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Emil Jahng April 11, 2016

T cell receptor affinity controls activation of competing CD4 T cells

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

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The immune system comprises many different types of cells. One class of cells, CD4 T cells, helps coordinate cell-mediated immune responses through binding interactions between their T cell receptors and antigenic peptide-MHCII complexes displayed on antigen presenting cells. Since each CD4 T cell is unique and has a stochastically generated T cell receptor, a host's population of CD4 T cells has a range of affinities for any given peptide-MHCII complex. Moreover, it has been shown that during an immune response, CD4 T cells with both high and low affinities for antigen can proliferate, with low affinity CD4 T cells displaying delayed proliferation compared to higher affinity cells. However, it is not known why this is the case. Here, we explore the interactions between high and low affinity CD4s with the hypothesis that high affinity CD4 T cells can help low affinity CD4 T cells proliferate. Our results provide evidence that these cells compete for access to activating factors on the surface of antigen-presenting cells. These results are consistent with others in demonstrating that competition can occur between T cells. Further investigation of such interactions may hopefully lead to a better understanding of the importance of the presence of low and high affinity CD4 T cells during a polyclonal helper T cell response.

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Abstract

The immune system comprises many different types of cells. One class of cells, CD4 T cells, helps coordinate cell-mediated immune responses through binding interactions between their T cell receptors and antigenic peptide-MHC II complexes displayed on antigen presenting cells. Since each CD4 T cell is unique and has a stochastically generated T cell receptor, a host's population of CD4 T cells has a range of affinities for any given peptide-MHC II complex. Moreover, it has been shown that during an immune response, CD4 T cells with both high and low affinities for antigen can proliferate, with low affinity CD4 T cells displaying delayed proliferation compared to higher affinity cells. However, it is not known why this is the case. Here, we explore the interactions between high and low affinity CD4s with the hypothesis that high affinity CD4 T cells can help low affinity CD4 T cells proliferate. Our results provide evidence that these cells compete for access to activating factors on the surface of antigen-presenting cells. These results are consistent with others in demonstrating that competition can occur between T cells. Further investigation of such interactions may hopefully lead to a better understanding of the importance of the presence of low and high affinity CD4 T cells during a polyclonal helper T cell response.

Introduction

Every day, we are exposed to a myriad of potentially disease-causing microbes, called pathogens. Nevertheless, many of us are able to remain relatively healthy thanks to our immune system—a set of

many different types of cells and molecules that work together to fight the pathogens that enter our bodies. One important component of the immune system consists of a class of cells called CD4 helper T lymphocytes (so called because they express a CD4 co-receptor). These cells are responsible for recognizing the presence of specific microbes as part of what we call the cell-mediated adaptive immune response.

CD4 T cells arise from lymphoid progenitor cells, which are initially produced in the bone marrow and then migrate to the thymus where they differentiate into immature thymocytes. In the thymus, each thymocyte that successfully matures expresses a unique TCR (via a process called TCR rearrangement) as well as either a CD4 or CD8 co-receptor (1). These cells then emigrate to secondary lymphoid compartments (e.g. the lymph nodes and spleen) to patrol the body. This transition is associated with these thymocytes becoming naïve T cells (2).

While patrolling the body, a given CD4 T cell can recognize the presence of a foreign invader and become activated if it receives at least two types of signals from another cell, called an antigen-presenting cell (or APC), which has already encountered the pathogen (3). When an APC encounters a pathogen, the APC can internalize it and utilize internal cell machinery to break down parts of the pathogen into peptides, which are subsequently loaded onto molecules called major histocompatibility complex class II (or MHC II) molecules for presentation on the surface of the APC (4). This TCR-peptide MHC II binding is the first signal the CD4 T cell needs to become activated. It is important to note that since each CD4 T cell has a unique TCR, there are CD4 T cells in the body that can recognize every possible antigen, but not every T cell can see every antigen.

The second type of signal a CD4 T cell needs is a costimulatory signal from antigen-presenting cells. APCs detect the presence of pathogens through receptors, called Pattern Recognition Receptors (or PRRs), that bind highly conserved molecular patterns of pathogens, called pathogen-associated molecular patterns (or PAMPs) (5). After such interactions between PRRs and PAMPs, APCs can

upregulate the expression of surface costimulatory molecules on their surfaces. This mechanism serves as a check against autoimmune disease because a T cell is much more likely to be activated when an APC recognizes that there is indeed a foreign pathogen present beforehand (5).

Once an individual T cell receives the required signals, it becomes activated and secretes cytokines such as interleukin-2—a growth factor that stimulates the expansion and differentiation of T cells (6). The proliferated T cells then mobilize an immune response, which can include differentiation of antibody-producing B cells or activation of pathogen-eating macrophages (6, 7).

The CD4 T cells in a given individual's body are able to recognize, and thus mobilize an immune response against a wide variety of pathogens. To understand how this is possible, we can consider a theory called the clonal selection hypothesis (1, 8). According to this theory, as mentioned previously, each individual CD4 T cell is actually unique because it develops a unique TCR. So as a whole, the population of CD4 T cells has many different TCRs that able to bind to different peptide MHC II complexes and thus recognize the presence of different pathogens. As the generation of TCRs is stochastic, T cells will have a range of affinities for peptide-MHC complexes (9). That is, some cells are more likely to bind to a particular peptide-MHC complex and are said to have higher affinity, while others are less likely to bind and have lower affinity. Another result of the stochastic generation of TCRs is that some T cells produced in the thymus actually wind up with receptors that can bind to self-antigens, again with high and low affinities. These cells must be eliminated because otherwise, they will mobilize an immune response to the body's own cells and cause autoimmune disease. The process by which self-reactive T cells are eliminated is called negative selection (10).

