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Effect of TIM-3 blockade on T CD8$^+$ and NK cells in ART-treated HIV-infected patients

Marta Sanz, Carolina Gutiérrez, Nadia Madrid-Elena, Sergio Serrano-Villar, María Jesús Vivancos, Alejandro Vallejo, Francisco Hernández and Santiago Moreno
Infectious Diseases Department, Ramon y Cajal Hospital, Madrid, Spain

Background: TIM-3 is a large transmembrane inhibitory receptor that is expressed in multiple cells of the immune system, including T-CD8$^+$ cells and NK cells. TIM-3 binds to Galectin-9, which has been described to be a potent mediator of HIV transcription and reactivation. We analyze the effect of TIM-3 blockade on the specific HIV-1 CTL response of T-CD8$^+$ cells and NK cells from HIV-infected-patients.

Methods:

- Ten patients were recruitied at the HIV Infection Clinic of the Infectious Diseases Service of the Ramón y Cajal Hospital, after giving informed consent.
- All the patients had plasma HIV RNA <1.7 log under ART for at least 12 months.
- For the co-cultures, CD4$^+$T, CD8$^+$T and NK cells were obtained from the PBMCs of the patients. Blood (200ml) was drawn by venipuncture, and PBMCs were isolated from the blood obtained. Subsequent isolation of the cellular subpopulations (CD4$^+$T, CD8$^+$T and NK) was performed by a positive selection magnetic separation. For this purpose, anti-CD4 antibodies were used for CD4$^+$T cells, anti-CD56 antibodies for NK cells and anti-CD8 antibodies for CD8$^+$ T lymphocytes.
- Cells were incubated with RPMI in addition to the culture of the CD4$^+$T cells. IL2 (10µl / ml) and PHA (1µl /ml) were added for activation for 2 days. The treatment of CD8$^+$T and NK cells was performed with the antibody against TIM-3 and incubated for 24h. After the incubation time, the co-cultures CD4$^+$: CD8$^+$, CD4$^+$: NK, CD4$^+$: CD8$^+$: NK (1: 1 ratio) was infected with HIV-1 BaL virus (5µl / million cells).
- The impact of TIM-3 blockade on the HIV-suppressive capacity of T-CD8$^+$ cells was determined by measuring p24 levels with Zeptometrix Kit in the supernatants at day 7 and day 10. Co-cultures without adding TIM-3 antibody were used as controls. To evaluate the impact of the natural ligand of TIM-3, p24 levels were compared in co-cultures with or without the addition of exogenous Galectin 9. Reduction of p24≥2log it means a good T-CD8$^+$-HIV specific T response (*Sáez-Cirión A. et al.*, Nature protocols 2010).

$$\text{Log}_{10} = \frac{\text{Mean p24ng/mL culture CD4+}}{\text{Mean p24ng/mL co-cultures CD4+:CD8+}}$$

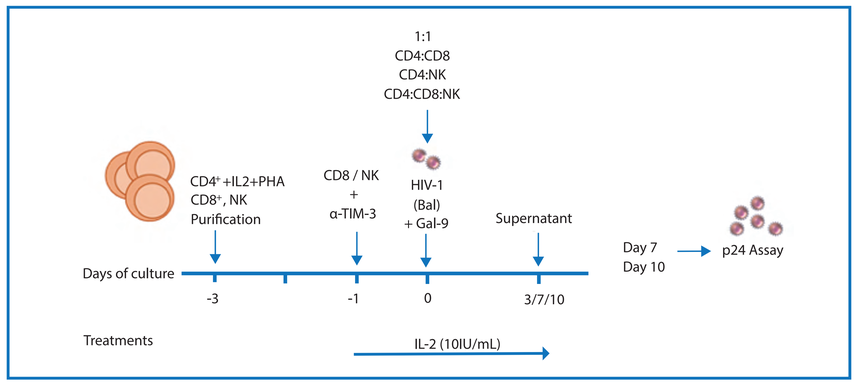


Figure 1. Scheme of co-culture.

Results: All the patients had HIV RNA<37 copies/ml, mean CD4$^+$ 661 cells/mm^3, mean CD8$^+$ 920 cells/mm^3 and mean nadir CD4$^+$ 327 cells/mm^3. The average time on ART was 88 months. All patients included in the study were men (table 1).

