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Characterizing monkeypox virus specific CD8+ T cell epitopes in rhesus macaques

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ABSTRACT

To characterize T cell epitopes in monkeypox virus (MPXV) infected rhesus macaques, we utilized IFNγ Elispot assay to screen 400 predicted peptides from 20 MPXV proteins. Two peptides from the F8L protein, an analog of E9L protein in vaccinia, were found to elicit CD8+ T cell responses. Prediction and in vitro MHC binding analyses suggest that one is restricted by Mamu-A1*001 and another by Mamu-A1*002. The Mamu-A1*002 epitope is completely identical in all reported sequences for variola, vaccinia, cowpox and MPXV. The Mamu-A1*001 epitope is conserved in MPXV and vaccinia, and has one residue substitution (V6 > I) in some cowpox sequences and all variola sequences. Given CD8+ T-cell epitopes from E9L were also identified in humans and mice, our data suggested that F8L/E9L may be a dominant pox viral protein for CD8+ T cell responses, and may be considered as a target when designing vaccines that target pox-specific T cell responses.

Introduction

Monekypox virus (MPXV) is a member of the *Orthopoxvirus* family that also includes variola virus (VARV), the causative agent of human smallpox, and vaccinia virus (VACV). Since cessation of broad VACV immunization, MPXV infections are now emerging as a public health concern. MPXV infection can cause up to 10% lethality in humans, and poses a risk to human health as an infectious agent and a potential biological weapon. MPXV infection resembles in many aspects the human clinical symptoms of smallpox, including fever, weight loss, lesion development, and death (Huhn et al., 2005; Jezek et al., 1987). To better understand the pathogenesis of MPXV and related orthopoxviruses, such as variola virus, and to provide a model to evaluate counter measures against these poxviruses, non-human primate (NHP) models of MPXV infection have been developed using aerosol (Zaucha et al., 2001), intranasal (Saijo et al., 2006), and intratracheal (Stittelaar et al., 2005, 2006), as well as intravenous (IV) (Johnson et al., 2011; Hooper et al., 2004) routes of exposure. The respiratory pathogen delivery routes are generally technically demanding and may also require specialized facilities and equipment, while the IV inoculation is technically easier and has been used successfully to evaluate the immunogeneicity and protective role of various forms of poxvirus vaccines against MPX infection in NHPs (Hooper et al., 2004; Edghill-Smith et al., 2005; Earl et al., 2004; Heraud et al., 2006).

MPXV-infected NHPs have symptoms resembling those of humans infected with monkeypox and smallpox. Macaques infected with MPXV generate both neutralizing antibodies (Johnson et al., 2011; Keasey et al., 2010) and T cell responses (Estep et al., 2011). Upon proteomic array analysis, while antibodies from MPXV-infected macaques and VACV-vaccinated humans recognized a few common proteins, antibodies from MPXV-infected macaques also recognized proteins that were not recognized by vaccinated human sera (Keasey et al., 2010). It is interesting to determine if this also applies to T cell responses. T cell responses, as the other major arm of adaptive immune responses, have also been shown to play a critical role in protection against poxvirus infection (Redfield et al., 1987; Howell et al., 2006; Xu et al., 2004; Snyder et al., 2004). The precise definition of T cell epitopes allows rigorous assessment of antigen-specific T cell responses in viral pathogenesis and in vaccine studies. Specifically in the case of poxviruses, a relatively large number of CD8+ and CD4+ T cell epitopes have been characterized in human and murine systems (Kennedy and Poland, 2007; Moutaftsi et al., 2010). However, only a few T cell epitopes have been reported in

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rhesus macaques infected with VACV WR (Walsh et al., 2009), and no specific epitopes have been reported for variola or monkeypox. Given the pivotal role of non-human primates for the study of poxvirus pathogenesis and counteracting agents, including vaccine studies, it is important to have specific reagents to study pox-specific T cell function in rhesus macaques. In the current study, we utilized an approach combining bioinformatic epitope predictions and Elispot immunological assays to identify the first CD8+ T cell epitopes for monkeypox virus recognized in rhesus macaques. Our results illustrate the feasibility of this approach to identify epitopes from biosafety level 3 (BSL-3) poxviruses.

Results

Selection of antigens for study

To identify candidate antigens for analysis, we compiled T cell recognition data from three sources. These included an analysis undertaken by Moutaftsi et al. (2010) to identify prevalently recognized VACV WR ORFs, a compilation and analysis of donor recognition data available in the immune epitope data base (IEDB) (Vita et al., 2010), and a study of CD8 T cell responses to VACV WR in *Mamu-A1*001* positive macaques (Walsh et al., 2009). While several orthopoxvirus antigens have been shown to elicit antibodies that are protective in challenge models, and linkage between CD4+ T cell recognition and antibody production following VACV has also been reported, for the present study we have selected only from ORFs for which CD8 T cell epitopes have been described.

