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Original article

Biological effect of varying peptide binding affinity to the *BoLA-DRB3*2703* allele

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Abstract – MHC class I and II molecules are immunoregulatory cell surface glycoproteins, which selectively bind to and present antigenic peptides to T-lymphocytes. Murine and human studies show that variable peptide binding affinity to MHC II molecules influences Th1/Th2 responses by inducing distinctive cytokine expression. To examine the biological effects of peptide binding affinity to bovine MHC (BoLA), various self peptides (BoLA-DQ and fibrinogen fragments) and non-self peptides from ovalbumin (OVA), as well as VP2 and VP4 peptides from foot and mouth disease virus (FMD-V) were used to (1) determine binding affinities to the *BoLA-DRB3*2703* allele, previously associated with mastitis susceptibility and (2) determine whether peptide binding affinity influences T-lymphocyte function. Peptide binding affinity was determined by a competitive assay using high affinity biotinylated self-peptide incubated with purified *BoLA-DRB3*2703* in the presence of various concentrations of competing peptides. The concentrations of non-self peptide required to inhibit self-peptide binding by 50% (IC50) were variable, ranging from 26.92 to > 320 μM. Peptide-specific T-lymphocyte function was determined by measuring DNA synthesis, cell division, and IFN-γ production in cultures of mononuclear cells from a *BoLA-DRB3*2703* homozygous cow. When compared to non-stimulated control cultures, differences in lymphocyte function were observed for all of the assessed parameters; however, peptide-binding affinity did not always account for the observed differences in lymphocyte function.

bovine / MHC class II / peptide binding affinity / lymphocyte function

1. INTRODUCTION

The major histocompatibility complex (MHC) class I and II molecules are immunoregulatory cell surface glycoproteins, which bind to and present

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antigenic peptides to T cells. Allelic variants are associated with specific diseases in all mammals, including cattle. The highly polymorphic bovine MHC *(BoLA)-DRB* gene, for example, has been implicated in the resistance and susceptibility to bovine leukemia virus [33], dermatophilosis [15] and mastitis [15, 24]. In one study of Canadian Holsteins, *BoLA-DRB3*1501/02* and *2703* alleles were associated with lower somatic cell scores and the occurrence of clinical mastitis, respectively [24]. Certain amino acid motifs found within the binding site, specifically in pocket four of the antigen-binding groove of various *BoLA-DRB3* alleles, were later demonstrated to confer susceptibility to *Staphylococcus* spp. mastitis, possibly by altering BoLA-DRB-peptide binding affinity [23].

In mice and humans, distinctive cytokine patterns [T helper 1 (Th1) *vs.* T helper 2 (Th2)], which affect disease outcome, are produced in the context of interactions between MHC and various peptides [19]. It has been established that while high affinity ligands for MHC class II (*e.g.* HLA DRA*0101) [10] may induce T helper cells to secrete IFN-γ and IL-2 (Th1 response), low affinity ligands can induce IL-4 and IL-5 (Th2 response) [13, 19]. It is speculated that high affinity interactions between peptides and MHC molecules can lead to increase in the number of stable MHC-peptide complexes on the surface of the antigen presenting cells, which augment the avidity of MHC-peptide-T-cell receptor (TCR) interactions [13]. It is plausible that susceptibility to bovine mastitis may be attributed, in part, to inappropriate Th1/Th2 immune responses resulting from differences in BoLA-peptide binding affinity that are governed by allelic variants of BoLA class II genes. The present study was designed to begin to address this possibility by (1) determining the binding affinities of various synthetic peptides from the core region of ovalbumin (OVA) and two foot-and mouth disease virus (FMD-V) peptides, VP-2 and VP-4, for the binding groove encoded by the mastitis susceptibility allele *BoLA-DRB3*2703*, and (2) determining whether *BoLA-DRB3*-peptide binding affinity influences T-lymphocyte function as determined by measuring DNA synthesis, cell division, and IFN-γ production.

2. MATERIALS AND METHODS

2.1. Peptides

Peptides were prepared by standard Fmoc solid phase chemistry and their identity was confirmed by mass spectrometry. Several self-peptides (BoLA-DQ and fibrinogen fragments), and non-self peptides (sperm whale myoglobin and mycobacterium heat shock protein fragments) previously eluted from *BoLA-DRB3*2703* molecules were selected for initial study based on sequences derived from mass spectrometry analysis of pooled peptides [26]. The assumption being that one or more of these peptides would bind with high affinity to

Table I. Peptides sequences and their respective concentration required to inhibit BoLA class II-self-peptide binding by 50% (IC50).

