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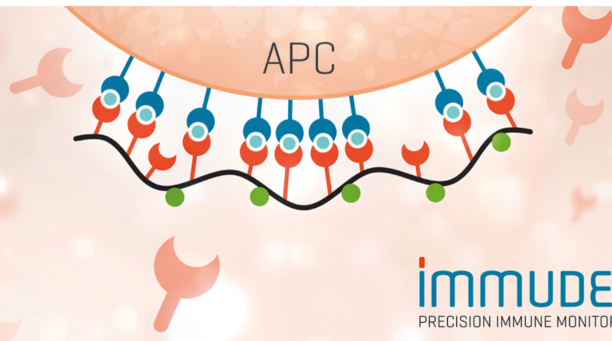

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Vaccinia Virus-Specific CD4$^+$ T Cell Responses Target a Set of Antigens Largely Distinct from Those Targeted by CD8$^+$ T Cell Responses[1]

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Recent studies have defined vaccinia virus (VACV)-specific CD8$^+$ T cell epitopes in mice and humans. However, little is known about the epitope specificities of CD4$^+$ T cell responses. In this study, we identified 14 I-A^b-restricted VACV-specific CD4$^+$ T cell epitopes by screening a large set of 2146 different 15-mer peptides in C57BL/6 mice. These epitopes account for ~20% of the total anti-VACV CD4$^+$ T cell response and are derived from 13 different viral proteins. Surprisingly, none of the CD4$^+$ T cell epitopes identified was derived from VACV virulence factors. Although early Ags were recognized, late Ags predominated as CD4$^+$ T cell targets. These results are in contrast to what was previously found in CD8$^+$ T cells responses, where early Ags, including virulence factors, were prominently recognized. Taken together, these results highlight fundamental differences in immunodominance of CD4$^+$ and CD8$^+$ T cell responses to a complex pathogen. ***The Journal of Immunology,*** **2007, 178: 6814–6820.**

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The CD4$^+$ T cells play a central role in the host defense against invading pathogens. Several recent studies showed a critical role of CD4$^+$ T cells for eliciting an efficient memory CD8$^+$ T cell response against acute viral infections. Memory CD8$^+$ T cells primed in the absence of CD4$^+$ T cell help are qualitatively impaired in their ability to mount a vigorous response to secondary encounter with Ag (1–4). CD4$^+$ T cells also help B cells to produce neutralizing Abs and play a critical role in generating B cell memory (5, 6). Finally, CD4$^+$ T cells can directly impede the spread of viruses through the secretion of antiviral cytokines such as IFN-γ that block viral replication, and cytotoxic functions have also been attributed to CD4$^+$ T cells (7, 8).

A clear role of CD4$^+$ T cells is apparent in infection with vaccinia virus (VACV)[3]. Immunization with VACV induces cellular immune responses that persist for >35 years (5, 9–11). T cell immunity is most important for VACV resistance in naive mice (12). In contrast, Ab responses play a major role in protection against lethal challenge with VACV in immunized mice (12) or monkeypox virus in primates (13).

In a recent study, a single modified vaccinia virus Ankara (MVA) inoculation protected mice deficient of B and CD8$^+$ T cells against lethal VACV intranasal challenge, whereas CD4 and MHC class II-deficient mice were poorly protected (14). Despite their importance, little is known about the breadth of VACV-specific CD4$^+$ T cell responses and the nature of the Ags recognized. In contrast, recent studies have defined the Ags and epitopes recognized by CD8$^+$ T cell responses following VACV infection in mice and humans (15–21). The breadth of CD8$^+$ T cell VACV responses is large with a total of 103 Ags recognized thus far in total, most of which are early Ags. CD8$^+$ T cells equally recognize VACV structural proteins, regulatory proteins, and virulence factors.

To understand the impact of CD4$^+$ T cell responses and the dynamic development of CD8$^+$ T cell responses, it is important to similarly define the breadth and nature of CD4$^+$ T cell responses.

Several studies have shown that the number of CD4$^+$ T cells involved in responses are of lower magnitude when compared with CD8$^+$ T cell responses, both in general and also in the case of VACV infection (22–25). It is currently unknown whether this lower magnitude is due to the recognition of fewer epitopes, but with each epitope being recognized by responses of similar magnitude. Alternatively, it is possible that the overall lower CD4$^+$ T cell response is reflective of a comparable number of epitopes, each associated with responses of lower magnitude.

