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Impact of Viral Dose and Major Histocompatibility Complex Class IB Haplotype on Viral Outcome in Mauritian Cynomolgus Monkeys Vaccinated with Tat upon Challenge with Simian/Human Immunodeficiency Virus SHIV89.6P▽†

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The effects of the challenge dose and major histocompatibility complex (MHC) class IB alleles were analyzed in 112 Mauritian cynomolgus monkeys vaccinated ($n = 67$) or not vaccinated ($n = 45$) with Tat and challenged with simian/human immunodeficiency virus (SHIV) 89.6P$_{cy243}$. In the controls, the challenge dose (10 to 20 50% monkey infectious doses [MID$_{50}$]) or MHC did not affect susceptibility to infection, peak viral load, or acute CD4 T-cell loss, whereas in the chronic phase of infection, the H1 haplotype correlated with a high viral load ($P = 0.0280$) and CD4 loss ($P = 0.0343$). Vaccination reduced the rate of infection acquisition at 10 MID$_{50}$ ($P < 0.0001$), and contained acute CD4 loss at 15 MID$_{50}$ ($P = 0.0099$). Haplotypes H2 and H6 were correlated with increased susceptibility ($P = 0.0199$) and resistance ($P = 0.0087$) to infection, respectively. Vaccination also contained CD4 depletion ($P = 0.0391$) during chronic infection, independently of the challenge dose or haplotype.

Advances in typing of the major histocompatibility complex (MHC) of Mauritian cynomolgus macaques (14, 20, 26) have provided the opportunity to address the influence of host factors on vaccine studies (13). Retrospective analysis of 22 macaques vaccinated with Tat or a Tat-expressing adenoviral vector revealed that monkeys with the H6 or H3 MHC class IB haplotype were overrepresented among aviremic or controller animals, whereas macaques with the H2 or H5 haplotype clustered in the noncontrollers (12). More recently, the H6 haplotype was reported to correlate with control of chronic infection with simian immunodeficiency virus (SIV) mac251, regardless of vaccination (18).

Here, we performed a retrospective analysis of 112 Mauritian cynomolgus macaques, which included the 22 animals studied previously (12), to evaluate the impact of the challenge dose and class IB haplotype on the acquisition and severity of simian/human immunodeficiency virus (SHIV) 89.6P$_{cy243}$ infection in 45 control monkeys and 67 monkeys vaccinated with Tat from different protocols (Table 1).

Study description. All animals were challenged intravenously with 10 ($n = 29$), 15 ($n = 68$), or 20 ($n = 15$) monkey infectious doses (MID$_{50}$) of SHIV86.6P$_{cy243}$ derived from a cynomolgus macaque inoculated with the original SHIV89.6P stock from rhesus monkeys (3, 5) (Table 1). The SIV RNA load in plasma and CD4 T-cell counts were determined as described previously (23).

MHC allele predictions were generated on the basis of the microsatellite profiles (26), with the alleles for class I and class II regions being inferred on the basis of established haplotype-allele associations (20, 26). As shown in Fig. 1A, the distribution of the seven major haplotypes (haplotypes H1 to H7) in vaccinated and control animals was balanced. Due to the presence of the H7 haplotype in only 3 out of 112 monkeys, it was excluded from the analyses.

As the factors investigated in this study may affect susceptibility to acute (1 to 4 weeks) or chronic (16 to 52 weeks) infection differently, these were analyzed separately. Since the plasma viral load is a good indicator of infection dynamics, monkeys were stratified into the aviremic group (<50 RNA eq/ml) or viremic group (>50 RNA eq/ml). During the chronic phase, the macaques were stratified as controllers (viremia of $< 5 \times 10^3$ RNA eq/ml and at least two determinations in which viral RNA was undetectable [<50 RNA eq/ml]) or viremic (persistent viremia, $> 5 \times 10^3$ RNA eq/ml). In chronic infection, the viral load set point was calculated as the mean of all values between the 16th and 52nd weeks, expressed as $\log_{10}$ number of copies/ml; a log value of $\log_{10}$ (50)/2 was assigned to viral loads below the cutoff (50 RNA eq/ml).