Peptide	IC50 (μM)
Self-peptide DQ (DVNEFRAVTPLGRPDAE)[1]	16.06
$OVA327_{[316-327]}$ (SSAESLKISQAV)[2]	> 320
$OVA332_{[322-332]}$ (KISQAVHAAHA)[2]	NB
$OVA335_{[323-335]}$ (ISQAVHAAHAEIN)[2]	26.92
$OVA336_{[325-336]}$ (QAVHAAHAEINE)[2]	> 320
$OVA339_{[323-339]}$ (ISQAVHAAHAEINEAGR)[2]	72.22
$FMD\text{-}VP2_{[74-88]}$ (PFGHLTKLELPTDHH)[1]	NB
$FMD\text{-}VP4_{[20-34]}$ (SIINNYYMQQYQNSM)[2]	46.46

Note: OVA = ovalbumin; FMD-VP = foot-and-mouth disease-virus peptide; NB = no binding; [1] Peptides Lab Queens University, Kingston, Ont.; [2] Dalton Chemical Laboratories, Toronto, Ont.

the *BoLA-DRB3.2*2703* molecule and could be used as the marker peptide in the binding assays. Selected self-peptides were biotinylated at the N-terminus after additional incorporation of 6-aminohexanoic acid as a spacer during synthesis. Immunodominant peptides from the core region of ovalbumin, including OVA 323–339, OVA 316–327, OVA 325–336, OVA 322–332, OVA 323–335 [17,22] and two viral peptides from the foot and mouth disease virus (FMDV), including VP2 74–88 and VP4 20–34 [7] were synthesized as competitor peptides (Tab. I).

2.2. BoLA class II-peptide binding assays

The BoLA class II-peptide binding assay was carried out as previously described [7]. Briefly, purified *BoLA-DRB3*2703* molecules were incubated with 10 μM of either biotinylated self-peptide DQ or fibrinogen [23], and a non-labeled synthetic competitor peptide (Tab. I) ranging in concentration from 0–640 μM. Biotinylated peptides were detected by enhanced chemiluminescence using the Western blot ECL enhancer (Amersham), Hyperfilm ECL (Amersham), and a Konica film processor. The relative band densities were calculated using Scion Image software (NIH). These values were used to calculate the concentration of the competitor peptide that inhibited self-peptide binding by 50% (IC50) (Prism, GraphPad Software Inc.). Each binding assay was repeated in two separate experiments. No differences were observed in Western blot analysis and, therefore, data shown here are derived from one representative experiment.

2.3. Peptide sequence alignment

Since previous analysis of naturally processed peptides eluted from *BoLA-DRB3.2*2703* has provided some information about the binding specificity of this allele [25], the OVA and FMD-V peptide sequences were aligned with the BoLA-DQ self-peptide, eluted from *BoLA-DRB3.2*2703* to identify similar amino acid residues between the synthetic and self-peptide sequences using the DIALIGN 2.1 program [18].

2.4. *In vitro* generation of peptide-specific T-lymphocytes

Blood-derived mononuclear cells (BMC) isolated from a Holstein cow homozygous for the *BoLA-DRB3*2703* were washed and suspended in culture medium (IMDM; Invitrogen life technologies, Burlington, Ont.) supplemented with FCS, penicillin, streptomycin, and β-mercaptoethanol. BMC were cultured as previously described with slight modification [7]. Briefly, BMC were primed *in vitro* with various concentrations (0, 2.5, 5.0, 10.0 and 20.0 $\mu g \cdot mL^{-1}$) of synthetic peptides (Tab. I), and after 7 days viable cells were enriched using the Shortman procedure [27], then cultured for an additional 10 days in 10 mL of complete IMDM supplemented with 100 units of recombinant human interleukin-2 (rhIL2; Serotec Inc., Raleigh, NC) to generate peptide-specific lymphocytes ($\geq$ 80% $CD2^+$ cells and no B-cells).