The aim of the present study was to probe the breadth and magnitude of VACV-specific CD4$^+$ T cell epitopes. By screening a large set of 15-mer peptides, we identified a total of 14 VACV-specific CD4$^+$ T cell epitopes derived from 13 viral Ags, which account for ~20% of the total response. The nature of the Ags recognized differed substantially from those recognized by CD8$^+$

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[3] Abbreviations used in this paper: VACV, vaccinia virus; WR, Western Reserve; SFC, spot-forming cells; LPS-Blasts, LPS-stimulated B lymphoblasts; DC, dendritic cell; ICCS, intracellular cytokine staining; SI, stimulation index; HCMV, human CMV.

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T cell responses. Whereas $CD8^+$ T cell responses are predominantly directed against early Ags, $CD4^+$ T cells most often recognize late Ags. These Ags include structural proteins and proteins involved in viral regulation, but thus far no epitopes derived from virulence factors have been identified.

Materials and Methods

Peptides and I-A^b-peptide-binding assays

A set of 2146 different peptides were synthesized as crude material by Pepscan Systems and Mimotopes and used in initial screening experiments. Of these synthetic peptides, 448 were initially selected based on the presence of putative I-A^b-binding patterns (26). However, in independent subsequent experiments, these patterns proved to be devoid of significant predictive value. In addition, the set contained 1698 peptides that were predicted to be potential DR1 binders and available for screening in our laboratory. Thus, these peptides represent a heterogeneous and effectively random peptide set. Altogether, these peptides account for 30.4% of the predicted transcribed sequences of VACV-Western Reserve (WR). The peptides are derived from 199 of the 218 annotated VACV genes (18 of the 19 open reading frames that are not represented in the set are <80 aa in length). Peptides from structural proteins, viral gene regulation proteins, and virulence factors were similarly represented (see supplemental Table I[4]). H-2 I-A^b class II MHC was purified, and peptide-binding assays were performed as previously described (27). MHC binding of the radiolabeled peptide was determined by capturing MHC-peptide complexes on the respective Ab-coated Lumitrac 600 plates (Greiner Bio-one) and measuring bound cpm using the TopCount (Packard Instrument) microscintillation counter.

Viruses

The VACV-WR strain was obtained from Bernard Moss (National Institute of Allergy and Infectious Diseases, Bethesda, MD) and grown in 143 TK^- cells.

Mice

C57BL/6 mice were obtained from The Jackson Laboratory and were used between 6 and 12 wk of age according to the National Institutes of Health guidelines and Institutional Animal Care and Use Committee-approved animal protocols.

Infection and immunizations

C57BL/6 mice were infected i.p. with 2×10^6 PFU VACV-WR in 0.5 ml of PBS. After 10 days, the mice were sacrificed and the splenocytes were used in either ex vivo IFN-γ-ELISPOT or intracellular cytokine staining (ICCS) assays as described below.

Stimulator cells

All cells were grown in complete RPMI 1640 medium containing 10% FBS (Gemini Bio-Products). In IFN-γ-ELISPOT assays, LPS-stimulated B lymphoblasts (LPS-Blasts), obtained by cultivating splenocytes in the presence of LPS (8.5 μg/ml) and dextran sulfate (7 μg/ml) (Sigma-Aldrich) for 3 days at 37°C, were used as stimulator cells. In ICCS assays, $CD11c^+$ dendritic cells (DC) were generated by s.c. injection of $5–10 \times 10^6$ melanoma cells that secrete FMS-like tyrosine kinase 3 ligand into C57BL/6 mice. Twelve to 14 days later, the splenocytes were removed and $CD11c^+$ DC were isolated by positive selection using CD11c MicroBeads (Miltenyi Biotec) according to their protocol.

