MF-59 adjuvant influence on the functions of gammadelta T cells in HIV-1+ adults immunized with influenza seasonal vaccine

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Key words

HIV-1 • Gammadelta T cells • Inflammatory cytokines

Summary

Introduction. *We previously reported that in HIV-1 infected patients circulating Vdelta1 T lymphocytes (Vδ1) increase and proliferate in vitro in response to Candida albicans (Ca). Herein, we analysed the effects of MF59 adjuvant on the Vδ1 T cell responses to hemagglutinin (HA) and Ca in HIV-1 seropositive and seronegative adults after influenzal vaccine, to clarify the molecular mechanisms triggered in vivo by an adjuvanted vaccine against influenza virus.*

Materials and methods. *58 seropositive (HIV-1+) and 48 seronegative (HIV-1-) subjects received influenzal vaccines containing or not the MF59 adjuvant. The follow-up of in vitro T cell proliferation and cytokine production (IL-17A, IL-22, IL-23, IL-6) to HA and Ca antigens were performed at different time points (at basal time and after 30 and 90 days from vaccination) by cytofluorimetric approaches.*

Results. *We confirmed that in HIV-1 infected individuals the Vδ1 T cell subset is expanded in HIV-1 infected individuals and moreo-ver the number of circulating Vδ1 T cells significantly enhanced in all HIV-1+ subjects on day 90 after influenza vaccination. Regard the follow-up of proliferative responses, the increments of CD3+ response to HA and Vδ1 T cells to Ca in HIV-1+ individuals were detectable earlier on day 30 for MF59-vaccinated patients, instead on day 90 post-vaccination in HIV+-vaccinated without MF59 adjuvant. Of note, production of IL-17A and IL-22, two cytokines with anti-fungal activity, in response to Ca was enhanced (for IL-17A) or restored (for IL-22) by vaccination in HIV-1+ donors, mainly using the MF59-adjuvanted vaccine. Moreover, after vaccination IL-23 and IL-6 production increased in response to HA in the HIV+ and HIV- groups vaccinated with MF59 adjuvant.*

Conclusions. *We suggest that in HIV-1 infected patients the circulating Vδ1 T lymphocytes reactive to Ca upon challenge with influenza virus vaccine receive an activating/enhancing signal mediated by cytokines triggered by the boost with HA antigen particularly in presence of MF59 adjuvant.*

Introduction

Gammadelta (γδ) T lymphocytes contribute to the first-line defense against several pathogens [1]. Two main subsets of γδ T cells are know [2]: Vδ2 T lymphocytes, circulating in the peripheral blood, which are involved in the response to mycobacteria, coxsakievirus B3 and herpes simplex virus type 2 [3, 4]; Vδ1 T cells, which are resident in the mucosal-associated lymphoid tissue and participate in the immunity against Listeria monocytogenes and cytomegalovirus [5, 6].

In HIV-1 infected patients, Vδ1 T lymphocytes are increased in periphery, as a result of mucosal depletion and recirculation [7, 8], whereas circulating Vδ2 T cells may decrease possibly due to reduced response to chemotactic stimuli or impaired survival [9, 10].

Both Vδ1 and Vδ2 T cells can produce interferon (IFN)-γ in response to damage signals [11]. It has been reported that resident γδ T lymphocytes can produce interleukin (IL)-17A, crucial for the control of intracellular pathogens and fungi [12].

Recently, we described in HIV-1 infected patients the expansion of a γδ T cell subset responsive to Ca and both IFN-γ and IL-17A-producing [13]. Moreover, in the presence of IL-23, IL-6 and transforming growth factor (TGF)-β, a subset of Vδ2 T lymphocytes can produce another mediator reported to contribute to Ca immunity in humans that is IL-22, besides IL-17A. On the other hand, sera levels of IL-22 are inversely correlated to the replication in vitro of HIV-1 subtype C, that accounts for 50% of all HIV-1 infections worldwide [14].

HIV-1-positive subjects represent a category of at-risk individuals due to their impaired immune responses; therefore influenza vaccination is recommended in order to reduce the risk of respiratory threatening complications. However, the immune response to influenza vaccines can be suboptimal or very low depending on their immunodeficient status. To fill this gap, vaccines containing adjuvants, such as the oil-in-water emulsion MF59, have been investigated and largely used in Europe since 1997, demonstrating a good safety profile and a higher immunogenicity than conventional non-adjuvanted vaccines, particularly in immunosuppressed

patients. In the last years, safety and immunogenicity of the MF59-adjuvated vaccine has been proved also in HIV-1 seropositive individuals [15]; nevertheless, the molecular mechanisms triggered in vivo by MF59, that enhances T cell specific response to hemagglutinin (HA), the major antigen of influenza virus, is still not fully understood, in particular in HIV-1-positive subjects.

