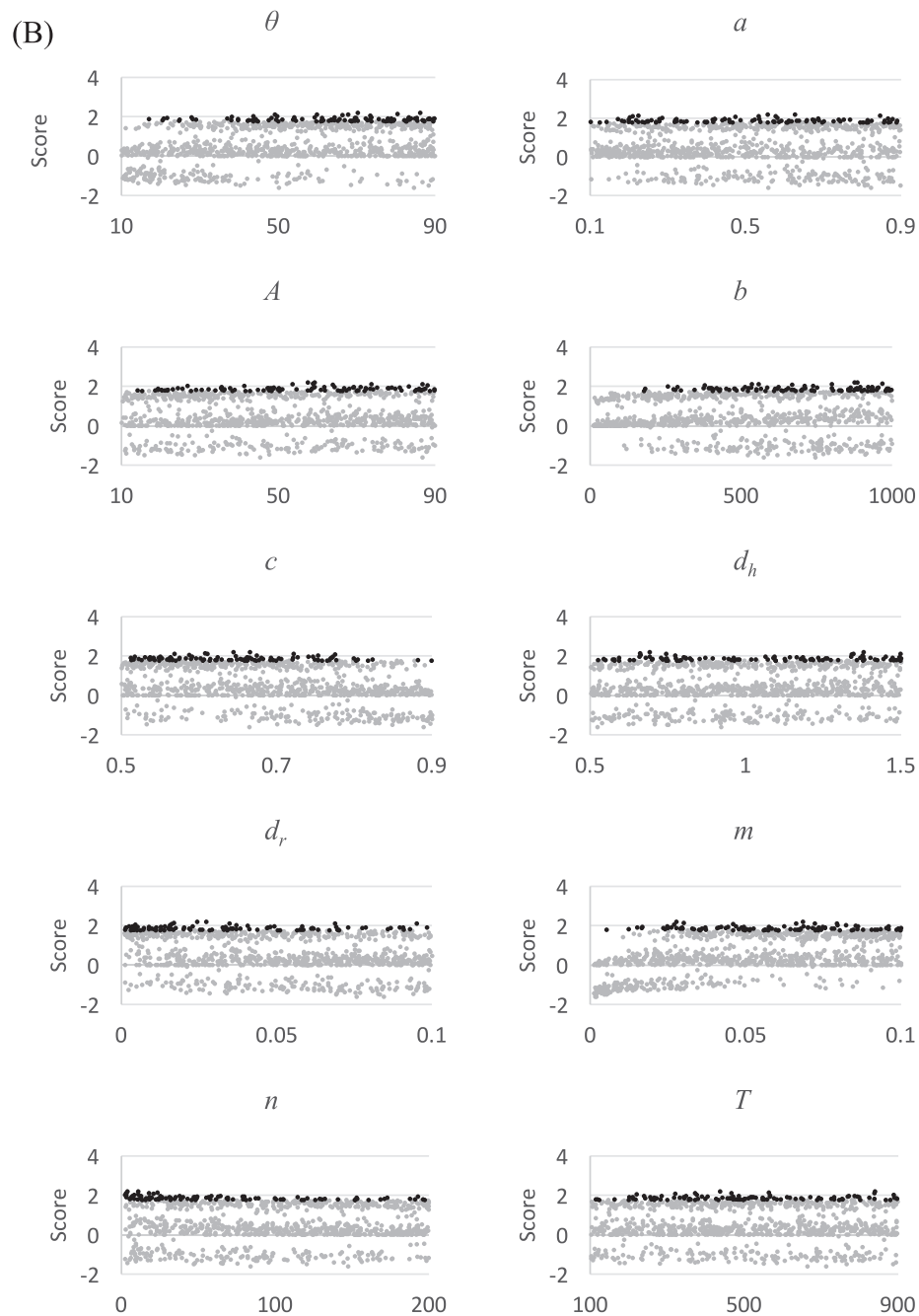


Fig. 6. Continued

that Amish people, who strictly follow traditional lifestyles, are less likely to have allergies and asthma than other farmers who have industrialized lifestyles. These observations likely correspond with increased numbers of neutrophils and decreased numbers of eosinophils but not with significant differences in Treg cell numbers. Hence, the present model may be improved by inclusion of additional cell types.

As with the coefficient n for numbers of nTreg cells, a positive initial value of $H(t)$ might be necessary for our model. Accordingly, the initial $H(t)$ value of 0 may be unrealistic because Th2 cells trigger humoral immune reactions for other kinds of harmful antigens, regardless of allergies. Thus, to consider this value, a constant term indicating the initial $H(t)$ should be added to Eq. (1b). This will elevate the dynamics of $H(t)$, leading to increased chances of allergic symptoms in patients with very large initial $H(t)$ values. This

is the same as lowering of the symptom threshold θ . Accordingly, because initial $H(t)$ values among individuals correspond with the threshold θ , the effects of threshold θ could be investigated in terms of initial $H(t)$ values.

Although the present model studied in this paper is very simple, it could be used to inform new methods of the therapy. However, the experimental values of each parameter and the mechanisms of differentiation of T cells remain unknown, warranting further experimental studies to develop more accurate and realistic models.