From the Moutaftsi et al. (2010) analysis, a list of the 23 most prevalently recognized VACV WR ORFs was compiled, where prevalence was defined as an ORF being recognized in the context of 3 or more different HLA or H-2 alleles. Next, we examined human CD8 T cell recognition data available in the IEDB. For this analysis we compiled the number of positive donor responses for each of the 218 VACV WR ORFs versus the total number of donors tested from all studies curated by the IEDB as of June 1, 2012. From these data a frequency of positive responses was calculated. Overall, 49 ORFs were identified that had response rates of 25% or greater (138 had at least one positive response). Finally, we tabulated a set of VACV WR ORFs described in a previous study that were recognized by CD8 T cells in vaccinated *Mamu-A1*001* macaques (Walsh et al., 2009). This identified 16 ORFs that were recognized in one or both of two animals studied.

Taken together, 70 ORFS were identified that were selected by one or another analysis. To limit the analysis to a more manageable set, we selected a set of 20 ORFs to include all ORFs identified in at least 2 of the 3 analyses described above, or that were recognized in both of the macaques studied in Walsh et al. (2009). This set of ORFs is listed in Supplemental Table 1.

Selection of peptides

Among the NHPs, we chose to work with those expressing *Mamu-A1*001* and *Mamu-A1*002*. Both of these alleles are prevalent in our cohort, and have been extensively characterized in previous studies of simian immunodeficiency virus infection in rhesus macaques (Loffredo et al., 2004; Allen et al., 2001, 1998). Furthermore, multiple efficient approaches for predicting peptide binders have been developed for both of these alleles (Peters et al., 2005, 2006).

To select peptides for *Mamu-A1*001* and *Mamu-A1*002*, we first analyzed the previously published CD8 T cell epitope data (Walsh et al., 2009; Loffredo et al., 2004; Allen et al., 2001, 1998) to determine the optimal peptide length(s) for consideration. We tabulated the number of epitopes identified for each allele, and determined their distribution as a function of peptide length. For both alleles it was found that the vast majority of epitopes defined previously were 9 residues in length (78 and 74%, respectively, for *Mamu-A1*001* and *Mamu-A1*002*). Because 9-mers represent the canonical epitope length for most class I alleles, we checked that the predominance of 9-mers in the cases *Mamu-A1*001* and *Mamu-A1*002* did reflect a selection bias. Accordingly, for each of the studies, we also tabulated the number of peptides synthesized of various lengths and then calculated the fraction of peptides examined that were eventually identified as T cell epitopes. As shown in Supplemental Table 2, 9-mers were more likely to be identified as epitopes than peptides of other sizes. We also note that the differences observed are likely to be conservative estimates, as each of the studies evaluated included only the top predicted or most canonical motifs for peptides of 8-, 10- and 11-residues, while for 9-mers broader selection criteria were considered to include peptides with poorer predicted affinities. Accordingly, for the present study, we have focused solely on 9-mer peptides.

Finally, to identify potential epitopes derived from the set of 20 antigens selected above, we scanned the corresponding MPXV Zaire 79 and Sierra Leone sequences utilizing the *Mamu-A1*001* and *Mamu-A1*002* consensus algorithms hosted by IEDB (www.iedb.org). Unique peptides were selected as described in the Materials and Methods. The final set selected represented the top 2.25%-scoring, 9-mer peptides amongst the 20 antigens in both MPXV isolates, further supplemented by ensuring that for each antigen at least 5 peptides or, if larger, 2.25% of the total peptides in the antigen, were included for study. Following this approach, 400 peptides (194 for *Mamu-A*001*, and 206 for *Mamu-A1*002*) were selected for further analysis (Supplemental Table 3).

Mapping epitope specific responses using Elispot and intracellular staining

We assayed PBMCs from two different MPXV-infected NHPs for each *Mamu-A* haplotype (Table 1). In the case of *Mamu-A1*001, we assayed PBMCs from animal A5E073, which was infected with the MPXV Sierra Leone strain, and DC11, which was infected with the MPXV Zaire 79. In the case of the *Mamu-A1*002* we utilized PBMCs from the DC22 and FLR macaques, both NHPs were infected with the MPXV Sierra Leone.

While DC11 succumbed to MPXV infection at day 14 post-infection, the remaining three NHPs survived to the end of the study (day 30). To identify specific epitopes, predicted peptide

Table 1
MPXV-infected NHPs and IFNγ Elispot results.

NHPs	Virus inoculation and dose (PFU)	*Mamu* haplotype	Day of necropsy	IFNγ Elispot no./1×10^6 PBMCs stimulated by peptides	IFNγ Elispot no./1×10^6 PBMCs stimulated by inactivated VACV	Peptides triggering IFNγ secretion
DC11	Zaire 79 1.5×10^7	A001	14	44	72	ISPDGCYSL
A5E073	Sierra Leone 2.5×10^6	A001	30	171	494	ISPDGCYSL
DC22	Sierra Leone 2.5×10^6	A002	30	180	317	LTFDYVVTF
FLR	Sierra Leone 2.5×10^8	A002	30	158	257	LTFDYVVTF