In this paper we show that the influenza vaccine containing the MF59 adjuvant influences the function of Vδ1 T lymphocytes in HIV-1 seropositive patients. Moreover, we observe an enhancement of cytokine production, including IL-17A and IL-22, in response to Ca, and IL-23 or IL-6 in response to HA, upon influenza virus vaccination with a vaccine containing the MF59 adjuvant.

Materials and methods

STUDY DESIGN, SUBJECTS AND VACCINATION

58 seropositive (HIV-1+) and 48 seronegative (HIV-1-) subjects were enrolled for influenza virus vaccination at the Rehabilitation Centre of San Patrignano (Rimini, Italy), provided informed consent and ethics committee approval of San Martino Hospital (EUDRACT code: 2006-001103-11). Both HIV-1+ and HIV-1- subjects, randomly assigned to group A or B, receive intramuscularly a single dose of an influenza virus vaccine with MF59 adjuvant (Fluad, n = 29 HIV-1+, n = 24 HIV-1-) or a subunit vaccine (AgrippalS1, n = 29 HIV-1+, n = 24 HIV-1-), respectively (both from Novartis Srl). The vaccines contained 15 μg of HA from A/New Caledonia/20/99 (H1N1), A/California/7/2004 (H3N2) and B/Shangai/361/2002 influenza virus. Blood samples were collected before vaccination (time 0) and at 30 or 90 days postvaccination (time 30 and time 90). The characteristics of HIV-1+ and HIV-1- subjects are summarized in Table I.

PHENOTYPIC ANALYSIS

The absolute number (cells/μl) of peripheral T-lymphocyte subsets were performed in HIV-1+ subjects at study entry and at 30 or 90 days postvaccination in single platform using a commercially available kit (Multiset, Becton Dickinson, BD). The frequencies of circulating γδT cell populations were evaluated with the anti-Vδ1 mAb A13 and the anti-Vδ2 mAb BB3 (both IgG1),

labelled with the fluorochrome Alexafluor594 prepared as described by manufacturer's instructions (Zenon Labeling Kit for mouse IgG1, Molecular Probes) and allophycocyanin-conjugated (APC) anti-CD3 mAb (BD). The percentage of γδ positive T cells was normalized for the T cell count in blood and expressed as number of cells/μl.

PROLIFERATION ASSAY

The proliferation of pheripheral lymphocytes to purified HA antigens (kindly supplied by Novartis Vaccines Srl) and Ca bodies was evaluated by a dye dilution assay [15]. Briefly, peripheral blood mononuclear cells (PBMC), obtained from Ficoll-Hypaque (Biochrom) gradient of heparinized venous blood, were stained with the carboxy-fluorescein diacetate succinimidyl ester (CFSE, Sigma Chemicals Co.) and seeded at $4 \times 10^5/200$ μl per well in duplicate in a flat-bottomed microtitre plate containing 20 μl of antigens (HA or Ca) in complete medium composed of RPMI1640 (Biochrom) with 10% autologous plasma. HA was used at 2 or 6 μg/ml final concentration; Ca was grown in RPMI 1640 medium for 2 days, washed twice in PBS, autoclaved and used at 10^6 bodies/ml final concentration in culture (body/cell ratio: 1/1). These concentrations, in preliminary titrations experiments, were optimal for PBMC stimulation. After 10 days (the last 7 days with IL-2), the cells were stained with Alexafluor594-A13 or Alexafluor594-BB3, followed by the allophycocyanin-conjugated (APC) anti-CD3 mAb (BD) and acquired by flow cytometry. Data were analysed on a FACS Canto cytometer (BD) using FACS DIVA software. Gates were set on live lymphocytes according to forward scatter and side scatter characteristics, 50,000 CD3+ T cells were collected for each sample. To estimate the frequency of proliferating cells (precursor frequency) in response to antigens, flow cytometric data files were analyzed with the "Proliferation Wizard" module using the ModFit LT version 3.0 computer program (Verity Software House Inc.).

