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► **To cite this version:**

Magali Rancez, S Logerot, S. Figueiredo, B Charmeteau-De-Muylder, I Bourgault-Villada, et al.. IL-7 as an adjuvant for mucosal vaccine development. 22nd International AIDS Conference (AIDS 2018), Jul 2018, Amsterdam, Netherlands. 2018. hal-03000425

HAL Id: hal-03000425

<https://hal.science/hal-03000425v1>

Submitted on 11 Nov 2020

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IL-7 AS AN ADJUVANT FOR MUCOSAL VACCINE DEVELOPMENT

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INTRODUCTION and OBJECTIVES

Development of mucosal immune responses, while certainly essential to allow protection against numerous sexually transmitted infections, is still hard to induce in most models. Despite some progresses in the development of adjuvants possibly helping for the setting of this kind of immunity, results in terms of protection as for induction of the local antibody production and specific T-cells remain largely disappointing.

We have showed that systemic injection of IL-7 stimulates chemokine-induced recruitment of circulating T-cells into the mucosa (*Blood* 2009, 114(4):816-25), and evidenced local IL-7 expression in acutely infected mucosae (*Front Immunol* 2017, 19:8:588).

In this study, we aimed at using recombinant glycosylated simian IL-7 (sIL-7) as an immunostimulating molecule for vaginal immune responses in vaccine protocols. We administered sIL-7 by spray directly on rhesus macaque's vaginal mucosa surface and quantified local transcription of 19 chemokines by qRT-PCR and cell infiltration by immunohistochemistry plus image analysis to define the optimal dose of sIL-7 to be sprayed on mucosal surface. Then, six macaques were immunized with antigens (Diphtheria Toxoid (DT) and the HIV-1 gp41-P1 peptide) applied directly on the vaginal mucosa, two days after either IL-7 or PBS administration. The immunizations were repeated thrice, four months apart, and the macaques were euthanized 2 weeks after the last immunization. Antigen-specific IgA and IgG productions were quantified in vaginal secretions by ELISA. Antigen-specific plasma cells were detected by reverse immunohistochemistry and by B-cell ELISPOT.

RESULTS

LOCAL IMPACT OF sIL-7 ADMINISTRATION BY SPRAY ON VAGINAL MUCOSA

Administration by vaginal spray of sIL-7 (10µg) induces the highest local chemokine transcription