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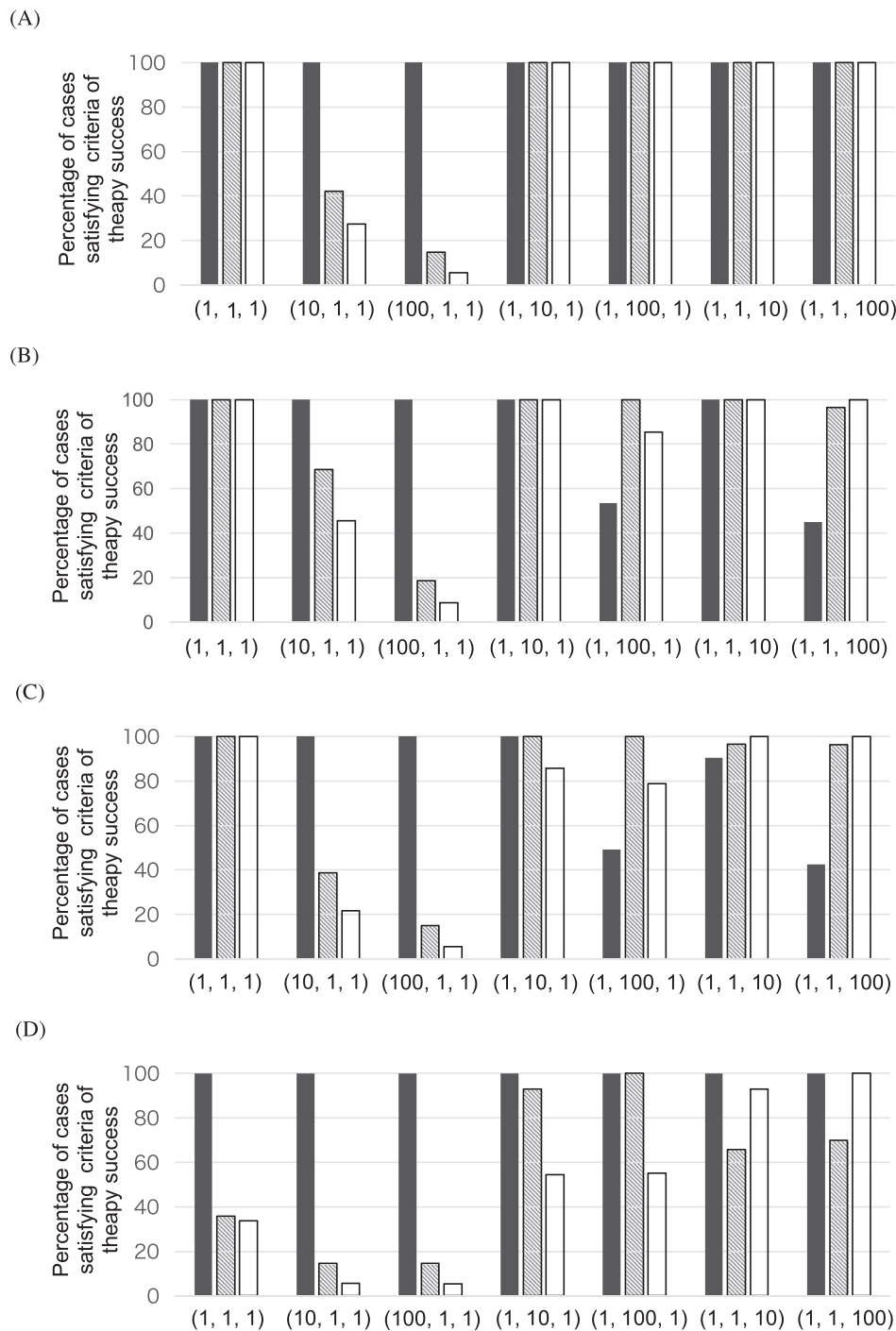


Fig. 7. Percentages of the cases in which $S_0 > 0$, $S_T \leq 0$, and $S_E \leq 0$ were satisfied. Percentages of cases in which the therapeutic success criteria $S_0 > 0$, $S_T \leq 0$, and $S_E \leq 0$ were satisfied are graphed as black bars, striped bars, and white bars, respectively. The weights of α , β , and γ are shown as w_α , w_β , and w_γ , respectively. (A) Result of runs of the standard scoring method (Eqs. (4), (5a), (5b) and (5c)); All runs of $(w_\alpha, w_\beta, w_\gamma) = (1, 1, 1)$, $(1, 10, 1)$, $(1, 100, 1)$, $(1, 1, 10)$, and $(1, 1, 100)$ satisfied all three criteria, as indicated by 100%. Runs of increasing weights for α , $(10, 1, 1)$ and $(100, 1, 1)$, show decreased percentages of the cases in which $S_T \leq 0$, and $S_E \leq 0$ are satisfied. (B) Result of runs of variation 1 (Eq. (A.1)) are shown. Runs of increasing weights for α , $(10, 1, 1)$ and $(100, 1, 1)$, show decreased numbers of cases satisfying $S_T \leq 0$ and $S_E \leq 0$. In cases with very large weights for β and γ , $(1, 100, 1)$ and $(1, 1, 100)$, the percentages of the cases satisfying $S_0 > 0$ were decreased, whereas in cases of $(1, 1, 1)$, $(1, 10, 1)$, and $(1, 1, 10)$ all runs satisfied all three criteria. (C) Results of runs of variation 2 (Eq. (A.2)) were similar to those of variation 1. Parameter weights $(w_\alpha, w_\beta, w_\gamma)$ of $(10, 1, 1)$ and $(100, 1, 1)$ decreased numbers of cases satisfying $S_T \leq 0$ and $S_E \leq 0$, and with $(1, 100, 1)$ and $(1, 1, 100)$, fewer cases satisfied $S_0 > 0$. (D) Runs of variation 3 (Eq. (A.2)) were performed with power of 0.5 instead of 0.1 in the standard scoring. For all combinations of weights, less than 100% of cases satisfied all three criteria. With $(w_\alpha, w_\beta, w_\gamma)$ weights of $(1, 1, 1)$, $(10, 1, 1)$, and $(100, 1, 1)$, percentages of cases that satisfied $S_T \leq 0$ and $S_E \leq 0$ were low. Increasing $(w_\alpha, w_\beta, w_\gamma)$ weights to $(1, 10, 1)$, $(1, 100, 1)$, $(1, 1, 10)$ and $(1, 1, 100)$ led to increased percentages of cases that satisfied $S_T \leq 0$ and $S_E \leq 0$.