CYTOKINE MEASUREMENT

Human IL-17A, IL-22, IL-23, IL-6 cytokines were measured in harvested supernatants (SN) before IL-2 adding, using the FlowCytomix Multiplex kit and BMS FlowCytomix software (Bender MedSystem), following manufacturer's instructions. Results are expressed as pg/ml for each cytokine.

Tab. I. Characteristics of HIV-1 seropositive and seronegative subjects.

	HIV-1+		HIV-1-	
Characteristics (at time 0)[1]	Group A (Fluad) (n = 29)	Group B (Agrippal) (n = 29)	Group A (Fluad) (n = 24)	Group B (Agrippal) (n = 24)
Age (mean ± SD)	31.4 ± 7.2	33.6 ± 6.9	39.4 ± 5.6	38.6 ± 6.3
Sex	19M, 10F	20 M, 9F	10M, 14F	9M, 15F
HIV-1 RNA copies/ml	2,607 ± 2,480	1,795 ± 1,610	na[2]	na[2]
CD4+ cells/mm³	371 ± 114	284 ± 105	na[2]	na[2]
CD4/CD8 ratio	0.47 ± 0.18	0.56 ± 0.19		
HAART[3]	20/29	18/29	na[2]	na[2]

[1] Viremia and CD4+ cell count did not vary significantly at time 30 or time 90 after vaccination; [2] na: not applicable; [3] HAART: highly active antiretroviral therapy.

STATISTICAL ANALYSIS

Statistical analysis was performed by the "analysis of the variance" (ANOVA) for repeated measures. We have first analysed the variance of each parameter in the HIV⁺ group, and then we have compared both HIV⁺ and HIV⁻ groups. The cut-off value of significance was .01.

Results

Circulating Vδ1 T cell number in HIV-1⁺ subjects increases after influenza vaccination. Pheripheral blood phenotypes, performed before vaccination (time 0) and at 30 or 90 days postvaccination, didn't show significant change for CD4⁺ and CD8⁺T subsets in HIV⁺ patients during the follow-up [15], but confirmed our previous observations [14] regard to γδ T cells. In fact the number of Vδ1 T lymphocyte resulted significantly higher in all HIV-1⁺ subjects than in HIV-1⁻ donors (p < 0.01) at basal time, at variance with Vδ2 T cells. Moreover the follow-up after influenza vaccination showed a significant enhancement of the Vδ1 circulating T cell subset in both HIV-1⁺ groups (vaccinated with or without MF59) on day 90. No variations of the Vδ2 T cell population in peripheral blood was observed at any time in either HIV-1⁺ or HIV-1⁻ donors (Fig. 1).
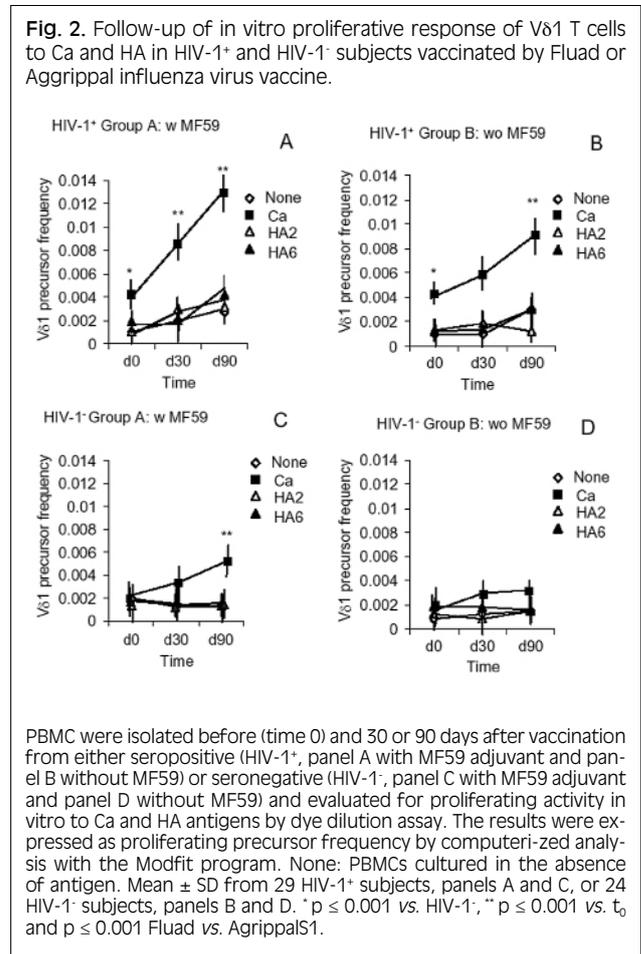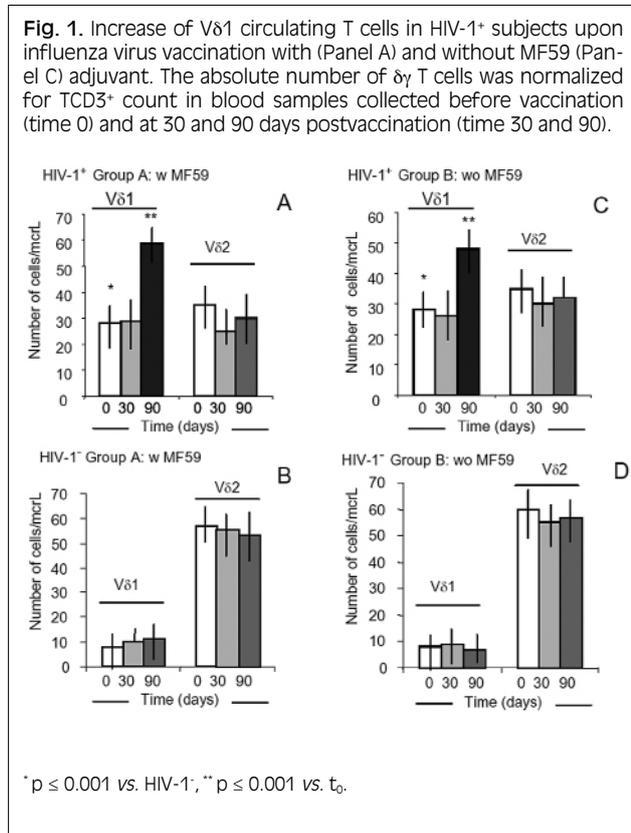
Vδ1 T cell reactivity to C. albicans (Ca) increases in HIV-1⁺ subjects immunized with MF59-adjuvanted influenza vaccine.

