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Our lab has demonstrated that encephalitogenic T cells can be effectively anergized by treatment with MHC variant peptides, which are analogues of immunogenic peptides containing an amino acid substitution at an MHC anchor residue. The MHC variant peptide of myelin oligodendrocyte glycoprotein (MOG)35–55 proves an effective treatment as it does not induce symptoms of experimental autoimmune encephalomyelitis and fails to recruit macrophages or MOG35–55-specific T cells to the CNS. In this study, we sought to characterize the signaling pathways required for the induction of anergy by building upon the observations identifying the tyrosine phosphatase SHP-1 as a critical regulator of T cell responsiveness. Motheaten viable heterozygous mice, which contain a mutation in the SHP-1 gene resulting in a reduction in functional SHP-1, were challenged with MOG35–55 or the MOG35–55 MHC variant 45D. These mice display symptoms of experimental autoimmune encephalomyelitis upon immunization with MHC variant peptide and have significant CNS infiltration of tetramer-positive CD4+ cells and macrophages, unlike B6 mice challenged with the variant peptide. The effects of SHP-1 are directly on the T cell as between the TCR and agonist or MHC variant. Rather, an unstable interaction between peptide and MHC attenuates the T cell autoreactive T cells are not anergized in vitro. Lastly, we demonstrate no distinguishable difference in the initial interaction with MHC variant peptide and have significant CNS infiltration of tetramer-positive CD4+ T cells in the initiation and propagation of anti-self response has made them therapeutic targets for treatment of MS.

One potential method to regulate myelin Ag-specific T cells is through the generation of altered peptide ligands (APLs) (3–5). These peptides have traditionally been defined as analogues of the immunogenic peptide that contain an amino acid substitution at a TCR contact residue without disrupting the interaction with MHC (6). It has been demonstrated that APLs can both anergize autoreactive T cells as well as skew the cytokine profile of this cell population from an inflammatory Th1 response to an anti-inflammatory Th2 response (3, 4). Although APLs proved successful in treating EAE (7, 8), patients undergoing APL therapy exhibited symptoms of hypersensitivity and the induction of T cells specific for the APL itself, since the peptide contains altered amino acids that contact the TCR. We have previously suggested an alternative therapeutic intervention with variant peptides that focuses on changing the interaction of the peptide with MHC instead of interaction with TCR. In contrast to the problems identified for APL therapy, MHC variant peptides of MOG 35–55 and PLP 139–151 were capable of regulating EAE by inducing anergy in polyclonal populations of encephalitogenic T cells without skewing to a Th2 phenotype (9, 10).

The findings that TCR antagonist variant peptides mediate their suppressive effects via the intracellular SHP-1 (11, 12) suggested to us that SHP-1 may also be influencing TCR signaling via other weak ligands such as MHC variant peptides. SHP-1 is a cytosolic protein tyrosine phosphatase expressed in all cells of hematopoietic lineage (13) and has been shown to negatively regulate T cell hyperresponsive thymocytes and peripheral T cells (23, 24).

The studies reported here extend our findings by investigating the mechanism of MHC variant peptide induced anergy. Using the me-v model, immunization of me-v heterozygous mice with MHC variant peptide results in the induction of EAE, which is a dramatic contrast to the complete absence of disease following its immunization in wild-type B6 mice. In vitro, we find that anergy was nullified in MOG 35–55-specific T cells from me-v heterozygous mice.
mice. Additionally, variant peptide:MHC interactions decay rapidly compared with the stable interaction of agonist and MHC resulting in a weak signal to the TCR and an up-regulation of SHP-1. These data highlight the importance of SHP-1 in the anergic phenotype of autoreactive T cells.

Materials and Methods

Mice

Female C57BL/6 and C57BL/6J-Ptpn6tm1v/v (me-v) mice (H-2b) were purchased from The Jackson Laboratory. Animals were housed in the Emory University Department of Animal Resources facility according to Institutional Animal Care and Use Committee protocols. Mice were used at 6–8 wk of age.

Peptides

MOG 35–55 (MEGVYRSPFSRVMHYRNGK) and 45D (MEGVYRSPFDVRVHLYRNGK) were purchased from Biosynthesis International. Peptides were analyzed by mass spectrometry and HPLC.