Ex vivo ELISPOT assay

The ELISPOT assays were performed as previously described (17), with some adaptations. Briefly, 1×10^6 LPS-Blasts were either peptide pulsed (10 μg/ml) or incubated with VACV-WR (multiplicity of infection = 5 or 10) for 2 h and washed once before used as stimulators. Two $\times 10^5$ $CD4^+$ T cells, isolated from 5 to 10 pooled spleens by positive selection using CD4 MicroBeads (Miltenyi Biotec), were cocultured with 1×10^5 peptide-pulsed or VACV-WR-infected LPS-Blasts. Each pool/peptide was tested in triplicate wells and the experimental values were expressed as the mean net spot-forming cells (SFC)/10^6 $CD4^+$ effector T cells ± SEM for each peptide. The net number of SFC/10^6 $CD4^+$ effector T cells was calculated as follows: [(number of spots against relevant peptide) − (number of spots against DMSO control)] × [(1×10^6)/(number of $CD4^+$ effector T cells/well)]. Responses against medium containing 1% DMSO (corresponding to the percent DMSO in a pool of 10 peptides, each at 10 μg/ml) were measured to establish background values that were subtracted from the experimental values. To determine the level of statistical significance, a Student's t test was performed using the mean of triplicate values of the response against relevant peptides vs the response against DMSO control. To estimate the signal-to-noise, we also calculated a stimulation index (SI) defined as (SFC experiment)/(SFC background). Pools yielding >20 mean net SFC/10^6 $CD4^+$ effector T cells, a $p < 0.05$, and a SI > 1.4 in two independent experiments were considered positive. Similarly, individual peptides yielding >20 mean net SFC/10^6 $CD4^+$ effector T cells, a $p < 0.05$, and a SI > 2 in two independent experiments were considered positive.

ICCS assay

$CD11c^+$ DC (1×10^6) were either pulsed with peptides (3 μg/ml) for 1 h in a 96-well plate or were infected with VACV-WR (multiplicity of infection = 5) between 10 and 18 h before the addition of $1–2 \times 10^6$ splenocytes (pooled from two to five mice that were immunized with VACV-WR for 10 days). Two hours later, brefeldin A (10 μg/ml) was added and cells were cultured for another 6–10 h before staining according to the protocol of the BD Fix/Perm Solution Kit (BD Biosciences) using anti-CD4-PerCP and anti-IFN-γ-FITC Abs (both from BD Pharmingen). At least $1.5–2 \times 10^6$ events per sample were collected using a FACSCalibur (BD Biosciences) and analyzed with FlowJo software (Tree Star) by gating lymphocytes on forward scatter × side scatter followed by gating on $CD4^+$ T cells to identify the IFN-γ-producing $CD4^+$ T cells. Background values were determined from samples pulsed with DMSO only (no peptide) and subtracted from the experimental values. At least three independent experiments were performed for each peptide or peptide pool. A peptide was considered positive if the average of the individual experiments was at least >1 SD above the background.

Results

Identification of H-2 I-A^b-restricted epitopes

A number of VACV-specific $CD8^+$ T cell epitopes have been described in the literature (15–21), but no epitope restricted by MHC class II molecules and recognized by $CD4^+$ T cells have been described. To fill this gap, a panel of 2146 peptides, 12–15 residues in size, were tested in IFN-γ-ELISPOT assays. Altogether, these peptides account for 30.4% of the predicted transcribed sequences of VACV-WR. The peptides are derived from 199 of the 218 annotated VACV genes (18 of the 19 open reading frames that are not represented in the set are <80 aa in length). Peptides from structural proteins, viral gene regulation proteins, and virulence factors were similarly represented (see Supplemental Table I).

The antigenicity of these 2146 peptides was tested in a standard IFN-γ-ELISPOT assay with $CD4^+$ T cells purified from pooled spleens of 5–10 C57BL/6 mice obtained 10 days following i.p. infection with VACV-WR. Specifically, a total of 215 pools, each consisting of ~10 peptides per pool, was tested as described in *Materials and Methods*. In initial screens, pools yielding >20 mean net SFC/10^6 cells and a SI (= [SFC experiment]/[SFC background]) >1.4, and a $p < 0.05$ in a standard t test in two independent experiments were considered positive. Accordingly, a total of 17 positive pools with SFC/10^6 values in the 20–240 range were identified (Fig. 1*A*). By contrast, none of the pools or peptides elicited positive responses in naive mice (data not shown).

Positive pools that fulfilled all three criteria mentioned above were deconvoluted to identify the individual peptide. A total of 18 peptides from 17 pools elicited positive responses (>20 SFC/10^6 cells, SI >2, and a $p < 0.05$; Fig. 1*B*). In four cases (marked by #), positive peptides were partially overlapping with each other, and the peptide associated with the highest response was selected for further analysis. This resulted in the identification of 14 putative $CD4^+$ T cell epitopes (marked by an asterisk in Fig. 1*B*). In

[4] The online version of this article contains supplemental material.

