

# Competition for antigenic sites during T cell proliferation: A mathematical interpretation of *in vitro* data

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**ABSTRACT** By fitting different mathematical T cell proliferation functions to *in vitro* T cell proliferation data, we studied T cell competition for stimulatory signals. In our lymphocyte proliferation assays both the antigen (Ag) availability and the concentration of T cells were varied. We show that proliferation functions involving T cell competition describe the data significantly better than classical proliferation functions without competition, thus providing direct evidence for T cell competition *in vitro*. Our mathematical approach allowed us to study the nature of T cell competition by comparing different proliferation functions involving (i) direct inhibitory T–T interactions, (ii) Ag-specific resource competition, or (iii) resource competition for nonspecific factors such as growth factors, and access to the surface of Ag-presenting cells (APCs). We show that resource competition is an essential ingredient of T cell proliferation. To discriminate between Ag-specific and nonspecific resource competition, the Ag availability was varied in two manners. In a first approach we varied the concentration of APCs, displaying equal ligand densities; in a second approach we varied the Ag density on the surface of the APCs, while keeping the APC concentration constant. We found that both resource competition functions described the data equally well when the Ag availability was increased by adding APCs. When the APC concentration was kept constant, the nonspecific resource competition function yielded the best description of the data. Our interpretation is that T cells were competing for “antigenic sites” on the APCs.

Competition between lymphocytes for stimulatory and survival signals is thought to play a pivotal role in the homeostatic control of the immune system. The steady-state population sizes of naive and memory T cell compartments (1–3), and of resting B cell and activated IgM-secreting B cell compartments (4, 5) are all independently regulated by cellular competition within each compartment. Due to competition between lymphocytes, cellular death rates and/or renewal rates are density-dependent functions of the peripheral population sizes (3, 6, 7). By such density-dependent mechanisms homeostasis is established.

There is a qualitative difference between the regulation of total T and B cell numbers. Although it has been shown that part of the B cell repertoire is maintained by competitive renewal in the periphery (5), B cells predominantly compete for survival signals (6). Memory T cells, however, mainly compete for stimulatory signals (7), affecting proliferation rates at high T cell concentrations. Here we study T cell competition and focus on the effect of high concentrations of T cells with the same specificity on the rate of T cell proliferation.

The nature of the signals for which T cells are competing remains elusive. Previous experiments have suggested that T cell competition is antigen (Ag)-specific. CD8<sup>+</sup> T cell competition was studied *in vivo* by reconstituting lethally irradiated mice with mixtures of precursor bone marrow cells from

normal nontransgenic and T cell receptor (TcR)-transgenic mice (7). It was shown that the proliferative capacity of the TcR-transgenic cells was diminished in the presence of other T cells, indicating that competition between T cells occurred. Moreover, nontransgenic cells appeared to have a selective advantage over TcR-transgenic cells in seeding the peripheral lymphoid tissue, suggesting that cells were competing for Ags.

Lymphocyte competition may, however, also act at a more global level, if lymphocytes compete for nonspecific factors such as growth factors, nutrients, or access to the surface of Ag-presenting cells (APCs). The fact that transgenic mice attain total peripheral lymphocyte numbers similar to those in normal mice has been interpreted as evidence for a global homeostatic control, acting independently of cell specificity (8). This argument was weakened by a mathematical model which showed that such equal total lymphocyte numbers could also be obtained when only an Ag-specific homeostatic control was taken into account (9). The experimental data (7, 8) thus fail to give a decisive answer about the nature of the factors controlling immune homeostasis. The advantage of an Ag-specific homeostatic control would be that the diversity of the immune system can be maintained. If all clonotypes were to compete for the same resource—e.g., a growth factor—the clonotype responding most vigorously would outcompete all other clonotypes (10).

In this paper we studied T cell proliferation, and in particular the nature of T cell competition, by fitting several mathematical proliferation functions to data from *in vitro* lymphocyte proliferation assays. Proliferation was measured both as a function of the Ag concentration and as a function of the number of T cells competing for Ag. The aim of this study was twofold: on the one hand to provide insights into the relative importance of inhibitory T–T interactions, Ag-specific resource competition, and nonspecific resource competition in T-cell proliferation, by mathematical analysis of *in vitro* data; on the other hand, to provide an experimental validation of several T cell proliferation functions that are frequently used in theoretical immunology. Briefly, our analysis shows that T cell proliferation functions allowing for T cell competition describe the experimental data significantly better than conventional noncompetitive saturation functions. This demonstrates that T cell competition plays a role *in vitro*. We show that most of the competition in our assays can be attributed to competition for antigenic sites on APCs.

## MATERIALS AND METHODS

**T Cells and Antigens.** The generation and maintenance of the CD4<sup>+</sup> Z1a T cell clone have been described previously (11).