Characteristics	Patients									
	1	2	3	4	5	6	7	8	9	10
Age(years)	50	63	33	57	53	33	38	61	26	52
CD4$^+$ nadir (cells/mm^3)	392	365	479	272	250	-	186	50	633	234
CD4$^+$ (cells/mm^3)	1029	504	522	970	773	700	603	502	774	417
CD4$^+$/CD8$^+$	1,07	1,04	0,8	0,7	0,99	1,2	0,4	0,6	0,8	0,4
HIV-1 RNA (copies/mL)	<37	<37	<37	<37	<37	<37	<37	<37	<37	<37
ART duration (months)	41	185	48	324	43	72	72	270	12	12
ART	RPV	RPV	DTG	DTG	RPV	DRV	EVG	DRV	DTG	EVG
	TAF	DTG	ABC	ABC	DTG	COBI	COBI	RTV	ABC	COBI
	FTC		3TC	3TC		FTC	FTC	3TC	3TC	FTC
						TAF	TAF			TAF

Table 1. Characteristics of study participants. RPV: Rilpivirina, TAF: Tenofovir alafenamida, FTC: Emtricitabina, DTG: Dolutegravir, ABC: Abacavir, 3TC: Lamivudina, DRV: Darunavir, COBI: Cobicistat, EVG: Elvitegravir.

There was a reduction of p24 ≥2log in the blocking conditions of TIM-3 compared to the control conditions. In the CD4$^+$: CD8$^+$ co-cultures, a low suppressive capacity of the CD8$^+$ T cells (mean of Agp24 of 0.9 log) was observed, but significantly improved after blocking TIM-3 with the antibody (mean of Agp24 of 2.4 log) and therefore a viral replication control. In the case of CD4$^+$: NK co-culture, blocking the Gal-9: TIM-3 interaction did not cause a decrease in Agp24 (mean Agp24 of 0.8 log versus 1.2 log with antibody). However, in co-cultures of CD4$^+$ T cells together with CD8$^+$ and NK, the blockade of TIM-3 in NK cells did not affect the CTL activity of CD8$^+$T cells, again observing a significant decrease when the interaction block Gal-9: TIM-3 (Agp24 mean of 1 log versus Agp24 mean of 2.3 log with antibody). On the other hand, we did not find significant differences between the results obtained at day 7 and day 10, nor between adding Gal-9 or without adding it.

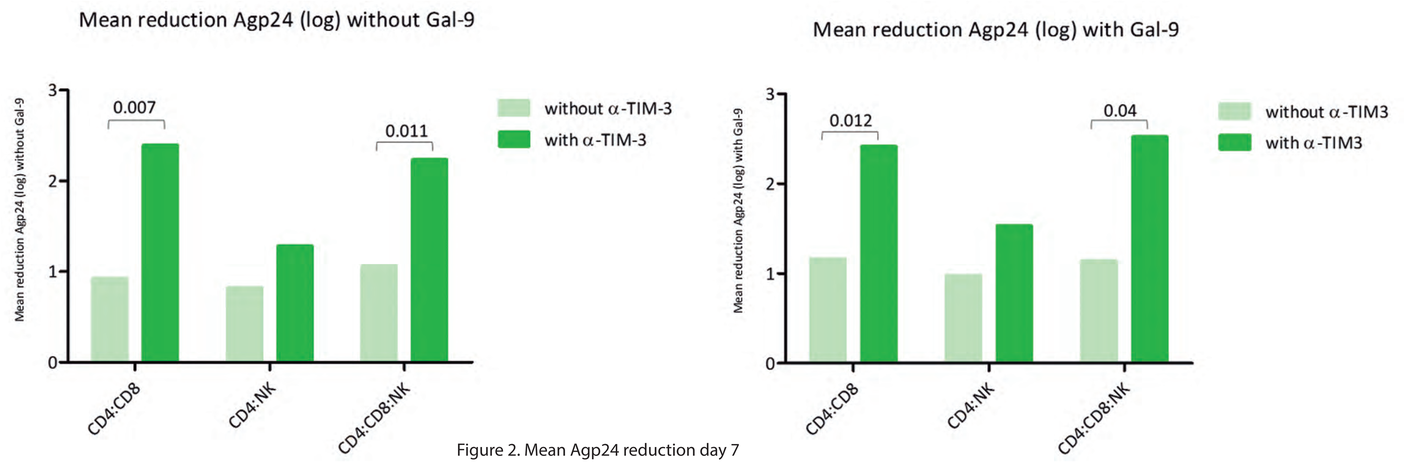


Figure 2. Mean Agp24 reduction day 7

Conclusions:

1.The blockade of TIM-3 restores the CTL response of the CD8$^+$ cells of ART-treated HIV-infected patients.
2.This same blockage in NK cells had no negative effects on the overall CTL response.
3.Since Gal-9 does not have any impact on the response, a combination of Gal-9/TIM-3 could be evaluated as an effective strategy in HIV-eradication.

Marta Sanz PhD student, contact email: marta_s17@hotmail.com