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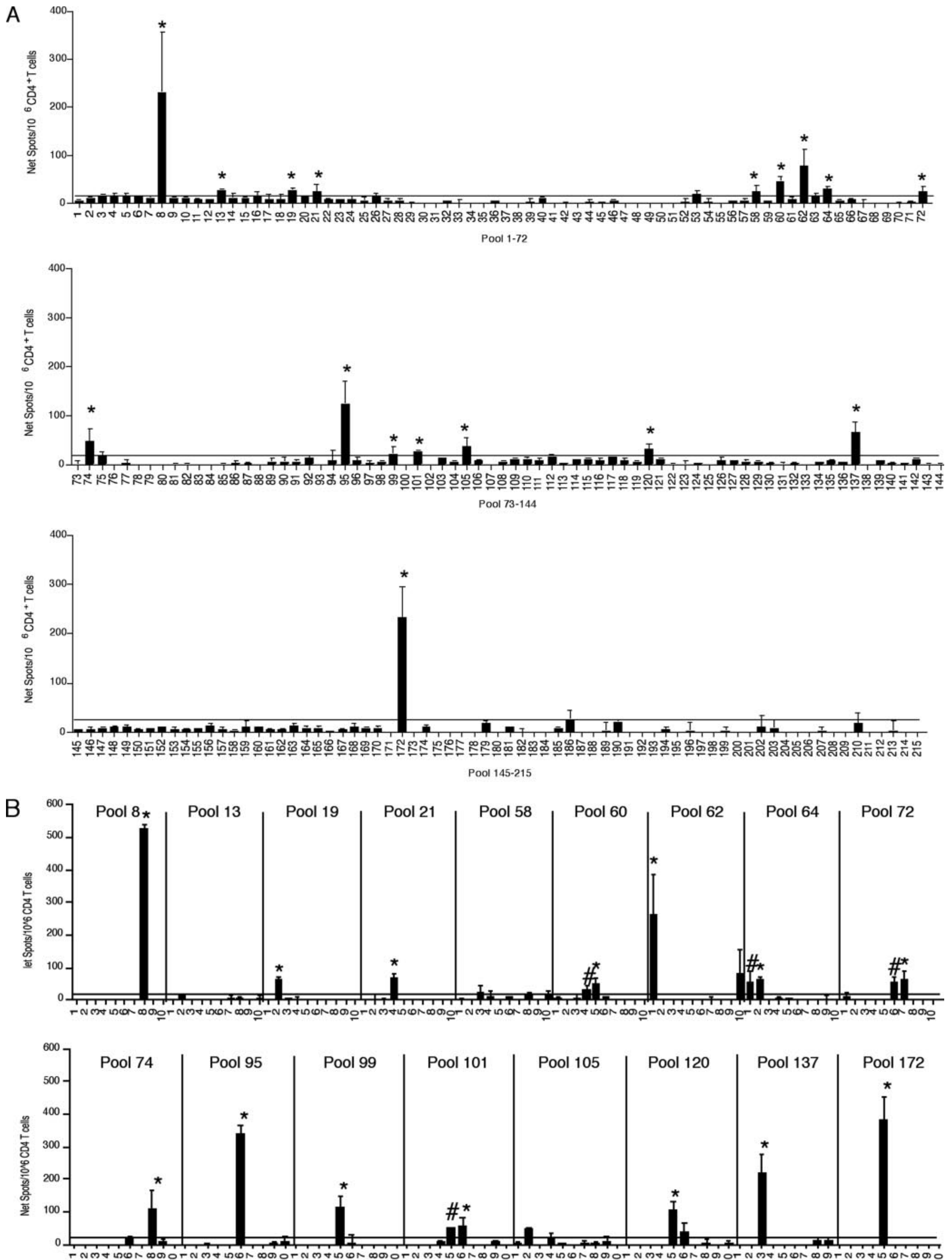

FIGURE 1. Identification of CD4$^+$ T cell epitopes from VACV-WR-immunized C57BL/6 mice by using IFN-γ ELISPOT assay. *A*, Screen of peptide pools. CD4$^+$ T cells purified from pooled spleens derived from 5 to 10 mice that were immunized with VACV-WR for 10 days were used to screen the 215 peptide pools (10 peptides/pool) as described in *Materials and Methods*. Pools yielding >20 mean net SFC/10^6 CD4$^+$ T cells,

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Table I. *Summary of VACV-WR-derived CD4$^+$ T cell epitope characteristics*