The purpose of this project was to investigate the interaction between high affinity and low affinity CD4 T cells in the same immune environment. As higher affinity TCRs have an increased likelihood of binding to peptide MHC II antigen, one might assume higher affinity T cells would be more likely to receive the signals needed to proliferate and, as a result, out-compete T cells with lower affinity

TCRs. However, it is known that both low and high affinity T cells exist during an immune response, suggesting that low affinity T cells may have some compensatory mechanisms or need different factors for survival than high affinity T cells (11). Our hypothesis is that high affinity T cells may help low affinity T cells proliferate. The null hypothesis is that high and low affinity T cells do not influence each other's proliferation.

Methods

2D micropipette assay for P25/85B: RBCs were isolated in accordance with the Institutional Review Board at Emory University and prepared as previously described (12). RBCs coated with various concentrations of Biotin-X-NHS (EMD) were coated with 0.5 mg/ml streptavidin (Thermo Fisher Scientific), followed by 1–2 μg of pMHC II monomer (NIAID TCR at Emory University). The pMHC (peptide-MHC)-coated RBCs were stained with anti-MHC class II PE antibody, and purified T cells were stained with anti-TCR PE antibody. The densities of I-Ab and TCR were calculated using BD QuantiBrite Beads (BD). The micropipette adhesion frequency assay was then preformed as previously described. In brief, a pMHC-coated RBC and T cell were placed on opposing micropipettes and brought into contact by micromanipulation for a controlled contact area (Ac) and time (t). The T cell was retracted at the end of the contact period, and the presence of adhesion (indicating TCR–pMHC binding) was observed by elongation of the RBC membrane. This T cell-RBC contact was repeated 30 times and the adhesion frequency (P_a) was calculated. The relative 2D affinity (A_cK_m) of each cell that had a P_a greater than 10% was calculated using the P_a at equilibrium (where $t \rightarrow \infty$) using the following equation: $A_cK_m = -\ln[1-P_a(\infty)]/(m_rm_l)$, where m_r and m_l reflect the receptor (TCR) and ligand (pMHC) densities, respectively. CD4 T cells from the spleens of P25 TCR-Tg mice were purified following manufacturer instructions using the CD4 T cell positive selection kit (Miltneyi Biotec).

Obtaining cells from mice: Splenocytes were obtained from 2D2 transgenic and P25 transgenic mice. All animals were housed in an Emory University Department of Animal Resources facility (Atlanta, GA) and used in accordance with an Institutional Animal Care and Use Committee–approved protocol.

Harvested spleens were processed into a single cell suspension in Fisherbrand Sterile Cell Strainer 100 µm Nylon Mesh filters in Hanks' Balanced Salt Solution with calcium & magnesium, then centrifuged at 1200 rpm for 5 minutes. All future centrifugations were performed at this speed and duration. Supernatant was removed, and 1 mL of ACK lysis buffer was added per sample of splenocytes for 1 minute in order to lyse red blood cells. 5 mL of R10 (RPMI 1640 media with L-glutamine mixed with 50 mL heat-killed FBS serum, 5 mL 2-mercaptoethanol, 5 mL L-glutamine, 5 mL HEPES, and 500 µL Gentamicin sulfate) was added to each sample before centrifuging to prevent excess lysis and was washed. Each sample was then resuspended in 10 mL of DPBS (Dulbecco's Phosphate-Buffered Saline without calcium & magnesium), and cell counts were performed using a hemocytometer.

CFSE staining: 2D2 splenocytes were resuspended to 1 x 10^7 cells/mL in PBS and incubated in a water bath at 37^{o}C for 10 minutes. CFSE (6mM) was added at 1:12000 dilution, and was incubated for 10 minutes at 37^{o}C, mixing the sample every two minutes. The samples were then washed with R10 media three times before the splenoctyes were counted with a hemocytometer. The splenocytes were resuspended to a concentration of 12 x 10^6 cells/mL in R10.

Cell Trace Violet staining: P25 splenocytes were resuspended to 5 x 10^6 cells/mL in PBS. Then, 1 µL of 5mM Cell Trace Violet solution per 1 mL PBS was added at 1:10 dilution (except for the first trial of the first assay, which was at 1:100 dilution), and the sample was incubated for 20 minutes at 37^{o}C. The sample was then washed with R10 once before splenoctyes were counted with a hemocytometer. The splenocytes were then resuspended to a concentration of 12 x 10^6 cells/mL in R10.

Peptide antigens: The peptide antigens used were $MOG_{35–55}$ (MEVGWYRSPFSRVVHLYRNGK), MTB $85B_{280-294}$ (FQDAYNAAGGHNAVF), GP_{61-81} (GLKGPDIYKGVYQFKSVEFDM), and NFM_{15-35} (RRVTETRSSFSRVSGSPSSGF). Each antigen was resuspended to a concentration of 40 µM in R10.