All 112 monkeys were evaluated for their susceptibility to

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TABLE 1. Summary of treatment, challenge dose, and outcome of infection in cynomolgus monkeys

Protocol code	No. of monkeys	Immunogen (dose)[a]	Adjuvant[b]	Schedule of immunization (wk)	Route[c]	Challenge[d] (MID_{50})	Virological outcome[e]			Reference(s) or source
							A	C	V	
ISS-ST	6	Tat (10)	Alum or RIBI	0, 2, 6, 12, 15, 21, 28, 32, 36	s.c., i.m.	10	4	1	1	4, 17
ISS-ST	1	Tat (6)	None	0, 5, 12, 17, 22, 27, 32, 38, 42, 48	i.d.	10	1	0	0	4, 17
ISS-PCV	3	pCV-tat (1 mg)	Bupivacaine + methylparaben	0, 2, 6, 11, 15, 21, 28, 32, 36	i.m.	10	3	0	0	6
ISS-ID	3	Tat (6)	none	0, 4, 8, 12, 16, 20, 24, 28, 39, 43, 60	i.d.	10	1	1	1	B. Ensoli, unpublished data
ISS-TR	6	Tat (10)	Alum-Iscom	0, 2, 6, 11, 16, 21, 28, 32, 36	s.c., i.d., i.m.	10	4	2	0	Ensoli, unpublished
ISS-TG[f]	3	Tat (10)	Alum	0, 4, 12, 22	s.c.	15	0		3	Ensoli, unpublished
ISS-TG	3	Tatcys22 (10)	Alum			15	0		3	Ensoli, unpublished
ISS-TG	4	Tatcys22 (10) + Gag (60)	Alum			15	0		4	Ensoli, unpublished
ISS-TG	4	Tat (10) + Gag (60)	Alum			15	0		4	Ensoli, unpublished
ISS-MP	3	Tat (10)	H1D-Alum	0, 4, 12, 18, 21, 38	s.c., i.m.	15	0	2	1	Ensoli, unpublished
ISS-MP	3	Tat (10)	Alum		s.c.	15	0	0	3	Ensoli, unpublished
ISS-GS	6	Tat (10)	H1D-Alum	0, 4, 12, 18, 21, 36	s.c., i.m.	15	1	3	2	Ensoli, unpublished
NCI-Ad-tat/Tat	7	Ad-tat (5×10^8 PFU), Tat (10)	Alum	0, 12, 24, 36	i.n., i.t., s.c.	15	2	3	2	Ensoli, unpublished
NCI-Tat	9	Tat (6 and 10)	Alum/Iscom	0, 2, 6, 11, 15, 21, 28, 32, 36	s.c., i.d., i.m.	15	2	4	3	12
ISS-NPT	3	pCV-tat (1 mg)	Bupivacaine + methylparaben-Iscom	0, 2, 8, 13, 17, 22, 28, 46, 71	i.m.	20	0	0	3	Ensoli, unpublished
ISS-NPT	3	pCV-tatcys22 (1 mg)	Bupivacaine + methylparaben-Iscom	0, 2, 8, 13, 17, 22, 28, 46, 71	i.m.	20	1	1	1	
Total vaccinated	67						19	17	31	
Naive	11	None	None	NA[g]	NA	10 or 15	1	3	7	
Control	34	None, Ad, or pCV-0	Alum, RIBI, H1D, Iscom or bupivacaine + methylparaben-Iscom		s.c., i.d., i.n., i.t., i.m.	10, 15, or 20	5	13	16	
Total controls	45						6	16	23	
Total	112						25	33	54	

[a] All animals were inoculated with the indicated dose of Tat plasmid DNA (pCV-tat [8], adenovirus-tat [Ad-tat] [27]) or protein, Gag protein, or empty vectors (pCV-0, adenovirus [Ad]) by the indicated route. Doses are in micrograms unless indicated otherwise.

[b] Alum, aluminum phosphate (4); RIBI oil-in-water emulsions containing squalene, bacterial monophosphoryl lipid A, and refined mycobacterial products (4); Iscom, immune-stimulating complex (4); H1D are biocompatible anionic polymeric microparticles used for vaccine delivery (10, 12, 25a).