Ag	Peptide Sequence	ELISPOT Assay[a] (SFC/10^6 CD4$^+$ T cells)	ICCS Assay[b] (% CD4$^+$IFN-γ^+) ± SD	I-A^b-Binding Affinity (IC_{50} nM)
A18R (49–63)	PKGFYASPSVKTSLV	62.8	0.04 ± 0.014	83
A20R (233–247)	DNIFIPSVITKSGKK	56.2	0.06 ± 0.026	332
A24R (402–416)	IHVLTTPGLNHAFSS	68.7	0.02 ± 0.013	18
A28L (10–24)	FFIVVATAAVCLLFI	103.7	0.01 ± 0.021	4081
D13L (486–500)	PKIFFRPTTITANVS	109.5	0.1 ± 0.046	19
D8L (238–252)	GEIIRAATTSPAREN	62	0.02 ± 0.014	122
E1L (117–131)	VLTIKAPNVISSKIS	51.2	0.03 ± 0.012	188
E9L (179–193)	PSVFINPISHTSYCY	260.3	0.1 ± 0.032	77
F15L (55–69)	TPRYIPSTSISSSNI	220.3	0.07 ± 0.036	353
H3L (272–286)	PGVMYAFTTPLISFF	337.8	0.1 ± 0.06	5.4
I1L (21–35)	LKAYFTAKINEMVDE	62.8	0.05 ± 0.039	362
I1L (7–21)	QLVFNSISARALKAY	315	0.13 ± 0.09	13.5
J4R (78–92)	DDDYGEPIIITSYLQ	114.5	0.04 ± 0.015	2266
L4R (176–190)	ISKYAGINILNVYSP	526.2	0.21 ± 0.08	128

[a] Peptide at 0.1 μg/ml.
[b] Peptide at 3 μg/ml.

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the case of three pools (pools 13, 58, and 105) no significant responses were identified. The sequences of the 14 epitopes identified, along with the corresponding name of the viral Ag and the observed average IFN-γ-ELISPOT response, are summarized in Table I.

Characterization of the H-2 I-A^b-restricted determinants by ICCS assays

In general, ICCS assays are more quantitative for vigorous responses, but less sensitive compared with ELISPOT assays. In this study, we used ICCS assays to obtain a quantitative estimate of the percentage of CD4$^+$ T cells recognizing the newly identified epitopes. Fig. 2*A* shows a representative experiment where all 14 peptides identified by ELISPOT assays induced IFN-γ production by CD4$^+$ T cells above background. The results from three independent experiments are summarized in Table I. The average responses for the 14 peptides ranged from 0.01 to 0.21% above background. All but one epitope (A28L) induced positive IFN-γ responses defined as being >1 SD above background and 7 of the 14 epitopes induced >2 SD above background (Table I).

H-2 I-A^b-binding capacity of identified epitopes

The C57BL/6 mice used in these experiments express a single MHC class II molecule, namely, I-A^b. Each peptide was tested for its capacity to bind purified I-A^b molecules in vitro (Table I). Significant binding to the relevant restriction element ranging from 5.4 to 4081 nM could be demonstrated for all of the epitopes. Consistent with previous studies in the context of MHC class II (28), 12 (88.9%) of the identified epitopes bound with high/intermediate affinity (≤1000 nM) to I-A^b molecules. The remaining two epitopes bound I-A^b molecules more weakly, with affinities of 2266 and 4081 nM but within the range observed for most MHC class II epitopes.

Taken together, these data further characterize the epitopes identified and support their putative I-A^b restriction, assigned on the basis of the recognition by CD4$^+$ T cells.

The identified epitopes account for 21% of the total CD4$^+$ VACV response

Next, we determined the fraction of the total VACV-specific CD4$^+$ T response attributable to the epitopes identified. To this end, the percentage of the total CD4$^+$ T cells responding to CD11c$^+$ DC stimulator cells that were pulsed with a pool containing all 14 identified peptides was determined as described in *Materials and Methods*. To quantify the total VACV-WR-specific CD4$^+$ T cell response, the same DC stimulator cells were infected with VACV-WR for 10–18 h before adding the effector cells consisting of spleens pooled from two to five day 10 VACV-WR-infected mice. To error on the side of comprehensiveness, the A28L peptide, while positive in the ELISPOT assay and also marginal but statistically not significantly positive in the ICCS assay, was included in the pool. A representative experiment of three performed is shown in Fig. 2*B*. It was found that 4.55% of the total CD4$^+$ T cells secreted IFN-γ following stimulation with VACV-WR-infected CD11c$^+$ DC. These data are comparable to what has been published in the literature (22, 25). The pool consisting of the 14 epitopes was recognized by 1.04% of the total CD4$^+$ T cell population. The average net response ([experimental value] − [background value]) of three experiments performed yielded 4.25% (SD = 1.18) for the total VACV-specific CD4$^+$ T cells and a net response of 0.89% (SD = 0.1) for the pool of 14 epitopes. These data suggest that 21% of the total VACV-WR-specific CD4$^+$ T cell response is attributable to the 14 epitopes we have identified.