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Briefly, the Z1a T cell clone was derived from the draining lymph nodes of a Lewis rat immunized in the hind footpads with guinea pig myelin basic protein (MBP) in complete Freund's adjuvant. T cell clone Z1a is reactive with the 72–85 amino acid sequence of MBP and with peptide 72–85<sub>S79A</sub>, an analogue of the native peptide which has a higher MHC class II RT1.B<sup>L</sup> binding affinity (12). T cells were cyclically restimulated *in vitro* for 3 or 4 days in the presence of irradiated (3,000 rads) thymocytes as APCs and 10  $\mu\text{g/ml}$  MBP, and propagated for 6 or 7 days. Cells were restimulated in Iscove's modified Dulbecco's medium (GIBCO), supplemented with 2% Lewis rat serum, 2 mM L-glutamine, (50  $\mu\text{M}$ ) 2-mercaptoethanol, and antibiotics. Propagation was performed in Iscove's modified Dulbecco's medium, supplemented with 2 mM L-glutamine, 2-mercaptoethanol, antibiotics, 10% fetal calf serum (FCS), 10% EL-4 supernatant (IL-2 source), and 1% nonessential amino acids. All experiments were also performed with the Lewis rat CD4<sup>+</sup> T cell clone A2b, specific for the 176–190 amino acid sequence of mycobacterial heat shock protein HSP65 (13, 14), yielding similar results (data not shown).

**T Cell Proliferation.** Proliferative responses of T cells were measured in triplicate cultures in flat-bottom microtiter plates (Costar). T cells were cultured at different concentrations in 0.2 ml of Iscove's modified Dulbecco's medium supplemented with 5% FCS, 2 mM L-glutamine, 2-mercaptoethanol, and antibiotics in the presence of irradiated (3,000 rads) thymocytes as APCs. To exclude any effects of free Ag or T–T cell presentation (15), T cells were incubated with APCs that had been prepulsed with peptide. APCs were prepulsed ( $6 \times 10^7$  cells per ml) with MBP 72–85<sub>S79A</sub> (or HSP65 176–190, data not shown) for 1.5 hr at 37°C (5% CO<sub>2</sub>) and thoroughly washed. In our first approach, APCs were prepulsed with a standard peptide concentration (100  $\mu\text{g/ml}$ ), after which T cells were incubated with different concentrations of APCs (varying from  $0.5 \times 10^6$  to  $2.5 \times 10^6$  cells per well). In the second approach, APCs were prepulsed with different concentrations of peptide (varying from 1 to 500  $\mu\text{g/ml}$ ), after which T cells were incubated with a standard concentration of APCs ( $1 \times 10^6$  cells per well). T cell concentrations varied from  $0.5 \times 10^4$  to  $32 \times 10^4$  cells per well. Total T cell proliferation was measured at 24 hr by addition of [<sup>3</sup>H]thymidine during the last 16 hr of a 24-hr culture period. Cells were harvested on fiberglass filters, and [<sup>3</sup>H]thymidine incorporation was measured by liquid scintillation counting.

**Statistical Procedures.** The optimal fits of the mathematical functions to the data were determined by using a generalized Gauss–Newton method to minimize the sum of the squared residuals (SSR) between the logarithms of the experimental and theoretical data. The logarithmic transformation was made because the experimental errors were likely to be proportional to the [<sup>3</sup>H]thymidine incorporation levels measured. To ascertain that the minima found were not reflecting local minima, the optimization procedure was repeated for various initial conditions. All conditions tested gave rise to the same minimal SSR and parameter values.

## T CELL PROLIFERATION FUNCTIONS

**Without Competition.** In theoretical studies of the immune system, it is common practice to describe T cell proliferation as a linear function of the concentration of Ag-specific T cells  $T$ , saturating over the concentration of Ag-presenting sites on APCs  $A$ :

$$T^* = \rho T \frac{A}{A + K}. \quad [1]$$

Here  $T^*$  is a measure of total T cell proliferation—i.e., the amount of [<sup>3</sup>H]thymidine incorporation in our experimental assays. The parameter  $\rho$  represents the maximum [<sup>3</sup>H]thymidine incorporation of T cells, and  $K$  is a saturation constant giving the concentration of antigenic sites at which the rate of

T cell proliferation is half-maximal. This saturation function describes the typical picture that is observed *in vitro*: total T cell proliferation increases with the Ag concentration until a certain plateau level is reached. In some experiments, T cell proliferation decreases at very high Ag concentrations, leading to log bell-shaped proliferation curves (16). Here we focused only on the first part of the curves, where T cell proliferation increases when the Ag concentration increases.

