Supplementary Text 1

Herein, we describe the procedure of the simulation conducted in our study.

Step 1: Setting the parameter values.

The parameter values are set as:

the fractions of Tconv and Treg in the immune system are given by $R_C = 0.9$, $R_R = 0.1$, respectively

the index k used to indicate the kind of T cells, that is Tconv or Treg, is given by $k \in \{C, R\}$

the number of types of TCR, $n = 9$

the index i used to indicate the type of TCR is given by $i \in \{1,2,3,,,\mathrm{n}\}$

the initial number of T cells in the lymph node, $Num^T = 1000$

the number of APCs, $Num^{APC} = 10$

the index j used to indicate an APC is given by $j \in \{1,2,3,,,Num^{APC}\}$

the effect of the IL-2 receptors inducing proliferation of Tconvs and Tregs are given by $E_C = 1$, $E_R = 5$, respectively

the parameter of proliferation, $A^{max} = 0.01$

the parameter of migration out of the lymph node is given by $B = 0.01 / Num^T$

the parameter of migration into the lymph node, $G = 0.01$

the parameter of amplification of the degree of T-APC interaction due to activation of APC-bound T cells, $alpha^{max} = 0.2$

the parameter of dissociation from APC, $beta^{max} = 0.005$

the parameter of new association of a T cell to APC, $gamma^{mini} = 0.01$.

the maximal extent of the change in dissociation probability (β) is M^b. $M^b = 0.1$ in most simulations except for that shown in Supplementary Figure 2, where M^b is indicated on the x-axis.

the maximal extent of the change in new association probability (γ) is M^g. $M^g = 10$ in most simulations except for that shown in Supplementary Figure 2, where M^g is indicated on the y-axis.

the affinities of Tconv and Treg are given by $\varepsilon_i = \frac{i}{n+1}$, $i \in \{1,2,3,,,\mathrm{n}\}$

the fraction of Tconv with i-th TCR affinity in the whole immune system is $WIS_{i,C}$

the fraction of Treg with i-th TCR affinity in the whole immune system is $WIS_{i,R}$

($WIS_{i,k}$ are set up in Step 2)

The dynamic variables are introduced as follows:

$LN_{i,C}$ is the number of Tconv with *i*-th TCR affinity in the lymph node.
$LN_{i,R}$ is the number of Treg with *i*-th TCR affinity in the lymph node.

$TAPC_{i,C}^{j}$ is the variable representing the degree of T-APC interaction between *j*-th APC and Tconv with *i*-th TCR affinity.

$TAPC_{i,R}^{j}$ is the variable representing the degree of T-APC interaction between *j*-th APC and Treg with *i*-th TCR affinity.

(Initial conditions of $LN_{i,k}$ and $TAPC_{i,k}^{j}$ are set up in Step 3. $LN_{i,k}$ changes during Step 4 and 5. $TAPC_{i,k}^{j}$ changes during Step 6.)

IL_j is the variable between 0 and 1 representing the amount of cytokine IL-2 produced by Tconv on *j*-th APC.

$\langle IL \rangle$ is the average of IL; $\langle IL \rangle = \frac{\sum_{j}^{Num^{APC}} IL_j}{Num^{APC}}$.

APC_j^a, APC_j^b, and APC_j^g are variables which represent the state of *j*-th APC. They are values between 0 and 1, and interpolate the effects of amplification of the degree of T-APC interaction (α, Step 6.4), dissociation (β, Step 6.5) and new association (γ, Step 6.2), respectively. The initial APC_j are set to 0, and a high Tconv/Treg ratio on *j*-th APC increases the variables up to 1 as calculated in Step 6.3. They are dynamic variables when Tregs are assumed to affect the state of APC, while they are fixed at 0 or 1 otherwise, depending on simulations.

CR_j is a binarized value representing Tconv/Treg ratio on *j*-th APC. CR_j is set to 1 (Step 6.3), if Tconv/Treg ratio in T-APC$_j$ interaction degree is higher than 9 at each time point. Otherwise, CR_j is set to 0.

Step 2: Setting the T cell fractions in the whole immune system in non-self and self conditions.