Nature of the Ags recognized by VACV-specific CD4$^+$ T cells

The 14 identified epitopes are derived from 13 different VACV proteins, whose characteristics are summarized in Table II. To examine whether these characteristics might be correlated with immunodominance for class II responses, we compared the structural features of the Ags recognized by CD4$^+$ T cell with the same features in the total VACV-WR proteome. Regarding the time of

a SI > 1.4, and a $p < 0.05$ in a standard t test in two independent experiments were considered positive. The asterisks indicate pools that were selected for further deconvolution. *B*, Deconvolution of the positive peptide pools. CD4$^+$ T cells purified from pooled spleens derived from 5 to 10 mice that were immunized with VACV-WR for 10 days were used to screen the individual peptides from the 17 positive peptide pools. Peptides yielding >20 mean net SFC)10^6 cells, a SI > 2, and a $p < 0.05$ in a standard t test in two independent experiments were considered positive. The asterisks indicate positive VACV-WR-derived epitopes. #, Partially overlapping peptides.

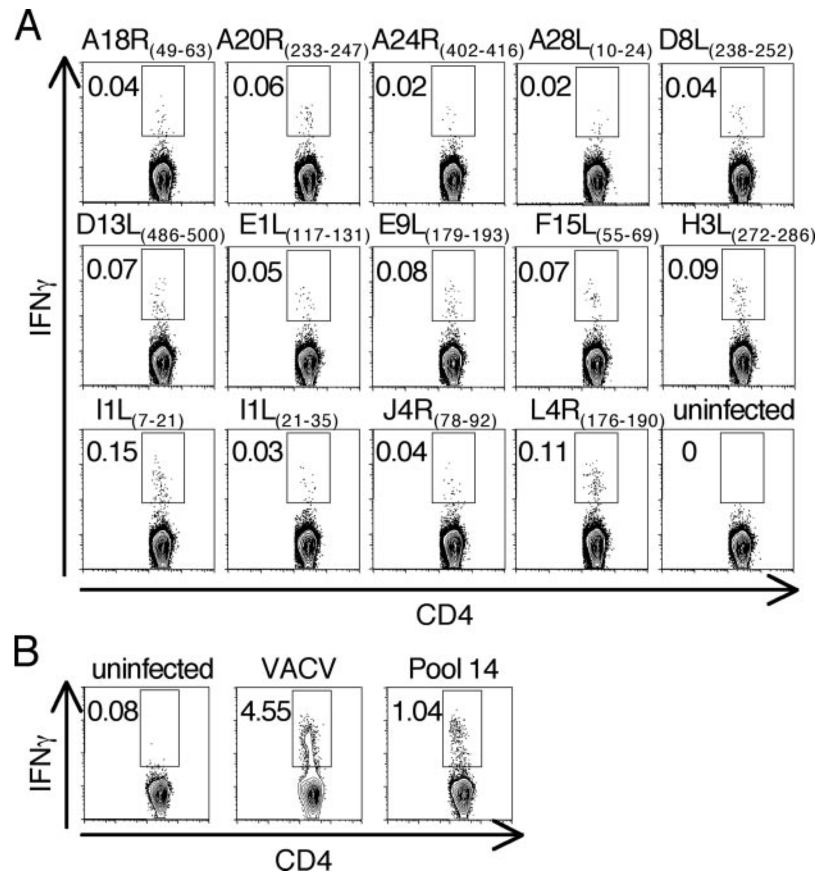

FIGURE 2. CD4$^+$ T cells recognition of VACV-derived H-2 I-A^b epitopes in ICCS assays. Positive peptides identified by IFN-γ ELISPOT assays were tested in ICCS assays using spleens pooled from two to five mice immunized with VACV-WR 10 days earlier as described in *Materials and Methods*. A peptide was considered positive if the average of the individual experiments resulted in at least >1 SD above background (DMSO sample). A representative experiment of three is shown. Percentages of CD4$^+$ T cells producing IFN-γ following stimulation with peptide-pulsed CD11c$^+$ DC (*A*) or CD11c$^+$ DC infected with either VACV-WR or pulsed with a pool of peptides containing all 14 epitopes (*B*) (pool 14).