Cells and reagents

MOG 35–55-specific T cell lines were generated by priming 6-wk-old C57BL/6 or me-v mice with 200 μg MOG 35–55 emulsified in CFA containing 1 mg/ml heat-inactivated Mycobacterium tuberculosis (H37RA; Difco) in the hind footpad and base of tail. Popliteal and inguinal lymph nodes were harvested on day 10. A total of 5 × 10^6 lymphocytes were incubated with 1 μM MOG 35–55 and 10 μg/ml IL-2 in a 24-well plate for 7 days. Culture media consisted of RPMI 1640 medium supplemented with 10% FBS (Mediatech). 2 mM t-glutamine, 0.01 M HEPES buffer, 100 μg/ml gentamicin (Mediatech), and 2 × 10^3 M 2-ME (Sigma-Aldrich).

Induction of anergy

MOG 35–55-specific T cells (2 × 10^5 per well) were incubated with irradiated syngeneic splenocytes (2000 rads; 5 × 10^5 per well), 10 pg/ml IL-2, and 10 μM 45D (or 1 μM MOG 35–55 as a control) in a 24-well plate. After 7 days in culture, live cells were separated over a Ficoll gradient (Mediatech) and restimulated in a 24-well plate with peptide and fresh APCs, as described above. After an additional 7 days in culture with fresh APCs and peptide, live cells were purified over a Ficoll gradient and were stimulated 18 h for an IL-2 ELISA.

Cytokine ELISA

MOG 35–55-specific T cells (2 × 10^5) were cultured with irradiated syngeneic splenocytes (5 × 10^6) in the presence of peptides for 18 h. Culture supernatant was added to microtiter plates coated with 50 μl of purified anti-IL-2 (5 μg/ml, clone JES6-1A12; BD Pharmingen), rIL-2 was used as a standard (BD Pharmingen). Captured cytokine was detected using biotinylated anti-IL-2 (100 μg/ml, 100 μl/well, JES6-5H4; BD Pharmingen) followed by alkaline phosphatase-conjugated avidin (Sigma-Aldrich) and p-nitrophenylphosphate substrate (Bio-Rad). Colorimetric change was measured at 405 nm on a Microplate Autoreader (Bio-Tek Instruments).

EAE induction

EAE was induced by immunization of female C57BL/6 or me-v mice with 200 μg of MOG 35–55 or 45D emulsified in CFA containing 5 mg/ml heat-inactivated M. tuberculosis (H37 RA; Difco) on days 0 and 7 s.c. in the hind flank. Mice also received 250 ng of Bordetella Pertussis toxin (List Biological Laboratories) i.p. on days 0 and 2. Mice were scored as follows: 0, no disease; 1, flaccid tail; 2, hind limb weakness; 3, hind limb paralysis; 4, forelimb weakness; and 5, moribund.

Tetramer staining

Cells were incubated with either MOG 38–49 or OVA 323–339 I-A^d tetramer at a concentration of 4 μg/ml for 3 h at 37°C. The cells were washed with buffer containing 1× PBS, 0.1% BSA, and 0.05% sodium azide. Cells were stained with anti-CD4-allophycocyanin (RM4.5) (BD Biosciences) for 30 min on ice. The percentage of tetramer-PE-positive cells was determined in CD4^+ populations.

Flow cytometric analysis of CNS

For analysis of lymphocytic infiltrates, mice with the highest disease score in each group at the time point indicated were sacrificed. Brains and spinal cords were isolated and dissociated in 10% FBS. Cells were resuspended in 100% Percoll (Sigma-Aldrich), underlayered with 70% Percoll, and centrifuged at 2500 rpm for 20 min. The interface was collected, and cells were incubated with MOG-tetramer-PE or CLIP-tetramer-PE for 3 h at 37°C. Cells were then surface stained with anti-CD4-allophycocyanin, with anti-CD45.2 FITC and anti-CD11b PerCP, and analyzed by flow cytometry.