2.5. Analysis of lymphocyte DNA synthesis

Peptide-specific T-lymphocyte DNA synthesis was assessed by re-stimulating the primed BMC with the same concentrations of peptide in 3–5 replicates per peptide per dose using *BoLA-DRB3*2703*-transfected L929 cells [26], treated with mitomycin C (Sigma, 80 $\mu g \cdot mL^{-1}$), as antigen presenting cells (APC). DNA synthesis was assessed after 5 days by measuring [^{3}H] thymidine incorporation (0.5 μCi) during the last 18 h of culture.

2.6. Analysis of cell division in T-lymphocyte sub-populations

BMC were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Eugene, OR) as previously described [9]. Labeling was performed just prior to *in vitro* peptide priming, at addition of rhIL2, and at the time of peptide re-stimulation. Adherent-autologous BMC, treated with mitomycin C (80 $\mu g \cdot mL^{-1}$), were used as APC for the peptide re-stimulation phase. $CD2^+$, $CD4^+$ and $CD8^+$ T-lymphocytes were identified using monoclonal antibodies IL-A43, IL-A11, and IL-A105, respectively (ILRAD Nairobi, Kenya), and then labeled with either biotinolated goat-anti-mouse $F(ab)_2$ IgG (H+L), or the respective isotype control, followed by streptavidin conjugated

phycoerythrin (PE; Cedarlane, Hornby, Ont.). The relative numbers of T-lymphocyte divisions were assessed by flow cytometry [12]. The $CD4^+$ and $CD8^+$ T-cells with high and low fluorescence intensities ($CD4^{+hi}$, $CD4^{+lo}$, $CD8^{+hi}$, $CD8^{+lo}$) were defined by comparison with the isotype control in the FL2/FL1 mode. Cell viability as assessed by trypan blue exclusion after the rhIL-2 expansion phase was > 90%. Lymphocyte stimulation with the mitogen, concanavalin-A (Con-A, Sigma, 5 $\mu g \cdot mL^{-1}$) was used as a positive control.

2.7. T-lymphocyte interferon-γ production

BMC were primed *in vitro* with OVA 335, 336, 339, or FMD-VP2 and VP4 peptides at a previously determined optimal antigen dose (5 $\mu g \cdot mL^{-1}$), then expanded with rhIL-2 as described above. Peptide-specific T-lymphocytes were re-stimulated with the same concentration of peptide using adherent-autologous BMC, treated with mitomycin C as APC. Aliquots of culture supernatant were removed 18 h after addition of rhIL-2 and at the peptide re-stimulation phase to assess relative amounts of IFN-γ produced by the peptide-specific T-lymphocytes. Lymphocyte stimulation with Con-A (5 $\mu g \cdot mL^{-1}$) was used as a positive control. Bovine IFN-γ was measured by ELISA according to the manufacturer's protocol (CSL Veterinary Inc., Victoria, Australia).

2.8. Statistical analyses

Band densities from Western blots following competitive peptide-binding assays were plotted against peptide concentration using Prism software (GraphPad Software Inc.) to calculate the concentration of competitor peptide that inhibited self-peptide binding by 50% (IC50).

Since variances for the DNA synthesis, IFN-γ, and most of the cell division data were homogeneous these data were analyzed by analysis of variance (ANOVA) using the Prism software (GraphPad Software Inc.). When significant differences ($P < 0.05$) were observed between treatments, planned contrasts were performed with the Dunnett's T-test, using the naïve cells (BMC that were neither primed, nor re-stimulated with the peptide) as the control group. The Kruskal-Wallis Test was used to analyze the non-parametric cell division data. When significant differences ($P < 0.05$) in these data were observed, contrasts were performed using the Dunn Test (GraphPad Software Inc.).

3. RESULTS

3.1. BoLA class II-peptide binding assays

An initial binding assay was performed to identify which peptide(s) had the highest *BoLA-DRB3*2703* binding affinity for use in the competitive binding