Since we reported that circulating Vδ1 in HIV-1-infected patients are expanded in vivo and previously we have been pointed out their response to Ca in vitro [13], we monitored the effect of influenza virus vaccination on Vδ1 proliferation to relevant antigen as HA and to fungal antigen as Ca (Fig. 2). The data showed a significantly higher Vδ1 proliferating precursor frequency (f) in response to Ca in HIV-1⁺ than in HIV-1⁻ individuals already at time 0 (mean f 0.0045 *vs.* 0.0019); nevertheless, we observed postvaccination an increment of in vitro Ca-specific reactivity more efficiently from HIV-1⁺ subjects receiving MF59-adjuvanted vaccine, whereas only a slight effect was observed in HIV-1⁻ subjects. Of note, the Ca-specific increment of Vδ1 T cells was faster in HIV-1⁺ subjects MF59-threated than HIV-1⁺ subjects immunized without adjuvant, that is the proliferative kinetic was similar to HA-specific trend evaluated in all the CD3⁺ αβ T cells of HIV-1⁺ patients immunized by Fluad [15]. The Vδ1 T cell response to HA was undetectable and superimposable to that of unstimulated cells, either in HIV-1⁺ or in HIV-1⁻ individuals (Fig. 2, panel A and B for HIV-1⁺, panel A and B for HIV-1⁻). No significant proliferation of Vδ2 T cells to either Ca or HA was observed in all the vaccinated groups.

IL-17A and IL-22 production to Ca increase after MF59-adjuvanted vaccination in HIV-1⁺ subjects.

We and others described that in particular Vδ1 T cells produced IL-17A in HIV-1-infected patients [13], while their IL-22 production is deficient [14]. Thus, we monitored the influence of vaccination on cytokine re-

Fig. 1. Increase of Vδ1 circulating T cells in HIV-1⁺ subjects upon influenza virus vaccination with (Panel A) and without MF59 (Panel C) adjuvant. The absolute number of δγ T cells was normalized for TCD3⁺ count in blood samples collected before vaccination (time 0) and at 30 and 90 days postvaccination (time 30 and 90).