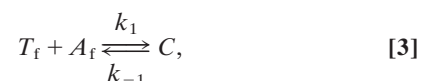
According to the conventional proliferation function of Eq. 1, doubling the number of T cells doubles total [<sup>3</sup>H]thymidine incorporation, regardless of the Ag availability. When Ag becomes limiting, this function may behave unrealistically, as T cells are expected to compete for the limiting antigenic resource. Thus, a T cell competition term may be an essential ingredient of T cell proliferation functions.

**Inhibitory T–T Interactions.** Several mechanisms have been described by which T cells may directly influence each others' proliferation/survival. By cytokine secretion (17) or consumption (18, 19), T cells may inhibit cell division of other T cells in the local environment. Alternatively, T cells may present Ags to other T cells, generally inducing the responding T cells to become anergic (15). If T lymphocytes indeed directly hinder each other by means of inhibitory T–T interactions, total T cell proliferation can be described by

$$T^* = \rho T \frac{A}{A + K} - \varepsilon T^2, \quad [2]$$

where T cell competition is modeled by the  $\varepsilon T^2$  term (see ref. 20 for an application). According to Eq. 2, a T cell approaches its maximum proliferation rate  $\rho$  when the Ag availability is large and the T cell population is small; the proliferation per T cell decreases when the total concentration of T cells increases.

**Ag-Specific Resource Competition.** We have previously proposed, and applied (21), an alternative proliferation function, based on the conjecture that T cells inhibit each other indirectly by competing for a limiting antigenic resource (22, 23). Such a function can be derived from the interactions between free T cells  $T_f$  and free antigenic sites on APCs  $A_f$ . When a T cell binds to a free antigenic site on an APC, it forms a complex  $C$  which may either dissociate or lead to T cell proliferation. Thus, a cellular immune response can be represented by the following interaction scheme:



where the constants  $k_1$  and  $k_{-1}$  are reaction rates, and new T cells are formed by proliferation proportionally to the number of T cell–Ag complexes  $C$ . In the *Appendix* we show that this scheme yields the following T cell proliferation function:

$$T^* = \rho T \frac{A}{A + cT + K}, \quad [4]$$

where  $A$  is the total concentration of antigenic sites (i.e.,  $A = A_f + C$ ), and  $T$  is the total concentration of Ag-specific T cells (i.e.,  $T = T_f + C$ ). In Eq. 4,  $c$  reflects the degree of T cell competition for Ag binding. If  $c = 0$  this function amounts to the conventional saturation function of Eq. 1. A  $c$  value larger than 0 would indicate that T cells are indeed competing for their antigenic resource. Competition between T cells thus results naturally from the decreasing Ag availability due to T cell–Ag complex formation.

**Nonspecific Resource Competition.** T cells may also compete for nonspecific resources, such as growth factors, nutrients, and/or access to the surface of APCs. To account for such an Ag-independent form of competition, we propose a third T cell proliferation function. Assuming that competition

for nonspecific resources affects the maximum proliferation rate of T cells, total T cell proliferation can be described by

$$T^* = \rho \frac{T}{1 + sT} \frac{A}{A + K}, \quad [5]$$

where  $s$  reflects the degree of T cell competition. Again, the proliferation per T cell decreases when the total concentration of T cells increases. Unlike the Ag-specific competition term of Eq. 4, the nonspecific competition term of Eq. 5 involves all T cell clones competing for the same nonspecific resource—e.g., all clones recognizing their antigen on the same APC.

## RESULTS AND DISCUSSION

The effect of T cell competition on proliferation was studied *in vitro*, by performing lymphocyte proliferation assays with the encephalitogenic CD4<sup>+</sup> T cell clone Z1a. In the assays, both the concentration of T cells and the concentration of Ag were varied. In a first approach, APCs were prepulsed with a standard concentration of peptide, after which increasing concentrations of prepulsed APCs, displaying the same ligand densities, were incubated with T cells.

To minimize any changes in the numbers of T cells in the wells during the experiment, proliferation had to be measured as early as possible. In a pilot study, [<sup>3</sup>H]thymidine incorporation for several combinations of Ag and T cell concentrations was measured at different time points (i.e., 17, 24, 40, 65, and 88 hr) after the start of the incubation period. At all time points T cell proliferation could be detected (data not shown). At the earliest time point (i.e., 17 hr), the dose–response curves of several T cell concentrations did not yet saturate as a function of the Ag concentration. From 24 hr onwards, the typically observed picture of proliferation saturating as a function of the Ag concentration was found (data not shown). Therefore, in all further experiments total T cell proliferation was measured by the [<sup>3</sup>H]thymidine incorporation 24 hr after incubation.

To study which of the T cell proliferation functions derived above could give the best description of the experimental data, the different functions were fitted to the results of the proliferation assays. For each of the proliferation functions the set of parameters giving the best fit to the data was computed by minimization of the SSR between the experimental data and the function studied.