[c] s.c., subcutaneous; i.m., intramuscular; i.d., intradermal; i.n., intranasal; i.t., intratracheal.

[d] All animals were inoculated intravenously with the indicated dose of the same SHIV89.6.P_{cy243} stock.

[e] According to the virological outcome upon challenge, monkeys were grouped as aviremic (A), controllers (C), or viremic (V).

[f] Because of the short follow-up, controller status could not be determined and all infected monkeys of the ISS-TG protocol were therefore considered viremic.

[g] NA, not applicable.

infection, but only viremic animals were considered for the analyses of acute ($n = 87$) and chronic ($n = 71$) infection. In the chronic phase, monkeys with follow-up at ≥28 weeks were further categorized as viremic ($n = 38$) or controllers ($n = 33$).

Susceptibility to infection. Upon challenge, 39 of 45 controls (87%) and 48 of 67 vaccinated animals (72%) became viremic. As shown in Table 2, the challenge dose did not affect susceptibility to infection in controls, while in vaccinated macaques, the percentage of aviremic monkeys increased to 68% after challenge with 10 MID_{50}, remaining comparable to that for the controls after challenge with 15 (12%) or 20 (17%) MID_{50} ($P < 0.0001$) (Table 2).

In the controls, the IB haplotype did not influence the infection acquisition rate, although a trend ($P = 0.0687$) for increased susceptibility was observed with the H1 haplotype (Table 2). In contrast, in vaccinated monkeys, the H2 and H6 haplotypes were associated with increased susceptibility ($P = 0.0199$) and resistance ($P = 0.0087$) to infection, respectively (Table 2), while the trend for an association of the H1 haplotype with increased susceptibility observed in the controls disappeared after vaccination ($P = 0.5821$).

The effects of the challenge dose and the IB haplotype on the likelihood of being aviremic were confirmed in a logistic regression model (the stepwise selection method was chosen to select the best-fit model, using maximum likelihood estimates) for controls and vaccinees. Specifically, in vaccinated animals the influence of the challenge dose (for 10 versus 15 to 20 MID_{50}, odds ratio = 16.2, 95% confidence interval [CI] = 4.0 to 65.6; $P < 0.0001$) and the H6 haplotype (odds ratio =7.2; 95% CI = 1.3 to 39.2; $P = 0.0215$) on the aviremic status remained significant, whereas the association of H2 with increased susceptibility to infection was lost.

Acute infection. The challenge dose and the IB haplotype had no significant impact on the peak viral load in control and