*p ≤ 0.001 *vs.* HIV-1⁻, **p ≤ 0.001 *vs.* t₀.

Fig. 2. Follow-up of in vitro proliferative response of Vδ1 T cells to Ca and HA in HIV-1⁺ and HIV-1⁻ subjects vaccinated by Fluad or Aggrippal influenza virus vaccine.

PBMC were isolated before (time 0) and 30 or 90 days after vaccination from either seropositive (HIV-1⁺, panel A with MF59 adjuvant and panel B without MF59) or seronegative (HIV-1⁻, panel C with MF59 adjuvant and panel D without MF59) and evaluated for proliferating activity in vitro to Ca and HA antigens by dye dilution assay. The results were expressed as proliferating precursor frequency by computeri-zed analysis with the Modfit program. None: PBMCs cultured in the absence of antigen. Mean ± SD from 29 HIV-1⁺ subjects, panels A and C, or 24 HIV-1⁻ subjects, panels B and D. *p ≤ 0.001 *vs.* HIV-1⁻, **p ≤ 0.001 *vs.* t₀ and p ≤ 0.001 Fluad *vs.* AgrippalS1.

lease induced by HA and Ca-stimulation in culture surnatants (SN) on day 3 harvested before (time 0) and 30 or 90 days after vaccination. First, we confirmed that in HIV-1+ individuals IL-17A production in response to Ca is stronger than in HIV-1- donors and detectable already at time 0 (Fig. 3, panel A vs. B, mean 400 pg/ml vs. 200 pg/ml). Second, in HIV-1+ subjects (Fig. 3, panel A) but not in HIV-1- donors (Fig. 3, panel B), Fluad vaccine enhanced this response significantly (> 1000 pg/ml at day 30 and 90 vs. 400 pg/ml). Also in this case the non-adjuvanted vaccine AgrippalS1 did not influence the IL-17A release in HIV-1+ patients (Fig. 3, panel A). No IL-17A production was observed in response to HA, both in HIV-1+ subjects (Fig. 3, panel A) and in HIV-1- donors (Fig. 3, panel B), regardless the type of vaccine used.

With regard to IL-22, its production Ca-induced was deficient in HIV-1+ subjects (Fig. 3, panel C, about 2000 pg/ml) compared with HIV-1- donors (Fig. 3, panel D, about 6000 pg/ml). However, influenza vaccine administration restored an efficient production of IL-22 to Ca already at 30 days, with a significantly higher efficiency of the MF59-adjuvanted vaccine (Fig. 3, panel C, mean 10000 pg/ml at day 30 after Fluad vs. 6000 pg/ml after AgrippalS1) that enhanced the amount of IL-22 secreted also in comparison to that of HIV-1- individuals (Fig. 3, panel C vs. panel D, mean 10000 pg/ml vs. 8000 pg/m at day 30). No significant IL-22 production was detected in response to HA, either in HIV-1+ subjects (Fig. 3, panel C) or in HIV-1- donors (Fig. 3, panel D), regardless the vaccine administered.

