The pigtail macaque MHC class I allele Mane-A*10 presents an immunodominant SIV Gag epitope: identification, tetramer development and implications of immune escape and reversion


Abstract: The pigtail macaque (Macaca nemestrina) is a common model for the study of AIDS. The pigtail major histocompatibility complex class I allele Mane-A*10 restricts an immunodominant simian immunodeficiency virus (SIV) Gag epitope (KP9) which rapidly mutates to escape T cell recognition following acute simian/human immunodeficiency virus infection. Two technologies for the detection of Mane-A*10 in outbred pigtail macaques were developed: reference strand-mediated conformational analysis and sequence-specific primer polymerase chain reaction. A Mane-A*10/KP9 tetramer was then developed to quantify CD8+ T lymphocytes primed by multigenic DNA vaccination, which have previously been difficult to detect using standard interferon-γ-based T cell assays. We also demonstrate mutational escape at KP9 following acute SIV infection. Mane-A*10+ animals have lower set point SIV levels than Mane-A*10− animals, suggesting a significant fitness cost of escape. These studies pave the way for a more robust understanding of HIV vaccines in pigtail macaques.

Key words: CD8+ T cells – major histocompatibility complex typing – tetramer – vaccines – viral escape

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Introduction

The escalating global spread of HIV makes the development of an effective vaccine an incredibly urgent task. Non-human primate models of AIDS are vital to the vaccine development effort. Commonly used models are the simian immunodeficiency virus (SIV) or chimeric simian/human immunodeficiency virus (SHIV) infection of rhesus (Macaca mulatta), cynomolgus (M. fascicularis) and pigtail (M. nemestrina) macaques.

CD8+ T cell responses significantly contribute to the control of natural HIV infection [27, 33, 36], and much attention is now focussed on understanding the underlying mechanisms of CD8+ T cell control [4, 15, 32] in order to harness them for vaccine and treatment strategies. During the course of natural HIV infection of humans, and also in animal models, the CD8+ T cell response is directed towards a limited number of defined viral epitopes [20, 35, 44, 45]. The characterization of these epitopes, and the discovery of the major histocompatibility complex (MHC) class I molecules that bind them, is fundamental to the detailed study necessary to understand how CD8+ T cells contribute to viral control.
The characterization of the common rhesus macaque MHC class I molecule Mamu-A*01 [34], and the study of its peptide-binding motif [1], have enabled detailed studies of specific immunodominant CD8+ T cell responses, particularly through the development of Mamu-A*01-peptide tetramers [2, 18, 28, 35, 42]. MHC typing of rhesus macaques is now a routine procedure, with the use of techniques such as PCR with sequence-specific primers (PCR-SSP) [26, 30] and more recently, reference strand-mediated conformational analysis (RSCA) [5], used to identify macaques expressing Mamu-A*01 and other known MHC class I alleles. Since these advances in the rhesus macaque model, Mamu-A*01+ animals have become much more scarce [11], thus providing significant imperative to develop alternative animal models such as the pigtail macaque.

We recently characterized an MHC class I allele in pigtail macaques, Mane-A*10, that restricts an immunodominant response to SIV Gag164-172 epitope KP9 [43]. The identification of this immunodominant epitope and its restricting MHC class I allele is an important advance for the pigtail macaque model of AIDS.

Interestingly, we recently described viral escape from T cell recognition at the Mane-A*10-restricted KP9 epitope in SHIV-infected macaques, involving a lysine to arginine substitution at position 2 of the epitope [19]. By mapping the rapid rate of escape through serially cloning plasma SHIV RNA, we estimated that KP9-specific T cells efficiently eliminate wild-type virus. Further, when we studied infection with an SHIV strain already escaped at KP9, we saw rapid reversion to wild-type sequence, suggesting that escape at KP9 causes a significant decrease in viral fitness [19]. Prior to the present report, escape at the KP9 epitope in SIV-infected macaques (rather than SHIV) has not been reported. Furthermore, the relative utility of KP9-specific T cells acting against wild-type SIV, compared with the reduction in viral fitness as a result of escape mutations, has not been previously studied.