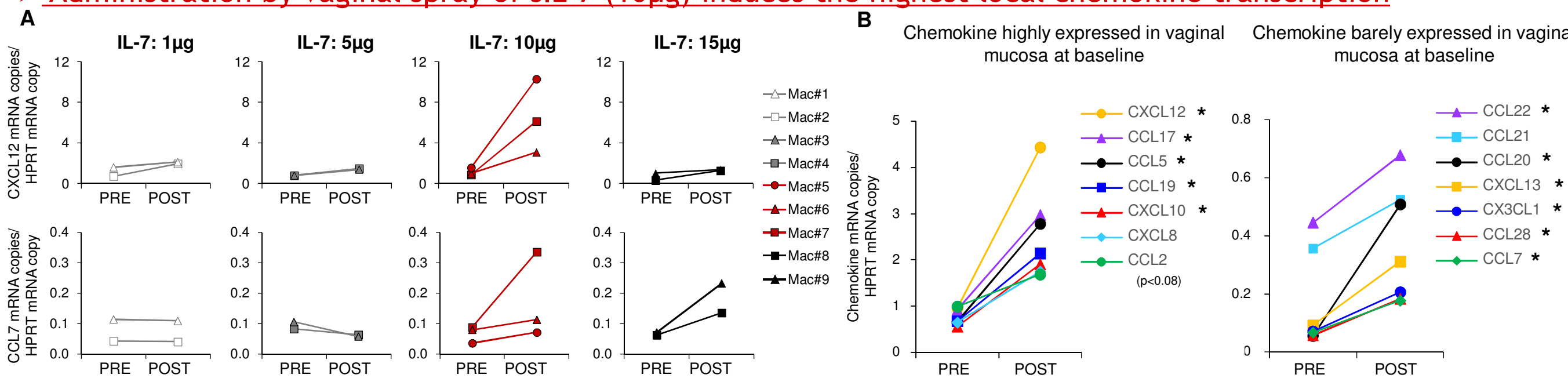


Fig 1: Impact of vaginal sIL-7 administration on chemokine transcription

(A) mRNAs coding for CXCL12 and CCL7, were quantified and normalized to HPRT mRNAs in each vaginal biopsies sampled one month before (PRE) and 48^h after the administration of 1µg (n=2), 5µg (n=2), 10µg (n=3) or 15µg (n=2) of sIL-7 (POST), by vaginal spray. Each point represents the median of a triplicate quantification in a given biopsy. (B) mRNAs coding for 19 chemokines, were quantified in triplicate and normalized to HPRT mRNAs in each vaginal biopsies sampled one month before (PRE) and 48^h after the administration of 10µg (n=3) and 15µg (n=2) of sIL-7 (POST), by vaginal spray. Each point represents the mean of all the quantifications performed per animal PRE and POST administration of the sIL-7. Four biopsies per animal PRE and POST administration. *: p<0.05 by the Wilcoxon Signed-Rank Test.

sIL-7 administration in vaginal mucosa stimulates T-cells, B-cells, and antigen-presenting cells (macrophages and myeloid dendritic cells) migration into the chorion

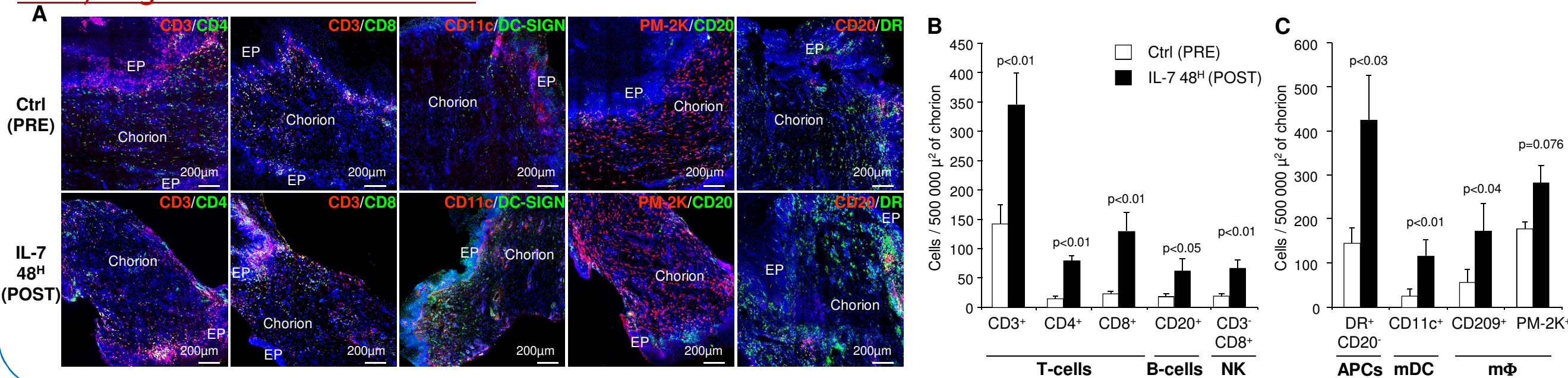


Fig 2: Immune cell recruitment into vaginal chorion of sIL-7 treated macaques

(A) Sections of monkey vaginal mucosa biopsies sampled one month before (Ctrl), or 48^h after (IL-7) the administration of 10µg of sIL-7 by vaginal spray were stained with DAPI. EP= Pluristratified Epithelium. (B, C) Cell quantifications by image analysis of IHF staining (expressed as number of cells per 500,000 µm² of chorion ± SEM) were realized from 20 to 32 vaginal mucosa biopsies (8 macaques, except for PM-2K+ cells quantification: 5 macaques) sampled one month before (Ctrl - white bars) and 48^h after (IL-7 48^h - black bars) the administration of 10µg of sIL-7. (Mann-Whitney U test).