IL-23 and IL-6 production in response to HA is enhanced after administration of MF59-adjuvanted influenza vaccine.
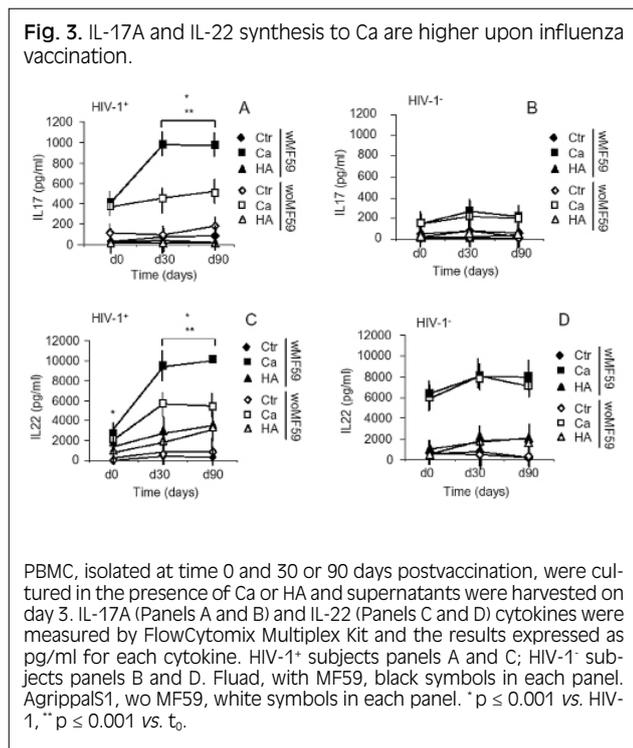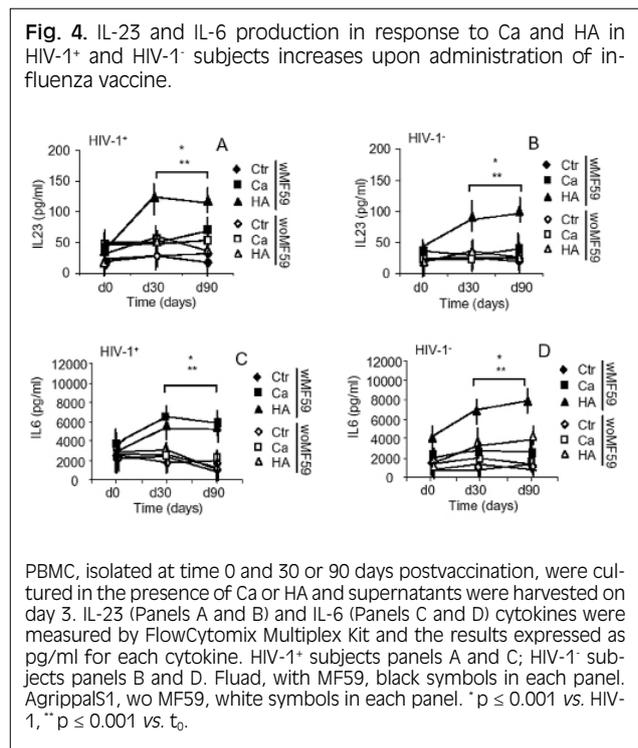
We evaluated the release in SN of IL-23 and IL-6 cytokines, required for IL-17A and IL-22 synthesis, before (time 0) and 30 or 90 days postvaccination upon stimulation with Ca or HA as above. Both in HIV-1+ and in HIV-1- subjects administration of MF59 adjuvanted vaccine led to an significant enhancement of IL-23 production in response to HA, detectable at day 30 and 90 (Fig. 4, panels A and B), the effect being more evident in HIV-1+ individuals. Interestingly, also for the HIV-1+ and in HIV-1- Fluad groups, the IL-6 synthesis was increased in HA-stimulated cultures upon influenza vaccination (Fig. 4, panels C and D). Moreover, IL-6 production was increased also in response to Ca in HIV-1+ donors receiving adjuvanted vaccine (Fig. 4, panel C).

Discussion

In this paper we show that: i) the influenza vaccine in HIV-1 seropositive individuals induce in vivo expansion of Vδ1 T lymphocytes that in vitro proliferate to Ca; ii) regard to cytokine studies, the MF59 adjuvanted vaccine influence more efficiently the cytokine profile in response to Ca (for IL-17A and IL-22) and to HA (for IL-23 or IL-6) in HIV-1+ subjects.

It is well known that the CD4 T helper subset is highly impaired in HIV-1-infected patients, leading to a defective control of viral infection and to an increased susceptibility to fungi and intracellular pathogens evasion. From this viewpoint, influenza vaccination should reduce the risk of respiratory threatening complications.

The availability of an adjuvanted vaccine, that enhances the immunogenicity of influenza virus vaccines, led to the possibility to administrate this vaccine in immuno-

Fig. 3. IL-17A and IL-22 synthesis to Ca are higher upon influenza vaccination.

PBMC, isolated at time 0 and 30 or 90 days postvaccination, were cultured in the presence of Ca or HA and supernatants were harvested on day 3. IL-17A (Panels A and B) and IL-22 (Panels C and D) cytokines were measured by FlowCytomix Multiplex Kit and the results expressed as pg/ml for each cytokine. HIV-1+ subjects panels A and C; HIV-1- subjects panels B and D. Fluad, with MF59, black symbols in each panel. AgrippalS1, wo MF59, white symbols in each panel. * p ≤ 0.001 vs. HIV-1, ** p ≤ 0.001 vs. t_0.

Fig. 4. IL-23 and IL-6 production in response to Ca and HA in HIV-1+ and HIV-1- subjects increases upon administration of influenza vaccine.