Preparation of assays: Four different types of assays were performed, and they are described in the order that their results are presented in. For the first assay, three wells were prepared in a sterile 12-well plate as follows after splenocytes and peptide antigens were prepared as above: 1st well—250 µL each of 2D2 splenocytes, P25 splenocytes, MOG, and 85B; 2nd well—250 µL each of 2D2 splenocytes, P25 splenocytes, MOG, and GP; 3rd well—250 µL each of 2D2 splenocytes, P25 splenocytes, GP, and 85B. The plate was then placed on a platform shaker for 10 minutes to ensure that the contents of each well were well mixed. The plate was then placed for approximately 48 hours in an incubator at 37°C. The second assay was conducted in the same manner as the first except NFM replaced MOG. The third assay was also conducted in the same manner as the first except that for each well, 250 µL of one of either the 2D2 or P25 cells was pipetted on the bottom of a Costar Transwell 24 mm, 0.4 µm Polyester Membrane, and then the other cells as well as the peptides were pipetted on top of the membrane.

For the fourth assay, 2D2 splenocytes, P25 splenocytes, MOG, and 85B were prepared as in the previous assays and then mixed in equal volumes. For the first two trials, each trial consisted of 4 wells in a sterile 96-well plate as follows: 1st well—100 µL splenocyte/antigen mix, 2nd well—99 µL splenocyte/antigen mix with 1 µL anti-MHC (Purified Anti-Mouse I-A(b) MHC Class II), 3rd well—99 µL splenocyte/antigen mix with 1 µL of 1:10 anti-MHC in R10, 4th well—99 µL splenocyte/antigen mix with 1 µL of 1:100 anti-MHC in R10. For each of the two remaining trials, two additional wells were prepared as follows: 1st additional well—98 µL splenocyte/antigen mix with 2 µL of 1:10 anti-MHC, 2nd additional well—98 µL splenocyte/antigen mix with 2 µL of 1:100 anti-MHC.

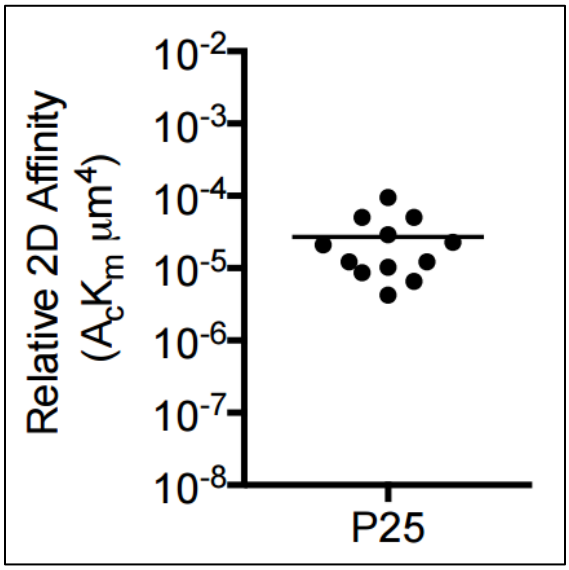
Flow cytometry and data analysis: After incubation, cells were centrifuged, and each sample was stained for 30 minutes on ice with 50 µL per sample of a fluorescent antibody mix (TCR PE Texas Red,

CD11b Percp, CD11c Percp, CD19 Percp, CD4 Brilliant Violet 510, Zombie Yellow 570, and CD69 Brilliant Violet 605) each at 1:100 dilution in DPBS. After 450 µL of FACS wash was added to each sample, the samples were collected on an LSR II (Becton Dickinson) and analyzed using Flowjo (Treestar). All gating of the resulting data was performed in Flowjo, some calculations were performed in Microsoft Excel, and all statistical analysis was performed in Prism 6 except for the power calculation performed on data from the fourth assay, which was done with G*Power 3.1.9.2. Proliferation indices were calculated as follows: Let G0 = number of cells resulting from zero divisions, G1 = number of cells resulting from 1 division, G2 = number of cells resulting from two divisions, G3 = number of cells resulting from 3 divisions, etc. Then, proliferation index = (total # divisions / # cells that went into division) = [(G1/2)*1 + (G2/4)*2 + (G3/8)*3 + …] / [(G1/2) + (G2/4) + (G3/8) + …]

Results

Figure 1:

A)



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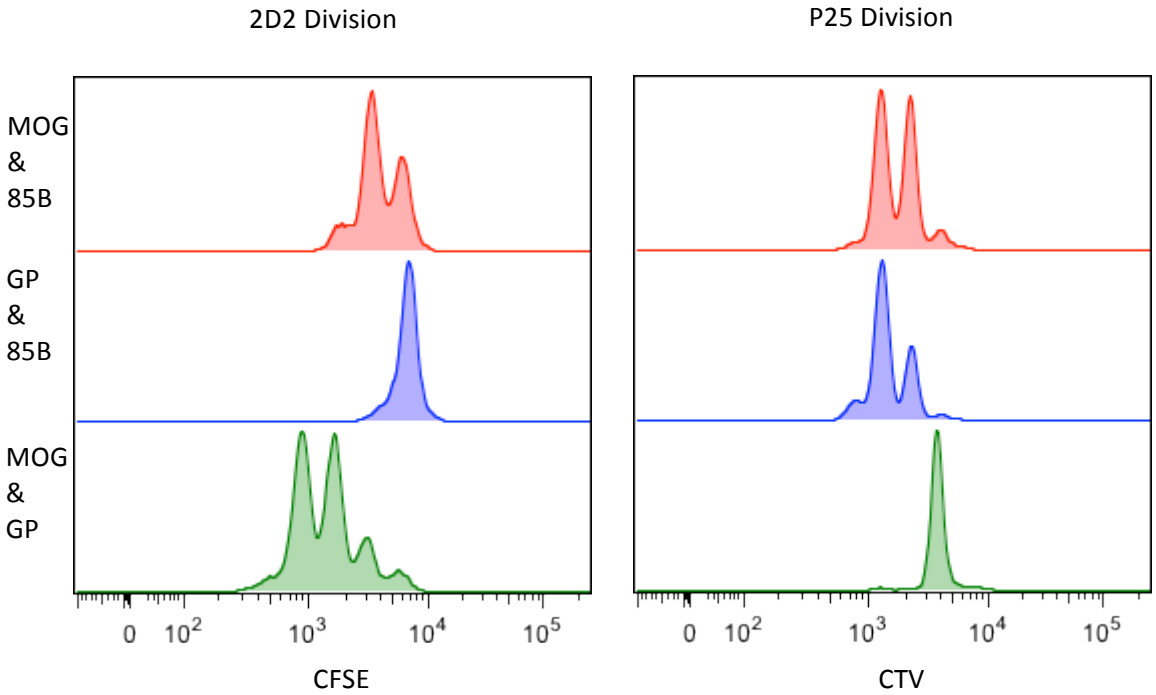


