



# Optimal T cell cross-reactivity and the role of regulatory T cells

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## AUTHOR HIGHLIGHTS

- A probabilistic model of T cell specificity was extended to include Tregs.
- We examined how Treg-suppression of dendritic cells affects immune performance.
- Probability of successful immune response is low for realistic T cell specificity.
- DC suppression alone is insufficient to provide an evolutionary benefit of Tregs.

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## ABSTRACT

The T lymphocytes of the adaptive immune system constitute a highly diverse repertoire of clones expressing a unique T cell receptor (TCR). It has been argued that TCRs are cross-reactive, meaning that one receptor can recognize a multitude of epitopes. Cross-reactivity between self and foreign epitopes can potentially lead to autoimmune responses. Regulatory T cells (Tregs) down-regulate immune reactions, and play an important role in the avoidance of autoimmunity. We use a probabilistic modeling approach to investigate how suppression of antigen-presenting dendritic cells (DCs) by Tregs influences the probability of mounting a successful immune response against a pathogen while remaining self-tolerant. For T cell cross-reactivity values close to experimental estimates, we find that the presence of Tregs increases this success probability somewhat. However, the probability of a successful immune response remains relatively low for these cross-reactivity values, and the probability of success is optimized when T cells are more specific and no Tregs are formed. We conclude that DC suppression on its own is insufficient to provide an evolutionary benefit of regulatory T cells. Rejecting one intuitively likely hypothesis for the function of Tregs thus narrows down the search for the mechanisms by which they are suppressing inappropriate immune responses.

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## 1. Introduction

The vertebrate adaptive immune system consists of a large, highly diverse repertoire of T and B lymphocyte clones, each characterized by a unique receptor. This receptor diversity is generated by somatic recombination during lymphocyte development. In this random recombination process, lymphocytes with specificity to any epitope are formed. Since this includes self-epitopes, a potentially harmful pool of self-reactive lymphocytes needs to become tolerant or down-regulated.

To prevent autoimmunity, maturing lymphocytes undergo a process known as clonal selection (Burnet, 1959). In the case of T cell development in the thymus, cells are first positively selected for a minimum affinity to self-peptide-MHC (pMHC) complexes to increase their interactions with the particular MHC molecules of their host. Thymocytes passing this positive selection step then undergo negative selection, in which lymphocytes recognizing self-pMHC with high

affinity become tolerant or are deleted by apoptosis (Kappler et al., 1987). By eliminating most self-reactive T cells in the thymus, negative selection accounts for a large reduction of the risk of autoimmunity. However, although many self-antigens are expressed in the thymus (Adamopoulou et al., 2013; Pinto et al., 2013), negative selection is unlikely to be complete. First, it is unlikely that all self-epitopes are presented in the thymus, and second, a lymphocyte need not be able to scan all presented self-epitopes during the limited selection period (Muller and Bonhoeffer, 2003). Indeed, it has been shown that self-reactive T cells are commonly present in the peripheral T cell repertoire (Sakaguchi and Sakaguchi, 1990; Jordan et al., 2000; Anderton and Wraith, 2002).

Incomplete self-tolerance induction poses strong constraints on lymphocyte specificity. A previous modeling study (Borghans et al., 1999) showed that incomplete tolerance induction requires that T cells should have a low degree of cross-reactivity,  $p$ , which is defined as the probability that a given T cell responds to a randomly chosen

epitope. If self-tolerance induction in the thymus would be complete, the optimal cross-reactivity is predicted to be inversely related to the number of self-epitopes (De Boer and Perelson, 1993). If tolerance induction is incomplete, however, the diversity of the potential repertoire becomes the most important parameter determining the optimal cross-reactivity (Borghans et al., 1999): the larger the potential repertoire, the lower the optimal cross-reactivity.

The cross-reactivity of T cells is experimentally observed as the precursor frequency, i.e. the fraction of the naïve repertoire responding to a novel epitope. These estimates vary around  $10^{-6} \leq p \leq 10^{-5}$  (Askonas et al., 1982; Owen et al., 1984; Merckenschlager et al., 1988; Mason, 1998; Blattman et al., 2002; Frankild et al., 2008; Ishizuka et al., 2009; Wooldridge et al., 2012). Although these observed frequencies are close to the theoretical estimates when self-tolerance induction is complete (De Boer and Perelson, 1993), they seem dangerously high when tolerance is incomplete (Borghans et al., 1999). Using a similar modeling approach, we here study whether regulatory T cells could help to prevent autoimmunity at the observed degree of T cell cross-reactivity.

Regulatory T cells (Tregs) are a subclass of CD4+ T lymphocytes that suppress rather than induce immune responses in the periphery. Tregs play a key role in the prevention of autoimmunity. Depletion of regulatory T cells has been shown to induce autoimmune diseases, while their reconstitution can prevent them (Sakaguchi et al., 1995). Tregs are developmentally classified as “natural” or “induced”. Natural Tregs form the majority and are produced in the thymus as a functionally mature and distinct population, whereas induced Tregs develop from normal naïve T cells in the periphery under tolerating milieus of antigenic stimulation, especially in suppressing cytokine milieus (Roncarolo et al., 2006). Thymal development of natural Tregs involves strong interactions with self-pMHC complexes (Sakaguchi et al., 2008; Hsieh et al., 2012), that would normally lead to negative selection and apoptosis (Stritesky et al., 2013). We here study natural Tregs that recognize at least one self-epitope in the thymus.

Activated regulatory T cells can suppress a wide range of immune cells including CD4+ and CD8+ T cells, natural killer (NK) and NKT cells, B cells and antigen-presenting cells (APCs) in vitro and in vivo (Sakaguchi et al., 2008). Many suppression mechanisms of regulatory T cells have been reported (reviewed in Vignali et al., 2008; Shevach, 2009), but the relative contributions of these mechanisms to the functioning of Tregs remain unclear. We here investigate the effect of Tregs on antigen presenting dendritic cells (DCs), for which at least two distinct suppression mechanisms have been suggested. First, the aggregation of Tregs on a DC may prevent conventional naïve T cells to access the DC and/or function as a sink for IL2 in the local micro-environment. Second, Tregs can directly down-regulate the expression of costimulatory molecules (CD80/86) on the DC (Tadokoro et al., 2006; Onishi et al., 2008). In both cases, the Treg can be thought to “shut down” the activated DC.

It has been argued that Tregs can down-regulate immune reactions to pathogens and cancer cells as well (Cools et al., 2007). Indeed, Tregs can be specific for pathogens (Zhao et al., 2011), and are able to disturb the early T cell response to these pathogens (Shafiani et al., 2010). It is not known how Tregs decide between immune reactions that should be suppressed and those that should not. The balance between their successful suppression of potentially dangerous autoimmune reactions, and their detrimental suppression of pathogen-specific immune reactions, is crucial in our understanding of the functioning and evolution of regulatory T cells.