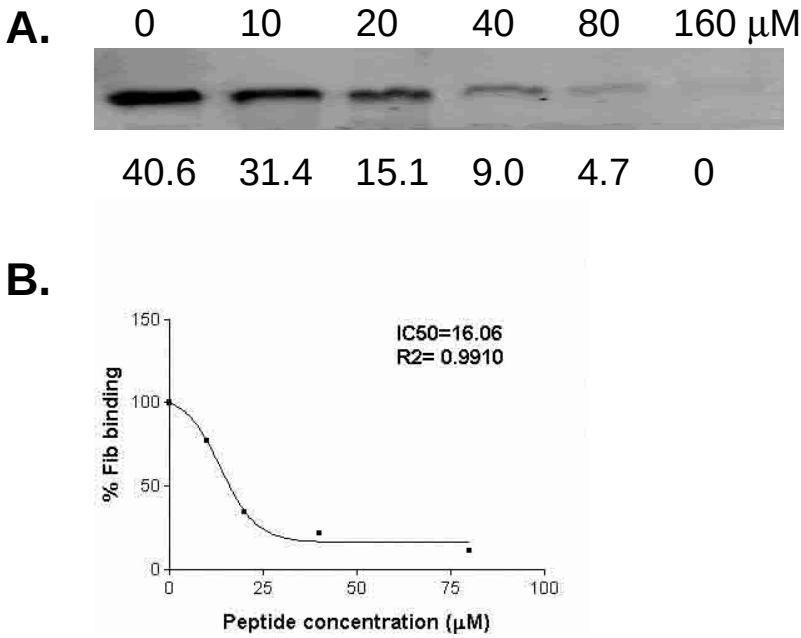

Figure 1. (**A**) A representative peptide binding assay for the *BoLA-DRB3*2703* class II molecule using biotinylated fibrinogen self-peptide (10 μM) at different concentrations of the competitor self-peptide, BoLA-DQ, and (**B**) determination of the concentration of BoLA-DQ required to inhibit fibrinogen-*BoLA DRB3*2703* binding by 50% (IC50).

assays. At an equivalent molarity, the band density for the BoLA-DQ fragment (Density = 58.2 pixels) was slightly greater than that of fibrinogen (Density = 47.8 pixels). Both of these self-peptides had much higher binding affinities than two non-self peptides, HSP (Density = 9.1 pixels) and SPM (Density = 7.8 pixels), previously eluted from *BoLA-DRB*2703*.

Figure 1A is a representative illustration of one of the BoLA class II-peptide binding assays. In this case, biotinylated fibrinogen self-peptide binding affinity for *BoLA-DRB3*2703* is shown in the presence of the competitor DQ self-peptide. The concentrations of DQ self-peptide were plotted against the fibrinogen band intensity in order to determine the IC50 for DQ as 16.06 μM (Fig. 1B). Based on these results, the BoLA-DQ peptide was selected as the biotinylated self-peptide for the competitive binding assays with OVA and FMD-V peptides. The IC50 for each of the synthetic competitor peptides are listed in Table I. The *BoLA-DRB3*2703*-peptide binding affinities from the highest to lowest for the various synthetic peptides were; OVA335 > FMD-VP4 > OVA339 > OVA 327, 336, and no binding could be detected for OVA 332 and FMD-VP2 peptides.

3.2. Peptide sequence alignment

Although, amino acid sequences associated with binding to *BoLA-DRB3*2703* were not absolute, complementary sequences were found between some of the peptides, capable of binding to this allele, and the DQ peptide. For the OVA peptides, complementary glutamic acid (E), isoleucine (I), asparagine (N) and E were found at positions 333, 334, 335 and 336, respectively. These amino acids aligned with amino acids of similar characteristics, aspartic acid (D), valine (V), N and E in the DQ peptide. The OVA 327 and OVA 332 peptides, which did not have this sequence similarity, bound weakly or not at all to *BoLA-DRB3*2703*. The FMD-VP4 peptide, with high binding affinity to *BoLA-DRB3*2703*, also had a sequence which is complementary to the N, E, phenylalanine (F) and arginine (R) sequence of the DQ. The FMD-VP2 peptide, which was not able to bind to *BoLA-DRB3*2703*, had complementary sequences with other regions of the DQ peptide (Tab. I).

3.3. DNA synthesis

When compared to naïve cell cultures (non-stimulated BMC), at various concentrations the OVA 335 (2.5–20.0 $\mu g \cdot mL^{-1}$) and OVA 339 (5.0 $\mu g \cdot mL^{-1}$) peptides, which have relatively high binding affinities, stimulated significant DNA synthesis of T-cells following peptide priming and re-stimulation (Tab. II). Conversely, OVA 327 (5.0–10.0 $\mu g \cdot mL^{-1}$) and FMD-VP4 (2.5–20.0 $\mu g \cdot mL^{-1}$) peptides, with low and high binding affinities respectively, when used at various concentrations, were associated with significantly lower T-lymphocyte DNA synthesis compared to BMC controls (Tab. II). DNA synthesis in the OVA 332, 336 and FMD-VP2 cultures was not significantly different from the control. All re-stimulated T-lymphocytes plus APC cultures responded with increased DNA synthesis when compared to the peptide-primed T-lymphocytes, without APC.