C)

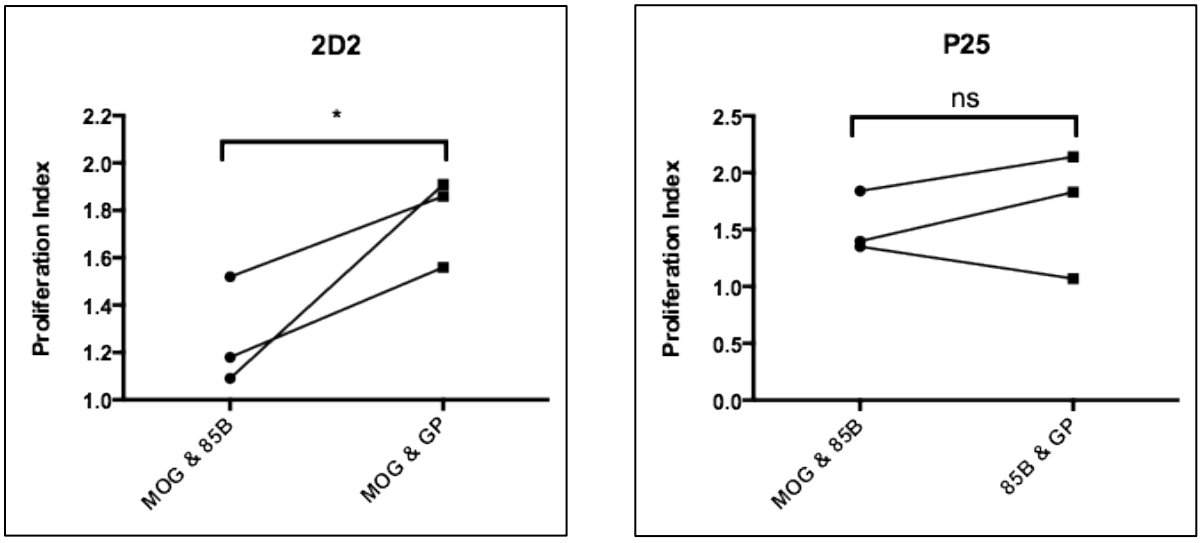


Figure 1: Affinity hierarchy controls T cell entry into the immune response. A) P25 TCR affinity for 85B antigen, as measured by 2D micropipette assay. B) Representative data showing 2D2 and P25 division as tracked by CFSE and CTV. C) Left: 2D2 proliferation indices when both 2D2 and P25 CD4 T cells were activated (MOG & 85B) compared with 2D2 proliferation indices when only 2D2 CD4 T cells were activated (MOG & GP). Right: P25 proliferation indices when both P25 and 2D2 CD4 T cells were activated (MOG & 85) compared with P25 proliferation indices when only P25 CD4 T cells were activated (85B & GP). One to two 2D2 and P25 spleens each were used per trial, and proliferation indices were calculated at approximately 48 hours of incubation.

Low affinity CD4 T cells display attenuated cell proliferation in competition with higher affinity CD4 T cells. To explore how TCR affinity controls the recruitment of T cells into an immune response, two TCR-transgenic T cell populations were analyzed for proliferation when both were activated versus when only one was activated. The two TCRs used were P25 and 2D2, with P25 transgenic CD4 T cells activated by 85B antigen, and 2D2 transgenic CD4 T cells activated by MOG antigen. P25 affinity for 85B was measured by 2D micropipette assay to be over three magnitudes of order higher than 2D2 affinity for