Probabilistic models have been used earlier to investigate the evolutionary and functional constraints on the adaptive immune system, in particular on T cell cross-reactivity (e.g. De Boer and Perelson, 1993; Borghans et al., 1999). Here, we extend this probabilistic framework to include Tregs by modeling the following

scenario. Consider a lymph node (LN) harboring a functional T cell repertoire of naïve T cells and natural Tregs. Activated DCs draining into this LN from an infected tissue present both foreign and self-epitopes on their surface, and will be scrutinized by most naïve T cells arriving in the LN. A conventional naïve T cell recognizing any of the epitopes presented on the DC will be activated and will proceed to mount an immune response to the pathogen or, in the case of a self-reactive T cell, against self. Once the DC is recognized by a Treg, we suppose that the DC is shut down, and loses its capacity to activate further naïve T cells. Then, a “successful” immune response is defined as an outcome in a draining LN where no self-reactive T cells have been activated, while at least one pathogen specific T cell has been triggered to mount an immune response by at least one of the activated DCs. The aim of this study is to calculate the probability of such a successful immune response, and study this probability as a function of T cell specificity and the presence or absence of Tregs. This model is used to study whether or not DC suppression by regulatory T cells provides an evolutionary benefit.

## 2. Model

To investigate the effects of the DC suppression mechanism described above, Tregs were implemented in a previous probabilistic modeling framework of T cell specificity (De Boer and Perelson, 1993; Borghans et al., 1999). These models calculate the probability of mounting a successful immune response,  $P_{suc}$ , upon encountering a pathogen. A successful immune response is defined as a response in which at least one of the foreign epitopes of the pathogen is recognized, while no potentially self-reactive T cells are activated. This probability depends on two processes: (i) formation of the T cell repertoire by negative selection and (ii) T cell activation by antigen bearing DCs.

### 2.1. Construction of the T cell repertoire

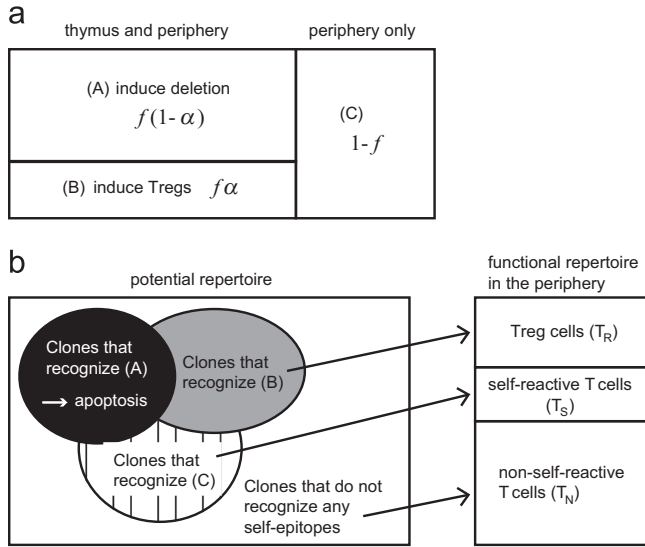
Following De Boer and Perelson (1993) and Borghans et al. (1999), we consider an animal with  $S$  different self-epitopes. Let each T cell clone have a chance  $p$  to recognize a randomly selected epitope. Hence, a low  $p$  value means that T cells are very specific, whereas a high  $p$  value corresponds to a high degree of cross-reactivity. Let  $R_0$  denote the size of the potential T cell receptor repertoire before negative selection. Since the probability that a randomly chosen T cell clone does not recognize a randomly chosen self-epitope is  $(1-p)$ , the expected repertoire size of non-self-reactive T cells, which do not recognize any of the  $S$  self-epitopes, is given by

$$T_N = R_0(1-p)^S. \quad (1a)$$

To avoid autoimmunity, clonotypes responding to self-epitopes are excluded from the T cell repertoire by negative selection. However, this selection is not complete. To allow for incomplete selection, we let  $f$  be the fraction of self-epitopes presented in the thymus. The size of the repertoire of potentially self-reactive T cells, which do not recognize any epitope presented in the thymus but do respond to at least one ignored self-epitope in the periphery, is then

$$T_S = R_0(1-p)^S(1-(1-p)^{(1-f)S}). \quad (1b)$$

Here,  $(1-p)^S$  is the probability that the T cell does not recognize any of the  $fS$  self-epitopes presented in the thymus, and the second term gives the probability that at least one of the  $(1-f)S$  ignored self-epitopes is recognized. Next, we consider the development of regulatory T cells upon recognition of a self-epitope in the thymus. Let some fraction,  $\alpha$ , of the self-epitopes presented in the thymus induce regulatory T cell formation, while the remaining fraction,  $(1-\alpha)$ , induces deletion. Hence, we divide



**Fig. 1.** (a) The distribution of self-epitopes. Of all self-epitopes,  $S$ , a fraction  $f$  is presented in the thymus, and of these presented epitopes a fraction  $\alpha$  induce regulatory T cell formation. The remaining fraction,  $f(1-\alpha)$ , are self-epitopes that induce deletion of T cell clones that recognize them. (b) Composition of the T cell repertoire. Since deletion is dominant, clones that recognize one or more of the  $f(1-\alpha)S$  “class A” self-epitopes will be deleted regardless of their recognition of other self-epitopes. Clones that recognize at least one of the  $f\alpha S$  “class B” self-epitope and no “class A” self-epitopes develop into regulatory T cells, while clones that recognize at least one of the  $(1-f)S$  ignored self-epitopes (“class C”) become potentially dangerous self-reactive T cells. Finally, non-self-reactive T cells develop from clones that do not recognize any of the  $S$  self-epitopes.