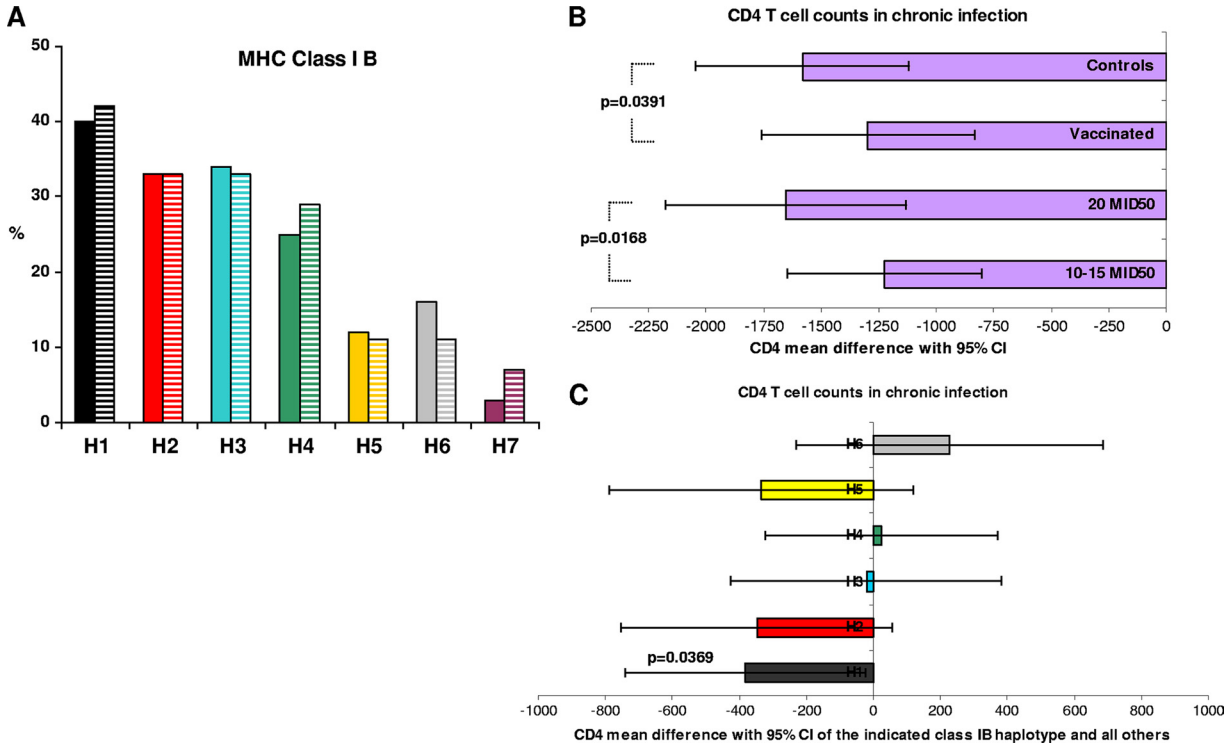

FIG. 1. (A) Frequency of MHC class IB haplotypes in 112 monkeys vaccinated with Tat (full-colored bars) or controls (zebra-striped bars). (B and C) Analysis of CD4 T cells in chronic infection in viremic macaques ($n = 71$) stratified by treatment (vaccinated, $n = 37$; controls, $n = 34$) and challenge dose (10 to 15 MID_{50}, $n = 58$; 20 MID_{50}, $n = 13$) (B) or class IB haplotype (H1, $n = 26$; H2, $n = 24$; H3, $n = 25$; H4, $n = 25$; H5, $n = 8$; H6, $n = 9$) (C). On the abscissa is reported the difference in CD4 T-cell counts pre- and postchallenge; least-squares means with 95% confidence intervals are shown (analysis of variance model).

Tat-vaccinated macaques. Notably, while CD4 T-cell loss in the controls was not affected by the challenge dose, it was significantly ($P = 0.0099$) contained in vaccinated monkeys inoculated with 15 MID_{50}. This was confirmed in a multivariate model (analysis of variance), which included the challenge dose and haplotype as explicative factors ($P = 0.0284$).

Chronic phase. Of the 71 viremic monkeys analyzed, 37 were controls and 34 were vaccinated. Of the 37 controls, 21 (57%)

TABLE 2. Univariate analysis of the effects of challenge dose and class IB haplotype on susceptibility/resistance to infection (aviremic or viremic) in control and vaccinated monkeys

Dose or haplotype	Controls			*P* value[a]	Vaccinated			*P* value[a]
	No. (%) of monkeys				No. (%) of monkeys			
	Total ($n = 45$)	Aviremic	Viremic		Total ($n = 67$)	Aviremic	Viremic	
MID_{50}								
10	10	3 (30)	7 (70)		19	13 (68)	6 (32)	
15	26	2 (8)	24 (92)		42	5 (12)	37 (88)	
20	9	1 (11)	8 (89)	0.2239	6	1 (17)	5 (83)	<0.0001
H1	19	5 (26)	14 (74)		27	9 (33)	18 (67)	
Non-H1	26	1 (4)	25 (96)	0.0687	40	10 (25)	30 (75)	0.5821
H2	15	0 (0)	15 (100)		22	2 (9)	20 (91)	
Non-H2	30	6 (20)	24 (80)	0.1575	45	17 (38)	28 (62)	0.0199
H3	15	2 (13)	13 (87)		23	6 (26)	17 (74)	
Non-H3	30	4 (13)	26 (87)	1.0000	44	13 (30)	31 (70)	1.0000
H4	13	2 (15)	11 (85)		17	3 (18)	14 (82)	
Non-H4	32	4 (12)	28 (88)	1.0000	50	16 (32)	34 (68)	0.3561
H5	5	0 (0)	2 (100)		8	2 (25)	6 (75)	
Non-H5	40	6 (15)	34 (85)	1.0000	59	17 (29)	42 (71)	1.0000
H6	5	0 (0)	5 (100)		11	7 (64)	4 (36)	
Non-H6	40	6 (15)	34 (85)	1.0000	56	12 (21)	44 (79)	0.0087

[a] Fisher's exact test.