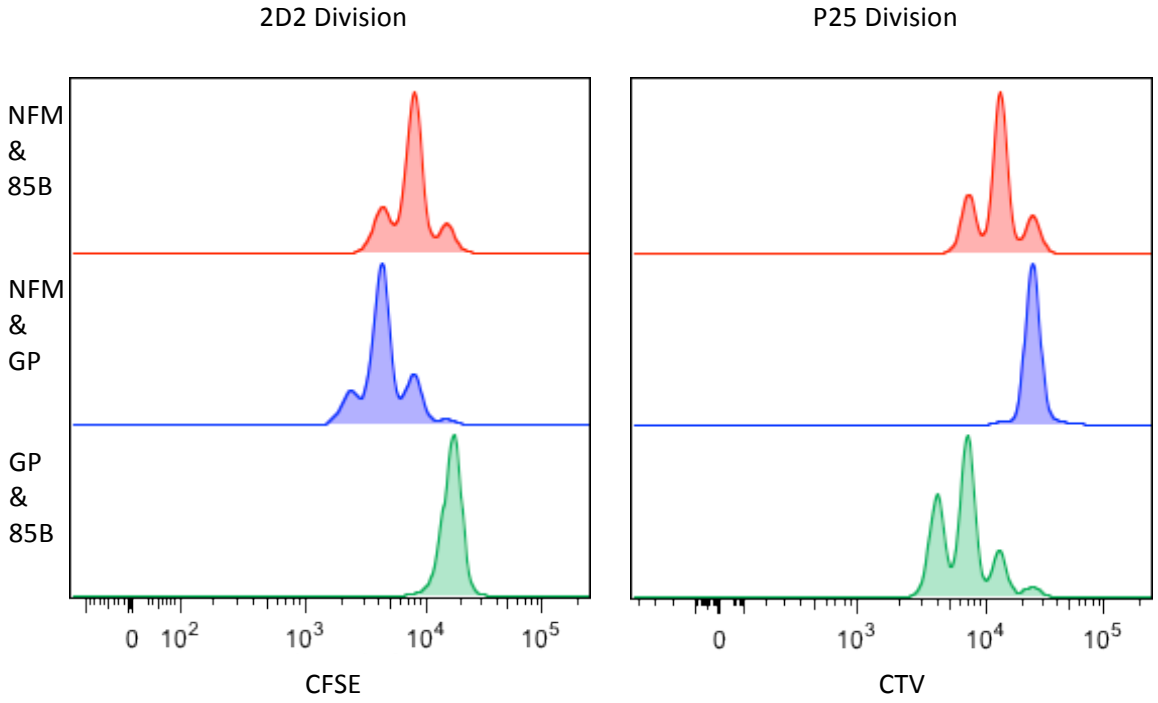
MOG (Figure 1A) (13). Thus, P25 CD4 T cells were the high affinity model and 2D2 CD4 T cells were the low affinity model. GP, a peptide which neither TCR-transgenic CD4 T cell recognized, was used as a control to prevent excess loading of the antigenic peptides when only one TCR-transgenic T cell population was being activated.

2D2 and P25 CD4 T cells were mixed together and given either 1) MOG and 85B antigen to activate both, 2) GP and 85B to activate P25 CD4 T cells only, or 3) MOG and GP to activate 2D2 CD4 T cells only. To track division, 2D2 CD4 T cells were labeled with CFSE, and P25 CD4 T cells were labeled with Cell Trace Violet (CTV). CFSE and Cell Trace Violet are fluorescent molecules that label intracellular components. When a given cell divides, the daughter cells become less fluorescent because the fluorescent molecules are split between the two. These cells in turn divide, creating another set of daughter cells that is even less fluorescent, and so forth. Thus, on the CFSE and Cell Trace Violet graphs, a given peak represents a set of cells that have undergone the same number of divisions, with the peaks to the left representing cells that have undergone a greater number of divisions. In the representative graphs shown, both 2D2 and P25 CD4 T cells displayed larger and a greater number of peaks to the left when activated alone than when both were activated (Figure 2B).

Statistical analysis revealed that the 2D2 CD4 T cells given MOG had a significantly lower proliferation index (total # divisions / # cells that went into division) in the presence of P25 CD4 T cells given 85B than in the presence of P25 CD4 T cells not activated with peptide antigen ($p=0.0396$, unpaired student's t-test, Figure 1C left). In contrast, the P25 CD4 T cells were not significantly affected by whether the 2D2 CD4 T cells were given MOG or not activated with peptide antigen ($p=0.6935$, student's unpaired t-test, Figure 1C right).

Figure 2:

A)



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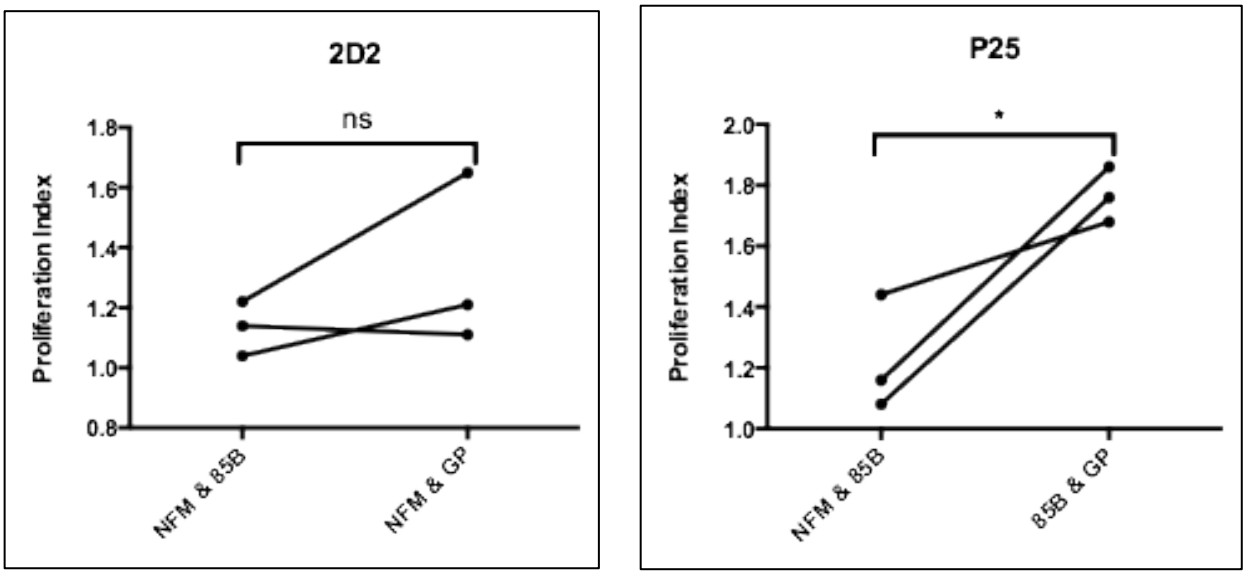