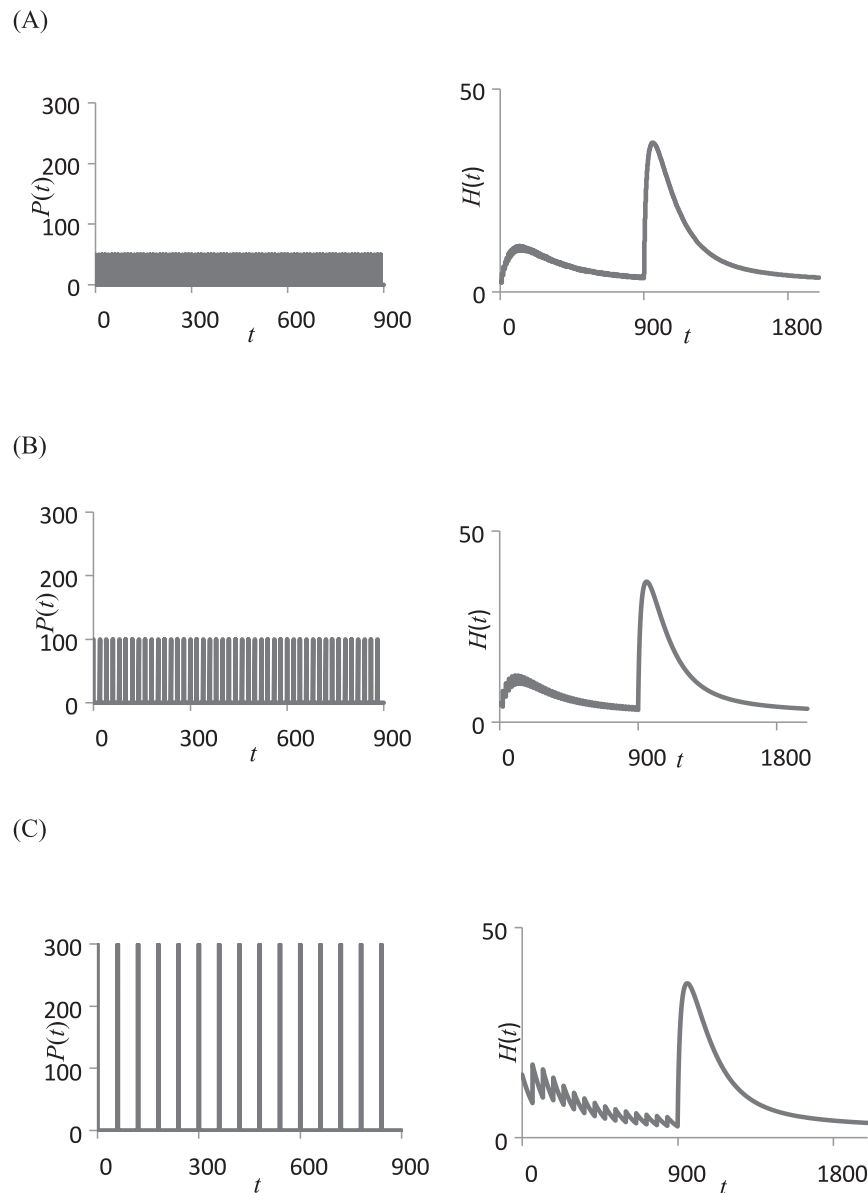


Fig. 8. Influence of intervals of administration. We changed the intervals of administration and examined the influence on therapeutic success. Total amounts doses were fixed at 4500 and the length of the therapy phase was $T=900$. (A) Administration of pollen in 90 doses of 50; (B) Administration of pollen in 45 doses of 100; (C) Administration of pollen in 15 doses of 300. The left graph shows temporal patterns of administration ($P(t)$) in the therapy phase; the right graph is for $H(t)$. The values of parameters A , b , c , d_h , d_r , m , n , and T were 50.0, 10.0, 0.5, 0.01, 0.001, 0.045, 200.0, and 900.0. Peaks of $H(t)$ during therapy and after therapy were (A) $\max_{t < T} H_w(t) = 11.275006$ and $\max_{t > T} H_w(t) = 36.801499$; (B) $\max_{t < T} H_w(t) = 12.252302$ and $\max_{t > T} H_w(t) = 36.775733$; (C) $\max_{t < T} H_w(t) = 17.399794$ and $\max_{t > T} H_w(t) = 36.695985$.

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Appendix A. Parameter sensitivity of relative peak height without therapy, in therapy phase and in exposure phase (S_o , S_T , S_E)

We investigated the parameter sensitivity of three relative peak heights, S_o , S_T and S_E in Eqs. (3a), (3b) and (3c), which determine score (Eqs. (5a), (5b) and (5c)). In $3^{10} = 59,049$ combinations of parameter, S_o , S_T and S_E was investigated and plotted in Fig. 5 against three level of 10 parameters. Larger b and c are likely to lead higher peak of $H(t)$. b represents sensitivity of naïve T cells to pollen and c represents the fraction of differentiation into Th2 cells. If these values are large, Th2 cells are more produced and

allergic symptoms are more likely to develop. Smaller d_h , m and n are likely to lead higher peak of $H(t)$. d_h , m and n represent decay rate of Th2 cells, the suppression effect of Treg cells and nTreg cells, respectively. If these values are small, Th2 cells are more produced and accumulated, and patients are likely to have symptoms. These are conditions that the patients are likely to have symptoms.