Because the experimental data included background [<sup>3</sup>H]thymidine incorporation, the proliferation functions first had to be extended with a term accounting for background proliferation. Background proliferation increased with the concentration of T cells. This proliferation was probably due to prior T cell stimulation, since the Z1a and A2b clones were maintained by a weekly phase of restimulation and expansion. In the absence of T cells, [<sup>3</sup>H]thymidine incorporation was low (<200 cpm). Assuming that background T cell proliferation was indeed due to prior T cell stimulation, we modeled it as a term independent of T cell–Ag complex formation. Thus, in the fitting procedure all functions described above were extended by adding the term  $\beta = b_T T + b_A A + b$ , accounting for background [<sup>3</sup>H]thymidine incorporation due to T cells, background incorporation due to APCs, and background incorporation in the absence of both T cells and APCs, respectively.

Fig. 1 summarizes the results. All panels represent the same set of experimental data, denoted by the symbols. Fig. 1 *Left* shows the total T cell proliferative responses, while Fig. 1 *Right* shows the same data expressed as the proliferative responses *per T cell*. From *Left* it can be seen that total T cell proliferation increased both with the concentration of T cells and with the APC concentration. If competition for T cell proliferation occurs, one would expect the proliferative responses *per T cell* to decrease when the T cell concentration increases. Indeed, Fig. 1 *Right* shows that T cells at high T cell concentrations had a lower proliferation rate per T cell

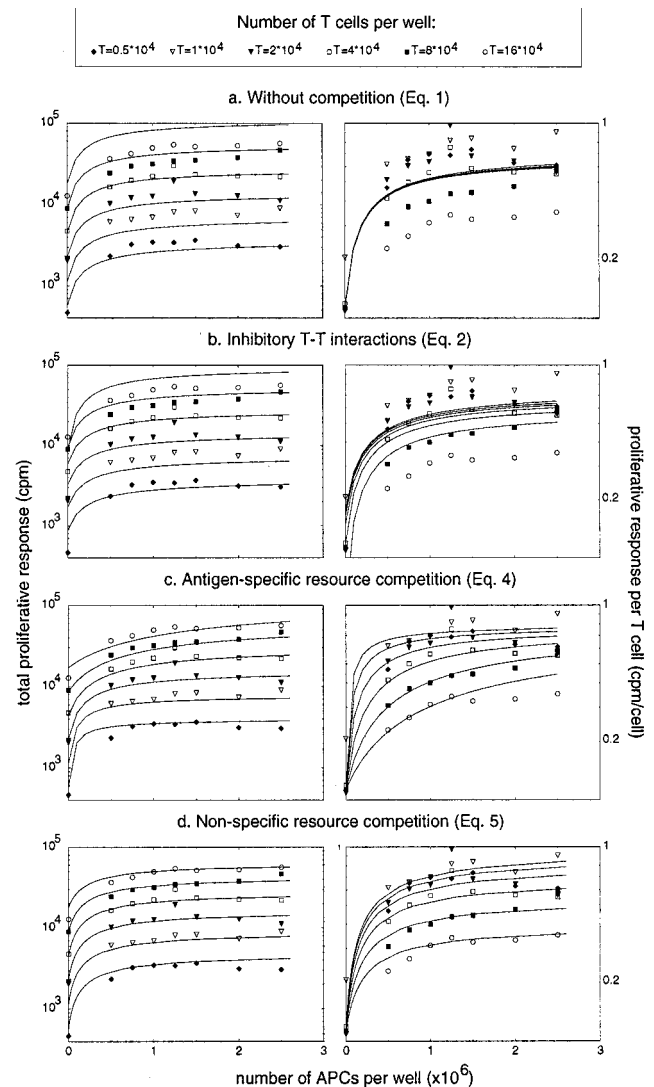


Fig. 1. Proliferative responses of different concentrations of Z1a T cells in response to different concentrations of equally prepulsed APCs. Graphs compare experimental results (in symbols) and best theoretical fits (curves). The experimental data were fitted to the conventional saturation function without T cell competition (Eq. 1; *a*), the proliferation function involving inhibitory T–T interactions (Eq. 2; *b*), the Ag-specific resource competition function (Eq. 4; *c*), and the nonspecific resource competition function (Eq. 5; *d*). On the *Left* the data are expressed as total proliferative responses, whereas on the *Right* the same data are expressed as proliferative responses per T cell. Parameters of the theoretical curves are listed in Table 1.

than cells at low T cell concentrations. Thus T cell competition played a role in our assays.