In non-self condition without negative or positive selection, the relative number of T cells with affinity $\varepsilon_i = \frac{i}{n+1}$ are given as

$$WIS_{i,k} = R_k \exp(n-i) \Big/ \sum_{l=1}^{n} \exp(n-l)$$

, where exp() indicates the exponential function, $\exp(n-i) \equiv e^{n-i}$. Self-antigens modify the TCR repertoire during development in the thymus. Negative selection reduces the fraction of T cells with high affinities to self-antigens. We assume that the reduction linearly correlates with the affinity down to 0.1 times. Some T cells with high affinities to self-antigens are positively selected to become Tregs. We assume that the probability of differentiation to Tregs linearly correlates with the affinity up to 100 times. To modify the affinity distribution through these positive and negative selections, the number of type $k \in \{C, R\}$ T cells with *i*-th TCR, $\exp(n-i)$, is multiplied by the following; $\left(\frac{Sel_{pos}^{k}(n-i)+i-1}{n-1}\right)\left(\frac{n-i+Sel_{neg}^{k}(i-1)}{n-1}\right)$. Here, we set the parameter values as follows; $Sel_{pos}^{C}=1$, $Sel_{neg}^{C}=0.1$ for Tconvs and $Sel_{pos}^{R}=0.01, Sel_{neg}^{R}=0.1$ for Tregs in self condition. In non-self condition, each *Sel* is set to 1. After this selection process, we normalize the Tconv and Treg fractions to 0.9 and 0.1, respectively, and set $WIS_{i,C}$ and $WIS_{i,R}$ for the fractions of Tconv and Treg with a particular affinity *i* in the whole immune system.

Step 3: Setting the initial conditions.

To prepare $LN_{i,k}$ that represents the number of each type of T cells in the lymph node, we randomly selected one T cell with index *i*,*k* depending on the fraction distribution (*WIS*), add 1 to the lymph node ($LN_{i,k}$), and repeat the process Num^{T} times. For simulations Supplementary Figure 2, five kinds of initial $LN_{i,k}$ are saved and used throughout the series of simulations.

The variable representing the degree of T-APC interaction between *j*-th APC and *k* type of T cells with *i*-th TCR affinity ($TAPC_{i,k}^{j}$) is set to 0 for any APC and T cell at the initial time-point.

The variables representing the amount of cytokine IL-2 produced by Tconv on *j*-th APC (IL_j) are set to 0 for any APC. The variables representing the state of *j*-th APC (APC_j^a, APC_j^b, and APC_j^g) are set to 0 at the initial time-point for any APC in simulation where α, β, or γ are labeled with H~L or L~H in the figure, which means that the initial APC state is set to be equivalent to that of APC with high Treg ratio and changes depending on the Tconv/Treg ratio on the APC. In some simulations where α, β, and γ are high, high, and low in the figure, APC_j^a, APC_j^b, and APC_j^g are fixed to 1. APC_j^a, APC_j^b, and APC_j^g are fixed to 0, when α, β, and γ are low, low, and high, respectively.

The time count is set to $t = 0$.

Step 4: Checking cell division.

One APC with index *j* is randomly chosen from 1 to Num^{APC}. Then one T cell with index *i,k* is randomly selected depending on the distribution of T-APC interaction degree ($TAPC^j$), if there are any T cells interacting with *j*-th APC. (Here, we assume that resources on an APC to activate T cells are limited and competed for among interacting T cells. This assumption would be supported by the facts that the number of APCs affects the degree of T cell proliferation.)

The selected T cell divides with a probability

$$\varepsilon_i \, E_k \, A^{max}(0.01 + 0.99 \, \langle IL \rangle),$$

where $\langle IL \rangle$ is the average of IL_j that represents the amount of cytokine IL-2 produced by Tconv on *j*-th APC; $\langle IL \rangle = \frac{\sum_j^{Num^{APC}} IL_j}{Num^{APC}}$. By adding 1 to $LN_{i,k}$, a successful division increases the number of T cells in the *k,i*-type population in the lymph node by one.

Step 5: Checking the migration of T cells into/out of the lymph node.

One T cell in the whole immune system migrates into the lymph node with a probability *G*. One T cell with index *i,k* is randomly selected depending on the distribution *WIS*. Then we increase one T cell with the selected type in the lymph node, by adding 1 to $LN_{i,k}$.