Micropipette assay

A previously described micropipette adhesion frequency assay (25) was modified to assess TCR affinity for peptide:MHC Ag and peptide dissociation from MHC molecules. In brief, a single MOG 35–55-specific T cell and a single RBC coated with purified peptide:MHC were aspirated by two micropipettes. MOG-specific polyclonal T cell lines derived from draining lymph nodes were cultured for 1 wk in vitro with 1 μM MOG 35–55. The peptide:MHC molecules were affinity purified from B cell membranes and exchanged with MOG or variant target peptides as previously described (26). The peptide:MHC was captured by a nonblocking anti-MHC class II Ab (KH74; BD Pharmingen) precoated on the red cell surface via chromium chloride coupling and the site density was measured by flow cytometry (25). Human RBCs were used in this assay as the surrogate APC since pMHC can be easily added to the cell membrane. The human RBCs also allow for specific assessment of only TCR:pMHC interactions because they lack the additional costimulatory and adhesives proteins that could interact with the mouse-derived T cells. The pair of cells were brought into contact using a computer-controlled piezoelectric actuator and held together for a controlled duration. Following pipette retraction, the cells either immediately separate (no adhesion) or remain bound as observed by elongation of the flexible RBC membrane (adhesion). The likelihood of adhesion for such a control contact was estimated from at least 100 consecutively adhesion cycles to determine the adhesion frequency and multiple cell pairs were tested to obtain a mean value and a SEM. Ten T cells that displayed binding to RBCs were used to determine the adhesion frequency. The initial slope of the generated binding curve (adhesion frequency plotted against time) is proportional to the on-rate, the steady-state adhesion frequency is related to the binding affinity, and the time required for the adhesion frequency to reach half the equilibrium adhesion level is inversely proportional to the off-rate (25). Affinity was calculated from equilibrium adhesion frequency as described (27).

Analysis of peptide:MHC stability was also analyzed using the micropipette assay. The functional ligand density may decrease over time if the peptide dissociates from the MHC molecule, which makes it incapable of binding to TCR, thereby reducing the adhesion frequency. Assuming first-order irreversible dissociation kinetics, the functional ligand density (mn) should decay exponential in time (t), mn = mn0 exp(−rt/τoff), where mn0 is the functional ligand density at r = 0 and t/τoff is half time for dissociation. The adhesion frequencies were measured at different times after the peptide: MHC-coated red cells were placed in infinitely dilute solution to allow peptide dissociation and 100 μM OVA 323–339 was used as a competitor peptide to prevent rebinding of MOG 35–55 to I-A^d. The measured decrease in adhesion frequency (Poff) over time data was fitted by the following equation to estimate the half-time;

\[
P_{off} = 1 - \exp\left[-A_K p_m m_0 \exp(-t/\tau_{off})\right]
\]

where \(m_0\) is the TCR site density, \(A_K\) is the contact area, and \(K_p\) is the two-dimensional binding affinity. The product \(A_Kp_m m_0\) and \(\tau_{off}\) were two curve-fitting parameters.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism (Software for Science). Mean clinical scores were analyzed using a one-way ANOVA. Adhesion frequency was analyzed with a Student’s t test.

Results

Immunization with MHC variant 45D results in the induction of EAE in me-v/+ mice

To identify the role of SHP-1 in the T cell response to MHC variant peptide, we assessed the ability of 45D to activate me-v heterozygous T cells in vivo. Me-v/me-v mice develop severe pneumonitis and subsequently die by 9 wk of age making this a difficult model in which to study EAE. However, mice heterozygous for the SHP-1 mutation (me-v+/+) reportedly have approximately one-half the amount of SHP-1 protein as wild-type mice, yet are phenotypically normal (28), enabling its use to examine the function of SHP-1 in EAE. To test this, B6 or me-v heterozygous
Table 1. The MHC variant peptide 45D induces EAE in me+/- mice

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Immunizing Peptide</th>
<th>Incidence</th>
<th>Day of Onset (Mean ± SEM)</th>
<th>Mean High Score* (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6</td>
<td>MOG 35–55</td>
<td>4/4</td>
<td>13.0 ± 0.6</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>me+/−</td>
<td>MOG 35–55</td>
<td>4/4</td>
<td>10.5 ± 2.0</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>B6</td>
<td>45D</td>
<td>0/6</td>
<td>19.7 ± 2.1</td>
<td>2.7 ± 0.4</td>
</tr>
</tbody>
</table>