the total repertoire of self-epitopes,  $S$ , into three partitions: (A) apoptosis-inducing ( $f(1-\alpha)S$  epitopes); (B) Treg-inducing ( $f\alpha S$  epitopes); and (C) non-presented ( $(1-f)S$  epitopes). This partitioning is illustrated in Fig. 1a. We assume that the deletion is dominant and that all clones that recognize at least one apoptosis inducing self-epitope execute apoptosis (Fig. 1b). Since regulatory T cells should recognize at least one epitope from the set of the  $\alpha f S$  Treg-inducing self-epitopes but none of the  $(1-\alpha)f S$  apoptosis inducing epitopes, the expected repertoire size of regulatory T cells is

$$T_R = R_0(1-p)^{(1-\alpha)fS}(1-(1-p)^{\alpha fS}). \quad (1c)$$

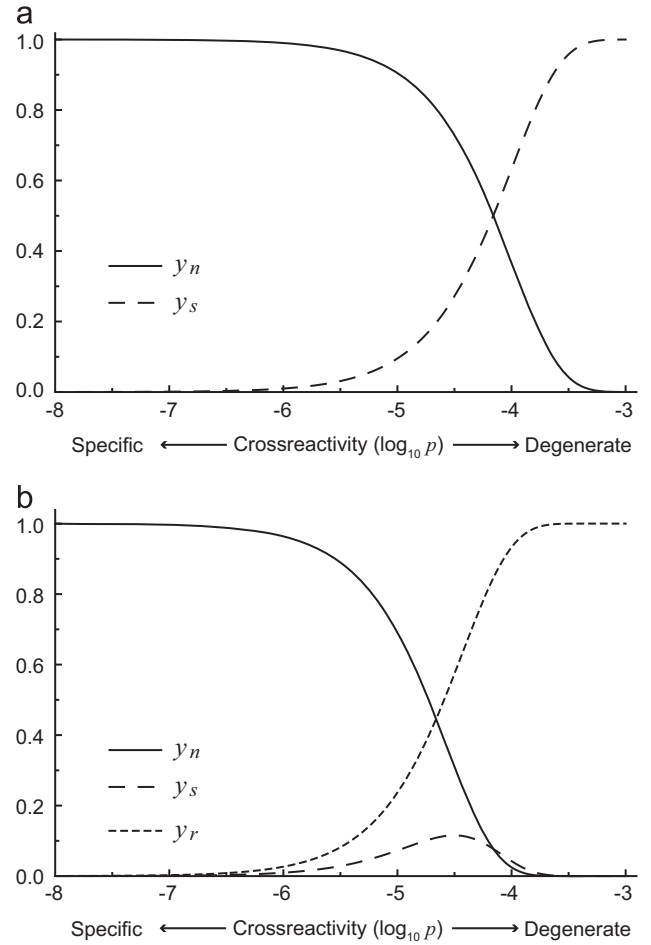
After negative selection, the functional repertoire consists of non-self-reactive, self-reactive, and regulatory T cells (Fig. 1b). Then, the peripheral fraction of each T cell type is easily calculated by dividing Eq. 1(a–c) by the functional repertoire size,  $R = T_N + T_S + T_R$ , yielding

$$y_N = (1-p)^{(1-(1-\alpha)fS)}, \quad (2a)$$

$$y_S = (1-p)^{\alpha fS}(1-(1-p)^{(1-f)S}), \quad (2b)$$

$$y_R = 1 - (1-p)^{\alpha fS}. \quad (2c)$$

These fractions provide the probability that a randomly picked T cell in the periphery is a non-self-reactive, a self-reactive, or a regulatory T cell, respectively. Fig. 2a and b shows how these fractions  $y_N$ ,  $y_S$  and  $y_R$  depend on the cross-reactivity  $p$  in the absence (Fig. 2a) and presence (Fig. 2b) of Tregs. When T cells are very specific (low  $p$ ), almost all T cells are expected to be non-self-reactive, but most of these cells will also not react to foreign epitopes. For cross-reactivity values around the experimental estimate ( $p \approx 10^{-5}$ ), we observe a small fraction of potentially dangerous self-reactive T cells (long dashed line in Fig. 2a). Inclusion of Tregs in the model decreases the size of this self-reactive fraction (long dashed line in Fig. 2b). This is at least partly

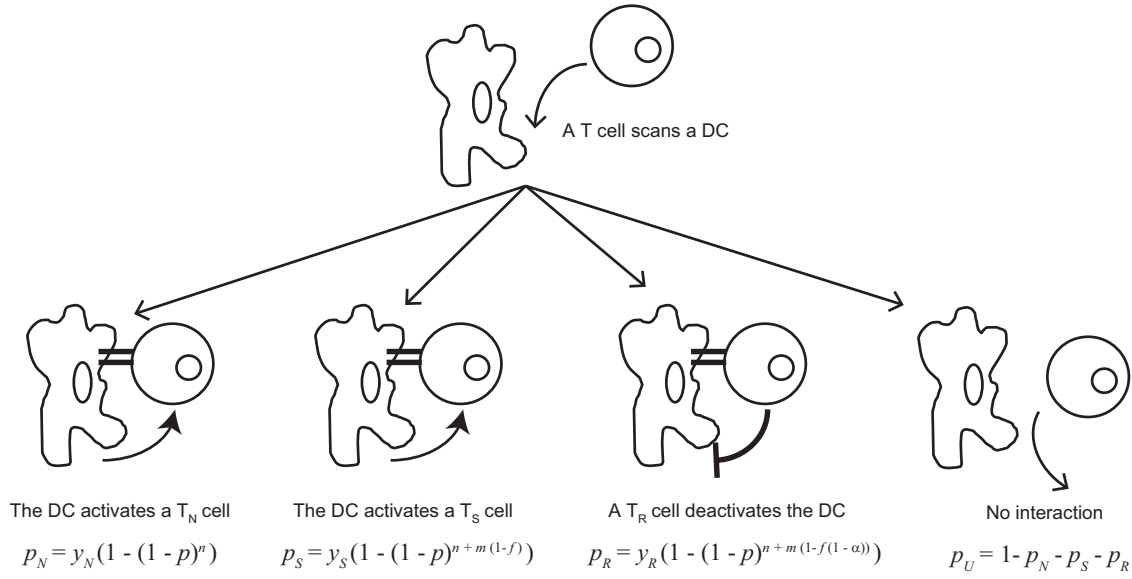


**Fig. 2.** The fraction of non-self reactive ( $y_N$ ), self-reactive ( $y_S$ ), and regulatory ( $y_R$ ) T cells in the periphery as a function of T cell cross-reactivity as calculated by Eq. (2). (a) In the absence of Treg formation ( $\alpha=0$ ) and (b) including Treg formation ( $\alpha=0.3$ ). For low cross-reactivity ( $p \approx 10^{-5}$ ), almost all cells are non-self-reactive. For a realistic cross-reactivity ( $p \approx 10^{-5}$ ), a small fraction of T cells is self-reactive. This fraction is lower in the presence of Tregs (b), which is at least partly due to dilution (see the main text). Other model parameters values are  $S = 10^5$  and  $f=0.9$ .

due to a dilution of the repertoire by Tregs, as the Treg model allows for the maturation of cells that would otherwise be deleted by negative selection. Therefore, allowing for Tregs increases the size of the functional repertoire  $R$ , and thereby reduces the relative contribution of the self-reactive T cells.