The identification of Mane-A*10 in outbred populations of pigtail macaques is now an important priority for researchers using this animal model. In addition, study of KP9-specific CD8+ T cells using Mane-A*10/KP9 tetramers will assist in understanding the characteristics of these T cells that contribute to viral escape and viral control. Here, we describe two alternative methods for MHC class I typing pigtail macaques – a Mane-A*10-specific PCR assay, and the further adaptation of RSCA – to augment the current, cumbersome methods of cloning and sequencing. In addition, we describe the design, development and validation of a Mane-A*10/KP9 tetramer, the first developed for the pigtail macaque, which will facilitate a specific and detailed analysis of KP9-specific CD8+ T cells.

Materials and Methods
Reference strand-mediated conformational analysis

The previously described RSCA method using an AlfExpress DNA Analyzer (Amersham Biosciences, Piscataway, NJ, USA) [43] was adapted for the characterization of MHC class I expression patterns in M. nemestrina on an ABI PRISM 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). Briefly, a 677-bp fragment of MHC class I cDNA was amplified with 1 μl of 25 μM phosphate-labelled forward primer (5’ RSCA-P; 5’-[phos]-GCTACGTGGACGACACGC-3’), an unlabelled reverse primer (3’ RefStrandR; 5’-CAGAACGCACCCACACCAGC-3’), and 1 U Phusion DNA polymerase (Finzymes, Espoo, Finland) in a 50-μl reaction. An initial 30 s 98°C step was followed by 25 cycles of 98°C for 5 s, 65°C for 1 s, 72°C for 20 s, then a final extension at 72°C for 5 minutes. Following amplification, samples were digested for 30 minutes at 37°C with 10 U lambda exonuclease (Epicentre, Madison, WI, USA) to yield single-stranded amplicons. Rhesus macaque alleles Mamu-A*15, Mamu-A*20 and Mamu-B*60 were prepared as reference strands under the same amplification conditions, using 6FAM, NED or HEX-labelled forward primers (5’-[fluorescent dye]GCTACGTGGACGACACGC-3’) and a phosphate-labelled reverse primer (5’-[phos]-CAGAACGCACCACACCACGC-3’), then exonuclease treated as described above. Heteroduplexes of the single-stranded cDNA amplicons and reference strands were created in a reaction involving 4 minutes at 95°C, 5 minutes at 55°C and 5 minutes at 15°C. The RSCA reactions were then desalted on a Sephadex G-50 column (Amersham Biosciences), dried down in a SpeedVac (Thermo Electron, Waltham, MA, USA), and loaded onto a 4.5% polyacrylamide gel on the ABI PRISM 377 DNA Sequencer (Applied Biosystems). Samples were electrophoresed for 12 hours at 30°C, 1200 V, 60 mA current and 10 W power. Data were analysed using DAx data acquisition and analysis software (Van Mierlo Software, Eindhoven, the Netherlands).

PCR-SSP assay
To design Mane-A*10-specific primers, all known M. nemestrina MHC class I allele sequences were...
aligned (Genbank: AY204715, AY204716, AY204723–AY204738, AY557348–AY557367). The alignment included several sequences that are likely to be further alleles but have not been found in enough clones to qualify for allele definition, in order to maximize the specificity of the primers for Mane-A*10. Regions unique to Mane-A*10 were identified, and candidate primers for the amplification of 100–200 bp fragments designed (using Primer Express Version 1.0, Applied Biosystems) to (i) have a melting temperature >70°C, and (ii) exhibit sequence mismatches with the non-Mane-A*10 alleles towards the 3’ end of the primer. Three primer sets were identified: set 1 (SSP1 forward: 5’-GGCCAACACACAGACC-TACCAGAGAG-3’, Tm 71.4°C; SSP1 reverse: 5’-TCTGGGTGCTAGGGCTAGTATTGATATGC-3’, Tm 74°C), set 2 (SSP2 forward: 5’-CGGGTC TCACACCTTCCAGAGGATGTAT-3’, Tm 71.3°C; SSP2 reverse: 5’-GGGTCCAGGAACGCGG- CCC-3’, Tm 73°C) and set 3 (SSP3 forward: 5’-GGGCCTCTCTTCCGGATATAG-3’, Tm 72.2°C; SSP3 reverse: 5’-GGCACTGCGCTCCACCG-TAAGT-3’, Tm 71.4°C).