- *B6 or me+/- mice were immunized with MOG 35–55 or 45D on days 0 and 7. Mice also received Pertussis toxin on days 0 and 2.
- **p < 0.001 between 45D-immunized B6 mice and me+/- mice as compared by one-way ANOVA test. *p < 0.05 for MOG 35–55-immunized me+/- mice compared to 45D-immunized me+/- mice.
- B6 mice immunized with 45D do not exhibit symptoms of EAE.

mice were challenged with either MOG 35–55 or the MHC variant 45D via the EAE induction protocol and monitored for clinical symptoms of paralysis. Confirming our previous findings, B6 mice immunized with MOG 35–55 develop severe paralysis (maximum clinical score of 3.8 ± 0.1), whereas 45D-immunized mice do not exhibit clinical symptoms of EAE (Table I and Fig. 1). The lack of disease induction with the MHC variant peptide was followed out to day 35 in this experiment and (Fig. 1A) confirms our previous characterization on the absence of symptoms out to day 180 (9). The me-v/+ SHP-1 heterozygous mice developed similar symptoms of disease as compared with B6 mice (maximum clinical score of 3.9 ± 0.1) when challenged with the wild-type peptide. In addition, the MHC variant peptide induced autoimmune symptoms in me-v/+ mice (maximum clinical score of 2.33 ± 1.0), which is in contrast to what was observed in B6 mice (p < 0.001) (maximum clinical score of 0) (Fig. 1). Although all of the me-v/+ mice immunized with 45D manifest with clinical symptoms of EAE (Fig. 1B), disease onset and severity were reduced relative to MOG 35–55-immunized mice (Fig. 1 and Table I). Following immunization with MOG 35–55, B6 mice exhibit symptoms of disease at day 13.0 ± 0.6, whereas me-v/+ mice show signs of EAE slightly earlier at day 10.5 ± 2.0. However, EAE symptoms are not observed in 45D-immunized me-v/+ mice until day 19.7 ± 2.1. Additionally, the mean high score in me-v/+ immunized with 45D is 2.6 ± 0.4 which is less than that observed with MOG 35–55-immunized mice. These results would be consistent with the reduced potency (>200-fold lower affinity for MHC) of the MHC variant peptide (9). In a separate set of experiments, induction of EAE with 200-fold less MOG 35–55 (1 µg) also led to a milder and delayed EAE disease course (Fig. 1C). However, me-v/+ mice immunized with 200 µg 45D had a significantly higher cumulative disease score compared with me-v/+ given 1 µg MOG 35–55 (Fig. 1C). Additionally, the me-v/+ phenotype had a significantly increased disease score when compared with the same B6 groups. We were unable to generate EAE or encephalitogenic T cells in me-v/-me-v mice as all mice died when given MOG 35–55 in CFA. In summary, MHC variant 45D induces EAE in mice containing a SHP-1 tyrosine phosphatase deficiency.

EAE clinical scores correlate to percentage of infiltrates

Potentially, the reduced SHP-1 levels in me-v/+ mice could change the responding autoimmune T cells or the infiltrating macrophage populations that mediate the immune attack in the CNS (Fig. 1). To further examine the response in these mice, we assessed the nature of the infiltrating cells. We have recently tracked the kinetics of MOG 35–55-specific T cells using a MOG 38–49: IAa tetramer (29) and found the peak percentage of CD4+ T cells (5–10%) occurring between days 18 and 20, followed by a decrease in the tetramer-positive cells to levels of 1–3%. At day 23, me-v/+ mice challenged with either MOG 35–55 or 45D and B6 mice given MOG 35–55 all had detectable MOG 35–55 tetramer-positive CD4+ T cells (1–2.75%) (Fig. 2). CLIP:IAa tetramer was used as a control and was subtracted as background from the MOG 35–55 tetramer-positive CD4+ T cell percentages that are reported. As expected, based on the absence of clinical EAE, B6 mice challenged with MHC variant peptide 45D or no peptide (control) displayed few MOG-specific T cells (0.6 and 0.08%, respectively) (Fig. 2). These Ag-reactive T cells recruit macrophages to the CNS, which are
responsible for the inflammation and demyelination leading to advanced disease progression (30, 31). We found that me-v/+ SHP-1 heterozygous mice challenged with MOG 35–55 or 45D and B6 mice given MOG 35–55 had similar levels of macrophages recruited to the CNS (6.06, 5.58, and 5.87%, respectively) compared with B6 mice given 45D (1.46%) or naive B6 mice (0.46%) (Fig. 2). Macrophages were identified by flow cytometry as the CD45high population of cells in the CNS. Thus, no unusual expansion of infiltrating T cells or macrophages was observed in the me-v/+ mice.