TABLE 3. CD4 T-cell count changes[a] in viremic control or vaccinated monkeys, stratified by challenge dose or class IB haplotype

Dose or haplotype	Controls			Vaccinated		
	Total no. of monkeys (n = 37)	Mean ± SE CD4 T-cell count change	P value[b]	Total no. of monkeys (n = 34)	Mean ± SE CD4 T-cell count change	P value[b]
MID_{50}s						
10	7	−1,386 ± 227	0.8395	6	−1,348 ± 357	0.7896
15	22	−1,223 ± 128	0.3723	23	−807 ± 96	0.0067
20	8	−1,453 ± 231		5	−1,465 ± 179	
Haplotypes						
H1	12	−1,604 ± 206		14	−1,144 ± 191	
Non-H1	25	−1,159 ± 100	0.0343	20	−898 ± 111	0.2445
H2	14	−1,406 ± 154		10	−1,178 ± 241	
Non-H2	23	−1,241 ± 130	0.4305	24	−925 ± 105	0.2684
H3	13	−1,280 ± 180		12	−815 ± 127	
Non-H3	24	−1,316 ± 121	0.8656	22	−1,100 ± 140	0.1882
H4	11	−1,100 ± 188		14	−994 ± 135	
Non-H4	26	−1,389 ± 115	0.1872	20	−1,003 ± 150	0.9641
H5	4	−1,266 ± 244		4	−1,419 ± 363	
Non-H5	33	−1,308 ± 109	0.8977	30	−943 ± 104	0.1372
H6	5	−954 ± 194		4	−535 ± 20	
Non-H6	32	−1,358 ± 109	0.1676	30	−1,061 ± 111	<0.0001

[a] Changes for the chronic phase were defined as the difference from the average value of multiple prechallenge determinations.
[b] Student's t test.

remained viremic and 16 (43%) became controllers (see Table S1 in the supplemental material). Of the 34 vaccinated monkeys, 17 remained viremic and 17 became controllers. Univariate analysis did not reveal significant effects of the challenge dose or IB haplotype on the viremic status (controller or viremic) in either controls or vaccinees (see Table S1 in the supplemental material), although a trend (P = 0.0570) to remain viremic was observed for vaccinees carrying H2, the IB haplotype associated with increased susceptibility to infection. A multivariate analysis (exact logistic regression model, backward-selection method) aimed at evaluating the effects of the MID_{50} and IB haplotype on the likelihood that viremic macaques became controllers confirmed the lack of significant associations in control monkeys, while in vaccinated macaques it strengthened the association of H2 haplotype with the viremic status (odds ratio = 0.1; 95% CI = 0.0 to 0.7; P = 0.0125) and disclosed a previously unnoticed association of the H5 haplotype with the viremic status (odds ratio = 0.1; 95% CI = 0.0 to 0.7; P = 0.0237).

The challenge dose did not significantly influence the viral load set point in control or vaccinated macaques, although there was a trend (P = 0.0560) for a lower set point in controls challenged with 15 MID_{50} (see Table S2 in the supplemental material). In controls, the H1 haplotype was associated with a higher viral load set point (P = 0.0280) (see Table S2 in the supplemental material) and greater CD4 T-cell loss (P = 0.0343) (Table 3). In vaccinees, trends for a higher (H2, P = 0.0942; H5, P = 0.0804) and a lower (H6, P = 0.0715) viral load set point were noticed (see Table S2 in the supplemental material). Further, CD4 T-cell loss was significantly contained in vaccinees challenged with 15 MID_{50} (P = 0.0067) or carrying H6 (P < 0.0001) (Table 3).