PBMC, isolated at time 0 and 30 or 90 days postvaccination, were cultured in the presence of Ca or HA and supernatants were harvested on day 3. IL-23 (Panels A and B) and IL-6 (Panels C and D) cytokines were measured by FlowCytomix Multiplex Kit and the results expressed as pg/ml for each cytokine. HIV-1+ subjects panels A and C; HIV-1- subjects panels B and D. Fluad, with MF59, black symbols in each panel. AgrippalS1, wo MF59, white symbols in each panel. * p ≤ 0.001 vs. HIV-1, ** p ≤ 0.001 vs. t_0.

deficient subjects, such as HIV-1^{+} individuals, reaching a good immune response. We previously found that influenza virus vaccination triggers a specific response in both HIV-1^{+} and HIV-1^{-} individuals; of note, the kinetic and intensity by CD3^{+}T cells were significantly more faster in HIV-1^{+} subjects treated with MF59-adjuvanted vaccine [15]. These data were confirmed in this report by the analysis of release of IL-23 and IL-6 cytokines to HA. Both of the inflammatory mediators are required for the production and release of IL-17A, important cytokine in the control of fungal infections, by recruiting neutrophils and macrophages to infected tissues. Along this line, upon vaccination IL-17A production to Ca by lymphocytes from HIV-1^{+} subjects was increased, in particular when the MF59-adjuvanted vaccine was used. With regard to IL-22, another anti-fungal cytokine, its deficient production in cultures stimulated with Ca from HIV-1^{+} subjects, was restored early after influenza vaccine administration, at significantly higher level by MF59-adjuvanted vaccine. We recently described in HIV-1 infected patients the expansion of a population of γδ T cells that respond to Ca and produce IL-17A, that might participate to anti-fungal immune response in vivo [13]. To date several evidences strongly suggest that IL-17A and IL-22 cytokines have a critical role in mucosal immunity to many extracellular pathogens and can also coordinate adaptive immunity to some intracellular pathogens. To this issue we confirmed a higher number of circulating Vδ1 T lymphocytes in HIV-1^{+} subjects before influenza vaccination, compared to healthy donors, that furtherly increases at 90 days postvaccination. Interestingly, we observed that Vδ1 T lymphocytes from HIV-1^{+} patients proliferate in vitro to Ca more efficien-tly after influenza vaccination than HIV-1 infected individuals, mainly with MF59-adjuvanted vaccine. Thus, it is tempting to hypothesize that the

Fig. 5. Work hypothesis.
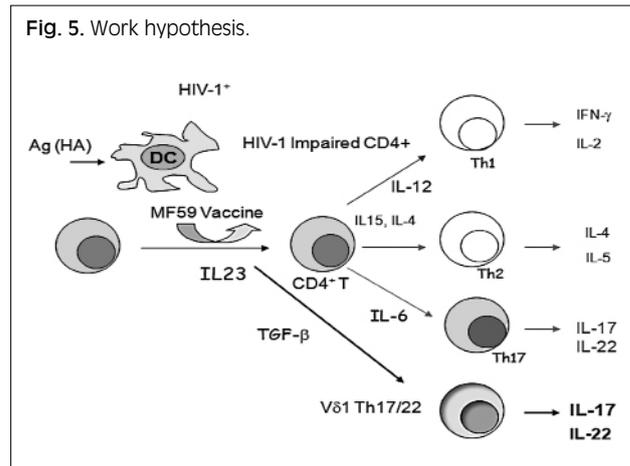
vaccine equipped with the adjuvant is able to overcome the hyporesponsiveness of CD4^{+} T cells in immunosuppressed HIV-1^{+} infected subjects, leading to release of cytokines needed for the development of cells producing anti-fungal cytokines, possibly represented by a subset of γδ T cells. The effector molecules IL-17A, IL-17F and IL-22 are produced by a third lineage of CD4^{+} Th cells (Th17), but can be produced also by γδ T cells. Human memory Th17 cells were found to react against Ca, and they also produce IL-22; recent data suggest that Th17 subset is impaired in immunosuppressed individuals.

Thus the expansion of a specific circulating γδ T cell population, capable of producing anti-fungal cytokines, enhanced by influenza vaccine administration, in HIV-1 infected subjects might participate in to the control of HIV-1 spreading and to the defence against opportunistic infections, possibly contributing to compensate the impairment of CD4^{+} T cells (Fig. 5).

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