3.4. Cell division in T-lymphocyte sub-populations

Cell division was evaluated during the production of peptide-specific T-lymphocytes at a previously determined optimal antigen dose (5 $\mu g \cdot mL^{-1}$). During the 7-day *in vitro* peptide priming phase, significant differences in cell division were not observed. However, after the 10-day rhIL-2 expansion phase, significantly ($P < 0.05$) more $CD4^{+hi\ and\ lo}$ and $CD8^{+hi\ and\ lo}$ T-cell division was observed in the FMD-VP2, and VP4 peptide-specific T-lymphocyte cultures, when compared to the naïve lymphocyte culture.

Following T-lymphocyte re-stimulation with peptide, using adherent-autologous BMC as APC, significant ($P < 0.05$) differences in cell division were also observed for specific T-lymphocyte sub-populations (Fig. 2).

Table II. Lymphocyte DNA synthesis following re-stimulation with peptide using BoLA transfected L-cells as antigen presenting cells.

Peptide concentration ($\mu g \cdot mL^{-1}$)	0	2.5	5	10	20
OVA327	1414 ± 104	1254 ± 69	$874 \pm 70^{**}$	$1110 \pm 62*$	1537 ± 45
OVA332	634 ± 52	722 ± 99	671 ± 54	804 ± 38	689 ± 56
OVA335	1153 ± 69	$515 \pm 105^{*}$	$3081 \pm 139^{**}$	$5006 \pm 97^{**}$	$3892 \pm 303^{**}$
OVA336	927 ± 53	1117 ± 65	1031 ± 74	1015 ± 124	920 ± 34
OVA339	437 ± 58	316 ± 30	$1104 \pm 142^{*}$	374 ± 58	302 ± 34
FMD-VP2	1315 ± 374	1874 ± 169	2026 ± 164	4399 ± 2055	1090 ± 149
FMD-VP4	2839 ± 184	$1155 \pm 283^{*}$	$2044 \pm 244^{*}$	2241 ± 141	$650 \pm 35^{**}$

Note: Data are presented as the mean $\pm$ SE cpm at each peptide concentration. Data that are significantly different from the naïve cultures are represented by * ($P \leq 0.05$) and ** ($P \leq 0.01$). These data are representative of two repeated assays for each peptide.

Less cell division, for example, was observed in all of the peptide-specific cultures for the $CD8^{+hi}$ T-lymphocyte population, when compared to the naïve lymphocyte culture; whereas, more cell divisions were observed for the OVA 336 and OVA 339 peptide-specific $CD8^{+lo}$ T-lymphocytes (Tab. III). More cell divisions were also observed in the $CD4^{+hi}$ T-lymphocyte sub-population in all but the FMD-VP4 peptide-specific cultures, but not in the $CD4^{+lo}$ sub-population (Fig. 2, Tab. III).

3.5. T-lymphocyte interferon-γ (IFN-γ) production

Lymphocyte function, assessed by measuring the ability of the various peptide-specific T-lymphocytes to produce IFN-γ, indicated that OVA 335, 336, 339, and FMD-VP2 stimulated culture supernatants contained less ($P < 0.05$) IFN-γ than the supernatant from the naïve cell culture 18 h into the rhIL-2 expansion phase. IFN-γ in the FMD-VP4 culture supernatant was not significantly ($P < 0.05$) different from those measured in the naïve culture supernatant at this time point (data not shown). Similar, but more dramatic, differences were observed after the various peptide-specific T-lymphocytes were re-stimulated with the peptide using adherent-autologous BMC as APC (Fig. 3). In each case (T-lymphocytes alone and T-lymphocytes re-stimulated with APC $+/-$ peptide) OVA 335 and 336 peptide-stimulated cultures, contained less ($P < 0.01$) IFN-γ than the naïve cell culture (Fig. 3, Tab. III). The FMD-VP2 and OVA 339 peptide-stimulated cultures also contained less ($P < 0.05$) IFN-γ than the naïve culture, but only when FMD-VP2 peptide-specific T-lymphocytes