The best theoretical fits between the total T cell proliferative responses and the proliferation functions derived above are denoted by the curves in Fig. 1 *Left*. It should be emphasized that in each panel all data were fitted simultaneously, explaining why the individual theoretical curves do not optimally fit the individual T cell concentration data sets. The parameter sets for which the optimal fits were obtained are given in Table 1. Although all four functions yielded a reasonable fit to the data, Fig. 1 *Right* exposes the shortcomings of both the conventional proliferation function without competition (Fig. 1*a*), and the function involving inhibitory T–T interactions (Fig. 1*b*). Both functions failed to account for the inhibitory effect of large T cell numbers on the proliferative response per T cell. Only the functions incorporating resource competition [either Ag-specific (Fig. 1*c*) or nonspecific (Fig. 1*d*)] yielded good descriptions of the proliferative responses

Table 1. Results of curve-fitting procedure of the first and second experimental approaches

Experimental approach	Function	$\rho$	$K_1$	$c_1$	$s$	$\varepsilon$	$b_T$	$b_A$	$b$	
1	No competition (Eq. 1)	0.56	$3.9 \times 10^5$				0.11	$4.2 \times 10^{-5}$	18	
	$T^* = \rho T \frac{A}{A + K_1} + \beta$	(0.10)	( $2.5 \times 10^5$ )				(0.01)	( $0.8 \times 10^{-5}$ )	(5.3)	
	Inhibitory T-T (Eq. 2)	0.53	$4.4 \times 10^5$			$7.8 \times 10^{-7}$	0.18	$4.3 \times 10^{-5}$	18	
	$T^* = \rho T \frac{A}{A + K_1} - \varepsilon T^2 + \beta$	(0.09)	( $2.4 \times 10^5$ )			( $1.6 \times 10^{-7}$ )	(0.02)	( $0.7 \times 10^{-5}$ )	(4.5)	
	Ag-specific resource comp. (Eq. 4)	0.65	$0.2 \times 10^5$	15.0			0.11	$4.2 \times 10^{-5}$	18	
	$T^* = \rho T \frac{A}{A + c_1 T + K_1} + \beta$	(0.06)	( $1.1 \times 10^5$ )	(2.7)			(0.01)	( $0.4 \times 10^{-5}$ )	(2.9)	
1	Nonspecific resource comp. (Eq. 5)	0.86	$3.8 \times 10^5$		$1.3 \times 10^{-5}$		0.11	$4.2 \times 10^{-5}$	18	
	$T^* = \rho \frac{T}{1 + sT} \frac{A}{A + K_1} + \beta$	(0.09)	( $1.4 \times 10^5$ )		( $0.2 \times 10^{-5}$ )		(0.01)	( $0.4 \times 10^{-5}$ )	(2.9)	
	2	No competition (Eq. 1)	0.18	$K_2$ 8.9	$c_2$			0.05		67
		$T^* = \rho T \frac{A}{A + K_2} + \beta$	(0.02)	(3.1)				(0.006)		(8.1)
		Inhibitory T-T (Eq. 2)	0.17	9.7			$1.1 \times 10^{-7}$	0.06		65
		$T^* = \rho T \frac{A}{A + K_2} - \varepsilon T^2 + \beta$	(0.02)	(3.2)			( $0.3 \times 10^{-7}$ )	(0.007)		(7.3)
Ag-specific resource comp. (Eq. 4)		0.21	4.5	$2.0 \times 10^{-4}$			0.05		65	
$T^* = \rho T \frac{A}{A + c_2 T + K_2} + \beta$		(0.02)	(2.3)	( $0.8 \times 10^{-4}$ )			(0.005)		(6.4)	
2	Nonspecific resource comp. (Eq. 5)	0.29	9.0		$0.9 \times 10^{-5}$		0.05		65	
	$T^* = \rho \frac{T}{1 + sT} \frac{A}{A + K_2} + \beta$	(0.02)	(1.7)		( $0.2 \times 10^{-5}$ )		(0.003)		(4.6)	

Parameters were estimated by minimizing the SSR between the logarithms of the total responses and the proliferation functions. Parameters giving the best fit to the data are followed by the corresponding standard deviations in parentheses. Background proliferation was modeled by the term  $\beta = b_T T + b_A A + b$ . In the second experimental approach, in which the concentration of APCs was not varied, no discrimination could be made between background proliferation due to APCs and background proliferation in the absence of APCs. Therefore both sources of background [<sup>3</sup>H]thymidine incorporation in the latter experiment were combined in the  $b$  term. Except for the  $K$  and  $c$  parameters, the parameter values of the two experimental approaches are of the same order of magnitude. The differences between the  $K$  and  $c$  parameters of the two approaches reflect the two different ways in which the Ag concentration was varied:  $K_1$  and  $c_1$  involve the number of antigenic sites per APC,  $n$ , whereas  $K_2$  and  $c_2$  involve the number of antigenic sites established per  $\mu\text{g}$  of peptide,  $m$ . Indeed, the estimates of the  $K$  parameters and the  $c$  parameters differ by the same order of magnitude—i.e., about 5 orders of magnitude. The parameters  $\rho$  and  $b_T$  are given in cpm/(cells per ml),  $b_A$  in cpm/(sites per ml),  $K_1$  and  $K_2$  in sites per ml,  $c_1$  and  $c_2$  in sites per cell,  $\varepsilon$  in cpm/(cells per ml)<sup>2</sup>,  $s$  in 1/(cells per ml), and  $b$  in cpm.

per T cell, indicating that T cells were competing indirectly, for shared resources.