Appendix B. Alternative scoring for therapeutic success

B.1. Weighting of components for therapeutic success

In the standard scoring system exemplified by Eqs. (4) and (5), α , β , and γ have equal impact. Hence, we considered various scenarios with higher importance of one of the three components.

Initially, we investigated the effects of increasing the impact of component α , leading to new scores of $score = 10\alpha + \beta + \gamma$ and $score = 100\alpha + \beta + \gamma$, and a standard score of $score = \alpha + \beta + \gamma$.

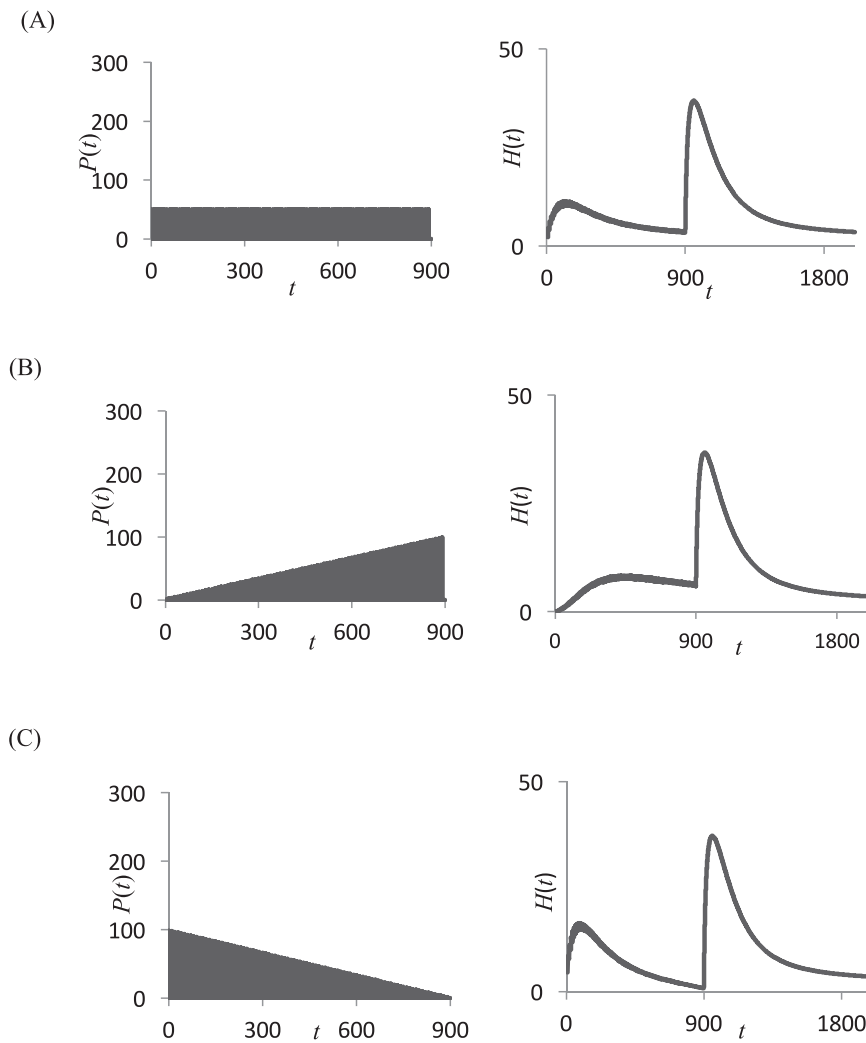


Fig. 9. Influence of patterns in the therapy phase. We examined the following three administration schedules: (A) 50 pollen constantly, (B) increasing administration with time in according to $a=0.1099t$, (C) decreasing administration with time according to $a=98.91-0.1099t$. The left graph shows changes in $H(t)$ with time, and the right graph shows changes in $P(t)$ with time. The parameters A , b , c , d_h , d_r , m , n , and T were 50.0, 10.0, 0.5, 0.01, 0.001, 0.045, 200.0, and 900.0, respectively. Peaks of $H(t)$ in therapy and exposure phase were as follows: (A) $\max_{t < T} H_w(t) = 11.275006$, $\max_{t > T} H_w(t) = 36.801499$; (B) $\max_{t < T} H_w(t) = 8.306327$, $\max_{t > T} H_w(t) = 36.97649$; (C) $\max_{t < T} H_w(t) = 16.31071$, $\max_{t > T} H_w(t) = 37.058121$.

The present computations changed with increased weight of α (Table 2A), and at α values of 1, 10, 100, $c=0.5$ was increased to $c=0.9$, and $d_h=1.5$ and $m=0.1$ were decreased to 0.5 and 0.001, respectively. Because α is an indicator of allergy without therapy, these changes increased the chances of allergic symptoms. In the result of $\text{score} = \alpha + \beta + \gamma$, all the parameter with top 10% high score achieved $\beta = 0$ and $\gamma = 0$, which implies that symptoms in and after therapy did not occur. On the other hand, enhancing the weight of alpha ($\text{score} = 10\alpha + \beta + \gamma$ and $\text{score} = 100\alpha + \beta + \gamma$), some cases achieved top 10% high score, even either or both of their β and γ are negative, due to very large effect of alpha. This caused change of the cases among the cases with different weight of alpha.

In a similar manner, we considered changes in the weight of β , and compared the results of $\text{score} = \alpha + 10\beta + \gamma$ and $\text{score} = \alpha + 100\beta + \gamma$ with those of the standard method $\text{score} = \alpha + \beta + \gamma$. We also considered changes in the weight of γ , in comparisons of the effects of $\text{score} = \alpha + \beta + 10\gamma$ and $\text{score} = \alpha + \beta + 100\gamma$ with the standard score. β and γ are penalties for having symptoms with therapy, and using the standard score, the top 10% of successful cases did not have symptoms with therapy ($\beta = 0$ and $\gamma = 0$).