## 2.2. T cell activation and successful immune response

Upon infection, DCs will become activated and migrate to draining lymph nodes, where they present self and foreign peptides on their MHC-complexes to scanning naïve T cells. Consider an activated DC in a draining LN, and let it present  $m$  unique self-epitopes, and  $n$  different foreign epitopes on its surface. For pathogens with a genome that is much smaller than that of their host (like viruses), we expect that  $m > n$ . When an activated DC encounters a T cell, which may be non-self-reactive, self-reactive or regulatory, one of four possible events takes place: (i) the encounter leads to the activation of a non-self-reactive T cell, (ii) the encounter leads to the activation of a self-reactive T cell, (iii) the DC is recognized by a regulatory T cell and deactivated, or (iv) the T cell does not recognize any of the  $m+n$  presented epitopes and remains inactive. These possibilities are depicted in Fig. 3.



**Fig. 3.** The four possible scenarios when a DC is scanned by a random T cell: (i) the encounter leads to the activation of a non-self-reactive T cell, (ii) the encounter leads to the activation of a self-reactive T cell, (iii) the DC is recognized by a regulatory T cell and deactivated, or (iv) the T cell does not recognize any of the presented epitopes and remains inactive. The occurrence of events (i)–(iii) requires both that the randomly selected T cell is of the appropriate type and that it recognizes at least one of the epitopes presented on the DC. This is reflected in the two terms constituting the probability of these events.

The occurrence of event (i) requires that a randomly encountered T cell is non-self-reactive and that it recognizes at least one of the  $n$  foreign epitopes on the DC. Therefore, the probability that a single T cell–DC encounter leads to activation of a non-self-reactive T cell (and therefore an immune response to the pathogen),  $p_N$ , is given by the product of these two probabilities,

$$p_N = y_N(1 - (1 - p)^n). \quad (3a)$$

To consider the probability of event (ii), we assume that all self-epitopes are presented on a DC with equal probability. Thus, a DC is expected to present  $(1 - f)m$  different self-epitopes not presented in the thymus, and the number of potential ligands of a self-reactive T cell on a DC is  $(1 - f)m + n$ . Because the probability of recognition for one epitope is  $p$ , the probability that a random T cell–DC encounter leads to activation of a self-reactive T cell is given by

$$p_S = y_S(1 - (1 - p)^{(1-f)m+n}). \quad (3b)$$

According to the same assumption, the number of potential ligands of a regulatory T cell is expected to be  $n$  foreign epitopes and  $(1 - (1 - \alpha)f)m$  self-epitopes, since Tregs potentially recognize any self-epitope that does not induce apoptosis in the thymus. Hence,  $p_R$ , the probability that a randomly encountered T cell is regulatory and recognizes at least one epitope on the DC, which leads to deactivation of the DC, is

$$p_R = y_R(1 - (1 - p)^{(1-(1-\alpha)f)m+n}). \quad (3c)$$

Finally, the probability of no cognate recognition in an encounter between a DC and a randomly selected T cell in the draining LN,  $p_U$ , is

$$p_U = 1 - p_R - p_S - p_N. \quad (3d)$$

As soon as a DC is recognized by a Treg, the DC will be shut down and lose its capacity to activate naïve T cells. Let  $k$  be the average number of different T cell clones that scan a single activated DC. In this model we set  $k = R = T_R + T_S + T_N$ , assuming that scanning is quick and a DC is scanned by the whole peripheral repertoire of T cell clones. The probability that no self-reactive T cell is activated in all these encounters, i.e. that the animal stays

tolerant to self, can be calculated as

$$\begin{aligned} P_t &= (p_N + p_U)^k + p_R \sum_{i=0}^{k-1} (p_N + p_U)^i \\ &= (p_N + p_U)^k + p_R \left( \frac{1 - (p_N + p_U)^k}{1 - (p_N + p_U)} \right), \end{aligned} \quad (4)$$

where the first term indicates the probability that the DC does not activate any Tregs or self-reactive T cells during its  $k$  encounters, while the second term provides the probability that the DC is shut down by a regulatory T cell before mounting an autoimmune response. In the same manner, the probability that none of the encounters leads to any immune response can be calculated as

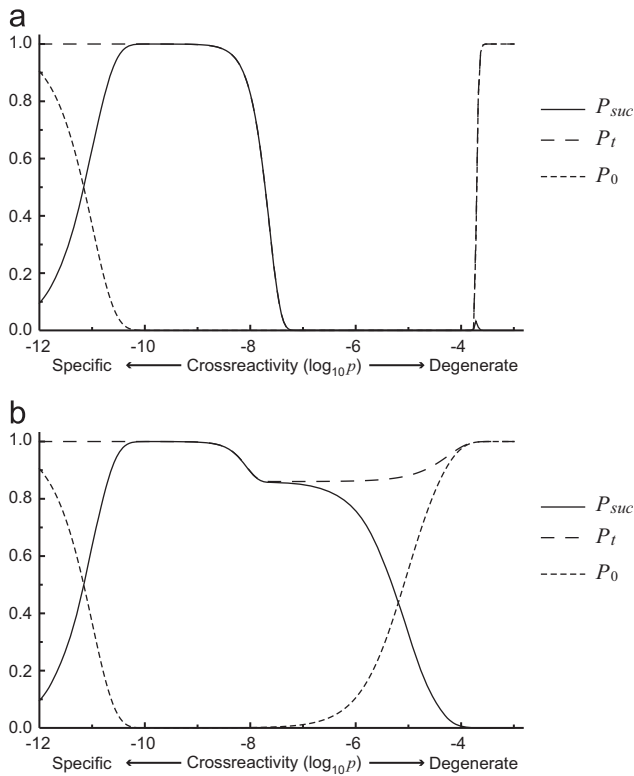
$$P_0 = p_U^k + p_R \left( \frac{1 - p_U^k}{1 - p_U} \right), \quad (5)$$

again including the probability that no recognition takes place in all  $k$  encounters (first term), and the probability that none of the encounters leads to activation of the T cell until the DC is shut down by a Treg (second term).

Now, suppose that there are  $q$  activated DCs present in the draining LN. We define an immune response as “successful” if none of these DCs elicits an autoimmune response, while at least one DC evokes an immune response to at least one foreign epitope. Then the probability of a successful immune response can be written as

$$P_{suc} = P_t^q - P_0^q. \quad (6)$$

i.e. the probability of staying tolerant to self, minus the probability that no reaction at all is mounted. To investigate the influence of regulatory T cells on the immune system, this probability  $P_{suc}$  was studied as a function of T cell specificity  $p$  and Treg induction likelihood parameter  $\alpha$ . Other parameter values were set to  $S = 10^5$  self-epitopes (Burroughs et al., 2004),  $R_0 = 10^{10}$  initial T cell clones (Borghans et al., 1999),  $f = 0.9$ ,  $m = 90$  self-epitopes,  $n = 10$  self-epitopes and  $q = 1$  DC.