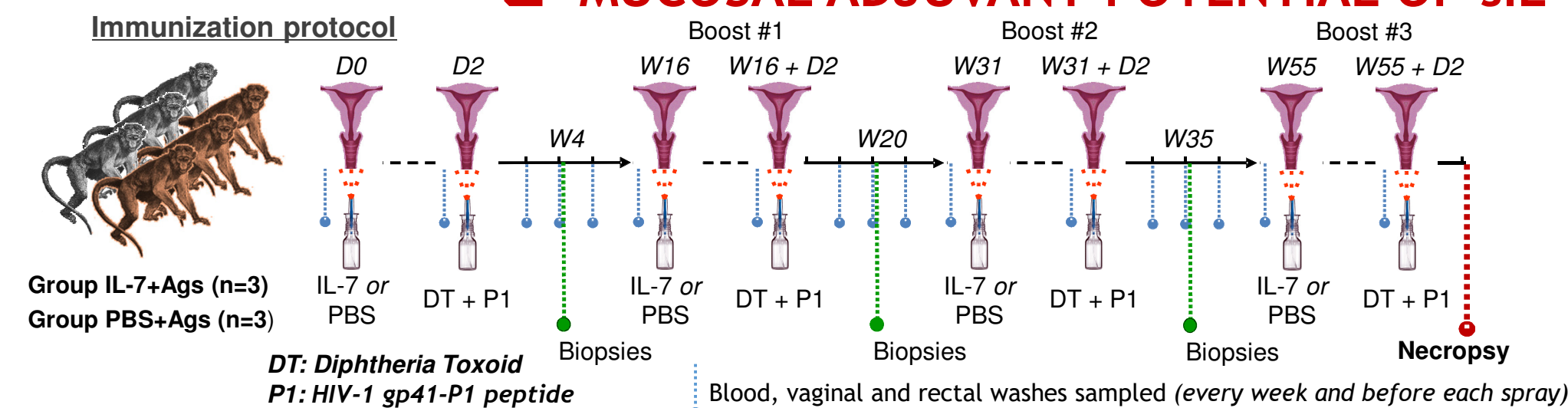
ACKNOWLEDGMENT

The authors thank the staffs of the Institut Pasteur (Paris, France) and Infectious Disease Models and Innovative Therapies (IDMIT, Fontenay-aux-Roses, France) Primate Centers. The authors greatly acknowledge Maryline Favier, Franck Letourneur and Pierre Bourdoncle, respectively heads of HistIM, GENOM'IC and IMAG'IC core facilities of the Institut Cochin. The authors thank Thomas Guilbert for IMAG'IC platform at the Institut Cochin for writing the routine for ImageJ image analysis software. The authors acknowledge Cytheris S.A. (now Revimmune Inc.), for R-sIL-7gly (sIL-7). This work was supported by ANRS, INSERM and Université Paris Descartes.

Presented at the 22nd International AIDS Conference – Amsterdam, the Netherlands

RESULTS

MUCOSAL ADJUVANT POTENTIAL OF sIL-7 in DT and P1 IMMUNIZATION



Local administration of sIL-7 have an adjuvant effect on mucosal DT & P1 immunization

