A High Rate of β7+ Gut-Homing Lymphocytes in HIV-Infected Immunological Nonresponders is Associated With Poor CD4 T-Cell Recovery During Suppressive HAART

Alexandre Girard, PharmD, MSc, Delphine Vergnon-Miszczycha, MD,*†*
Anne-Emmanuelle Depincé-Berger, MD,‡ Xavier Roblin, MD, PhD,*§ Frederic Lutch, MD, PhD,*†*
Claude Lambert, MD, PhD,‡ Nicolas Rochereau, PhD,* Thomas Bourlet, PharmD, PhD,*‖*
Christian Genin, MD, PhD,‡ and Stéphane Paul, PhD*‡*

Objective: Correlation between GALT homing markers on lymphocytes and the low blood CD4 T-cell reconstitution in immunological nonresponders (INRs) has been studied.

Design: Thirty-one INRs, 19 immunological responders (IRs), and 12 noninfected controls were enrolled in this study. INRs were defined by an undetectable plasma viral load RNA less than 40 copies per milliliter and $CD4^+$ T-cell count <500 cells per cubic milliliter in at least 3 years.

Methods: A complete peripheral and mucosal lymphocyte immunophenotyping was performed on these patients with a focus on the CCR9, CCR6, and α4β7 gut-homing markers.

Results: A highly significant upregulation of α4β7 on INRs peripheral lymphocytes compared with that of IRs has been observed. This upregulation impacts different lymphocyte subsets namely $CD4^+$, $CD8^+$, and B lymphocytes. The frequency of $\beta7^+$ Th17 and Treg cells are increased compared with IRs and healthy controls. The frequency of $\beta7^+$ $CD8^+$ T cells in the blood is negatively correlated with integrated proviral DNA in rectal lymphoid cells in contrast to $\beta7^+$ $CD4^+$ T cells associated with HIV integration.

Conclusions: Alteration of lymphocyte homing abilities would have deleterious effects on GALT reconstitution and could participate to HIV reservoir constitution. These results emphasize the great interest to consider α4β7-targeted therapy in INR patients to block homing of lymphocytes and/or to directly impair gp120-α4β7 interactions.

Key Words: INR, HIV, α4β7 integrin, gut homing, lymphocytes

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INTRODUCTION

Intestinal $CD4^+$ T cells are rapidly and profoundly depleted in the peripheral blood of treated individuals with HIV.[1–4] $CD4^+$ T cells of immunological nonresponder (INRs) patients are poorly restored in the blood and also at the mucosal level despite effective highly active antiretroviral therapy (HAART).[1] However, INRs have undetectable plasma residual viremia.[5] Accordingly, AIDS seems to progress faster in INRs compared with immunological responders (IRs) due to chronic immunological activation.[6] During HIV infection, equilibrium between Treg and Th17 lymphocytes must be conserved for avoiding deleterious response.[7] In untreated patients and also in INRs, the levels of activated circulating but not mucosal Tregs increase in comparison to HAART-treated patients.[8,9]

The ability of HIV to bind to α4β7 integrin via gp120 recognition enables to colonize GALT and participate to intestinal reservoir constitution.[10,11] $\alpha4\beta7^+$ $CD4^+$ T cells are very permissive and highly depleted in the early stages of infection and also during infection.[10] A high frequency of blood $CD4^+$ $\beta7^+$ T cells is well correlated with gut and rectal $CD4^+$ $\beta7^+$ T cells. Macaques controlling plasma viremia GALT $\beta7^+$ $CD4^+$ T-cell frequency is only partially restored.[12] The delay of initiate HAART seems to reduce the blood and mucosal immune reconstitution.[13] In INRs, impoverishment of thymus functions might be due to substantial central depletion mechanisms. Residual viral replication, higher immune activation, modulation of galectin-9/TIM3 axis, and decrease in IL7-R expression on naive and thymic $CD4^+$ T cells that is involved in thymocyte development could also participate in CD4 depletion into lymphoid tissues.[14–19] We asked in this study whether