Figure 2: Competition between 2D2 and P25 CD4 T cells depends on TCR affinity. A) Representative data showing 2D2 and P25 division as tracked by CFSE and CTV. B) Left: 2D2 proliferation indices when both 2D2 and P25 CD4 T cells were activated (NFM & 85B) compared with 2D2 proliferation indices when

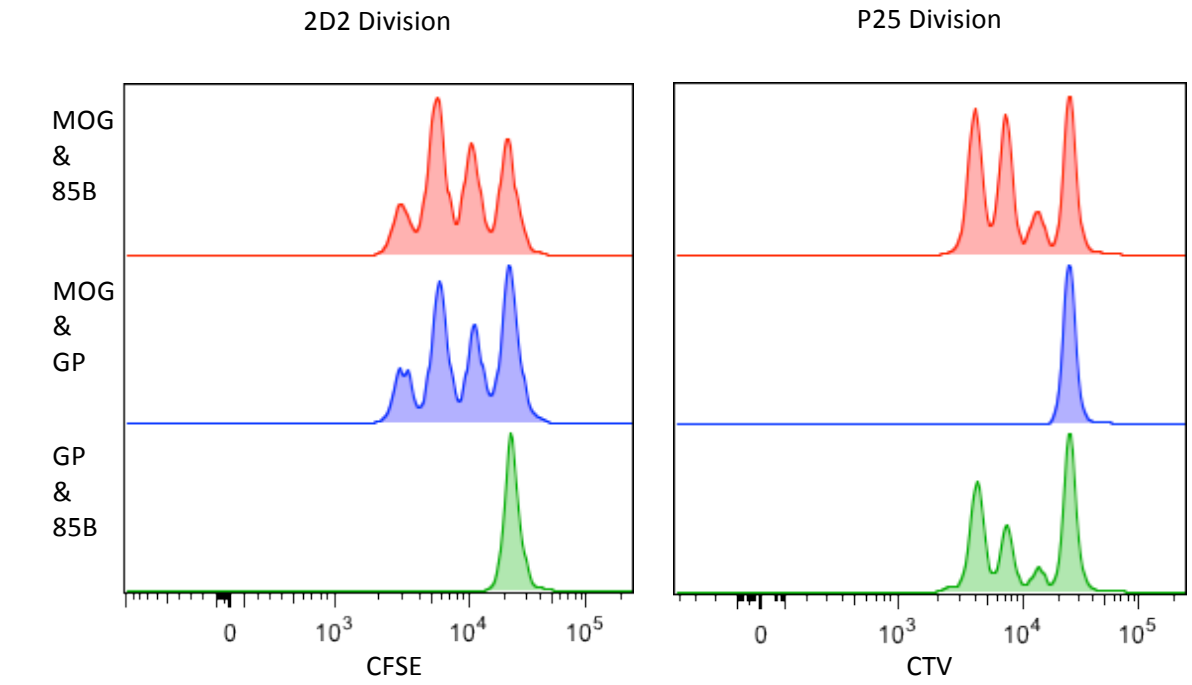
only 2D2 CD4 T cells were activated (NFM & GP). Right: P25 proliferation indices when both P25 and 2D2 CD4 T cells were activated (NFM & 85) compared with P25 proliferation indices when only P25 CD4 T cells were activated (85B & GP).

Observed competition between 2D2 and P25 CD4 T cells is based on TCR affinity for peptide-MHC II.

One potential explanation of the results in Figure 1C is that the difference in affinity for antigen was not a factor, but rather the 2D2 CD4 T cells were simply not as good as P25 CD4 T cells at proliferating for some other reason. For example, it is known that different transgenic T cells have different levels of reactivity (14). To test the hypothesis that the competition observed in the first assay was due to affinity, the previous experiment was repeated, except that 2D2 CD4 T cells were given an antigen that they have a higher affinity for—NFM—so that their affinity for antigen was no longer low compared to P25 affinity for 85B (13). When 2D2 CD4 T cells were given NFM instead of MOG, they did not exhibit a significantly different proliferation index regardless of whether the P25 CD4 T cells were given 85B or not stimulated with peptide antigen ($p=0.3358$, student's unpaired t-test, Figure 2B left). Interestingly, the P25 CD4 T cells given 85B exhibited significantly attenuated proliferation in the presence of 2D2 CD4 T cells given NFM compared to 2D2 CD4 T cells not activated with peptide antigen ($p=0.0111$, student's unpaired t-test, Figure 2B, right).