Primers specific for the constitutively expressed CCR5 gene were used as a positive control. The CCR5 primer set amplifies a 239-bp fragment: (SSP CCR5 Forward: 5’-GGCTGTGTTTGTGGCTCTTCTTCAGGA-3’, Tm 72.9°C; SSP CCR5 Reverse: 5’-TCTCACAGGCGTGTGCTCTTCT- TTCT-3’, Tm 72.3°C).

First strand cDNA was prepared from total RNA extracted from macaques with known Mane-A*10 status [43]. PCR amplification of 0.9 μl of cDNA or 5.3 ng of MHC clone DNA was carried out with 0.375 U of AmpliTaq Gold DNA polymerase (Applied Biosystems) in a 15-μl reaction containing 1x PCR buffer II (Applied Biosystems), 1.5 mM MgCl₂ (Applied Biosystems), 0.2 mM dNTPs (Promega, Madison, WI, USA) and 0.8 μM of each sequence-specific or CCR5 forward and reverse primer. The amplification conditions for the SSP sets (SSP1, SSP2 or SSP3) were: 95°C for 5 minutes, followed by 30 cycles of 94°C for 20 s, 72°C for 20 s and 72°C for 20 s, with a final extension at 72°C for 7 minutes, using a GeneAmp 9700 Thermal Cycler (Applied Biosystems). Amplification of CCR5 DNA was performed as above but with an annealing temperature of 60°C. A no-template reaction and an irrelevant Mane-B*10 clone (GenBank: AY557355) served as negative controls for all primer sets. Cloned Mane-A*10 (GenBank: AY557348) and Mane-A*16 (GenBank: AY557354) were used as positive controls. Amplicons (5 μl) were visualized on 1.5% TBE agarose gels and sized against pGEM DNA markers (Promega).
200 μl whole blood stained with a 1:400 dilution of tetramer for 45 minutes at room temperature in the dark. Counter-staining with anti-CD3-FITC (BD, clone SP34), anti-CD4-PerCP (BD, clone L200) and anti-CD8-APC (BD, clone SK1) was then conducted at room temperature in the dark for 45 minutes. Erythrocytes were lysed with 2 ml 1x FACS Lysing Solution (BD) for 10 minutes at room temperature incubation and washed twice in FACS buffer. Acquisition and analysis were performed as described above.

SIV infection and analysis of viral escape

Eight naïve pigtail macaques were infected intravenously with SIVmac251 as previously described [43]. Intracellular IFN-γ secretion was assessed by flow cytometry using 200 μl whole blood incubated with overlapping SIVmac239 Gag 15-mer peptides (kindly supplied by the National Institutes of Health AIDS Research and Reference Reagent Program) at 1 μg/ml/peptide, using protocols described elsewhere [19, 43]. SHIV RNA was extracted from plasma at each timepoint, and cDNA generated as previously described [19]. The Gag region containing the KP9 epitope was then amplified using SIV Gag-specific primers 4 and 5 and Phusion DNA polymerase (Finnzymes) using conditions as previously outlined [19]. The amplicons were then cloned into the pGEM-T Easy vector (Promega), transformed into competent JM109 E. coli (Promega), and individual clones sequenced using the BigDye Terminator v3.1 chemistry (Applied Biosystems).

Results

Mane-A*10 typing of pigtail macaques by RSCA

The RSCA is a sensitive method to characterize multiple MHC class I alleles. The technique involves heteroduplexing known labelled rhesus macaque reference alleles with unknown pigtail macaque cDNA. We have previously described the use of RSCA to MHC class I genotype pigtail macaques through the use of rhesus macaque fluorescent reference strands and an AlFExpress DNA Analyser [43]. While this technique is sensitive and useful for detecting multiple unknown MHC class I alleles in pigtail macaques, it is limited by the fact that only one reference strand can be used in each lane, and that the size standard has to be run in a separate lane from the samples. Using three separate reference strands per animal, and leaving lanes for the external size standards, throughput is limited to 12 animals per gel. This technique has recently been adapted to the ABI PRISM 377 DNA Sequencer, which enables four separate fluorescent traces to be analysed per lane (K. Krebs, Z. Jin, R. Rudersdorf, A. Hughes, D.H. O’Connor, submitted for publication). This significantly increases the throughput potential of the RSCA technique, as samples need to be run with multiple fluorescent reference strands in order to eliminate false positives. With 96 lanes available, up to 96 animals can be analysed in a single gel.