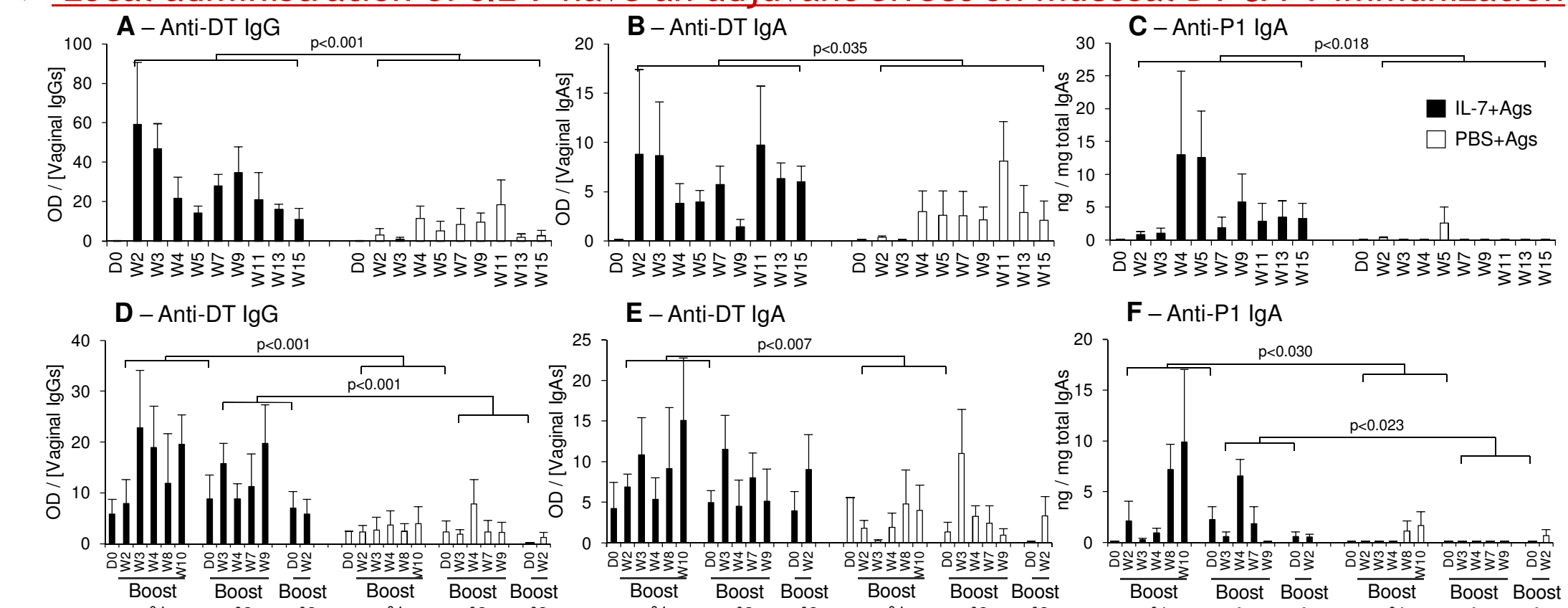


Figure 3: Local sIL-7 treatment allows vaginal mucosa to mount a specific immune response and boost lead to its maintenance

Specific anti-DT IgGs (A, D) and IgAs (B, E), and anti-P1 IgAs (C, F), were quantified by ELISA in vaginal secretions of 6 rhesus macaques that received vaginal administration of either 10µg of sIL-7 (black bars, n=3) or PBS (white bars, n=3), followed at day 2 by antigen administration (DT and P1). Similar immunizations were repeated thrice, every 4 months. Results are expressed as optical density over IgG or IgA concentration at any time-point for the 3 animals from the same group in any given sample for specific anti-DT (A-B, D-E) or as ng of specific anti P1 per mg of total IgAs (C, F). Bars and error bars represent means ± SEM. Non interpretable data due to menstruations were excluded. (MANOVA with Fisher test). D0: Administration of sIL-7 or PBS; W: Week post-antigens administration.

sIL-7-dependent immunization protocol induces ectopic lymphoid follicles genesis in vaginal mucosa