A multivariate model (analysis of variance) that included vaccination, challenge dose, and haplotype as explicative factors was then applied to CD4 T-cell counts. Animals challenged with 10 or 15 MID_{50} were cumulated, since the results for these two groups did not significantly differ (P = 0.2649). This analysis showed that CD4 loss was influenced by both vaccination (P = 0.0391) and challenge dose (10 to 15 versus 20 MID_{50}, P = 0.0168) (Fig. 1B). In addition, the association of the H1 haplotype with severe CD4 T-cell loss was confirmed (P = 0.0369) (Fig. 1C).

Thus, both the infectious dose and the MHC IB haplotype are important when the vaccine effects in monkey studies are evaluated. However, the impact of the challenge dose on infection susceptibility and severity appears to be negligible in control animals, in substantial agreement with our former data (4). Similarly, the influence of class IB haplotypes in controls was limited to H1 in acute infection (increased susceptibility, P = 0.0687) and chronic infection (viral load set point, P = 0.0280; CD4 loss, P = 0.0343).

In contrast, both the infectious dose and IB haplotype significantly affected the protection offered by the vaccine. In particular, vaccination significantly reduced infection acquisition at 10 MID_{50} but not at 15 or 20 MID_{50} (P < 0.0001), underscoring the importance of using in vaccine efficacy studies challenge doses not exceedingly higher than those responsible for transmission in humans (22).

MHC-encoded host factors, including immune response genes, contribute importantly to susceptibility to human immunodeficiency virus (HIV)/SIV infection and progression (1, 7, 19), confounding the interpretation of efficacy afforded by vaccine candidates (13). The associations in rhesus monkeys of Mamu-A*01, Mamu-B*08, and Mamu B*17 with resistance to SIV represent good examples of this influence (15, 21, 24, 25). The results of our studies with cynomolgus macaques suggest a different scenario. In fact, while the impact of IB haplotypes on infection susceptibility/resistance was very limited in the controls, in the vaccinees, haplotypes H2 and H6 were associated with increased susceptibility (P = 0.0199) and resistance (P =

0.0087), respectively, to becoming viremic (Table 2), a finding confirmed for haplotype H6 in a multivariate analysis.

Once they were infected, vaccinees did not differ from controls with respect to peak viremia, regardless of the challenge dose or IB haplotype, whereas CD4 T-cell loss was contained during acute infection ($P = 0.0099$) in vaccinees challenged with 15 MID_{50}. In contrast, a relevant influence of these factors was evident in chronic infection. In fact, a significant containment of CD4 T-cell loss was apparent in vaccinees but not in control animals challenged with 15 MID_{50} ($P = 0.0067$) (Table 3), the most-represented challenge dose group ($n = 45$), confirming the preservation of CD4 T-cell counts observed during acute infection with 15 MID_{50} ($P = 0.0099$). Further, vaccinated macaques challenged with 10 to 15 MID_{50} exhibited significantly milder CD4 T-cell losses than those challenged with 20 MID_{50} ($P = 0.0168$) (Fig. 1B). A protective role for vaccination was also suggested by comparing CD4 T-cell losses in the controls and vaccinees, regardless of the challenge dose ($P = 0.0391$) (Fig. 1 B).

With regard to the impact of the IB haplotype, both haplotypes H2 and H5 were significantly associated in multivariate analysis with the viremic status in chronic infection, suggesting poor containment. The association of haplotype H1 with increased susceptibility to infection and a worse chronic infection observed in the controls was not found in vaccinated macaques, suggesting a protective effect of vaccination. Taken together, these associations suggest that IB haplotypes do not influence *per se* infection susceptibility or progression but, rather, affect responsiveness to vaccination and immunity to the virus.

Analysis of the immune responses in macaques with known IB haplotypes is warranted to confirm this hypothesis and provide relevant information for the development of monkey models suitable to evaluate both pathogenesis and vaccine efficacy. Notably, on the basis of these preclinical data, Tat has been tested in phase I preventative and therapeutic trials (2, 9–11, 16) and is being evaluated in a phase II therapeutic trial (http://www.hiv1tat-vaccines.info/).

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