**Fig. 4.** The probabilities of remaining self-tolerant ( $P_t$ ), not mounting any immune response ( $P_0$ ), and of a successful immune response ( $P_{suc}$ ) as a function of T cell cross-reactivity as calculated by Eqs. (4), (5), (6) respectively. (a) In the absence of Treg formation ( $\alpha = 0$ ), and (b) including Treg formation ( $\alpha = 0.3$ ). The success probability is maximal for relatively low cross-reactivity values. In the absence of Tregs, the system loses its ability to remain self-tolerant at high cross-reactivity (Panel (a): low  $P_t$  for high  $p$ ). At these high  $p$ -values the system remains self-tolerant if Tregs are present (Panel (b):  $P_t$  remains high over the entire  $p$ -range), but then the success probability is low because almost all immune reactions, including those to the pathogen, are suppressed (Panel (b): high  $P_0$  for high  $p$ ). Other model parameter values are  $R_0 = 10^{10}$ ,  $S = 10^5$ ,  $f = 0.9$ ,  $m = 90$ ,  $n = 10$ ,  $k = R$  (the functional repertoire size) and  $q = 1$  DC.

### 3. Results

#### 3.1. Probability of a successful immune response

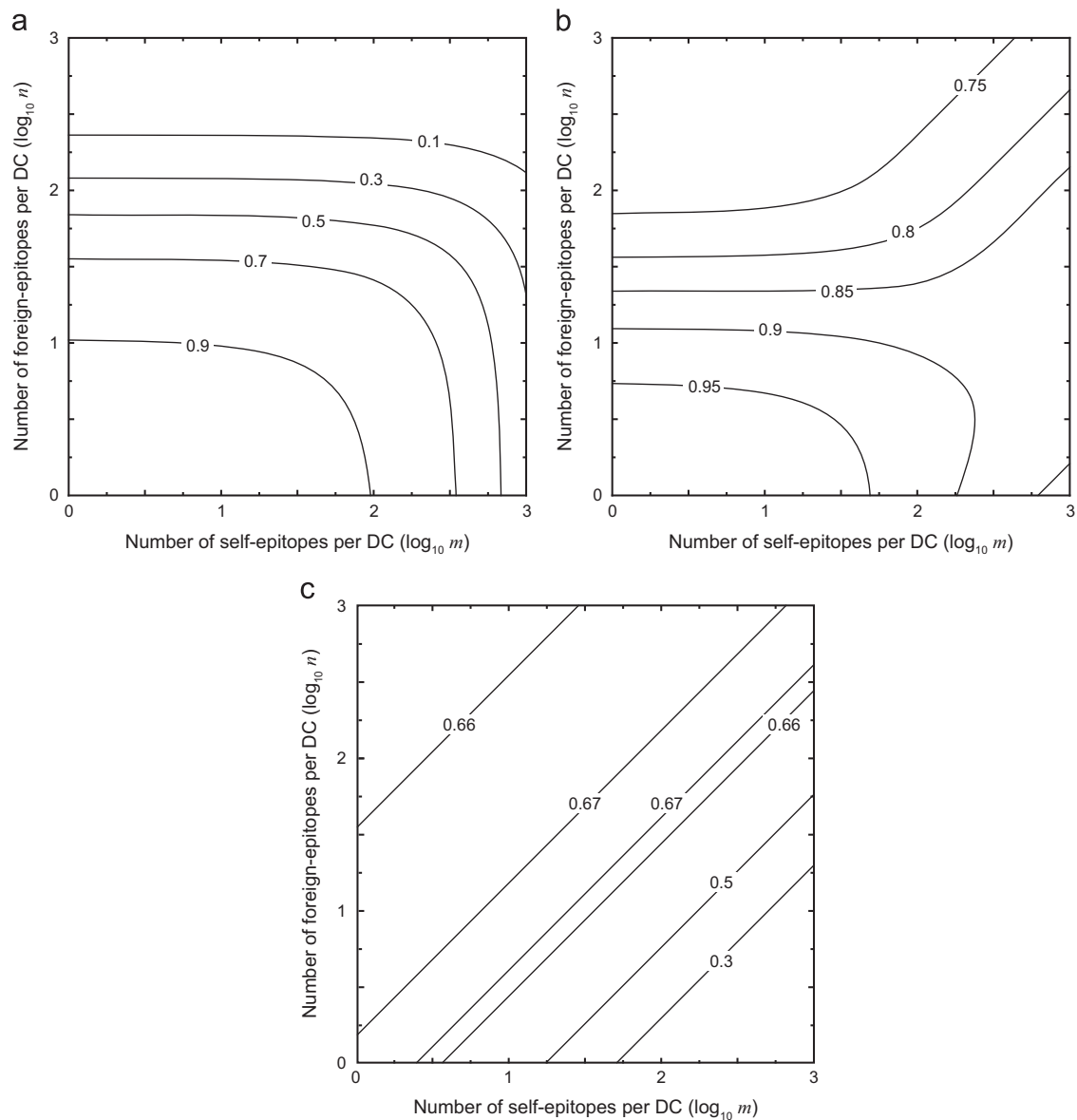
First, we consider the model without Treg formation ( $\alpha = 0$ ). Fig. 4a shows the probabilities  $P_t$ ,  $P_0$  and  $P_{suc}$  as functions of the cross-reactivity parameter  $p$  for this scenario. At very high T cell specificity, i.e. small  $p$ , the system is likely to remain tolerant to self (high value of  $P_t$ ) but will typically do so by not mounting any immune response at all (high value of  $P_0$ ). Therefore, the probability of mounting a successful immune response,  $P_{suc}$ , is low for very high T cell specificities (De Boer and Perelson, 1993; Borghans et al., 1999). On the other hand, when T cells are highly degenerative (high values of  $p$ ),  $P_{suc}$  is also low. For the parameter range  $10^{-7} < p < 10^{-4}$ , this is caused by a low probability of remaining tolerant  $P_t$ . For even higher cross-reactivity values ( $p > 10^{-4}$ ), most of the cells are deleted during negative selection and the remaining functional repertoire size  $R$  is insufficient to mount a successful immune response. For  $R_0 = 10^{10}$ , the probability of a successful immune response is maximized around  $10^{-10} < p < 10^{-8}$ , where these two opposing factors are at balance (Borghans et al., 1999). This cross-reactivity value is several orders of magnitude smaller than experimental estimates. One problem is that we currently have no good estimates for the diversity of the T cell repertoire, and a potential repertoire of  $R_0 = 10^{10}$  clones could be a too high estimate. According to Borghans et al. (1999) the optimal cross-reactivity value increases

when  $R_0$  decreases. Nevertheless, this work suggests that under incomplete tolerance induction, the optimal cross-reactivity could be much lower than what is experimentally observed (Borghans et al., 1999).