Figure 3:

A)



B)

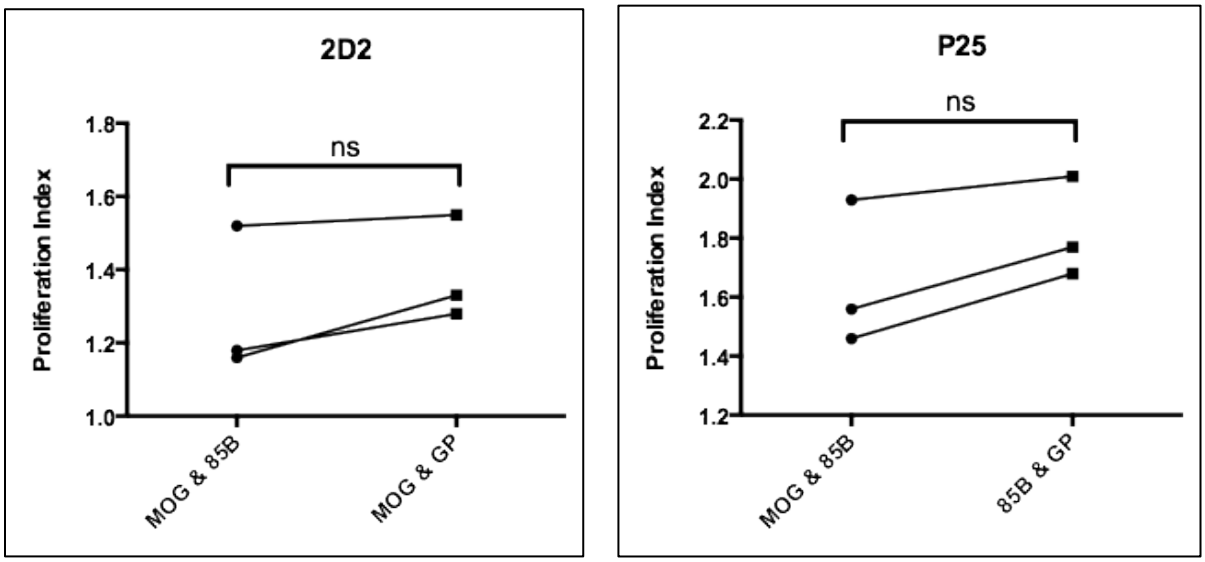


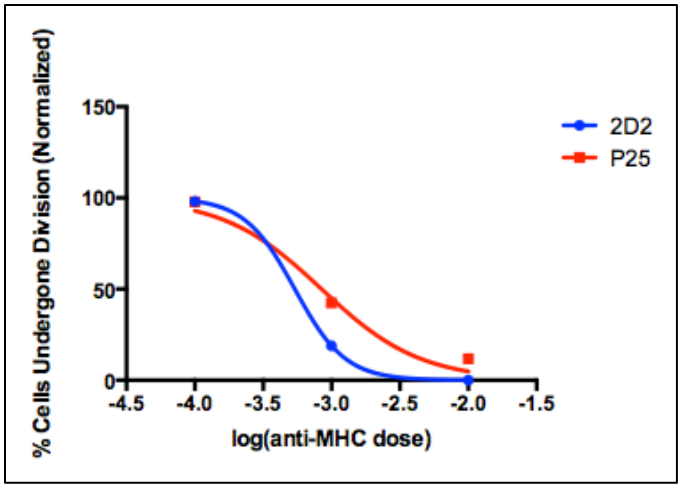
Figure 3: TCR affinity competition requires cell surface contact. A) Representative data showing 2D2 and P25 division as tracked by CFSE and CTV. B) Left: 2D2 proliferation indices when both 2D2 and P25 CD4 T cells were activated (MOG & 85B) compared with 2D2 proliferation indices when only 2D2 CD4 T cells were activated (MOG & GP) and transwells separated 2D2 and P25 splenocytes. Right: P25

proliferation indices when both P25 and 2D2 CD4 T cells were activated (MOG & 85) compared with P25 proliferation indices when only P25 CD4 T cells were activated (85B & GP) and transwells separated 2D2 and P25 splenocytes.

TCR affinity-based competition is based on cell-to-cell contact. It was hypothesized that perhaps low and high affinity CD4 T cells were competing for a soluble growth factor, such as IL-2, which affects proliferation (6). If this was indeed then case, then low and high affinity CD4 T cells would display competition if they were separated with a membrane that let such soluble factors pass but prevented cell-to-cell contact. However, when a filter was used such that 2D2 and P25 splenocytes were kept separate during activation, 2D2 CD4 T cells given MOG did not exhibit a significantly different proliferation index regardless of whether P25 CD4 T cells were given 85B or not activated with peptide antigen (p=0.5236, student's unpaired t-test, Figure 3B left). Likewise, P25 CD4 T cells given 85B did not exhibit a significantly different proliferation index depending on whether 2D2 CD4 T cells were given MOG or not activated with peptide antigen (p=0.3829, student's unpaired t-test, Figure 3B right).

Figure 4:

A)



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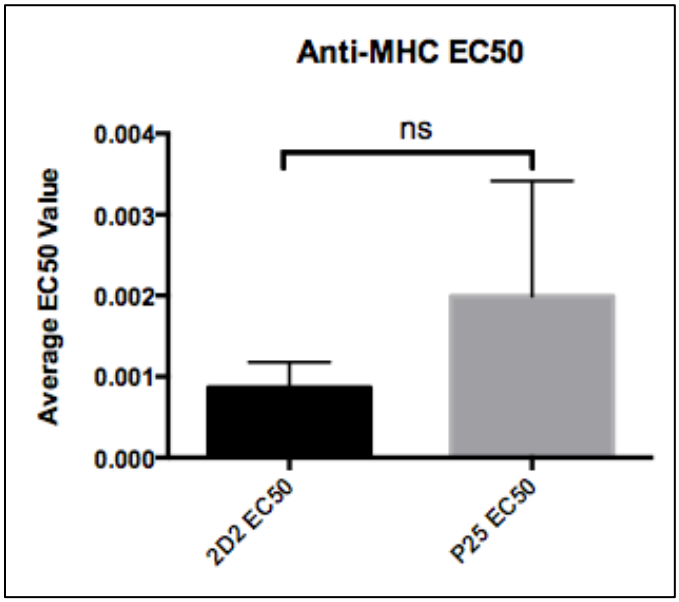

Figure 4: There is a trend, but not statistical significance, that low affinity CD4 T cells are more sensitive than high affinity CD4 T cells to MHC block. A) Representative data showing how increasing the anti-MHC dose affects the proportion of CD4 T cells that undergo division. Data were normalized relative to the division that occurred in control wells (to which no anti-MHC was added). B) Comparison of the average EC50 for 2D2 and P25 CD4 T cells, EC50 being the dose of anti-MHC necessary to give a half-maximal response (maximal response being a normalized 0% of cells undergoing at least one division).