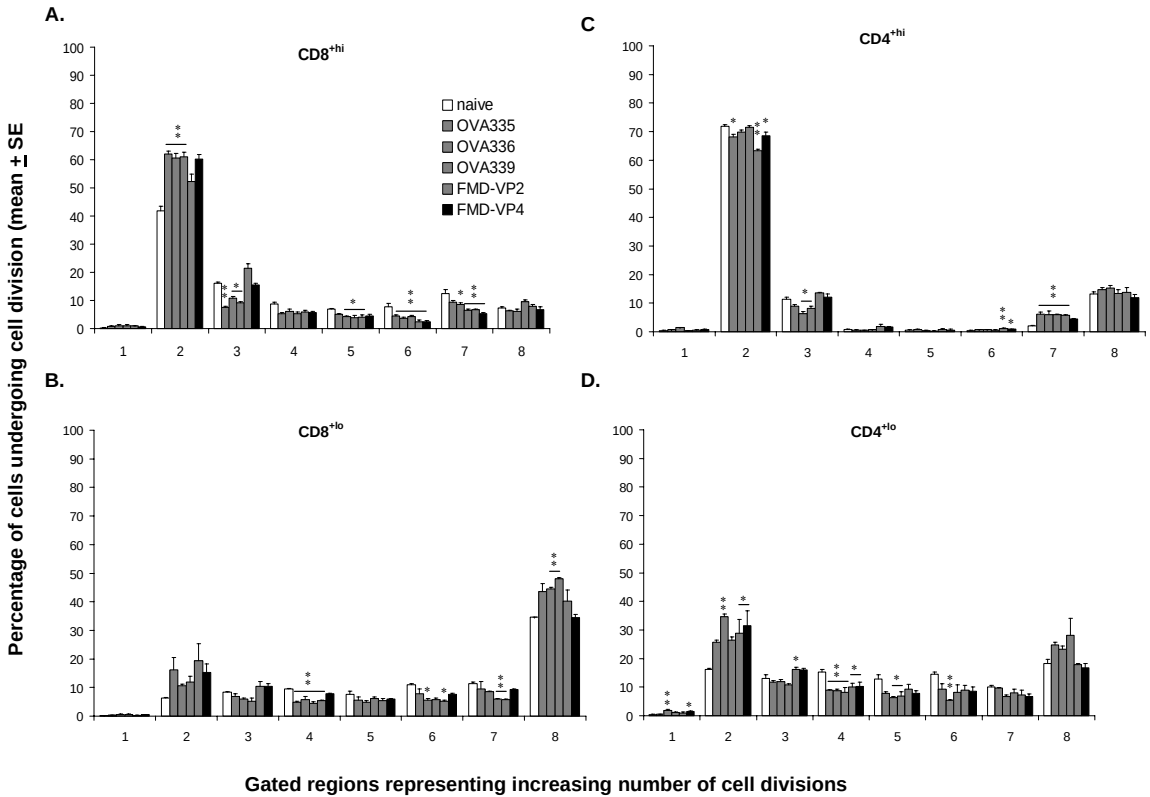

Figure 2. Percentage of $CD8^+$ high (**A**) and low (**B**), and $CD4^+$ high (**C**) and low (**D**) peptide-specific T-lymphocytes at various stages of cell division after five days of re-stimulation with peptide using adherent-autologous BMC as APC. The percentage of peptide-specific lymphocytes within each gated region was compared to the corresponding percentage of naïve lymphocytes to determine statistical significance ($* = P \leq 0.05$, $** = P \leq 0.01$). Data are presented as the mean ± SE.

Table III. Synopsis of peptide-DRB3*2703 binding affinities and T-lymphocyte functions following *in vitro* priming and subsequent re-stimulation with peptide.