We analysed amplified cDNA from a total of 79 pigtail macaques by RSCA. Of these, 13 were definitively positive for Mane-A*10, with peaks corresponding to the Mane-A*10 clones with all three rhesus macaque MHC class I reference strands tested. Traces from two of these Mane-A*10+ animals are shown in Fig. 1 in comparison with a cloned Mane-A*10 allele. Both animals were recently vaccinated against SIV Gag and confirmed to present the KP9 epitope (data not shown). In addition, both have been shown to be Mane-A*10+ by PCR-SSP (Fig. 3 and data not shown).

![Fig. 1. Identification of Mane-A*10 expression by reference strand-mediated conformational analysis.](image-url)

Raw trace data from a Mane-A*10 clone and cDNA from macaques 6370 and 6276 are displayed. Each sample has been heteroduplexed to three separate reference strands, as indicated on the right-hand side of each trace. Mane-A*10 mobility with the Mamu-A*15-FAM reference strand is indicated with the blue box, Mamu-A*20-NED indicated in black, and the Mamu-B*60-HEX in green. Arrows below the traces indicate the presence of a peak in each respective trace that corresponds to the Mane-A*10 clone.
PCR with Mane-A*10 SSP

A PCR-based assay for the detection of the Mane-A*10 allele will be a valuable tool for the bulk screening of macaques, being quicker and more efficient than the current methods of cloning and sequencing, and less resource intensive than RSCA. We designed three primer sets with specificity for regions across the Mane-A*10 sequence encoding the polymorphic α1 and α2 domains (Fig. 2). The primers were designed to have high melting temperatures (>70°C) to increase specificity, mismatches with non-Mane-A*10 alleles towards the 3′ end, and to amplify short (100–200 bp) fragments. All primers, except the set 2 forward primer, are specific for both Mane-A*10 and the closely related allele, Mane-A*16. The Mane-A*16 sequence differs from Mane-A*10 by only two nucleotides within the regions studied [43]. Both mismatches between Mane-A*10 and Mane-A*16 are contained within the region covered by the set 2 forward primer, the only primer not specific for Mane-A*16 (Fig. 2). The set 1 forward primer is the least specific, detecting Mane-A*10, Mane-A*16, Mane-A*08 and Mane-A*14, though the reverse primer is specific only for Mane-A*10 and Mane-A*16 (Fig. 2).

All three primer sets were able to detect Mane-A*10 in four animals shown to be Mane-A*10+ by RSCA (Fig. 3). None of the three primer sets detected any product in four Mane-A*10− animals. Interestingly, there was no detectable difference between the three primer sets when used on cDNA from Mane-A*10− animals, suggesting that the
Mane-A*10 identification, tetramer development and viral escape

**Fig. 3.** Validation of specificity of sequence-specific primers. cDNA from four Mane-A*10+ and four Mane-A*10− macaques was amplified using each of the three PCR-SSP primer sets. As negative controls, a no-template sample (NT) plus a clone of allele Mane-B*10 were also amplified, with Mane-A*10 and Mane-A*16 clones acting as positive controls. pGEM markers are shown flanking the samples on either side. Amplicons from PCR-SSP primer set 1 (161 bp), primer set 2 (131 bp) and primer set 3 (173 bp) are shown in panels A–C respectively. Panel D shows amplification of a 239-bp fragment of CCR5, demonstrating that the cDNA from all eight cDNA samples was intact. The Mane allele clones do not contain the CCR5 sequence.

Relative specificity of the three sets for Mane-A*10 is equivalent. When tested on cloned MHC alleles, none of the three primer sets detected Mane-B*10, and all three sets detected both the Mane-A*10 and Mane-A*16 clones. Unexpectedly, even primer set 2, which had 3’ mismatches for Mane-A*16 in the reverse primer, was able to detect the Mane-A*16 clone DNA, though the band appears to be weaker than that of the Mane-A*10 clone.