protein expression during the VACV replication cycle, five Ags (38.5%) are expressed at early time points and eight (61.5%) are expressed during late stages of the viral replication cycle. These data suggest a trend of preferential recognition of late Ags by CD4$^+$ T cells. This is in contrast to what was previously observed for CD8$^+$ T cells, where Ags expressed early are preferentially recognized in a statistically significant manner (17).

In terms of function, category assignments for 12 of 13 Ags were available. A total of six (50%) Ags was structural proteins, six (50%) were viral genome regulation proteins, and none (0%) were virulence factors. The lack of recognition of virulence factor Ags compared with their overall frequency in the vaccinia genome is borderline statistically significant ($p = 0.058$, Fisher's exact test). This is in contrast to what is observed for VACV-specific CD8$^+$ T cell responses where 5 (17.9%) of 18 Ags recognized are virulence factors (17), corresponding to their proportional representation in the genome.

Discussion

Little is known about CD4$^+$ T cells generated in response to VACV infection. In this study, we report the identification of 14 different VACV-specific CD4$^+$ T cell epitopes by screening a large set of 15-mer peptides.

In VACV-immunized humans, the overall CD4$^+$ T cell response is lower than the CD8$^+$ T cell response (9). In C57BL/6 mice, the CD4$^+$ T cell responses are also up to 10-fold lower than

Table II. *Structural characteristics of recognized VACV Ags*

VACV-WR[a]	Ag[b]	Protein Length	Time of Expression[c]	Function	Life Cycle
VACWR138	A18R	494	Early	Transcription elongation regulator	Viral gene regulation
VACWR141	A20R	427	Early	DNA polymerase processivity factor	Viral gene regulation
VACWR144	A24R	1165	Early	RNA polymerase subunit, RPO132	Viral gene regulation
VACWR151	A28L	147	Late	IMV protein	Structural protein
VACWR118	D13L	552	Late	IMV membrane protein	Structural protein
VACWR113	D8L	305	Late	IMV membrane protein	Structural protein
VACWR057	E1L	465	Late	poly(A) polymerase, large subunit	Viral gene regulation
VACWR065	E9L	1007	Early	DNA polymerase	Viral gene regulation
VACWR054	F15L	159	Late	—	—
VACWR101	H3L	325	Late	IMV membrane protein	Structural protein
VACWR070	I1L	313	Late	Virion core DNA-binding protein	Structural protein
VACWR096	J4R	186	Early	RNA polymerase subunit, RPO22	Viral gene regulation
VACWR091	L4R	252	Late	Core DNA-binding protein, VP8	Structural protein

[a] Defined by Poxvirus Bioinformatics Resource Center (www.poxvirus.org).
[b] Defined by McGraith et al. (48).
[c] Predicted by E. J. Lefkowitz (unpublished data).

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CD8$^+$ T cell responses (22, 25). Our study reveals that the number of the CD4$^+$ T cells that responded to each epitope is lower compared with the response of CD8$^+$ T cells directed against VACV epitopes (17, 21). The breadth of Ags and epitopes recognized by CD4$^+$ T cell responses is large (Tables I and II). This number is still lower than the number of CD8$^+$ T cell epitopes (14 vs 49) and is likely a reflection of the fact that the totality of epitopes has not yet been identified, since the epitopes identified thus far only account for ~20% of the total VACV CD4$^+$ T cell response. Thus, our data are most compatible with overall lower CD4$^+$ T cell responses due to comparable numbers of CD4$^+$ and CD8$^+$ T cell epitopes but lower numbers of CD4$^+$ T cells responding to each epitope.

We would like to point out as a caveat that our study thus far only addressed IFN-γ-producing T cells. Additional CD4$^+$ T cell epitopes may exist that do not induce IFN-γ, but do trigger production of other cytokines, such as IL-2, IL-4, or IL-5. Further studies will address these issues.