MHC variant peptide does not induce anergy in MOG 35–55-specific me/v+ T cells

We have previously demonstrated the potent anergic effects of MHC variant peptides in the PLP and MOG EAE models (9, 10). To begin to elucidate the mechanism behind this T cell anergy, we sought to determine whether anergy was induced in autoreactive T cells with reduced levels of SHP-1 following treatment with the MHC variant peptide. To this end, MOG 35–55-specific T cells were generated in wild-type and me-v/+ mice through immunization with the autoantigen. Lymph nodes were isolated from these mice and Ag-reactive T cells were cultured in the presence of MOG 35–55 for 18 h. IL-2 secretion was measured by ELISA of supernatants from MOG 35–55-or 45D-treated T cells 18 h after restimulation with MOG 35–55. This data is representative of two individual experiments.

In contrast, MOG 35–55-specific T cells from me-v/+ mice secrete significant amounts of IL-2 (1668 pg/ml) when stimulated with MOG Ag and also following treatment with the MHC variant peptide (1963 pg/ml) (Fig. 3). B6 APCs sufficient in SHP-1 were used for restimulation of the T cells, isolating the difference in response to MHC variant peptide to the T cells. Therefore, these data demonstrate that the anergic phenotype induced by the MHC variant peptide 45D is prevented on reduction of SHP-1.

TCR binding affinity and peptide:MHC stability

Two key issues over the effects of the MHC variant peptide concern whether the amino acid changes alter the recognition of the peptide:MHC surface by the TCR or the stability of the peptide:MHC complex. A micropipette adhesion frequency assay was used to assess these two possible outcomes. This assay has been successfully used to measure two-dimensional binding affinity and kinetic rates for FcγR-IgG interactions (25–30), selectin-ligand interactions (32, 33), and integrin-ligand interactions (34). In addition, we recently reported on its use to assess binding of CD8 to MHC class I (27). A major advantage for the micropipette assay is that it allows for in situ measurements of binding affinity of TCR for pMHC and the stability of the pMHC complex in intact cells (two-dimensional binding) as opposed to purified proteins (three-dimensional binding).

To interrogate the two-dimensional adhesion frequency binding kinetics for TCR:pMHC interactions, MOG 35–55-specific T cells (TCR density of 142/mum2) were attached to the end of one microtipette, while RBC coated with pMHC ligands (507/mum2) were attached to a second micropipette. The pair of cells were brought into contact using a computer-controlled piezoelectric actuator and held together for a controlled period of time. Following pipette retraction, the cells either immediately separate (no adhesion) or remain bound as observed by elongation of the flexible RBC membrane (adhesion). The likelihood of adhesion for such a control contact was estimated from 100 consecutive adhesion cycles to determine the adhesion frequency and 10 adhesive cell pairs were tested to obtain a mean value and a SEM. In the previous experiments, the adhesion frequency was measured over a range of contact durations to determine the kinetic rates of the interaction between the receptor-ligand pair the densities of which were varied to assess the effect of mass action (25). In this study, the contact duration was set at 5 s, sufficiently long for TCR-peptide:MHC
binding to reach steady-state equilibrium. Similar equilibrium adhesion frequencies were observed between MOG 35–55-specific T cells and MOG 35–55:I-A\(^b\) (64.8 \pm 9.3) or 45D:I-A\(^b\) (59.1 \pm 10.7) coated RBCs (\(p = 0.35\)) (Fig. 4A), which corresponds to similar effective two-dimensional binding affinities of 1.45 \pm 0.37 and 1.24 \pm 0.32 \(10^{-5}\) \(\mu m^2\), respectively (\(p = 0.67\)). This finding indicates that the MHC variant peptide does not significantly alter initial TCR recognition of the antigenic complex.