Mane-A*10/KP9 tetramer analysis of PBMC and whole blood

The development of a Mane-A*10 tetramer, complexed with the immunodominant SIV Gag epitope, KP9, facilitates the analysis of KP9-specific CD8+ T cells in vaccinated and SIV-infected pigtail macaques. The Mane-A*10 tetramer was constructed using standard E. coli expression systems and conjugated with PE. To validate the tetramer, we first analysed a frozen PBMC sample from an animal, 4664, from a timepoint where a large response to KP9 had been detected by ICS for IFN-γ [14, 19, 43]. A large tetramer-positive population was detected in this sample, accounting for 8.88% of all CD8+ T lymphocytes (Fig. 4A), a similar magnitude to that detected by ICS on fresh blood (9.8%) [19, 43].

Most tetramer analysis described in the literature is conducted on PBMC [9, 31, 37, 42], but the preparation of PBMC requires considerable cell handling and results in significant cell loss. We therefore adopted whole blood protocols we used for intracellular IFN-γ staining [14] for tetramer analysis. These protocols are simpler and facilitate the analysis of rare populations such as tetramer-positive populations on small volumes of blood as there is no significant cell loss.

Initial experiments were carried out on macaques receiving DNA only or DNA/fowlpoxvirus vaccinations against SIV Gag [14]. We have previously shown that although these DNA vaccines prime for boosting with viral vectors such as fowlpoxvirus, the level of immunity after DNA vaccination alone is difficult to detect using standard IFN-γ ICS or ELISpot (enzyme-linked immunosorbent spot-forming cell assay) techniques [14, 13, 16, 25]. Tetramer analysis was used to investigate the level of KP9-specific CD8+ T lymphocytes in Mane-A*10+ vaccinees after DNA-only or DNA/fowlpoxvirus vaccination. The background level of tetramer-positive cells in an unvaccinated Mane-A*10+ animal was negligible, demonstrated by staining 200 μl of whole blood from animal 6167 (Fig. 4B, left panel). This animal was
vaccinated with an empty plasmid construct, and has clearly not elicited a KP9-specific response. Animal 5614 had been DNA-vaccinated against SIV Gag three times at 4-week intervals and was analysed with the Mane-A*10/KP9 tetramer 1.5 weeks after the last vaccine. Tetramer staining shows a small but clearly discernable tetramer-positive population at this time, accounting for 0.15% of CD8+ T lymphocytes (Fig. 4B, middle panel). Another Mane-A*10+ animal, macaque 5396, was vaccinated at 4-week intervals: twice with SIV Gag-expressing DNA, then boosted once with a fowlpoxvirus expressing SIV Gag. Blood from this animal was also analysed 1.5 weeks after the last vaccine, and displayed a larger and more distinct tetramer-positive population (1.95% of CD8+ T lymphocytes; Fig. 4B, right panel) compared with that detected in the DNA-only vaccinated animal.

Immune escape at KP9 following SIVmac251 infection: importance of viral load

We have previously reported that a response to KP9 correlates with a lower viral load during acute SIV infection [43], and separately, that immune escape occurs at the KP9 epitope in SHIV-infected animals, resulting in a significant fitness cost to the virus [19]. The mechanism mediating SIV control in Mane-A*10+ animals is not clear. Do Mane-A*10+ animals produce a CD8+ T cell response that effectively dampens viral replication, or does the fitness cost to the virus caused by the escape mutation result in deficient replication? To further probe the relationship between KP9-specific CD8+ T cell responses and control of SIV replication, we followed eight pigtail macaques infected with SIVmac251, including five Mane-A*10+ animals and three Mane-A*10+ animals (Table 1). These macaques were typed for Mane-A*10 primarily by RSCA as described above, and following SIVmac251 infection, we studied their CD8+ T cell responses to SIV Gag (by intracellular staining for IFN-γ) and to the KP9 epitope (by Mane-A*10 tetramer, as above). The proportion of viral clones bearing wild-type or mutant sequence at the KP9 epitope was determined by cloning and sequencing of serial viral RNA samples derived from plasma.

The viral loads of the Mane-A*10+ animals were significantly lower than those of the Mane-A*10+ animals (P < 0.05 [43]), and correlate with a higher retention of CD4+ T cells, even at later timepoints (e.g. week 21 post-infection) (Table 1). All three
Mane-A*10+ animals displayed vigorous responses to the KP9 epitope when measured by ICS (data not shown), with even greater responses detected using the Mane-A*10/KP9 tetramer (Table 1). The Mane-A*10− animals showed negligible Gag responses, and no KP9-specific responses, emphasizing the immunodominance of the KP9 epitope within Gag.