Synthetic peptide	Binding affinity [a]	DNA synthesis [b]	Cell division [c]				IFN-γ [d]
			$CD4^{+hi}$	$CD4^{+lo}$	$CD8^{+hi}$	$CD8^{+lo}$	
OVA 327	Low	↓	–	–	–	–	–
OVA 332	Low	ND	–	–	–	–	–
OVA 335	High	↑	↑	ND	↓	ND	↓
OVA 336	Low	ND	↑	ND	↓	↑	↓
OVA 339	High	↑	↑	ND	↓	↑	↓
VP-2	Low	ND	↑	ND	↓	↓	↓
VP-4	High	↓	ND	ND	↓	ND	↑

Note: Arrows represent significant ($P \leq 0.05$) increases or decreases as compared to the naïve (no peptide-priming and re-stimulation) blood mononuclear cell (BMC) control. ND = no difference as compared to the naïve BMC control. [a] Designated high and low peptide binding affinities for *BoLA-DRB3*2703* are determined based on comparison to the high binding self peptide BoLA-DQ (Tab. I). [b] DNA synthesis based on ^{3}H-thymidine incorporation was used as an indicator of lymphocyte proliferation. [c] The relative number of cell divisions following peptide re-stimulation was assessed by labeling with carboxyfluorescein diacetate succinimidyl ester dye. Lympocyte sub-populations were determined using anti-bovine CD monoclonal antibodies. [d] Bovine interferon-γ was determined using a commercial ELISA test.

were cultured alone, and when OVA 339 peptide-specific T-lymphocytes were re-stimulated with APCs plus peptide. When FMD-VP4 peptide-specific T-lymphocytes were cultured alone and re-stimulated with APC $+/-$ peptide, IFN-γ was dramatically greater ($P < 0.01$) than those measured in the naïve cell culture (Fig. 3). For all cultures, IFN-γ was the greatest when T-lymphocytes were cultured alone. With the exception of FMD-VP4, the Con-A positive control enhanced IFN-γ production for all of the different peptide-primed cultures (Fig. 3).

4. DISCUSSION

Polymorphic residues within the peptide-binding cleft of MHC are important for peptide binding affinity and specificity and therefore, regulate T-cell recognition by influencing either MHC-peptide or MHC-TCR interactions [32]. It has been established for mice and man that while high affinity ligands for MHC class II molecules may induce T helper cells to secrete IFN-γ (Th1 response) [10], low affinity ligands may induce the secretion of IL-4 and IL-5 (Th2 response) [13, 19]. In addition to playing different roles in

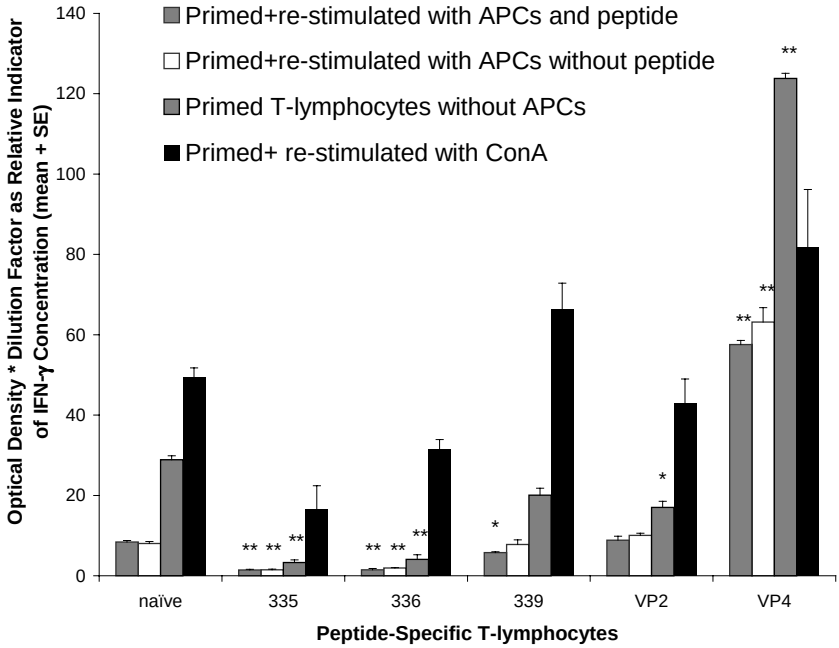

Figure 3. Relative concentration of IFN-γ in the supernatants of various peptide-specific T-lymphocyte cultures. IFN-γ was measured in lymphocyte culture supernatants, 18 h after re-stimulated with and without peptide using adherent-autologous BMC as APC, in peptide-primed T-lymphocytes alone, and in peptide-primed T-lymphocytes that were re-stimulated with Con-A. For each treatment, IFN-γ in the peptide-specific lymphocyte cultures was compared to the corresponding amount in the naïve lymphocyte cultures. Data are presented as the mean optical density $\times$ dilution factor $\pm$ SE. ($* = P \leq 0.05$, $** = P \leq 0.01$).

host defense, inappropriate Th1/Th2 responses have also been associated with certain diseases [21].