Next, regulatory T cells were included in the model by allowing a fraction of peptides presented in the thymus to induce Treg formation ( $\alpha > 0$ ). Results for these settings are shown in Fig. 4b. In contrast to the case without Tregs, we now observe that the probability to keep tolerant to self,  $P_t$ , remains high over the entire range of  $p$ . Hence, the inclusion of regulatory T cells leads to an increase of self-tolerance at high T cell cross-reactivity. However, if cross-reactivity is too high, the probability of a successful immune response remains low because Tregs will quickly prevent all immune responses (high value of  $P_0$ ). For a limited range of intermediate values of cross-reactivity, which includes the empirical estimates of  $p \approx 10^{-5.5}$ , the probability of a successful immune response  $P_{suc}$  increases somewhat with the addition of Tregs to the model (Fig. 4). This will be further explored in the next section. Note, however, that in order to mount successful immune responses to several subsequent pathogens, the probability of one successful response should be close to one (as is obtained for low cross-reactivity). Hence, the success probabilities in the  $p \approx 10^{-5}$  parameter region seem too low to allow for long-term survival.

Concluding, both in the scenario with and without Tregs an optimal degree of T cell cross-reactivity was found, consistent with previous work (Borghans et al., 1999). However, the mechanism by which this optimum arises depends on the presence or absence of Tregs. If Tregs are absent, the probability of a successful immune response at high cross-reactivity is low because of high probability of autoimmunity. In the presence of Tregs, high cross-reactivity will cause these Tregs to shut down all immune responses, therewith preventing adequate response to pathogens. Note that we found qualitatively similar results when varying all other model parameters, i.e.  $f, m, n$ , and  $k$ , with somewhat different optimal values for  $p$  and  $P_{suc}$  (results not shown).

Fig. 5 shows how the probability of a successful immune response,  $P_{suc}$ , depends on the number of self ( $m$ ) and foreign ( $n$ ) epitopes presented on a DC. First, we consider the model at the low, but optimal T cell cross-reactivity of  $p = 10^{-8}$  (Fig. 5a–b). In the absence of Tregs (Fig. 5a), we observe that not only the number of self-epitopes  $m$  but also the number of foreign epitopes  $n$  on the DC should be small to optimize  $P_{suc}$ . This is because self-reactive naive T cells can also become activated by foreign epitopes, which they recognize with probability  $p$ . Fig. 5a suggests that a small number of foreign epitopes is enough to trigger an immune response, and that a higher amount of foreign epitopes per DC mostly increases the risk of autoimmunity. In the presence of Tregs, this effect is less strong (Fig. 5b). Both for high  $m$  and high  $n$  values, higher success probabilities are obtained. Furthermore, for large values of  $n$ , i.e.  $n > 30$ , the success probability increases with increasing  $m$ . Since a DC presenting a large number of foreign epitopes is likely to activate self-reactive naive T cells as well, timely inhibition of this DC will reduce the risk of autoimmunity, and presenting a high number of self-epitopes will increase the probability of recognition and deactivation by a Treg. All together, these results suggest that Tregs allow for higher values of both  $m$  and  $n$ . Finally, we consider the model for  $p = 10^{-5.5}$ , which is around experimentally estimated values (Fig. 5c). Under these settings, we find again that increasing the number of self-epitopes,  $m$ , is protective when a DC is expressing more foreign epitopes,  $n$ . However, none of these success probabilities is high enough to ensure long-term survival (as mentioned above). In the absence of Tregs,  $P_{suc}$  is close to zero for all  $m$  and  $n$  values (result not shown). Summarizing, Fig. 5 illustrates that the partial benefit of Tregs found in the previous section does not depend on our specific choice of  $m$  and  $n$ .



**Fig. 5.** Contour plot of the probability of mounting a successful immune response  $P_{suc}$  as a function of the number of self- ( $m$ ) and foreign- ( $n$ ) epitopes per DC. (a)  $\alpha = 0$  and  $p = 10^{-8}$ , (b)  $\alpha = 0.3$  and  $p = 10^{-8}$ , and (c)  $\alpha = 0.3$  and  $p = 10^{-5.5}$ . When we set  $\alpha = 0$  and  $p = 10^{-5.5}$ ,  $P_{suc}$  is close to zero irrespective of the values of  $m$  and  $n$  (not shown). In the absence of Tregs, the success probability decreases with increasing values of  $m$  and  $n$  because of an increased risk of autoimmunity. The presence of Tregs allows for much higher values of  $m$  and  $n$ . Other model parameters are  $R_0 = 10^{10}$ ,  $S = 10^5$ ,  $f = 0.9$ ,  $k = R$  and  $q = 1$ .

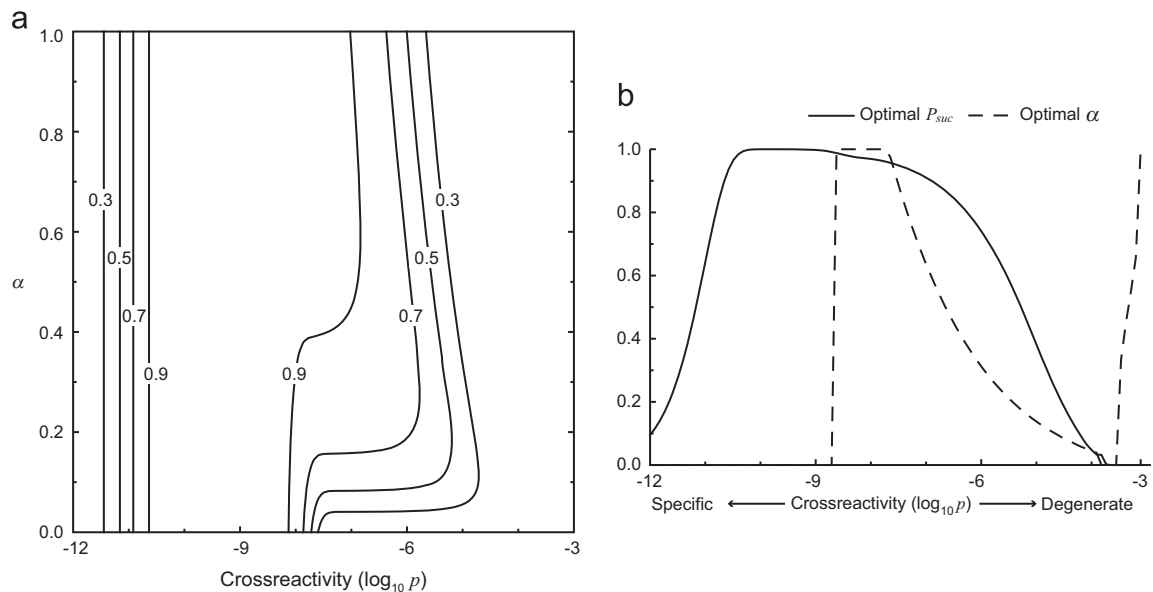
### 3.2. Optimal cross-reactivity and Treg induction

To further investigate the influence of Tregs, the probability of a successful immune response  $P_{suc}$  was plotted in the  $p$ - $\alpha$  plane (Fig. 6a). We observe four distinct regions along the T cell specificity parameter  $p$ , i.e. the horizontal axis. For either very low ( $p < 10^{-11.5}$ ) or high ( $p > 10^{-5}$ ) cross-reactivity, the success probability is low regardless of the presence of Tregs (indicated by  $\alpha$ ). The reasons underlying this low success probability were discussed in the previous section. In the region  $10^{-11.5} < p < 10^{-8.5}$ , the chances of mounting a successful immune response do not depend on the presence of Tregs (i.e. the value of  $\alpha$ ). Since the probability of mounting autoimmune reactions is very low in this region (see the  $P_t$  curve in Fig. 4a), we would indeed expect hardly any effects of Tregs here.

