Poster #363

Linking pig-tailed macaque major histocompatibility complex class I haplotypes and cytotoxic T lymphocyte escape mutations in SIV infection

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Introduction

- The influence of MHC class I alleles and CTL immunity in HIV infection have been well characterised in humans at the population level (1).
- Simian immunodeficiency virus (SIV) infection progression in macaques, resembles HIV infection progression in humans.
- Therefore, macaques are a valuable resource to study immunity to HIV infection and in devising candidate vaccines.
- Indian rhesus macaques *(Macaca mulatta*) infected with SIV is the most well studied non-human primate model of HIV infection.
- However, our macaque model is the pig-tailed macaque *(Macaca nemestrina*) due to the export ban placed in 1978 restricting the availability of rhesus macaques to most researchers, but it has a similar infection progression as rhesus macaques.
- In pig-tailed macaques, the immune pressure created by CTLs result in the emergence of 3 known escape mutations in the virus: KP9 (K165R) in Gag, and KSA10 (S95P) and KVA10 (K116E) in Tat which are all associated with the Mane-A1*084 allele (2).
- Some escape mutations arise with a fitness cost, such as the Gag KP9, but compensatory mutations such as V145A may negate this effect (3).
- Many other escape mutations, however, are likely to be driven by other MHC class I haplotypes and are yet to be characterised.

Aims

1.Characterise the MHC class I haplotypes expressed by our pig-tailed macaque colony

2.Study the evolution of SIV in a large cohort of pig-tailed macaques

3.Define the MHC class I haplotypes that contribute towards CTL escape and infection progression

Materials and methods

Animals: 44 naïve pig-tailed macaques were challenged with SIV_{mac251}. The infection progression was monitored by employing viral load assays and peripheral blood CD4+ T cell counts from week 0 to week 23/64 (sampling mean = approximately 15 weeks post challenge).

Samples: Cellular RNA and viral RNA were extracted from frozen macaque PBMCs and infected macaque plasma samples collected in EDTA vacuettes respectively.

Whole genome amplification: The whole viral genome was amplified from viral RNA by RT-PCR in four over-lapping fragments. The stock virus was also sequenced as a control. Fragments from a single genome were pooled and libraries were made for the individual macaques using the Illumina Nextera XT dual indexing kit. Libraries were sequenced by employing the Illumina MiSeq system.

MHC class I typing: RT-PCRs were used to amplify MHC class I alleles from cellular RNA and macaques were genotyped using 454 Roche pyrosequencing in a Junior run 430.

Data analysis: MHC class I haplotyping data was analyzed as described previously by the Wisconsin Primate Research Centre (4). Viral sequencing data was analyzed using Geneious 7.0.2. Briefly, sequencing data from a single sample was paired, trimmed, aligned to the reference sequence built from the stock sequence reads and single nucleotide polymorphisms (SNPs) were called at a cut-off point of 5%. SNPs were associated with macaque haplotypes using specifically designed in-house pipelines.

ICS assays: To validate the above approach, 3 pig-tailed macaques with different Mane-A and –B haplotypes were challenged with SIV_{mac239}. Whole blood was stimulated with 15-mer overlapping peptides that span a SNP associated with a Mane haplotype and peptide responses were measured by intracellular cytokine staining assays (ICS) as previously described (5).

Results

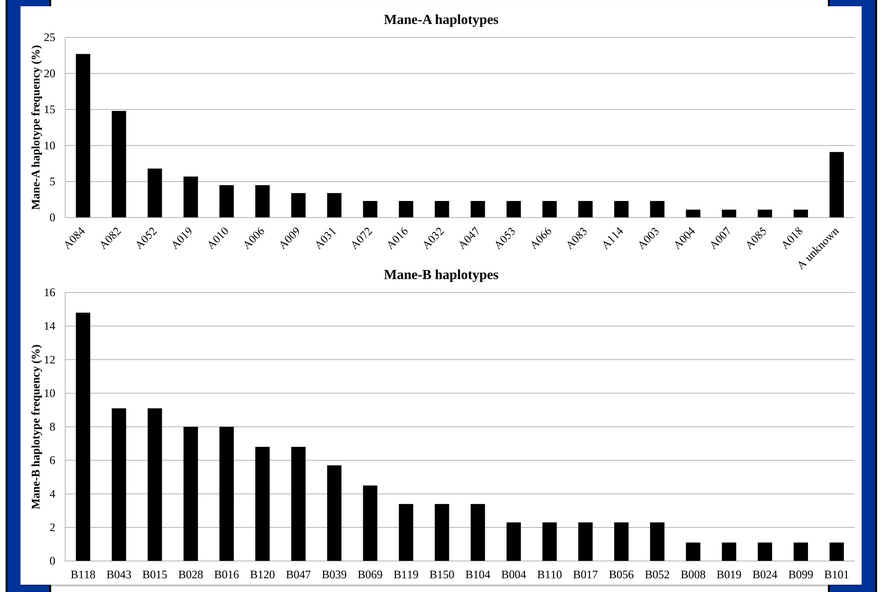


Figure 1. MHC class I genotyping data of the 44 pig-tailed macaques infected with SIV_{mac251}.