Understanding immune responses in MHC-defined cattle may shed light on the underlying mechanisms that lead to MHC-associated disease resistance, and effective peptide vaccines. To enhance our understanding of the molecular interactions between bovine MHC II and peptides, the present study sought to determine the binding affinities of various synthetic OVA and FMD-V peptides to *BoLA-DRB3*2703*, an allele previously associated with mastitis susceptibility. T-lymphocyte function was also assessed as a means of defining peptide presentation, and recognition by the T-cell receptor.

Various OVA and FMD-V peptides have previously been used as immunogenic epitopes to define antigen presentation, the kinetics of peptide-MHC binding, and assess antibody responses of cattle and mice [3, 4, 7, 11, 17, 22, 31]. FMD-VP2 and VP4, for example, were both demonstrated to bind with high

affinity to *BoLA-DRB3*1101* and *BoLA-DRB3*0201*, respectively. Moreover, their high binding affinity was consistent with their ability to stimulate lymphocyte proliferation [7]. In the present study, OVA peptides were demonstrated to have binding affinity for *BoLA-DRB3*2703* ranging from 26.92 to > 320 μM. DRB3*2703 binding affinity of FMD-VP2 was not detectable, and VP4 had a binding affinity of 46.46 μM. Following re-stimulation of T-lymphocytes, the high affinity peptides, OVA 339 and 335, were associated with significantly increased DNA synthesis and cell division of $CD4^{+hi}$ lymphocytes, but decreased IFN-γ production compared to the naïve cell controls (Tab. III). On the contrary, the high DRB*2703-binding FMD-VP4 peptide was not associated with increased DNA synthesis or cell division, but had significantly greater IFN-γ compared to the naïve control. Following re-stimulation of T-lymphocytes, peptides with lower DRB*2703-binding affinities tended to have lower or no differences in DNA synthesis, cell division and IFN-γ production (Tab. III). A greater number and variety of peptides will need to be studied to confirm any associations between peptide binding affinities for DRB3*2703 and T-cell function.

The analysis of the MHC class II eluted peptides has generally been less informative than the analysis of MHC class I-associated peptides because they are more variable in length, can extend beyond the binding groove, and may not have identified anchor residues [1]. Additionally, most of the binding energy originates from the α-carbon backbone of the peptides for MHC class II molecules, whereas for MHC class I molecules, the binding energy is derived from the interaction between a few peptide anchor residue side chains and the MHC pocket [5, 28]. Taken together, these data suggest that instead of using simple motifs to assess the probable contribution of each peptide residue to MHC II binding, a different approach, possibly utilising a computer scoring algorithm to scan proteins and to create a hierarchy of likely peptide ligands, may be required [5, 8].

Although these data suggest some association between high or low peptide binding affinity for *BoLA-DRB3*2703* and differences in T-lymphocyte function (Tab. III), it is not absolute. This may not be surprising, since the peptides used in this study were ranked according to their binding affinity for the *BoLA-DRB3*2703* molecule, alone. Other variables including, peptide degradation, binding affinity for the T-cell receptor, expression of co-stimulatory and adhesion molecules, T-cell receptor avidity, surface density of MHC class II-T-cell receptor complexes, cell-cycle and length of differentiation time have also been demonstrated to influence MHC class II-T-cell receptor mediated T-lymphocyte function [2, 6, 11, 16, 20, 29, 30]. Our initial interpretation of the decreased DNA synthesis in the FMD-VP4 (high binding) peptide-specific T-lymphocytes was that there might be a hole in the T-cell repertoire. However, the high levels of IFN-γ production by this lymphocyte culture clearly indicate that this is not

the case. This may be an example whereby T-cell effector cytokine expression was attained independently of the cell cycle [14]. It is also possible that the OVA 339 and FMD-VP2 cultures, which produced relatively little IFN-γ, were actually Th2 polarized sub-populations [6].

5. CONCLUSION

The present study determined the binding affinities of several OVA and FMD-V peptides for *BoLA-DRB3*2703*. Although these peptides elicited unique T-lymphocyte responses and effector cytokine production, it is not absolutely clear that high and low peptide binding affinity for *BoLA-DRB3*2703* alone accounts for observed differences in T-lymphocyte function.

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