Statistical analysis of the SSRs of the different proliferation functions yielded that all proliferation functions involving T cell competition gave a significantly better fit to the data ( $F$  test,  $P < 0.001$ ) than the conventional saturation function without competition (see Fig. 2). In Fig. 2 the Ag-specific resource competition of Eq. 4 is denoted by A, the nonspecific resource competition function of Eq. 5 by N, the inhibitory T-T interaction function of Eq. 2 by T, and the conventional saturation function of Eq. 1 by C. Solid arrows denote model extensions giving significantly better fits to the data, while dashed arrows denote extensions that did not lead to significantly better fits. It was of interest to determine whether extension of the competition functions with an extra competition term could significantly improve the fit to the experimental data. To this end, three new proliferation functions, each combining two of the competition terms described above (denoted by NA, AT, and NT) were fitted to the experimental data. Fig. 2 shows that once the proliferation function involved resource competition (A or N), the fit to the experimental data could not be significantly improved by adding another competition term. The proliferation function involving inhibitory T-T interactions (T), however, could be improved by incorporating either form of resource competition. Thus the SSR analysis demonstrates that T cell competition was mainly due to resource competition.

To study the nature of the resources for which the T cells were competing, the analysis was repeated with a different experimental approach. APCs were incubated at a standard concentration with Z1a T cells, after prior prepulsing with

increasing concentrations of peptide, leading to increasing ligand densities on the APCs. The results are summarized in Fig. 3. Because of the wide range of Ag concentrations, the results of the second experimental approach were plotted on a logarithmic horizontal axis. This explains the sigmoid shape of the proliferation curves. The proliferation function involving nonspecific resource competition (Fig. 3d) gave the best description of the data. Both the standard saturation function without competition (Fig. 3a) and the competition function involving inhibitory T-T interactions (Fig. 3b) failed to describe the inhibitory effect of large T cell concentrations on the proliferation per T cell, demonstrating again that resource competition plays a role *in vitro*.

The SSR analysis of the second experimental approach is summarized in Fig. 4. Extending the conventional saturation function of Eq. 1 (C) with inhibitory T-T interactions (T) did not significantly improve the fit to the data, whereas extension with a resource competition term (A or N) again did. Interestingly, the nonspecific resource competition function (N) now gave a much better fit than the Ag-specific resource competition function (A), and it was the only competition function that could not be significantly improved by extension with another competition term. Extension with a term accounting for nonspecific resource competition significantly improved the fit of the Ag-specific competition function ( $A \rightarrow NA$ ). Thus, nonspecific resource competition must have played a significant role in the second experimental approach. Increasing the Ag availability by increasing the concentration of presented peptides on APCs (approach 2) apparently differs from increasing the Ag availability by increasing the concentration of APCs presenting peptides (ap-

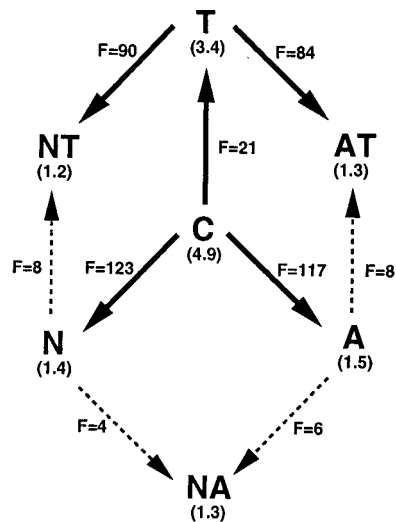


FIG. 2. Statistical comparison of the SSRs of the different proliferation functions of the first experimental approach, with SSRs in parentheses. Arrows represent different model extensions and are accompanied by the corresponding  $F$  values. Solid arrows denote model extensions that significantly improved the fit to the experimental data ( $F$  test,  $P < 0.001$ ); dashed arrows denote extensions that did not lead to significantly better fits ( $P > 0.001$ ). C represents the conventional saturation function without competition (Eq. 1), T the inhibitory T–T interaction function (Eq. 2), A the Ag-specific resource competition function (Eq. 4), and N the nonspecific resource competition function (Eq. 5). The proliferation function denoted by NA combines both forms of resource competition, AT combines Ag-specific competition and inhibitory T–T interactions, and NT combines nonspecific resource competition and inhibitory T–T interactions.

proach 1). Our interpretation is that T cells are competing for antigenic sites on APCs—i.e., for spaces on APCs where T cells can bind to their specific Ag without being disturbed by surrounding T cells. Because the concentration of APCs was fixed in the second experimental approach, there was a limited number of APC sites T cells could bind to. Prepulping with higher peptide doses might thus have increased the peptide concentration *per antigenic site*, but might have failed to increase the actual Ag availability for T cells.