In contrast to the affinity, the stability of these pMHC complexes varies greatly. To determine the stability of pMHC, excess Ag was removed from the system, and the RBCs were used at ensuing time points to allow for dissociation of the ligand. OVA 323–339 was added to the media as a competitor peptide to limit rebinding of the MOG peptides to MHC class II. After that, the dissociation time course was determined by adhesion frequency measurement at various time, as the replacement of MOG 35–55 or 45D by OVA 323–339 would result in a pMHC nonreactive to the MOG 35–55-specific T cells, thereby reducing the functional ligand density and, in turn, reducing the specific adhesion frequency. Although there was still strong adhesion between MOG 35–55-specific T cells and MOG 35–55-coated RBCs (53.5 \pm 8.9) at 8 h following removal of Ag from the system, the adhesion frequency between T cells and RBCs coated with 45D:I-A\(^b\) rapidly declined, with an adhesion frequency of 19.9 \pm 7.5 at 8 h post-Ag removal (Fig. 4B). Curve-fitting of these data with Equation 1 returns a half-life for 45D:I-A\(^b\) of 5.5 h, which is significantly less than the half-life of MOG 35–55:I-A\(^b\) (>2 days). We did not carry out the stability analysis of MOG:I-A\(^b\) beyond 2 days but it had not appreciably decreased at that point (data not shown). In summary, these data demonstrate that the affinity between the TCR and wild-type or MHC variant peptides is similar, yet the variant peptide rapidly dissociates from MHC, indicating that the energy observed in MOG 35–55-specific T cells cultured on 45D is due to the decreased stability of the pMHC complex.

**Discussion**

Anergy in T cells can be induced using several distinct methods including costimulation blockade, calcium flux, anti-CD3, APLs, and MHC variant peptides (35–39). Although all of these methods induce decreased levels of T cell division and IL-2 production, they may mediate the observed anergic properties through distinct molecular mechanisms (37, 40). The anergic effect has been characterized as changes in CD3 \(\xi\) phosphorylation, altered MAPK signaling, changes in NFAT, and p27kip (41, 42). For MHC variant peptides, our data indicates that SHP-1 mediates the anergic effect (Figs. 1–3) and should be added to the list of signaling proteins involved in T cell anergy. This is the first report indicating a role for SHP-1 in anergy of CD4\(^+\) T cells. We have also identified a role for SHP-1 in CD8\(^+\) T cells responding to a viral escape mutant epitope of lymphocyctic choriomeningitis virus, which is an MHC variant peptide (F. J. Schnell, N. Alberts-Grill, B. D. Evavold, submitted manuscript). Germain and colleagues (12) suggested that SHP-1 is recruited to the TCR following engagement with weak ligands, resulting in the inactivation of Lck. This model would be supportive of SHP-1 as a mediator by which weak ligands suppress T cell activation. Additionally, the requirement of SHP-1 for inhibition of responses by TCR antagonists indicates that SHP-1 can have potent dampening effects (11). The amount of SHP-1 levels may also be important for mediating autoimmune disease as Deng et al. (43) reported enhanced EAE disease severity, more frequent relapses, and decreased survival following immunization of B10.PL me-\(v\) mice with myelin basic protein Ac 1–11. A further role for SHP-1 in the autoimmune T cell response is supported by the recent report detailing decreased SHP-1 gene activity in PBMC’s of MS patients (44). Together, our results and the published findings highlight SHP-1 as an important point of control for autoimmune T cell responses.