Therefore, increasing the weights of β and γ did not change the results from those with the standard score.

In conclusion, successful c , d_h , and m values changed depending on the weight of α (Table 2A). Specifically, increasing the weight of α corresponds with initial choices of participants with severe symptoms, which may lead to different selections of patients for whom allergen immunotherapy is successful than when the 3 components are considered equally.

B.2. Variations of scoring methods

We considered the following 3 variations of scoring methods:

- (1) Application of $H(t)$ peaks that are smaller than θ to the score,
- (2) not considering scores with negative values, and (3) changing the power from 0.1 to 0.5. We also changed weights for α , β , and γ as in Section B.1.

Variation 1: Incorporation of $H(t)$ values below the threshold into the score

To consider peaks of $H(t)$ that were below the threshold, we defined α , β , and γ as follows:

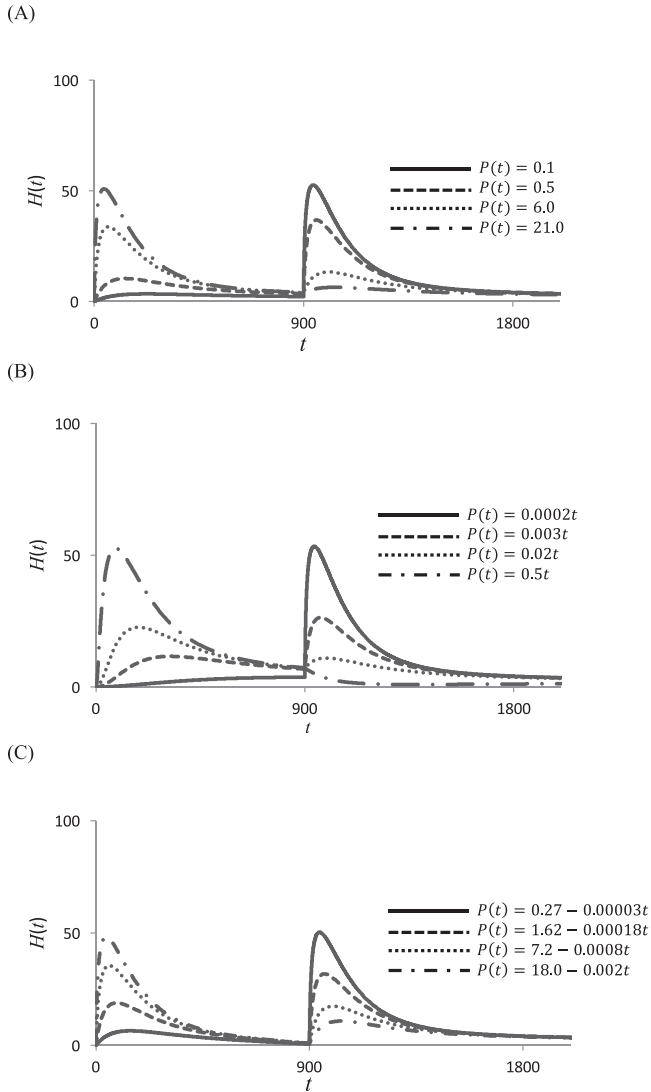


Fig. 10. Influence of changes in total doses during constant administration of pollen. (A) Total pollen doses $P(t)$ in Eq. (3) were 0.1, 0.5, 6.0, and 21.0 and changes in $H(t)$ with time are graphed with fixed parameters A, b, c, d_h, d_r, m, n , and T of 50.0, 10.0, 0.5, 0.01, 0.001, 0.045, 200.0, and 900.0, respectively. Peaks of $H(t)$ in therapy and exposure phases were as follows: $P(t) = 0.1$, $\max_{t < T} H_w(t) = 3.435564$ and $\max_{t > T} H_w(t) = 52.656663$; $P(t) = 0.5$, $\max_{t < T} H_w(t) = 10.375391$ and $\max_{t > T} H_w(t) = 36.828364$; $P(t) = 6.0$, $\max_{t < T} H_w(t) = 33.687784$ and $\max_{t > T} H_w(t) = 13.351538$; and $P(t) = 21.0$, $\max_{t < T} H_w(t) = 50.844979$ and $\max_{t > T} H_w(t) = 6.431192$. (B) We represented doses as $P(t) = st$ ($s > 0$) and varied these with slope s values of 0.0002, 0.003, 0.02, and 0.5. Graphs show changes in $H(t)$ with time. The parameters A, b, c, d_h, d_r, m, n , and T were 50.0, 10.0, 0.5, 0.01, 0.001, 0.045, 200.0, and 900.0, respectively. Peaks of $H(t)$ in therapy and exposure phases were as follows: $s = 0.0002$, $\max_{t < T} H_w(t) = 3.740696$ and $\max_{t > T} H_w(t) = 53.367095$; $s = 0.003$, $\max_{t < T} H_w(t) = 11.644834$ and $\max_{t > T} H_w(t) = 26.332285$; $s = 0.02$, $\max_{t < T} H_w(t) = 22.661222$ and $\max_{t > T} H_w(t) = 10.94445$; and $s = 0.5$, $\max_{t < T} H_w(t) = 52.591565$ and $\max_{t > T} H_w(t) = 7.263804$. (C) We present total doses as $P(t) = u - st$ ($s > 0$ and u is constant) and varied the slope s at -0.00006 , -0.00018 , -0.0008 , -0.002 . Graphs show changes in $H(t)$ with time. The parameters A, b, c, d_h, d_r, m, n , and T were 50.0, 10.0, 0.5, 0.01, 0.001, 0.045, 200.0, and 900.0, respectively. Peaks of $H(t)$ in therapy and exposure phases were as follows: $s = -0.00006$, $\max_{t < T} H_w(t) = 6.45453$ and $\max_{t > T} H_w(t) = 50.320351$; $s = -0.00018$, $\max_{t < T} H_w(t) = 18.848269$ and $\max_{t > T} H_w(t) = 31.80507$; $s = -0.0008$, $\max_{t < T} H_w(t) = 35.47852$ and $\max_{t > T} H_w(t) = 17.374652$; and $s = -0.002$, $\max_{t < T} H_w(t) = 48.134032$ and $\max_{t > T} H_w(t) = 10.789832$.