Each T cell in the lymph node either migrates out or dies with a probability *B*. Each value in *LN* decreases, based on a binomial distribution.

Step 6: Checking the condition of T-APC interaction.

For each APC with index *j*, the following steps are performed.

Step 6.1: Checking death of the APC

An APC is replaced by a new APC at a probability of $1/10^4$. In this probability, *j*-th APC takes the initial conditions regarding the T cell interaction ($TAPC^j = 0$) and parameter conditions ($APC_j = 0$ in simulation where they are not fixed parameters but variables).

Step 6.2: Checking the new association of a T cell to the APC.

One T cell in the lymph node newly interacts with APC_j with a probability

$$gamma^{mini} \, M^g / \left(1 + \, (M^g - 1) \, APC_j^g \, \right),$$

where M^g is the extent of change. In most simulations except for Supplementary Figure 2, $M^g = 10$.
To determine the type of the associating T cell, we randomly select one T cell with index *i,k* depending on the distribution *LN*, and increase one interaction degree of the selected type of T cell on *j*-th APC, by adding 1 to $TAPC_{i,k}^{j}$.

Step 6.3: Checking the parameter conditions of the APC.
If Tconv interaction degree over Treg interaction degree on *j*-th APC are higher than the original Tconv/Treg ratio (R_C / R_R = 9), CR_j is set to 1. Otherwise, CR_j is set to 0. Based on the Tconv/Treg ratio on the APC, the APC condition is calculated:

$$APC_j^a = 0.9\, APC_j^a + 0.1\, CR_j$$
$$APC_j^b = 0.9\, APC_j^b + 0.1\, CR_j$$
$$APC_j^g = 0.9\, APC_j^g + 0.1\, CR_j$$
$$IL_j = 0.999\, IL_j + 0.001\, CR_j$$

In some simulations, Tregs are assumed not to regulate the probabilities of amplification, dissociation, or new association for the change of T-APC interaction degree. When α, β, and γ are set as high, high, and low, respectively, APC_j^a, APC_j^b, and APC_j^g are fixed to 1. APC_j^a, APC_j^b, and APC_j^g are fixed to 0, when α, β, and γ are set as low, low, and high, respectively.

Step 6.4: Checking the amplification of the degree of T-APC interaction.
One T cell on *j*-th APC increases the degree of T-APC interaction stochastically due to affinity-dependent activation. We randomly select one T cell with index *i,k* depending on the distribution of T-APC interaction degree ($TAPC^j$). With a probability

$$\varepsilon_i\, alpha^{max}\left(0.1 + 0.9\, APC_j^a\right),$$

we increase one interaction degree of the selected type of T cell, by adding 1 to $TAPC_{i,k}^{j}$.

Step 6.5: Checking the dissociation of T cells from the APC.

Any T cells on *j*-th APC decrease the degree of T-APC interaction by dissociation with a probability

$$beta^{max}\left(M^b + (1 - M^b)\, APC_j^b\right),$$

where M^b is the extent of change. In most simulations except for Supplementary Figure 2, $M^b = 0.1$.

Each value in $TAPC^j$ decreases, based on a binomial distribution.

Step 7: Set t = t +1 and go to Step 4.

Repeat the cycle until $t = 10^5$.

Supplementary Text 2

Herein, we show a demonstration of $\langle\varepsilon\rangle \approx \varepsilon_h$ and $f_h \approx 1$ at the equilibrium, when $\alpha\Delta\varepsilon >> \gamma$ in the mathematical model of the degree of T-APC interaction. The symbol $\Delta\varepsilon$ is the difference between the highest affinity and second-highest affinity among the T cell populations.

The average affinity can be calculated from the fraction f_h with the affinity ε_h and the fraction (1- f_h) with the affinities less than (ε_h - $\Delta\varepsilon$): $\langle\varepsilon\rangle \leq \varepsilon_h f_h + (\varepsilon_h - \Delta\varepsilon)(1 - f_h)$. This is the same: $\Delta\varepsilon(1 - f_h) \leq \varepsilon_h - \langle\varepsilon\rangle$.

On the other hand, from equation (e4), one has that $\alpha(\langle\varepsilon\rangle - \varepsilon_h) + \gamma > 0$, which implies $\varepsilon_h - \langle\varepsilon\rangle < \gamma/\alpha$.