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From the *Groupe Immunité des Muqueuses et Agents Pathogènes, Université de Saint-Etienne, Université de Lyon; †Service de Maladies Infectieuses et Tropicales; ‡Laboratoire d'Immunologie; §Service d'Hépato-gastroentérologie; and ‖Laboratoire de Virologie, Centre Hospitalo-Universitaire Nord, Saint-Etienne, France.
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Correspondence to: Stéphane Paul, PhD, Immunology Laboratory, CIC INSERM 1408, GIMAP EA3064, Campus Santé Innovations–Faculté de Médecine, 10 Rue de la Marandière, BP 80019, 42270 Saint-Priest En Jarez (e-mail: stephane.paul@chu-st-etienne.fr).

intestinal homing alterations on lymphocytes could participate to the low reconstitution observed in INR patients.

RESULTS

Higher α4β7 and CCR9 Expression CD4 Peripheral T Cells in INRs

As expected, the rate of $CD4^+$ T cells in the blood is lower in INRs than in the group of IRs (34.9% ± 12.1% vs 44.9% ± 11.3%, $P = 0.065$) (Fig. 1A) (see Table S1, Supplemental Digital Content, http://links.lww.com/QAI/A787). The frequency of $\beta7^+$ $CD4^+$ T cells is significantly increased in INRs compared with IRs and healthy control (HC) (30.4% ± 10.3% vs 44.9% ± 11.3%, $P < 0.0001$). No significant difference was observed between IRs and HC. Surprisingly, an increase of peripheral $\beta7^+$ $CCR9^+$ $CD4^+$ T cells has been only observed in INRs in contrast to IRs (12.4% ± 6.1% vs 5.3% ± 4.2%, $P < 0.0001$) and HC (12.4% ± 6.1% vs 6.6% ± 3.1%, $P = 0.0009$) (Fig. 1A).

Higher Proportion of Peripheral $\beta7^+$ $CCR5^+$ $CD4^+$ T Cells in INRs

Apparently, INRs are more sensitive to infection with CXCR4 HIV-1 strains (see Table S1, Supplemental Digital Content, http://links.lww.com/QAI/A787).[14] The frequency of peripheral $CXCR4^+$ $CD4^+$ T cells is decreased in INRs compared with IRs (59.8% ± 13.9% vs 69.7% ± 11.3%, $P = 0.0117$), in contrast to $CCR5^+$ $CD4^+$ T cells which are increased in the same level range (27.5% ± 10.9% vs 18.9% ± 11.8%, $P = 0.026$) (see Figure S1A, Supplemental Digital Content, http://links.lww.com/QAI/A787). The frequency of $CCR5^+$ $\beta7^+$ $CD4^+$ T cells increased between INRs and HC, whereas that of $CXCR4^+$ $\beta7^+$ $CD4^+$ T cells did not (see Figure S1A, Supplemental Digital Content,