The study of viral sequence at the KP9 epitope shows that the wild-type KP9 sequence is maintained in all of the Mane-A*10− animals at both early (week 8) and late (week 17/21) timepoints following SIVmac251 infection (Table 1). In two of three Mane-A*10+ animals, viral escape was detected at week 8 following infection, and by week 21, all three Mane-A*10+ animals displayed mutant sequence at the KP9 epitope. The predominant escape mutant detected was the previously described K165R mutation [19]. Macaque 5424, the Mane-A*10+ animal that did not display any viral escape at week 8, also had the lowest viral load of all the animals.

**Discussion**

Pigtail macaques have emerged as an important alternate non-human primate model for the study of HIV vaccines. The MHC class I allele, Mane-A*10, is common in outbred pigtail macaques and restricts an immunodominant response to the SIV Gag epitope, KP9. Importantly, the presence of Mane-A*10, and a response to the KP9 epitope, confers a significantly lower viral load during acute SIVmac251 infection [43]. Furthermore, rapid immune escape has been shown to occur during primary infection at the KP9 epitope following both SHIV infection [19] and SIV infection, emphasizing the efficacy of KP9-specific CD8+ T cells. This data now provides a valuable framework for the study of immunodominance, CD8+ T cell dynamics and phenotypes, and viral escape. Mane-A*10− macaques infected with virus containing mutant sequence at the KP9 epitope rapidly revert to wild-type sequence [19]. This reversion to wild-type sequence implies that the escape mutation comes at a fitness cost to the virus, as it is only maintained in the presence of a KP9-specific immune response. This paper further describes methods for the detection of Mane-A*10 and its associated cellular immune responses in outbred pigtail macaques, including a novel sequence-specific PCR assay, and the development and validation of a Mane-A*10/KP9 tetramer, first developed for the pigtail macaque model of AIDS. We also explored the relationship between SIV viral load and the occurrence of immune escape at the KP9 epitope.

The RSCA represents a powerful and accurate technique for MHC class I typing pigtail macaques. The RSCA method using the ABI 377 DNA Sequencer has already been used to identify shared MHC class I alleles from cynomolgus macaques (K. Krebs, Z. Jin, R. Rudersdorf, A. Hughes, D.H. O’Connor, submitted for publication) and to select pigtail macaques for vaccine trial groups, with equal numbers of Mane-A*10+ animals assigned to
each group. The current RSCA method is still, however, limited by the lack of an appropriate size standard and differences in gel-to-gel sample mobilities, leading to some ambiguities that have had to be resolved through cloning and sequencing. The further development of appropriate size standards is a high priority and will be necessary to ensure that accurate mobility tracking and minimization of lane-to-lane variability can be achieved.

In contrast to RSCA, PCR-SSP assay is a comparatively straightforward and simple method for the detection of \( \text{Mane-A}^*10 \). The PCR-SSP assay will be very useful as a method for bulk screening of macaques for \( \text{Mane-A}^*10 \), as it is much less resource intensive than RSCA, making it suitable for use in macaque breeding colonies both in developed and in less developed countries. The PCR-SSP assay requires further validation to ensure its accuracy, which will involve studying much larger numbers of pigtail macaques with known genotypes, plus cross-referencing with techniques such as RSCA and MHC class I cloning and sequencing. All three of the PCR-SSP primer sets described here detect \( \text{Mane-A}^*10 \) as well as the very closely related allele, \( \text{Mane-A}^*16 \). This is the case even for primer set 2, which has a forward primer with a 3’ mismatch for \( \text{Mane-A}^*16 \). \( \text{Mane-A}^*16 \) was originally characterized in a macaque which responded to KP9, but did not have the \( \text{Mane-A}^*10 \) allele, suggesting that \( \text{Mane-A}^*16 \) may also present the KP9 epitope [43]. The Mane-A*16 protein has since been shown to re-fold around the KP9 epitope during tetramer production, which provides further evidence that \( \text{Mane-A}^*16 \) can elicit a KP9-specific response (data not shown). It is therefore of interest to know whether animals carry the \( \text{Mane-A}^*16 \) allele, although the impact of the more rare \( \text{Mane-A}^*16 \) allele on the outcome of SIV infection is not yet known. The specificity of the PCR-SSP assay for \( \text{Mane-A}^*10 \) could potentially be increased through the adaptation of the assay to real-time PCR, involving the incorporation of a fluorescent probe that binds to a third \( \text{Mane-A}^*10 \)-specific sequence within the amplified fragment. Taken together, the PCR-SSP and RSCA techniques for MHC class I typing provide important advances for the pigtail macaque model of human AIDS.