In summary, our model implies $\Delta\varepsilon(1 - f_h) \leq \varepsilon_h - \langle\varepsilon\rangle < \gamma/\alpha$.

Finally, if $\alpha\Delta\varepsilon >> \gamma$ then $\gamma/\alpha \ll \Delta\varepsilon < 1$ and hence: $\gamma/\alpha\,(1 - f_h) \ll \Delta\varepsilon(1 - f_h) \leq \varepsilon_h - \langle\varepsilon\rangle < \gamma/\alpha \ll 1$. From this, it follows that $(1 - f_h) \ll 1$, that is $f_h \approx 1$,

and $\varepsilon_h - \langle\varepsilon\rangle \ll 1$, that is, $\langle\varepsilon\rangle \approx \varepsilon_h$.

Supplementary Text 3

Herein, we describe a mathematical model that represents the change in T cell numbers in a lymph node.

We constructed a macroscopic model of T cell numbers in an individual compartment (e.g. lymph node). We assumed that the T cell numbers increase with activation-dependent cell division and migration into the lymph node, and decrease by migrating out of the lymph node or death (the middle layer in Fig. 4). While we focused on the stable tolerant state or events just after exposure to foreign antigens, we could use the microscopic model of T-APC interaction in the main text to determine the cell-division rate. The lymph node in this macroscopic model is equivalent to the T cell pool in the microscopic T-APC interaction model. We assumed that division of T cells in *i*-th T cell population is proportional to TCR-signaling intensity, represented in the first term of the right-hand side of equations (e2) as $\frac{\alpha\varepsilon_i x_i}{\sum_j x_j} \equiv \alpha\varepsilon_i f_i$. Thus, the cell-division rate can be expressed by $AE_i\alpha\varepsilon f_i$, where AE_i is a proportional constant representing cell division due to the intensity of TCR signaling. The symbol *A* includes the amount of inflammatory cytokines and the number of APCs in the lymph node. The symbol E_i denotes the characteristics of the population, e.g., Tconv or Treg. In addition, as proliferation takes longer than changes in T-APC interaction degree (days versus minutes), we estimate $f_i \equiv$

$\frac{x_i}{\sum_j x_j}$ at the equilibrium of the T-APC interaction model as shown in equation (e4), $f_i \approx \frac{\gamma p_i}{\alpha(\langle\varepsilon\rangle - \varepsilon_i) + \gamma}$. Then, the time evolution of the number of T cells belonging to the *i*-th T cell population, X_i, in a lymph node was modeled as equation (e5).

$$\frac{d}{dt} X_i = \frac{\alpha\varepsilon_i\gamma AE_i}{(\alpha(\langle\varepsilon\rangle - \varepsilon_i) + \gamma)} \frac{X_i}{\sum_j X_j} - BX_i + GP_i \quad \text{(e5)}$$

The parameter p_i, which is the fraction of the *i*-th T cell population in the T cell pool, was replaced by $\frac{X_i}{\sum_j X_j}$. The symbol *B* is the rate of decrease in the number of T cells due to migration or death per unit time. The symbol *G* represents the overall rate of T cell migration into the lymph node per unit time, and P_i is the fraction of the *i*-th population in the whole immune system. Strictly speaking, $\langle\varepsilon\rangle$ changes depending on the T cell fractions in the lymph node, $\frac{X_i}{\sum_j X_j}$, however we estimated that $\langle\varepsilon\rangle$ calculated from $\frac{X_i}{\sum_j X_j}$ at time *t* approximates to that at $t + \Delta t$ at least in the stable tolerant state where X_i change little.

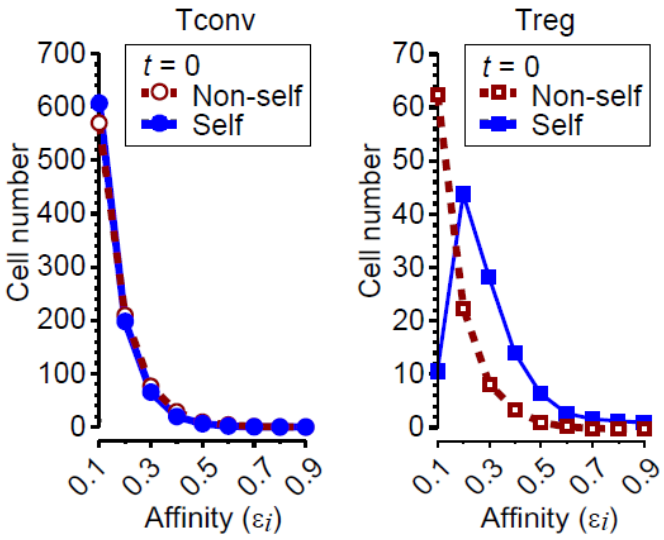
If a binding affinity satisfies the condition (e6), the *i*-th T cell population proliferates at a rate higher than that at which it decreases.