Table 1. Validation of the study approach to link pig-tailed macaque MHC class I haplotypes with CTL escape mutations by using previously defined CTL responses restricted by the Mane-A1*084 allele.

Protein	Epitope	Region of epitope within protein	No. of Mane-positive mutation-positive animals/total no. of Mane-positive animals	No. of Mane-negative mutation-positive animals/total no. of Mane-negative animals	*P* value (two-tailed)	Amino acid change	Escape motif[a]
Gag	KP9	164–172	11/16	0/28	5.7E−07	K → R	KRFGAEVVP
Tat	KSA10	87–96	6/16	0/28	1.1E−03	A → T	KKAKTNTSSA
	KSA10	87–96	6/16	0/28	1.1E−03	S → T or S → P	KKAKANTSTA or KKAKANTSPA
	KSA10	87–96	5/16	0/28	4.0E−03	A → T	KKTKANTSSA
Tat	KVA10	114–123	9/16	0/28	1.6E−05	K → E	EKETVEKAVA
	KVA10	114–123	7/16	0/28	3.0E−04	K → E	KEETVEKAVA

[a] The mutated amino acids are in boldface and underlined.

Table 2. Novel SIV_{mac251} SNPs linked to MHC class I haplotypes in pig-tailed macaques.

Haplotype	Protein	Nucleotide position	No. of Mane-positive mutation-positive animals/total no of Mane-positive animals	No. of Mane-negative mutation-positive animals/Total no. of Mane-animals	*P* value[a] (two tailed)	Amino acid change(s)	Wild-type motif[b]
Mane-A*010	Gag	2179	2/4	0/40	0.0064	P → S	EALKEALAPVPIPFAAAQQRG
	Pol	4405	2/4	0/40	0.0064	N → S	CPTESESRLVNQIIEEMIKKS
	Pol	4408	2/4	0/40	0.0064	Q → R	PTESESRLVNQIIEEMIKKSE
	Env	7483	2/4	0/40	0.0064	D → E	NRTYIYWHGRDNRTIISLNKY
	Env	7493	2/4	0/40	0.0064	I → V	IYWHGRDNRTIISLNKYYNLT
	Env	8019	2/4	0/40	0.0064	T → I	VTSLIANIDWTDGNQTNITMS
Mane-A*032	Gag	2179	2/2	0/42	0.0011	P → S	EALKEALAPVPIPFAAAQQRG
	Pol	4405	2/2	0/42	0.0011	N → S	CPTESESRLVNQIIEEMIKKS
	Pol	4408	2/2	0/42	0.0011	Q → R	PTESESRLVNQIIEEMIKKSE
	Env	7483	2/2	0/42	0.0011	D → E	NRTYIYWHGRDNRTIISLNKY
	Env	7493	2/2	0/42	0.0011	I → V	IYWHGRDNRTIISLNKYYNLT
	Vif	5863	2/2	0/42	0.0064	N → D	NPTWKQWRRDNRRGLRMAKQN
Mane-A*053	Nef	9676	2/2	0/42	0.0011	S → Y, S → F	RHYLMQPAQTSKWDDPWGEVL
Mane-A*082	Env	7001	5/13	0/31	0.0012	T → A, T → S, T → P	LTKSSTTTAPTTTTAASKIDM
	Env	7033	8/13	5/31	0.0087	M → I	TTTTAASKIDMVNETSSCITH
Mane-A*114	Gag	2477	2/2	0/42	0.0011	Q → R	PAVDLLKNYMQLGKQQREKQR
Mane-B004	Vif	5935	2/2	1/41	0.0032	K → E	DKQRGGKPPTKGANFPGLAKV
	Env	6996	2/2	1/41	0.0032	A → V	WGLTKSSTTTAPTTTTAASKI
	Env	7017	2/2	2/42	0.0064	A → G A → V	TTTAPTTTTAASKIDMVNETS
Mane-B015	Env	9236	4/8	1/36	0.0024	L → V	RIRQGLELTLL
	Env	7280	3/8	0/36	0.0042	T → A	QESCDKHYWDTIRFRYCAPPG
Mane-B016	Gag	1360	3/6	0/38	0.0015	I → V	KVKHTEEAKQIVQRHLVVETG
	Env	6789	3/6	0/38	0.0015	G → D	WGTTQCLPDNGDYSELALNVT
Mane-B017	Nef	9568	2/2	0/42	0.0011	I → T	DWQDYTSGPGIRYPKTFGWLW
Mane-B028	Env	7049	3/7	0/37	0.0027	S → P	SKIDMVNETSSCITHDNCTGL
	Env	9105	4/7	3/37	0.0075	Q → R	YGWSYFQEAVQAGWRSATETL
	Nef	9105	4/7	3/37	0.0075	K → E	MGGAISRRRSKPAGDLRQRLL
Mane-B039	Env	7027	3/5	2/39	0.0070	I → M	APTTTTAASKIDMVNETSSCI

Table 2 continued.