In the second experimental approach, it would be more appropriate to explicitly model the concentration of antigenic sites  $A$  as a saturation function of the peptide concentration used for prepulping the APCs (see *Appendix*). When such a saturation is substituted in Eq. 4 a proliferation function is obtained that has the same qualitative behavior as the nonspecific resource competition of Eq. 5, explaining why the latter function described the data better than the Ag-specific resource competition function of Eq. 4. Because in the first experimental approach the number of antigenic sites  $A$  increased linearly with the concentration of APCs, both functions gave a good fit to the data in Fig. 1. Additionally, the fact that the data fitting in our two experimental approaches gave qualitatively different results suggests that T cells were not merely competing for resources such as growth factors or nutrients in the medium. If T cells were competing for such APC-independent resources, one would expect Figs. 2 and 4 to be similar. Finally, when the analysis was repeated with arthritogenic A2b T cells, the same qualitative picture as Figs. 2 and 4 was obtained. This result confirms that T cells compete for antigenic sites on APCs.

### CONCLUSION

By mathematical analysis of data from T cell proliferation assays in which both the concentration of Ag-presenting sites and the concentration of T cells were varied, we have shown evidence for T cell competition *in vitro*. Our results are in full agreement with *in vivo* data demonstrating that the prolifer-

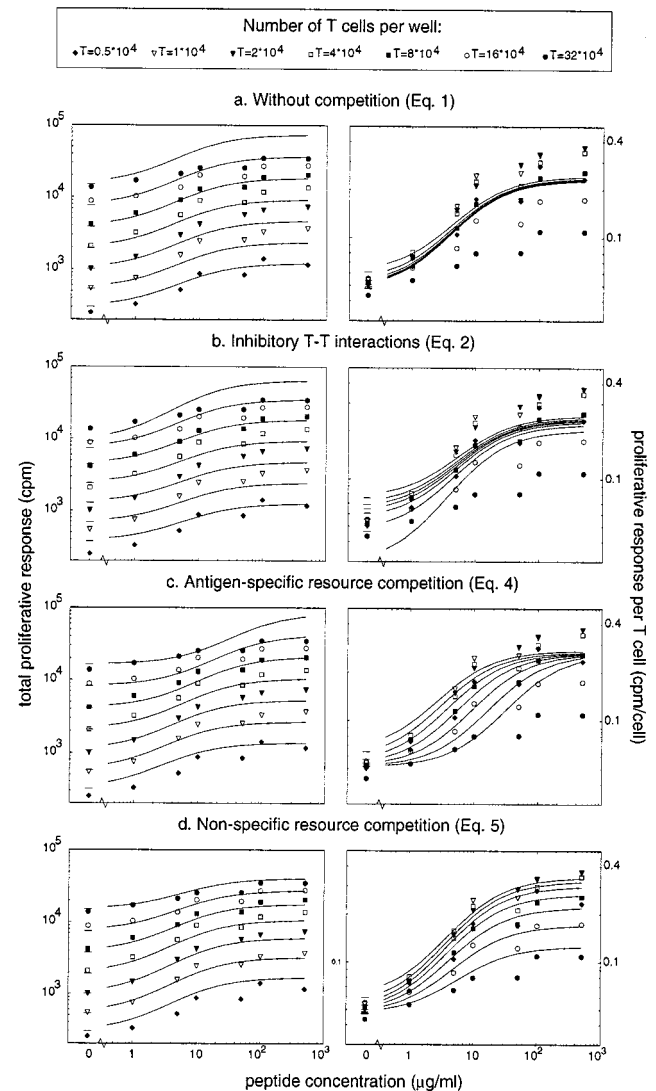


FIG. 3. Proliferative responses of different concentrations of Z1a T cells in response to a standard concentration of APCs prepulsed with different concentrations of MBP 72–85S79A. For details see the legend of Fig. 1.

ative capacity of T cells is influenced not only by the Ag availability but also by the presence of other T cells—i.e., T cell competition (2, 3, 7). In theoretical models of the immune system, T cell competition terms are often applied for their stabilizing effect on T cell population sizes (24). The results presented here provide an experimental validation for the use of such T cell competition terms.