$$\varepsilon_i > \frac{(\alpha\langle\varepsilon\rangle + \gamma)B\sum_j X_j}{\alpha\left(B\sum_j X_j + \gamma AE_i\right)} \quad \text{(e6)}$$

This proliferation threshold means that the sum of the first two terms of the right-hand side of equation (e5) is a positive value. It is largely dependent on the T cell fractions on APCs. If amplification of existing T-APC interaction with positive feedback is the major

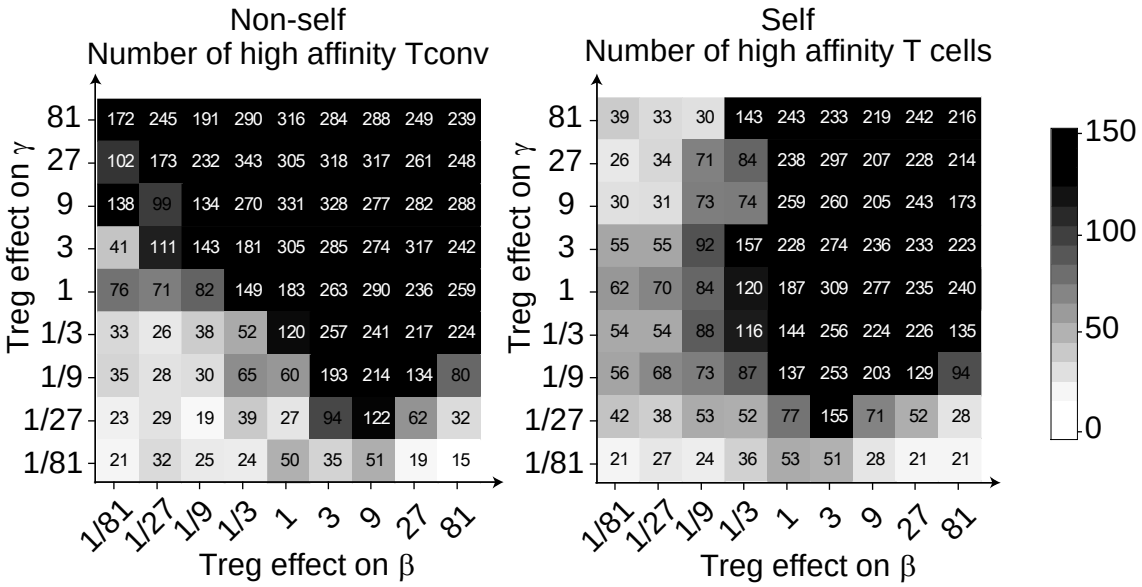
contributor to the increase in the degree of T-APC interaction, $\alpha\Delta\varepsilon \gg \gamma$, T cells with the highest affinity ($i = h$) dominate the interactions on APCs (Fig. 6A and Supplementary Text 2), which means $f_h \approx 1$, and the first terms of the right-hand side of equation (e5), which is $AE_h\alpha\varepsilon_h f_h$, approaches $AE_h\alpha\varepsilon_h$. The proliferation threshold for T cells with the highest affinity is $\varepsilon_h > \frac{BX_h}{\alpha AE_h}$. The threshold values for other T cells with lower affinities are $\varepsilon_{i\neq h} > \frac{\varepsilon_h B\sum_j X_j}{B\sum_j X_j + \gamma AE_i}$, due to $\langle\varepsilon\rangle \approx \varepsilon_h$. On the other hand, if new association of T cells to APCs is more important for the increase in T-APC interaction degree, $\alpha \ll \gamma$, the number of rare T cell populations with high affinity does not always increase ($X_i \to \frac{GP_i\sum_j X_j}{B\sum_j X_j - \alpha\varepsilon_i AE_i}$) and the proliferation threshold values are $\varepsilon_i > \frac{B\sum_j X_j}{\alpha AE_i}$, due to $\frac{x_i}{\sum_j x_j} \approx \frac{X_i}{\sum_j X_j}$. To illustrate these results, we considered three T cell populations with a particular given affinity (P_1 = 0.01, rare specific T cells), intermediate affinity (ε_2 = 0.2, P_2 = 0.1, self-reactive T cells), or low affinity (ε_3 = 0.01, P_3 = 0.89, non-specific T cells) and calculated the proliferation threshold based on the condition (e6) in Supplementary Figure 3. The proliferation threshold for the 1st rare specific T cells to increase in number was plotted against the cell-division parameter A. The high thresholds in a region with low A and high γ in Supplementary Figure 3 indicate inhibition of rare T cells with high affinity to proliferate in this parameter condition. The low thresholds in a region with high A and high γ indicate that T cells