$$\alpha = \begin{cases} |S_o|^{0.1} & \text{if } S_o \geq 0 \\ -|S_o|^{0.1} & \text{if } S_o < 0 \end{cases}$$

$$\beta = \begin{cases} |S_T|^{0.1} & \text{if } S_T < 0 \\ -|S_T|^{0.1} & \text{if } S_T \geq 0 \end{cases} \quad (\text{A.1})$$

$$\gamma = \begin{cases} |S_E|^{0.1} & \text{if } S_E < 0 \\ -|S_E|^{0.1} & \text{if } S_E \geq 0 \end{cases}$$

We calculated scores as the sum of these components (score = $w_\alpha \alpha + w_\beta \beta + w_\gamma \gamma$) with w_α , w_β , and w_γ as weights of each component at 1, 10, and 100. We calculated the scores for 3^8 parameter sets according to this method.

In this method, $H(t)$ values that were smaller than the threshold for developing symptoms were included in the score, assuming that $H(t)$ values below the threshold can be detected. Using this method, the strength of therapeutic suppression of symptoms can be evaluated even if symptoms do not occur.

With increasing weights of α , c , d_h , and m tend to reflect allergic symptoms, as in Section 4.1 (Table 2B(i)). However, increasing weights of β , a , b , and n result in changes of the main value included in the successful group (Table 2B(ii)). Finally, as the weight of γ gets larger, the most included value of A , b , and n changes (Table 2B(iii)), as described below.

Variation 2: Not considering reductions of scores depending on undesirable conditions for therapeutic success. We calculated scores for 3^{10} parameter sets according to following equations (A.2): α, β , and γ were defined using $[x]_- = x$ if $x \leq 0$, $[x]_- = 0$ if $x > 0$:

$$\alpha = ([S_o]_+)^{0.1}, \quad \beta = ([S_T]_-)^{0.1} \text{ and } \gamma = ([S_E]_-)^{0.1} \quad (\text{A.2})$$

In this method, only the degree of appropriateness for therapy is evaluated, as shown in Table 2C (i), (ii), and (iii). These results are similar to those following (1) application of H values that are smaller than θ to the score. As the weight of α gets larger, the tendencies of c , d_h , and m change (Table 2C(i)), as the weight of β gets larger, tendencies of a , b and n change (Table 2C(ii)), and increases in the weight of γ lead to changes in the tendencies of A , b and n (Table 2C(iii)).

Variation 3: Using the power function of 0.5 in the score

Scores are calculated according to the following equations:

$$\alpha = ([S_o]_+)^{0.5}, \quad \beta = -([S_T]_+)^{0.5} \text{ and } \gamma = -([S_E]_+)^{0.5}. \quad (\text{A.3})$$

The effect of very large $H(t)$ peaks was smaller than the standard score. (Eq. (3); Table 2D). As the weight of α was increased, tendencies of c , d_h , and m changed, and as the weight of β increased, tendencies of a changed, whereas increased weights of γ had little effect.

Among three variations, with the weight of β increased, the small a (0.1) tends to be included in the successful group. Too large amount of pollen dose as therapy, large a , can be risky because it may cause symptoms, and thus small amount of pollen dose is desirable if weight for β is very large.

Results of variations 1 and 2 show similar tendencies (Table 2B and C). Specifically, in addition to the changes of c , d_h , and m with increased weights of α , similar to the results of the standard method, changes in the weight of β resulted in changes of a , b and n and changes in the weight of γ resulted in changes of A , b and n .

If the weight for β is very large, small b (10) and large n (200) are more frequently included in the top 10% of successful cases. Since b represents the number of naïve T cells that differentiate per unit of pollen, when b is large, even mild therapy promotes differentiation of naïve T cells into Th2 cells, indicating negative control for β . Similarly, n represents the number of nTreg cells, which

are produced regardless of differentiation of naïve T cells. Accordingly, nTreg cells prevent symptoms in the early stage of therapy when few iTreg cells have been produced.

With increased weight of γ , the small A ($A=10$) tends to be included in the successful group. Because γ is an indicator of therapeutic effect during the exposure phase, greater importance of γ diminishes the preferred environmental pollen exposure. Thus, to prevent symptoms in the exposure phase, this approach may be used to treat patients who are unlikely to experience symptoms during years when pollen production is small.

Appendix C. Relative peak heights (S_0 , S_T and S_E) and score when parameters are randomly sampled from uniform distribution

In addition to setting three levels of parameters in Section 3, we derived parameters from uniform distribution to perform minute investigation of parameter dependence. We derived 1000 combinations of parameters and plotted the values of three relative peaks (S_0 , S_T and S_E) (Fig. 6A) and score (Fig. 6B).