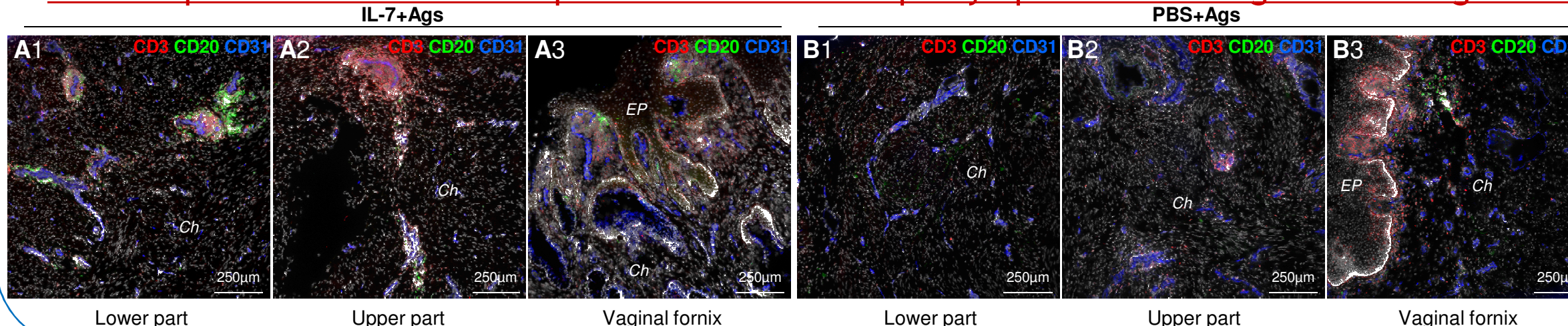


Fig 5: Ectopic lymphoid follicles in vaginal mucosa of sIL-7 treated macaques at necropsy

Sections of monkey vaginal mucosa, lower part (A1, B1), upper part (A2, B2), and vaginal fornix (A3, B3), sampled at necropsy (2 weeks after the 3rd boost) from sIL-7-treated (A, IL-7+Ags) or control (B, PBS+Ags) macaques were labeled for CD3 (Alexa-Fluor[®] 546), CD20 (Alexa-Fluor[®] 488) and CD31 (Alexa-Fluor[®] 633), respectively. Nuclei were stained with DAPI. EP= Pluristratified Epithelium; Ch= Chorion.

CONCLUSION

We here evidenced that 10µg of sIL-7, administered by spray on rhesus macaques vaginal mucosa, stimulates local transcription of CCL2, CCL5, CCL7, CCL17, CCL19, CCL20, CCL21, CCL22, CXCL10, CXCL12, CXCL13, and CX₃CL1 by 48^h after administration. Local expression of these chemokines leads to massive mDC, macrophages, NK, CD4⁺ T-, CD8⁺ T-, and B-cells infiltration into the vaginal mucosa.

Healthy rhesus macaques immunized with a cocktail of antigens (Diphtheria Toxoid + the HIV-1 gp41-P1 peptide) directly applied on the vaginal mucosa two days after vaginal sIL-7 (10µg) administration developed an earlier and stronger mucosal antigen-specific immune response as compared to animals receiving the antigenic cocktail alone. Indeed, during the immunization protocol, sIL-7 pre-treatment promoted then sustained a robust production of antigen-specific IgAs and IgGs in vaginal secretions, as detected by ELISA.

By attracting immune cells, local sIL-7 administration prepares the mucosal immune system, gathering conditions that result in enhanced antigen-specific vaginal immune responses. Moreover, throughout the immunization protocol, CXCL12, CXCL13, CCL19 and CCL21 known to contribute to tertiary lymphoid organ formation and organization (*Nat Immunol* 2006, 7: 344-53), which could support the induction of immune responses, in particular by sustaining productive immunoglobulin class switching in germinal centers, were increased only in the vaginal mucosa of sIL-7 treated macaques, and sIL-7 pretreatment favored the formation of tertiary lymphoid organs genesis in the vaginal mucosa. Besides, high levels of IgAs in the vaginal secretions are produced by mucosally localized plasma cells as evidenced by reverse IHF. CXCL12 is known as mucosal chemoattractant molecule for IgA- and IgG-secreting plasma cells (*J Exp Med* 2002, 196(1):65-75), suggesting its role in mucosal infiltration of DT- and P1-specific plasma cells. Finally, we evidenced an increased frequency of B-cell response in peripheral blood.

Altogether, these data demonstrate the capacity of sIL-7 to be used as a mucosal adjuvant leading to enhanced mucosal antibody response, a very promising strategy to confer protection to numerous sexually transmitted infections.