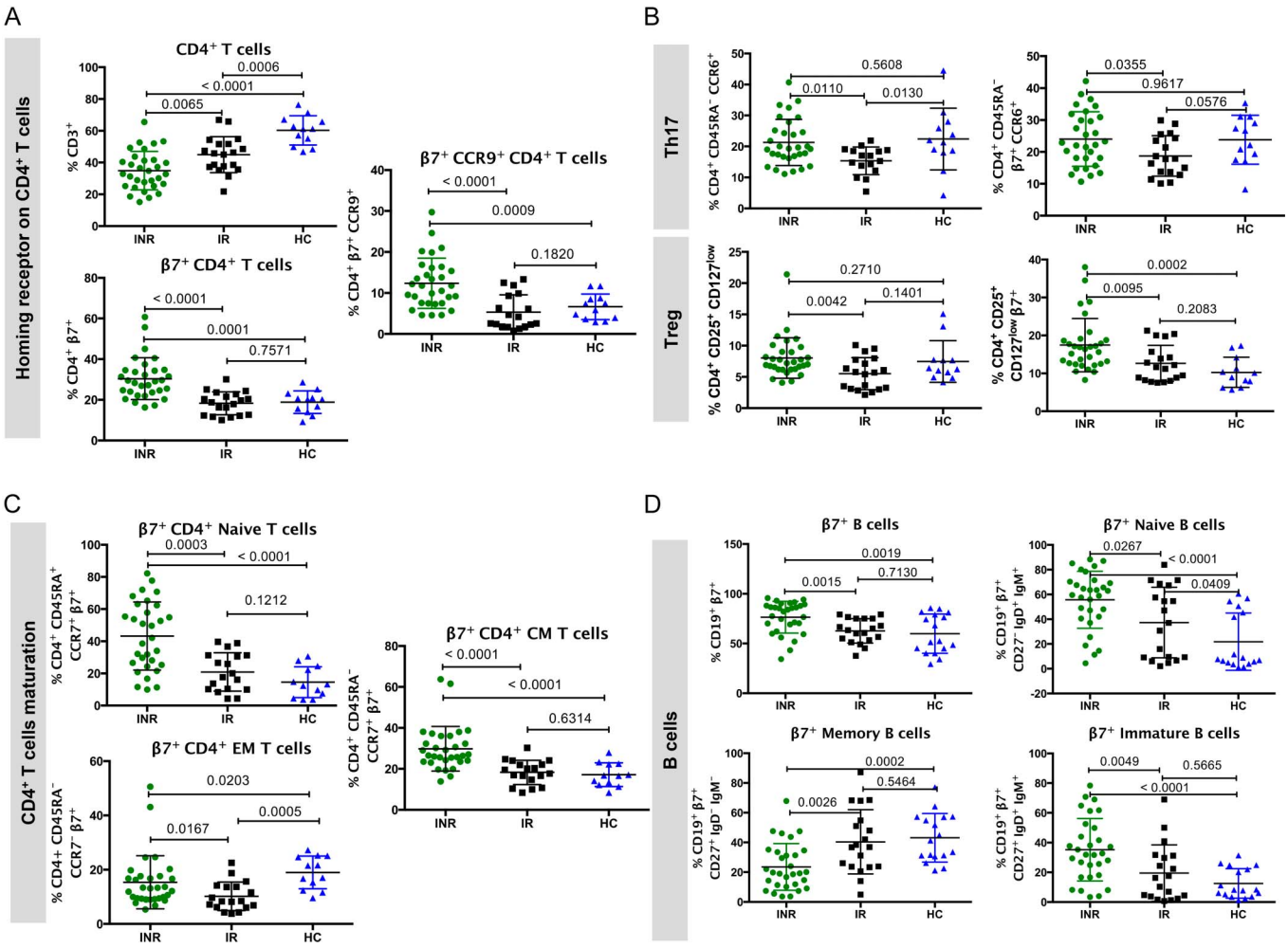

FIGURE 1. Induction of α4β7 gut homing integrin on $CD4^+$, $CD8^+$ T cells, B cells in blood T cells in INRs. A, Comparison of frequencies of $CD4^+$ T cells, $\beta7^+$ $CD4^+$ T cells, and $\beta7^+$ $CCR9^+$ $CD4^+$ T cells between INRs (circles), IRs (squares), and HC (triangles). B, Expression of gut-homing receptors on Th17 and Treg cells in INRs, IRs, and HC. C, Frequencies of $CD4^+$ T-cell maturation in the three groups. D, $\beta7^+$ $CD19^+$ B cells and some steps of B-cell maturation: naive, memory, and immature $\beta7^+$ $CD19^+$ B cells. Each dot represents a single individual. *P* value is indicated (Mann–Whitney test).

http://links.lww.com/QAI/A787). This is consistent with a specific CXCR4+ β7+ CD4+ T cell depletion in INRs by X4 HIV-1 strains[14] or a relative increase in CCR5+ cells in IRs due to immune activation. This would be also associated with a relative decrease in CXCR4+ cells.