Our data suggests a direct role of SHP-1 in regulating T cell anergy and induction of EAE of myelin-specific T cells, yet it is possible that SHP-1 may mediate alternative effects in the cells of the immune system. For example, me/me or me-v/me-v mice deficient in SHP-1 show altered effects in thymocyte development highlighted by increased positive selection, negative selection, and the generation of regulatory T cells (14, 16, 18, 45). It should be noted that the most dramatic effects of SHP-1’s role in T cell development occur in mice homozygous for loss of functional SHP-1, while me/+ heterozygous mice were less affected. We were unable to determine the role of these T cells in EAE as the me-v/me-v mice died rapidly upon challenge with peptide in CFA (unpublished observations). To determine whether the SHP-1 heterozygous mice deviate in their response to variant peptide compared with wild-type B6, we tracked the infiltrating Ag-specific CD4\(^+\) T cells and macrophages into the CNS (Fig. 2). All mice displaying EAE symptoms had detectable levels of MOG:IA\(^b\) tetramer-positive T cells at day 23. Consistent with the presence of MOG-specific CD4\(^+\) T cells, similar levels of infiltrating macrophages were found in the CNS of B6 mice immunized with MOG 35–55 or me-v/+ mice immunized with the MHC variant 45D (Fig. 2). Therefore, no difference was observed in the extent of T cell or macrophage infiltration in SHP-1 heterozygous mice. This data does correlate disease symptoms to immune cellular infiltrates (Fig. 1) and further support the hypothesis that SHP-1 is necessary for peptide induced effects.

To further pinpoint SHP-1 as the modulator of MHC variant peptide anergy, we demonstrated that the MHC variant peptide failed to diminish the production of IL-2 in T cells derived from me-v/+ mice (Fig. 3). This was in contrast to our data (Fig. 3) (9, 10) demonstrating reduction in IL-2 production and T cell proliferation, two hallmarks of anergy. Although SHP-1 is expressed in all cells of hematopoietic origin, the effect of SHP-1 in MHC variant peptide induced anergy is specific to the T cells since the anergy was induced and assessed using B6 APC with normal SHP-1 levels. Thus, the in vitro data indicates that SHP-1 is an essential modulator in MHC variant peptide induced anergy.

Our previous analysis of the MHC variant peptide 45D found that this peptide possessed a 200-fold decrease in affinity for I-A\(^b\), but the binding kinetics of TCR for pMHC or the half-life of the peptide in complex MHC were not determined. Traditional APLs with mutations at TCR contact residues affect T cell recognition of the variant peptide in a clone-specific manner. When used in vivo, APLs induce additional T cell specific for the mutated peptide. This limitation of APLs to specific TCR clones highlights one of their inherent drawbacks for therapeutic use (9, 46). In contrast to the clone-specific effects of APLs, MHC variant peptides induce anergy in polyclonal populations of cells, suggesting conservation of TCR recognition. The micropipette experiments demonstrated a similar two-dimensional binding affinity of MOG 35–55-specific T cells for MOG 35–55 or 45D loaded MHC (Fig. 4A). Instead of an appreciable alteration in TCR recognition, the MHC variant peptides lead to incomplete activation of T cells as the peptide MHC complex rapidly decays. This was an important finding, indicating alterations in MHC contact residues of an antigenic peptide can lead to conserved recognition of peptide (MOG 35–55 and 45D) by the TCR.

The dissociation rate of the peptide from the MHC molecule is a determining factor affecting the outcome of T cell activation for agonist and weak agonist peptides (47–49). Our lab has shown that...
peptide and HMC molecule results in decay over time with minimal TCR engagement at later time points. This difference in the stability as compared with the antigenic ligand leads to a signaling cascade regulated by the tyrosine phosphatase SHP-1. Therefore, future therapies to target SHP-1 could lead to effective treatment for autoimmunity and induction of T cell tolerance.

Disclosures

The authors have no financial conflict of interest.

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FIGURE 4. MOG 35–55-specific TCR possesses a similar affinity for MOG 35–55 and 45D, yet the stability between 45D and MHC rapidly decays over time. A single MOG 35–55-specific T cell and a single RBC coated with peptide:MHC were aspirated by two micropipettes and brought into contact for 5 s. Adhesion between the TCR and pMHC complex can be detected by a deflection in the RBC membrane. For each experiment, 10 different cell pairs were tested with 100 consecutive adhesion cycles. The 45D does not alter initial binding through the TCR as the adhesion frequency is similar to that obtained with agonist loaded MHC (A). However, the adhesion frequency between MOG-specific T cells and 45D:MHC is significantly reduced over time (B) revealing a decreased half-life.
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