Our mathematical approach enabled us to discriminate between three qualitatively different forms of T cell competition—i.e., direct inhibitory T–T interactions, Ag-specific resource competition, and nonspecific resource competition. The best description of the experimental data was obtained with proliferation functions involving resource competition. Comparison of two different experimental approaches, in which the antigenic ligand concentration was controlled differently, indicated that T cells were mainly competing for antigenic sites on APCs. T cell competition for antigenic sites thus seems to be a phenomenon arising naturally from the interactions of T cells with their ligands, which should be taken into account in both experimental and theoretical studies of T cell proliferation. The relative importance of competition for a site on an APC, and competition between cells binding the same MHC-peptide complex, remains to

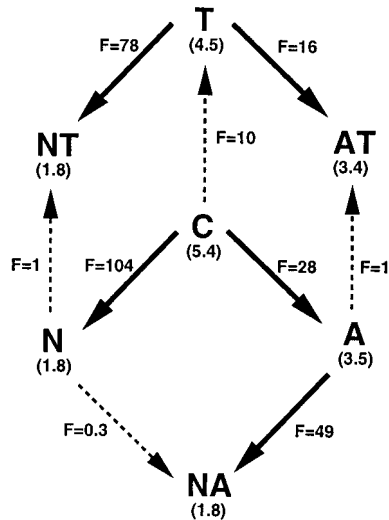


Fig. 4. Statistical comparison of the SSRs of the different proliferation functions of the second experimental approach. For details see the legend of Fig. 2.

be elucidated by developing models and experiments involving multiple T cell clones competing for multiple antigens.

**APPENDIX**

**Derivation of the Ag-Specific Competition Function.** The T cell interaction scheme (3) can be described by the following differential equation for the T cell–Ag complexes *C*:

$$\frac{dC}{dt} = k_1 T_f A_f - k_{-1} C. \tag{6}$$

Following Borghans *et al.* (23), we make a quasi-steady state (QSS) approximation for the T cells in complex (*C*), and substitute the equations for the total concentration of T cells  $T = T_f + C$ , and the total concentration of antigenic sites  $A = A_f + C$  into Eq. 6, giving:

$$\frac{dC}{dt} = k_1((T - C)(A - C) - KC) = 0, \quad \text{where } K = \frac{k_{-1}}{k_1}. \tag{7}$$

If the concentration of T cells and Ags in complexes *C* is small (23) compared with the total concentration of T cells *T*, and compared with the total concentration of antigenic sites *A*, the *C*<sup>2</sup> terms in Eq. 7 can be neglected, yielding:

$$C \approx \frac{AT}{A + cT + K}, \quad \text{where } c = 1. \tag{8}$$

According to Huisman and de Boer (25), this *c* value becomes a parameter that may deviate from *c* = 1 if T cell proliferation is modeled as a multistep process in which a T cell–Ag complex (*C*) first becomes an activated T cell, which subsequently proliferates to form two free T cells *T<sub>f</sub>*. For maximal simplicity, we have left out this activated T cell stage, but we do allow for *c* being a parameter that can be estimated freely. Since total T-cell proliferation is assumed to be proportional to the total number of T cell–Ag complexes *C*, total [<sup>3</sup>H]thymidine incorporation can be modeled by  $T^* = \rho C$ , i.e., by Eq. 4.

**Transforming Peptides and APCs into Antigenic Sites.** To become stimulated, a T cell has to bind to an APC and interact with the appropriate MHC–peptide complex. Because only a limited number of T cells can bind to one APC at any time, T cells compete for “sites” on APCs where T cells can bind and Ag is

presented (9). In the first experimental approach, the antigenic site concentration (*A*) is increased by adding APCs, and is thus proportional to the APC concentration:  $A = nA_c$ . Here *A<sub>c</sub>* represents the concentration of APCs and *n* the number of antigenic sites per APC. In the second approach, however, it is more appropriate to model the antigenic site concentration as a saturation function of the peptide concentration:

$$A = m \frac{A_p}{A_p/h + 1}, \tag{9}$$

with *A<sub>p</sub>* denoting the peptide concentration used to prepulse the APCs, *h* denoting the peptide concentration at which the antigenic site concentration is half-maximal, and *m* representing the number of antigenic sites established per μg of peptide at low peptide concentrations. Because substitution of Eq. 9 into the Ag-specific resource competition function of Eq. 4 yields a competition function that is very similar to the nonspecific resource competition function of Eq. 5, but involves one more parameter than Eq. 5, we refrained from substituting Eq. 9 when we fitted the experimental results of Fig. 3. Instead we substituted the linear domain of Eq. 9, i.e.,  $A = mA_p$ .

Since both *n* and *m* are unknown, we have scaled the *c* and *K* parameters by dividing both the numerator and the denominator of the proliferation functions of Eqs. 1, 2, 4, and 5 by *n* in the first experimental approach and by *m* in the second approach. In the fitting procedure we have thus estimated  $c_1 = c/n$  and  $K_1 = K/n$  for the first experimental approach and  $c_2 = c/m$  and  $K_2 = K/m$  for the second experimental approach. This explains the differences in *c* and *K* in the first and second experimental approach (see the legend of Table 1 for further details).

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