The observed pattern of Ag recognition from VACV CD4$^+$ T cell responses, overrepresentation of late vs early Ags and of structural proteins vs virulence factors, is most consistent with virions being a prevalent source of Ags for CD4$^+$ T cell recognition. CD4$^+$ T cells are usually primed and activated by professional APC such as DC following uptake of exogenous Ag and presentation of processed peptides via MHC class II molecules (29). Therefore, the lack of recognition of virulence factors by CD4$^+$ T cells could be explained by the near complete absence of virulence factors in the virion (30). It is likely that DC take up debris from apoptotic or necrotic VACV-infected cells that might contain VACV virulence factors that theoretically could be presented by DC via MHC class II molecules. Our results suggest that this source of antigenic materials is not functionally relevant in the case of class II responses to VACV. Our results also suggest that direct APC infection and viral replication is apparently not a major source of antigenic material for class II responses, since early proteins and virulence factors are significantly underrepresented.

The pattern of Ags recognized by CD4$^+$ T cells differs substantially from the pattern of Ags recognized by CD8$^+$ T cells on the same murine strain. An interesting finding of our study is that virulence factors representing 22% (22 of 110) of VACV proteins of known function were not found to be recognized by CD4$^+$ T cells. This is in sharp contrast to what is observed for VACV-specific CD8$^+$ T cells, where 17.9% (5 of 28) of recognized Ags are virulence factors, comparable to their overall representation in the viral genome (17). The peptide set we have tested accounts for the same fraction (30.3%) of the genome encoding for virulence factors as the other proteins (structural and genome regulation proteins, 30.4%). This finding suggests fundamental differences in CD4$^+$ and CD8$^+$ T cell responses to VACV. However, we would like to point out that this underrepresentation does not exclude that some virulence factors might contain some as yet unidentified epitopes.

Even more comprehensive studies have been performed for other viruses (31–33), including the complex pathogen human CMV (HCMV) by using overlapping peptides spanning the entire genome (34). Interestingly, HCMV virulence factors were targeted with similar frequencies by both CD4$^+$ and CD8$^+$ T cell responses. VACV is eliminated following acute infection, whereas HCMV causes a lifelong latent/persistent infection and this may result in a continuous exposure to peptides derived from virulence factors.

Little correlation appears to exist between the binding affinity of the epitopes shown in Table I and the their "placement" in the immunodomination hierarchy. That binding beyond a certain threshold often does not correlate strongly with immunodominance, in both class I and class II contexts, has been pointed out in several studies (17, 35–47).

Optimization regarding the peptide epitope length or sequence has not been performed. Given the open ends of the class II-binding pocket and the necessity of flanking residues to provide a stable complex, 15-mer peptides have proven to be a very good length of peptide in CD4 contexts. Shorter, "optimal" peptides, limited to the exact residues recognized by T cell specificities, often lack the necessary flanking residues for high-affinity binding.

The peptide with the lowest affinity (A28L) was also the peptide positive in the ELISPOT assay but only marginal in the ICCS assay. This peptide contains a cysteine and might become oxidated. We performed MHC-binding experiments in the presence of 2-ME but no differences were noted. Because this peptide is remarkably hydrophobic, it is also possible that its low solubility may yield somewhat discrepant results in the various assays.

Any linkage of viral Ag recognition by CD4$^+$ and CD8$^+$ T cells seems unlikely, as only 4 of the 43 (A18R, D13L, E9L, and J4R) Ags targeted by CD8$^+$ T cells are also recognized by VACV-specific CD4$^+$ T cells (Table II). This is in concordance with a model of CD4$^+$ T cell help for CD8$^+$ T cells not depending on the recognition of the same antigenic protein, but rather any protein derived from the same pathogen.

Finally, we would like to point out that the Ags most dominantly recognized by CD8$^+$ T cell responses would not necessarily be most effective in inducing CD4$^+$ T cell responses. This situation might result in generation of "helpless" CTL with impaired memory development (2). To address this point, we are currently examining whether CD4$^+$ T cell responses can be induced by vaccination with Ags recognized as dominant in the context of CD8$^+$ T cell responses (such as B8R). We are also investigating whether any association exists between Ags recognized by Ab responses and CD4$^+$ T cells.

In conclusion, our data demonstrate divergence between Ags recognized in CD4$^+$ and CD8$^+$ T cell responses and illustrate how the full picture of adaptive immune responses against poxviruses is only revealed by the definition of the various Ags and epitopes recognized.

Disclosures

The authors have no financial conflict of interest.

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