In Fig. 6A, the figures in the left row represent plots of S_0 , relative peak of $H(t)$ without therapy. Larger A and b value are likely to lead higher S_0 , and smaller m and n , are likely to lead higher S_0 . The figures in the middle row represent plots of S_T , relative peak of $H(t)$ in therapy. The larger a and m parameter values are likely to lead higher S_T , and the smaller θ and m are likely to lead higher S_T . The figures in the right row represent plots of S_E , relative peak of $H(t)$ after therapy. In c , the larger parameter value is likely to lead higher S_E , and in a and m , the smaller parameter value is likely to lead higher S_E .

In Fig. 6B, the plots of score in Eqs. (4), (5a), (5b) and (5c) are shown against each of 10 parameters. In each figure, three clusters can be seen; upper, middle and lower layer of plots. This structure derived from the definition of score; we consider the function of 0.1 power of relative peak heights (Eqs. (5a), (5b) and (5c)) and α , β , or γ approaches a constant value if S_0 , S_T or S_E is very large, respectively. In upper layer, successful cases with score by alpha and without penalties of beta and gamma are plotted. In middle layer, the cases with score of alpha and one penalty for either beta or gamma are plotted and lower layer includes the cases with both penalty of beta and gamma. The black dots represent the cases with top 10% score among 1000 trials. In Section 3, we investigated the cases with top 10% high score among 59,049 combinations of parameters. Similar tendency was observed between the black dots in Fig. 6B and the result of Section 3 in Fig. 3; large value of θ , a , b and m , and small value of c , d , and n are likely to be included in top 10% successful cases.

Appendix D

We defined therapy as successful if $S_0 > 0$, $S_T < 0$, and $S_E < 0$. Some of our scoring methods gave high scores even in unsuccessful cases, in which one or more of the three criteria were violated. Thus, for each scoring method (standard method, variations 1, 2 and 3), we calculated percentages of cases in which the criteria $S_0 > 0$, $S_T < 0$, and $S_E < 0$ are satisfied.

We represented weights of α , β , and γ as w_α , w_β , and w_γ , respectively. In these computations, α , β , and γ are functions of S_0 , S_T , and S_E (see Eqs. (5a), (5b), and (5c)). Black bars indicate percentages of cases satisfying $S_0 > 0$, striped bars represent percentages of the cases satisfying $S_T < 0$, and white bars represent percentages of the cases satisfying $S_E < 0$. Bar graphs correspond with the weights w_α , w_β , and w_γ .

In the standard scoring method (Fig. 7A), percentages of successful cases in terms of S_T and S_E decreased with increasing w_α .

Prioritization of S_0 may violate the conditions for S_T and S_E , leading to failure of therapy.

In the variations 1 and 2 (Fig. 7B and C), high priority of S_0 (w_α , w_β , $w_\gamma = 10, 1, 1$ or $100, 1, 1$) and extremely large weights of S_T and S_E (w_α , w_β , $w_\gamma = 1, 100, 1$ or $1, 1, 100$, respectively), may violate one or two criteria, and may lead to errors in choices of patients.

In the variation 3 (Fig. 7D), the three components were weighted equally (1, 1, 1) and could lead to failure to choose patients with sufficient margins of safety in therapy and exposure. Because the power was 0.5 (Eq. (A.3)), one component could be very large and achieve a high score even if the other two criteria are violated. Increasing weights of β and γ improves this situation, although power of 0.5 may be insufficient to weaken very large values of one component if the three components are weighted equally.

Appendix E. Temporal patterns of administration

E.1. Intervals and amounts of pollen doses

Frequency of treatments with pollen extract is an important aspect of therapeutic schedules. We set I as the dosing interval (time t between successful treatments with pollen extract). We also considered (A; $I=10$) administration of pollen 90 times at 50 (Fig. 8A), (B; $I=20$) 45 times at 100 (Fig. 8B), and (C; $I=60$) 15 times at 300 (Fig. 8C). Under all three conditions the total amount of pollen taken by the patient is 4500, and the whole phase for the therapy is constant at $T=900$ days. We examined the two peak values of $H(t)$ to indicate the magnitude of symptoms. If the total administered amount was the same, symptoms induced by therapy tended to be more severe when I was larger, reflecting larger doses given less frequently. In contrast, symptoms during the exposure phase did not vary with I .

E.2. Constant, increasing, and decreasing doses

- (i) *Gradual changes in daily doses over time (constant, increasing, or decreasing)*

Fig. 9 illustrates comparisons of (A) fixed pollen doses of 50 (Fig. 9A), (B) increasing doses with time following $P(t)=0.1099t$ (Fig. 9B), and (C) decreasing pollen doses with time following $P(t)=98.91-0.1099t$ (Fig. 9C). Total doses were the same for each treatment regimen. Treatments with increasing doses (B) suppressed symptoms during therapy most successfully, whereas method (C) caused the most severe symptoms during therapy. During the exposure phase, symptoms were most severe with method (C). Hence, method (C) produces worse outcomes than methods (A) and (B). Moreover, increasing doses (B) with time tended to gradually suppress $H(t)$ peaks during the exposure phase without causing symptoms during therapy.

- (ii) *Total doses of treatment schedules*

Treatments with larger amounts of pollen before exposure to environmental pollen should lead to greater accumulation of Treg and enhanced resistance to pathogens. Therefore, total doses should correspond with therapeutic effects. However, administration of large amounts of pollen during the therapy phase increases the risk of symptoms during the therapy phase. Thus, we examined the effects of changing total doses in each of the treatment schedules (A), (B), and (C).