Competition for TCR access to peptide-MHC interactions is trending, but not statistically significant in controlling for T cell expansion. Considering the result that cell-to-cell contact is required for competition between low and high affinity CD4 T cells, it was hypothesized that perhaps there was competition for access to peptide-MHC II on the APC surface, with high affinity CD4 T cells being better at competing for that access. If this was indeed the case, then it would be expected that by reducing the amount of MHC on the surface, one could expect differing activation between TCRs with different affinities, with the lower affinity CD4 T cells displaying a more pronounced attenuation of division before

the high affinity CD4 T cells did. However, when 2D2 CD4 T cells activated with MOG and P25 CD4 T cells activated with 85B were mixed and treated with increasing doses of anti-MHC II antibody to block access, there was no significant difference in the EC50 values ($p=0.2109$, student's unpaired t-test with Welch's correction, f-test $p=0.0335$, Figure 4B). However, there was a trend for 2D2 EC50 values to be less than P25 EC50 values with $n=4$ trials each for 2D2 and P25 anti-MHC experiments. Moreover, a power calculation utilizing the data obtained from this assay thus far with desired power set to 0.8 indicated that a larger sample size of $n=12$ trials each for 2D2 and P25 anti-MHC II experiments would be needed to adequately test this hypothesis (15).

Discussion

The results indicate that rather than high affinity CD4 T cells helping low affinity CD4 T cells proliferate, high and low affinity CD4 T cells compete with each other for access to activating factors on the APC surface. The first assay in which 2D2 CD4 T cells activated with MOG and P25 CD4 T cells activated with 85B were mixed without any barriers between the cells suggests that in terms of proliferation, low affinity CD4 T cells are more sensitive to competition with high affinity CD4 T cells than vice versa.

The second assay in which 2D2 CD4 T cells were activated with NFM instead of MOG indicates that indeed, differences in affinity were responsible for how well the CD4 T cells competed in the first assay. Specifically, when 2D2 CD4 T cells were no longer low affinity relative to P25 CD4 T cells, the 2D2 CD4 T cells no longer displayed sensitivity to competition in terms of proliferation. An unexpected result was that the P25 CD4 T cells that suffered significantly attenuated proliferation when in competition with the activated 2D2 CD4 T cells. Although it makes sense that the P25 CD4 T cells would lose their competitive advantage, the results of the assay are puzzling since it is not clear why P25 CD4 T cells

would be at a competitive disadvantage—P25 CD4 T cell affinity for 85B is not less than 2D2 CD4 T cell affinity for NFM. Perhaps the result may be due a peculiarity to the transgenic models. However, further investigation is needed to elucidate the causes of this particular effect.

The third assay in which 2D2 CD4 T cells were activated with MOG, P25 CD4 T cells were activated with 85B, and 2D2 and P25 splenocytes were separated by a transwell indicates that the competition observed in the first assay was for access to something on the surface of antigen presenting cells. If the high and low affinity CD4 T cells had been competing for say, a secreted growth factor (e.g. IL-2), which would have been able to pass through the transwell, one would have expected similar results to the first assay. However, this was not the case—the low affinity CD4 T cells no longer showed increased sensitivity to competition with high affinity CD4 T cells. An explanation is that due to the transwell, both low and high affinity CD4 T cells had their own exclusive APC source and thus no longer had to compete for activating factors on the APC surface.

The fourth assay in which 2D2 and P25 CD4 T cells were treated with anti-MHC shows that there is a trend of low affinity CD4 T cells displaying a lower mean EC50 (i.e. greater sensitivity to) anti-MHC dose than high affinity CD4 T cells, although this result is not statistically significant. However, a power calculation suggests that competition for access to peptide-MHC II is a factor, but the sample sizes used in the assay were too small.

Since the appropriate models were not available at the time the experiments were conducted, one weakness of these assays was that the low and high affinity models used were specific for different antigens, and thus, direct competition the same peptide-MHC II complexes was not a factor that was tested. Nevertheless, these results are consistent with literature indicating that T cells exhibit competition with each other. For example, competition for physical access to antigen and antigen-presenting cells has been observed for CD8 T cells (16, 17). Moreover, our results are consistent with

findings that CD4 T cells can compete for complexes on the surface of antigen presenting cells even when the T cells are of differing specificities (18).

Currently, our lab is conducting follow-up experiments examining the effects that differential access to costimulatory molecules may have on CD4 T cell competition. If costimulatory factors indeed play a role in mediating CD4 T cell competition, this may have implications with respect to the effects of not only higher affinity CD4 T cells curtailing lower affinity CD4 T cell proliferation, but also potential, although not as strong, reverse effects during an immune response. However, further studies are needed.

Conclusion

Although the results do not support the initial hypothesis that high affinity CD4 T cells can help low affinity CD4 T cells proliferate, the data do support the idea that high and low affinity CD4 T cells compete for activating factors on the APC surface.

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