Finally, in the region  $10^{-8.5} < p < 10^{-5}$  we observe a positive effect of Tregs because the  $\alpha$ -value that optimizes  $P_{suc}$  is greater

than zero. This is also shown in Fig. 5b, where the optimal  $\alpha$  and the corresponding optimal  $P_{suc}$  are plotted as function of the degree of cross-reactivity  $p$ . If T cells have an intermediate specificity, Tregs can increase the probability of a successful immune response. However, maximal success probabilities are rather low for this intermediate cross-reactivity, and  $P_{suc}$  remains maximal around  $10^{-10} < p < 10^{-9}$  (with an optimal  $\alpha$  equal to zero). As mentioned in the previous section, the probability of mounting a successful immune response when exposed to one pathogen,  $P_{suc}$ , needs to be close to one (as found for the more optimal settings) to survive attacks by several pathogens. Therefore, the Treg function included in this modeling framework on its own seems insufficient to provide an evolutionary benefit for Treg formation.

We also performed a parameter search on the model to search for situations where Tregs are truly advantageous. The situation where the maximal  $P_{suc}$  on the  $p$ - $\alpha$  plane is obtained when  $\alpha = 0$ ,



**Fig. 6.** (a) Contour plot of the probability of mounting a successful immune response  $P_{suc}$  as a function of T cell cross-reactivity  $p$  and the Treg induction parameter  $\alpha$ . (b) The optimal probability of a successful immune response  $P_{suc}$  given by the optimal  $\alpha$  that maximizes  $P_{suc}$  for each value of  $p$ . For a relatively low cross-reactivity ( $p < 10^{-9}$ ), the optimal value of  $P_{suc}$  does not depend on the presence or absence of Tregs ( $\alpha$ ). If T cells are realistically cross-reactive ( $p \approx 10^{-5.5}$ ), the addition of Tregs improves the probability of a successful immune response somewhat. However,  $P_{suc}$  values in this region remain low. Other model parameter values are  $R_0 = 10^{10}$ ,  $S = 10^7$ ,  $f = 0.9$ ,  $m = 90$ ,  $n = 10$ ,  $k = R$  and  $q = 1$ .

was very common. For example, lowering the fraction of self-epitopes presented in the thymus,  $f$ , increases the importance of preventing autoimmunity, potentially increasing the chances of positive Treg effects. However, in the current framework this still proved insufficient to reach high success probabilities. Since we would certainly expect to find multiple activated DCs in a draining LN, we also varied the number of activated DCs bearing foreign epitopes in the LN,  $q$ . The results for 200 activated DCs ( $q = 200$ ) are shown in Fig. 7. In this scenario, we require that mounting an autoimmune response via any DC is avoided, while activation by only one DC is sufficient to mount an immune response to the pathogen. Therefore, this again increases the importance of avoiding autoimmunity in the process, and we might expect that this creates more possibilities for a positive effect of Tregs. This is partly reflected in Fig. 7b. When  $p < 10^{-9}$ , which is unrealistically low, high success probabilities are reached for  $\alpha = 1$ . However, the benefit of Tregs is very minimal in this “unrealistic” region because for much lower values of  $\alpha$  the probability of success is very similar, i.e. the contour lines in Fig. 7a are almost parallel to the vertical axes.

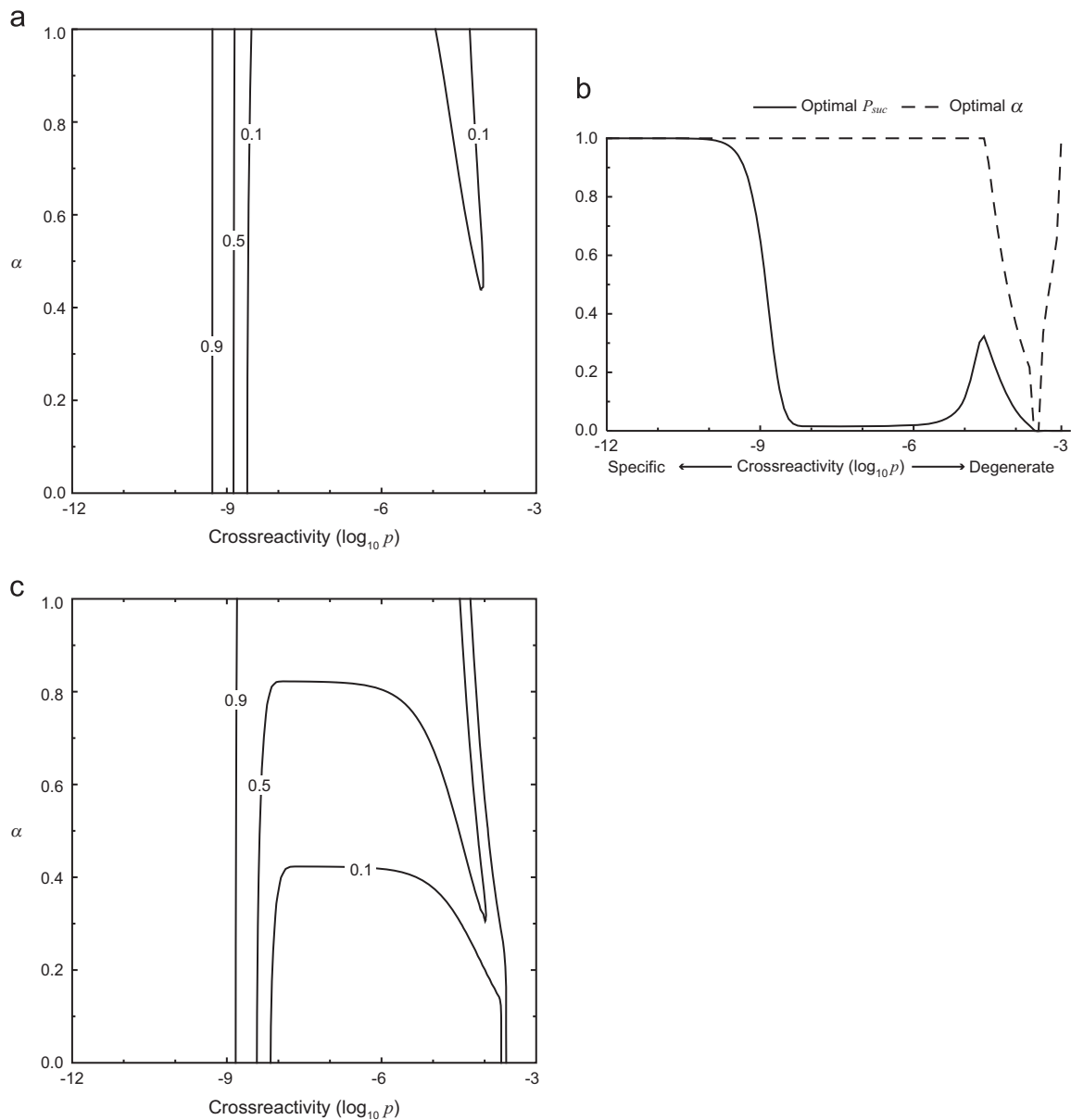
In the region with more realistic cross-reactivity values, an opposite effect was found, as adding more DCs to the model removes most of the benefits of Tregs at higher cross-reactivity (Fig. 7a). Interestingly, a positive effect of Tregs in the realistic range of cross-reactivity values reappears if we assume that very few self-epitopes are ignored in the thymus by choosing a higher value of  $f$ , for instance  $f = 0.98$  (Fig. 7c). Our interpretation is that if 10% of self-peptides is ignored during negative selection ( $f = 0.9$  in Fig. 7a–b) and multiple DCs are present, the risk of autoimmunity becomes so high that the Treg pool is not large enough to prevent the activation of at least one self-reactive T cell. If we increase  $f$  as done in Fig. 7c, the number of self-reactive T cells decreases, while the number of Tregs increases. This shifts the balances in favor of the Tregs, such that they then can suppress all self-reactive T cells. Taken together, these results show that the DC suppression mechanism of Tregs is rather weak, as Tregs using this mechanism can only suppress autoimmune reactions if the self-reactive T cell pool is small, and even then success probabilities are too low to ensure long-term survival.