Initially, we adopted a constant dose and made observations at $P(t)=0.1, 0.5, 6.0$, and 21.0 (Fig. 10A). Single doses need to be within a certain range to optimize effects and side effects. At

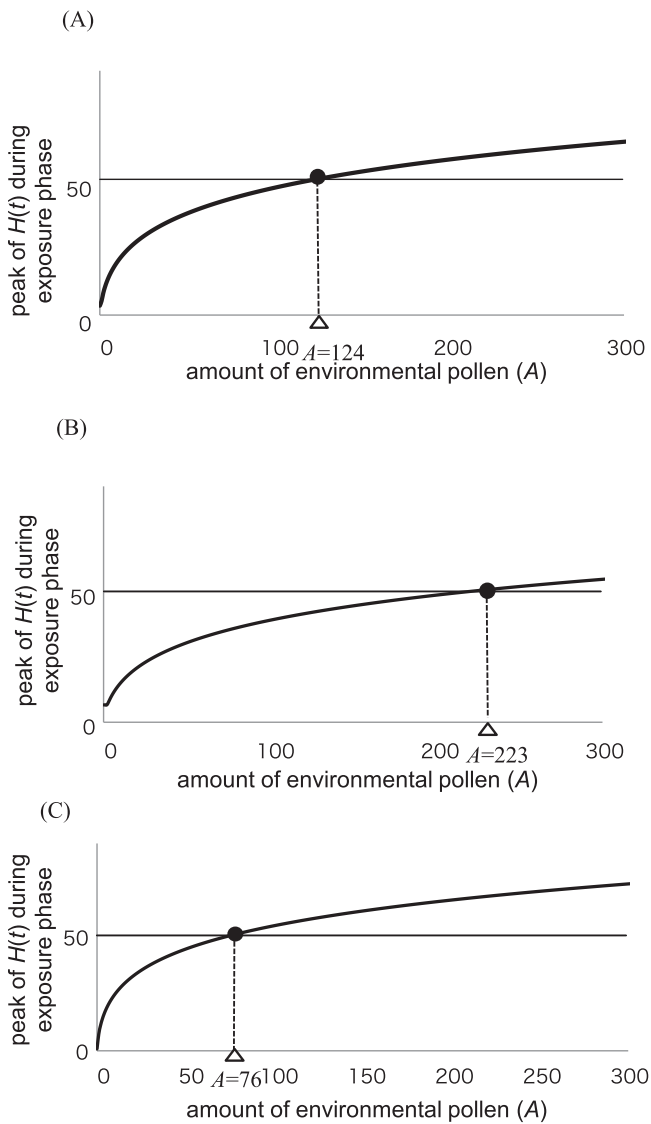


Fig. 11. Suppressive effects of constant and increasing pollen treatments. The administration schedules $P(t)=0.4705$, $P(t)=0.000204t$, and $P(t)=0.5202-0.0000578t$ were chosen to represent constant, increasing, and decreasing doses, respectively. $H(t)$ peaks in the therapy phase were all close to 10. The vertical line shows $\max_{t>T} H_w(t)$ values and the horizontal line shows the environmental pollen exposure A . Using gradual changes in A , we determined the maximum value of A for which $\max_{t>T} H_w(t)$ was kept at less than or equal to the threshold θ . The method that achieves the larger A value should lead to better resistance to pollen exposure. (A) For $P(t)=0.4705$, $H(t)$ reached the threshold θ at around $A=124$, (B) for $P(t)=0.000204t$, $H(t)$ reached the threshold θ at around $A=223$, and (C) for $P(t)=0.5202-0.0000578t$, $H(t)$ reached the threshold θ at around $A=76$.

$P(t)=0.1$, the total dose was too small to suppress symptoms after therapy, whereas at $P(t)=21.0$, single doses were too large and caused symptoms. At $P(t)=0.5$ and 6.0 , both peaks of $H(t)$ did not exceed the threshold θ and the therapy was successful.

Subsequently, we examined the effects of gradual dose increases following $P(t)=st$ (where s is speed $s>0$) instead of a constant value, and observed changes in slope s (Fig. 10B). s was optimized for therapeutic efficacy with no side effects. When $s=0.0002$, symptoms were not suppressed after therapy, whereas successful therapy was achieved with $s=0.003$ and 0.02 and $s=0.5$ caused symptoms.

In further computations, we examined the effects of gradual dose decreases in the shape of $P(t)=s(T-t)$ (where s is the speed

of increase) and increased total amounts with s (Fig. 10C). The dose reached zero at T : $P(T)=0$ and when $s=0.00003$. Hence, the therapy was too weak to suppress symptoms during the exposure phase. When $s=0.00018$ and 0.0008 , $H(t)$ peaks were low in both therapy and exposure phases. When s was 0.002 , the peak in the exposure phase was the highest and the risk of symptoms during therapy was the largest.

(iii) Therapeutic effects under conditions of symptom control

Suppressive effects were compared between constant (Fig. 9A), increasing (Fig. 9B), and decreasing pollen doses (Fig. 9C) to determine which method suppresses symptoms at larger values of A (daily pollen dose during the exposure phase). We assumed that higher pollen dispersal would produce better therapeutic effects and represented the methods as $P(t)=0.4705$, $0.000204t$, and $0.5202-0.0000578t$, respectively. All treatment schedules produced symptoms with degrees of 10.

Subsequently, we represented methods (a), (b), and (c) as $P(t)=19.85$, $0.0397t$, and $0.5202-0.0000578t$, respectively, and compared symptom suppression with increasing pollen dispersal. Under gradual increases of released pollen (A), H reached the threshold θ (Fig. 11A), and at $P(t)=0.4705$, H reached the threshold θ at around $A=124$. When $P(t)=0.000204t$, H reached θ at around $A=223$ (Fig. 11B) and when $P(t)=0.5202-0.0000578t$, H reached θ at around $A=76$ (Fig. 11C). Therefore, increasing doses with time suppressed symptoms during larger environmental pollen exposures than for the other treatment schedules. These computations indicate that increasing dose schedules has therapeutic effect on greater pollen dispersal than other methods.

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