with moderate affinities might proliferate in this situation, explaining the experiment results that Tregs under strong stimulatory conditions augmented allogeneic T cell proliferation (Fig. 1, 2).

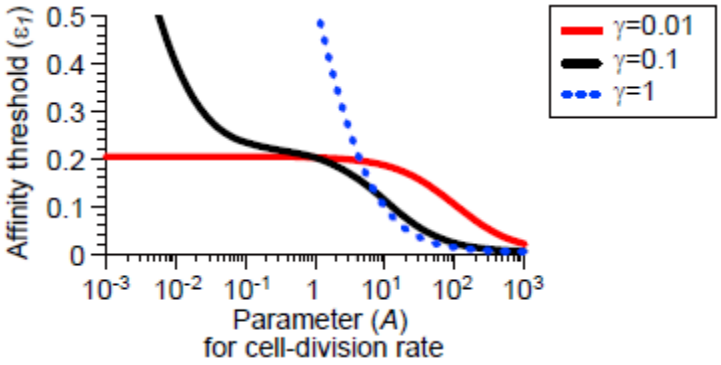


Supplementary Figure 1. The presumed TCR-affinity distributions in simulations.

The TCR affinity (ε_i) distributions of Tconvs and Tregs in the lymph node at the initial time-point (t = 0) in non-self and self conditions. The mean initial T cell numbers in 15 × 4 simulations used in Fig. 5C-F are shown. The data are same to those shown in Fig. 5A-B. The distributions are almost equivalent to the presumed affinity distributions in the whole immune system. Those in self condition are affected by positive and negative selections, as calculated in Step 2 in Supplementary Text 1.



Supplementary Figure 2. Increase in the number of high affinity T cells in the simulation model where Tregs control β and γ. The ratio of Treg/Tconv on an APC is assumed to regulate the dissociation probability (β) and the association probability (γ) between 1 and the indicated extents on the axes. When the Treg/Tconv ratio on an APC < 1/9, the probabilities change from the indicated extent to 1. The condition in Fig. 5C is equivalent to the data on (1, 1) and Fig. 5H are those on (1/10, 10). The mean numbers of high affinity ($\varepsilon_i \geq 0.5$) Tconv in non-self condition and those of high affinity T cells in self condition at $t = 10^5$ in five simulations are shown. Large expansions in the high affinity Tconv population in non-self condition and small expansions of the high affinity T cell population in self condition were observed only where high Treg/Tconv ratio on each APC was assumed to decrease β and increase γ (upper left regions).



Supplementary Figure 3. The proliferation threshold for the T cell population to increase in the lymph node in the macroscopic mathematical model. The threshold of affinity for increase in the number of specific T cells among three T cell populations; specific T cells (P_1 = 0.01, ε_1 is indicated on the Y axis), self-reactive T cells (P_2 = 0.1, ε_2 = 0.2), and non-specific T cells (P_3 = 0.89, ε_3 = 0.01). The x-axis indicates the parameter A value for cell-division rate. $\alpha = 1$, $B\sum_j X_j = 1$. E_i = 1, which means that the difference of cell-division rate due to cell types (e g. Treg or Tconv) is ignored in this calculation. Details are described in Supplementary Text 3.