Haplotype	Protein	Nucleotide position	No. of Mane-positive mutation-positive animals/total no of Mane-positive animals	No. of Mane-negative mutation-positive animals/Total no. of Mane-animals	*P* value[a] (two tailed)	Amino acid change(s)	Wild-type motif[b]
Mane-B043	Vif	5917	3/8	0/36	0.0043	G → S	KQNSRGDKQRGGKPPTKGANF
	Nef	9402	5/8	5/36	0.0091	T → A	VPVMPRVPLRTMSYKLAIDMS
Mane-B056	Pol	4618	2/2	1/42	0.0032	R → K	KELVFKFGLPRIVARQIVDTC
Mane-B069	Gag	1880	2/4	0/40	0.0064	R → K	WIQLGLQKCVRMYNPTNILDV
	Tat	8808	2/4	0/40	0.0064	I → V, I → L	KPISNRTRHCQPE
	Env	8804	2/4	0/40	0.0064	T → A	FSSPPSYFQQTHIQQDPALPT
	Env	8808	2/4	0/40	0.0064	H → R, H → P	SSPPSYFQQTHIQQDPALPTR
Mane-B104	Tat	6408	2/3	0/41	0.0032	G → R	DATTPESANLGEEILSQLYRP
	Env	8019	2/3	0/41	0.0032	T → I	VTSLIANIDWTDGNQTNITMS
Mane-B118	Env	8981	4/13	0/31	0.0053	G →A	FSNCRTLLSRAYQILQPILQR
Mane-B119	Pol	2538	2/3	0/41	0.0032	D → N	RKQREALQGGDRGFAAPQFSL
	Env	9089	2/3	0/41	0.0032	F → V	LTYLQYGWSYFQEAVQAGWRS
	Env	9093	2/3	0/41	0.0032	Q → R	TYLQYGWSYFQEAVQAGWRSA
	Nef	9089	2/3	0/41	0.0032	I → M	MGGAISRRRSKPAGD
	Nef	9093	2/3	0/41	0.0032	R → G	MGGAISRRRSKPAGDLR
	Tat	6364	2/3	1/41	0.0094	C → Y	SLESSNERSSCISEADATTPE
	Env	7931	2/3	1/41	0.0094	V → I	IRQHNTWHKVGKNVYLPPRE
Mane-B150	Vpr	6055	2/3	0/41	0.0032	M → I	YLCLIQKALFMHCKKGCRCLG

[a] *P* values are uncorrected (Fisher's exact test).
[b] The mutated amino acids are in boldface.

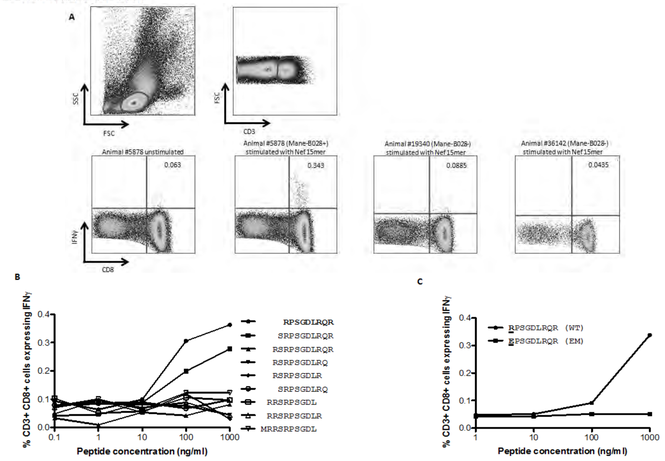


Figure 2. SIV Nef-specific CD8+ T cell response predicted by linkage analysis. (A) Whole blood from 3 SIV-infected macaques was stimulated by 3 SIV Nef 15-mer peptides spanning the site of a putative Mane-B028-restricted CTL response identified by the MHC-I–SIV mutation linkage analysis. Gated CD3+ CD8+ lymphocytes were studied for IFNγ expression, with the 1 Mane-B028+ macaque showing a positive response and 2 Mane-B028- macaques showing no response. SSC, side scatter; FSC, forward scatter. (B) The minimal Nef-specific CTL epitope was mapped to a 9-mer peptide using serial dilutions of overlapping 9- to 11-mer peptides. (C) The putative CTL escape mutation (R to E at position 1) identified in the linkage analysis was evaluated for whether it abrogated CTL recognition.

Conclusions

- Mane-A1*084 occurs at a high frequency in this population of pig-tailed macaques from Indonesia and Australia.
- Next-generation data from Illumina MiSeq sequencing is capable of demonstrating SIV CTL escape mutations that occur in the viral genome of infected pig-tailed macaques.
- There are novel potential CTL escape mutations that occur in macaques with certain Mane-A alleles and Mane-B haplotypes.
- A larger cohort will be more beneficial for studying low frequency haplotypes.
- It is important to define pig-tailed macaque genetics that contribute to SIV progression, in order to perform insightful CTL vaccine studies.

Literature cited

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