The development of a functional Mane-A*10/KP9 tetramer is another significant development for the use of pigtail macaques as an animal model for AIDS research. Highly sensitive, tetramer analysis can detect and quantify very small numbers of antigen-specific CD8\(^+\) T cells [3, 31]. Apart from enabling more sensitive and accurate quantification, tetramer analysis also facilitates the phenotyping of antigen-specific CD8\(^+\) T cells without requiring \textit{in vitro} stimulation. Tetramer analysis will also be very useful for the measurement of mucosal immune responses, which are typically difficult to measure in pigtail macaques with standard IFN-\(\gamma\)-based T cell assays due to high levels of background activation [24]. Furthermore, tetramer-positive CD8\(^+\) T cell populations can be sorted by flow cytometry for further analysis, including phenotyping, study of gene expression patterns by microarray and the induction of cellular regulatory and transcriptional networks [8, 17, 23]. We recently showed that DNA vaccination alone primes for protection against SHIV challenge almost as effectively as a DNA/fowlpoxvirus regimen, despite negligible immunogenicity post-vaccination as assessed by standard assays such as ELISpot and ICS [14]. The Mane-A*10/KP9 tetramer now provides a powerful tool for isolating the very small antigen-specific populations elicited by DNA vaccination in order to phenotype them and dissect what characteristics may contribute to their efficacy following viral challenge.

Viral escape occurs during acute and chronic phases of HIV and SIV infection [6, 7, 10, 22, 38, 40, 41], presenting a formidable challenge to the development of an effective HIV vaccine. While mutation at dominant CD8\(^+\) T cell epitopes can undermine immune control of viral replication, it is becoming apparent that escape mutations can carry significant fitness costs to the virus, as indicated by the reversion to wild-type sequence that occurs in the absence of immune pressure [19, 21, 29, 39]. We show here that viral escape occurs at the KP9 epitope in SIV\(\text{mac251}\)-infected Mane-A*10\(^*\) macaques. Interestingly, escape at KP9 occurred at different rates in the animals studied, and in all cases did not occur completely. In two of three animals (macaques 5284 and 5715), escape was detected by week 8 post-infection and in the third (macaque 5424), escape was not detected until later. In all three animals, the K165R escape variant accounted for only 80% of viral clones sequenced at week 21, with the less common P172S mutation persisting in 20% of clones from animal 5284. These results contrast with the more rapid and complete escape observed at the KP9 epitope in animals challenged with SHIV\(\text{SF162P3}\) [19]. Macaque 5424 had a lower viral load than the other two Mane-A*10\(^*\) macaques, suggesting that the CD8\(^+\) T cell response may have constrained viral replication sufficiently to prevent the emergence of the escape variant until later in infection. KP9-specific T cells constrain viral replication by
acting on cells infected with wild-type virus, resulting in variable rates of escape at the KP9 epitope. Combined with the rapid reversion of KP9 escape variants seen in Mane-A*107 animals [19], this provides a defined scenario in which to dissect the influence of T cell immunity on viral load, and on the rate and extent of viral escape in pigtail macaques.

In summary, this paper describes the MHC class I typing of pigtail macaques, an increasingly common animal model for HIV infection, the development of the first MHC class I tetramer for use in this model and an investigation of the effect of immune escape on SIV viral loads. Together, these developments represent a considerable advance for the use of pigtail macaques in HIV research, providing the opportunity to undertake detailed study of CD8+ T cells directed towards the immunodominant KP9 epitope. MHC class I typing for Mane-A*10 will also enable the selection of animals for future vaccine trials, facilitating a more robust comparison of vaccine regimens and better understanding of CD8+ T cell immunity to SIV and SHIV.

References