#### 4. Discussion

We have examined how the probability of mounting a successful immune response depends on the degree of T cell cross-reactivity and regulatory T cell induction by extending the probabilistic modeling frameworks of previous studies (De Boer and Perelson, 1993; Borghans et al., 1999). Earlier theoretical considerations have suggested that the empirically estimated level of cross-reactivity is too high to keep self-tolerance (Borghans et al., 1999; Mason, 2001), giving a possible explanation for the existence of regulatory T cells (Mason, 2001). Furthermore, many different acting mechanisms by which Tregs suppress immune reactions have been reported, and their relative contributions to Treg-functioning are largely unknown. The model presented here allows us to test the influence of one such suppression mechanism, namely the suppression of activated DCs.

The model shows that regulatory T cells can cause a slight improvement in the probability of mounting a successful immune response when T cells have a realistic cross-reactivity. There are two factors involved in this improvement. First, Tregs dilute the proportion of self-reactive T cells in the periphery (see Fig. 2b). If we then assume that only a certain number of T cell clones can scan a DC while it is active, a lower fraction of self-reactive T cells will lower the probability that one of these self-reactive cells gets activated and mounts an autoimmune reaction. Second, regulatory T cells can suppress DCs by shutting them down. This reduces the risk of DCs activating self-reactive T cells, but potentially comes at the cost of a lower probability of mounting a successful response against the pathogen. Although the model shows a slight improvement in the success probability at realistic T cell cross-reactivity, the optimal immune system functioning is still found to occur in the absence of Tregs ( $\alpha = 0$ ) at a higher specificity (lower  $p$ ).

For T cell specificities close to the experimental estimates ( $10^{-6} < p < 10^{-5}$ ), the probability of mounting a successful immune response against a given pathogen remains relatively low, even though it is slightly increased in the presence of Tregs. To stand a chance against multiple pathogenic attacks, this probability should be close to one. Even in the presence of Tregs, we were unable to reach such high success probabilities in the  $p \approx 10^{-5}$  range.



**Fig. 7.** Contour plot of the probability of the successful immune response  $P_{suc}$  as a function of  $p$  and  $\alpha$  for  $f=0.9$  (a) and for  $f=0.98$  (c), and the optimal values of  $P_{suc}$  and  $\alpha$  for each cross-reactivity  $p$  for  $f=0.9$  (b) for a scenario with many DCs ( $q=200$ ). Allowing for many DCs in the model removes most of the benefits of Tregs at realistic cross-reactivity values (Panel a–b). The partial beneficial effect of Tregs reappears if the number of ignored self-epitopes is reduced (Panel c). Other model parameters are the same as in Fig. 6.

Therefore, we conclude that the DC suppression by regulatory T cells as implemented here is insufficient to allow for a fully functional immune system at experimentally estimated T cell cross-reactivity. Although we found that producing Tregs was the best solution when there are  $q=200$  activated DCs in the draining lymph node, this happened only for unrealistically low values of the cross-reactivity, and hardly depended on the Treg induction parameter  $\alpha$  (Fig. 7).

The conclusion that Tregs have little effect on the success probability could partly be due to the relatively simple implementation of the different self-epitopes in our model. Since in our model regulatory T cells respond to self-epitopes that are present in the thymus, and potentially self-reactive T cells recognize self-epitopes that are only expressed in the periphery, Tregs and self-reactive T cells are expected to react to different sets of self-epitopes. The shutting down of DCs in the periphery can only occur when a Treg recognizes a self-epitope that is present in the thymus. In the current framework, there is therefore no way in

which Tregs could be primed to specifically shutting down DCs that carry potentially dangerous self-epitopes. Such preferential recognition could be included if we assume that Treg-inducing self-epitopes are not a random set of self-epitopes (as assumed in the current work), but rather a selected set associated with self-epitopes that are not presented in the thymus. This could potentially increase the positive effect of regulatory T cells. However, it remains unclear whether these two sets of self-epitopes share functional properties that can be distinguished by the TCRs.

Concluding, we have shown how a probabilistic modeling framework can be used to describe Treg functioning and to test hypotheses about functional mechanisms and their possible evolutionary benefit. We found that the suppression of DCs slightly improves immune performance for realistic cross-reactivities, but that this functional mechanism on its own is insufficient to fully explain a potential evolutionary benefit of Tregs. The probabilistic modeling framework that we have used here can be a powerful

tool to study hypotheses on the functioning of Tregs in the immune system.

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