Increase of β7+ Th17 and Treg Populations in INR Patients

The ability of Th17 and Treg cells to migrate to the intestine was compared in the different groups (see Table S1, Supplemental Digital Content, http://links.lww.com/QAI/A787). The frequency of INRs peripheral memory CD4+ CCR6+ Th17 is significantly increased compared with that of IRs (21.3% ± 7.4% vs 17.7% ± 6.9%, $P = 0.0110$). No difference was observed between IRs and HC (21.3% ± 7.4% vs 22.4% ± 10%, $P = 0.5608$) (Fig. 1B). INRs peripheral β7+ Th17 cells are increased in contrast to that of IRs (23.5% ± 8.8% vs 19.5% ± 7.0%, $P = 0.0355$). No difference was observed between IRs and HC (23.5% ± 8.8% vs 23.8% ± 7.7%, $P = 0.9617$). Regulatory T-cell frequency, defined as $CD3^+CD4^+CD127^{low}CD25^+$ cells, is also higher in INRs than in IRs (8.0% ± 3.2% vs 5.5% ± 2.5%, $P = 0.0042$). β7 expression on Treg is highly increased in INRs compared with IRs and HC (17.4% ± 7.0% vs 12.6% ± 4.8%, $P = 0,0095$) (Fig. 1B).

Upregulation of α4β7 on Matured CD4+ T Cells in INRs Patients

Frequencies of naive, central memory (CM), and effector memory (EM) CD4+ T cells are not modified between IRs and INRs (data not shown) (see Table S1, Supplemental Digital Content, http://links.lww.com/QAI/A787). By contrast, naive β7+ CD4 T cells are significantly increased in INRs compared with IRs and HC (43.2% ± 21.2% vs 20.9% ± 11.9% and 14.6% ± 9.6%, $P = 0.0003$ and $P < 0.001$), identically, frequencies of CM β7+ CD4 T cells (29.8% ± 10.9% vs 18.3% ± 5.9% and 17.1% ± 5.9%, $P = 0.0001$ and $P = 0.0001$) and EM β7+ CD4 T cells (15.4% ± 9.8% vs 10.2% ± 5.3% and 19.0% ± 6.0%, $P = 0.0167$ and $P = 0.0203$) are higher than IRs and HC (Fig. 1C). The proportion of EM CD4+ T cells in INRs and in IRs patients was lower than in HC, suggesting that CM CD4+ T cells were probably depleted before their maturation.

Higher Proportion of Peripheral β7+ CD8+ T in INR Patients

In HAART-infected patients, an increase in peripheral CD8 T cells and a lower CD4/CD8 ratio are linked to morbidities (see Table S1, Supplemental Digital Content, http://links.lww.com/QAI/A787). The frequency of CD8+ T cells in INRs and IRs was significantly higher than that in HC (55.8% ± 13.3% and 47.7% ± 10.8% vs 29.3% ± 6.9%, $P < 0.001$ and $P < 0.001$, respectively). Furthermore, a higher frequency of CD8+ T cells was also observed in INRs compared with IRs ($P = 0.0252$) (see Figure S2A, Supplemental Digital Content, http://links.lww.com/QAI/A787). The frequency of β7+ CD8+ T cells increase significantly in INRs compared with that in IRs (42.4% ± 14.5% vs 27.2% ± 10.6%, $P = 0.009$) and HC (42.4% ± 14.5% vs 23.4% ± 8.8%, $P = 0.0002$). Finally, we showed that β7+ CM CD8+ T cells and β7+ CD8+ EM T cells are also decreased in INRs compared with IRs (see Figure S2B, Supplemental Digital Content, http://links.lww.com/QAI/A787).

Increase of Peripheral β7+ B Cells May Also Participate to the Emergence of the GALT HIV Persistence in INR Patients

An increase of β7+ CD19+ B cells was also observed in INRs in comparison to IRs and HC, respectively (76.4% ± 16% vs 62.7% ± 12.2% and 59.8% ± 19.7%, $P = 0.015$ and $P = 0.0019$) (Fig. 1D). A higher proportion of β7+ naive B cells ($CD19^+CD27^-IgD^+IgM^+$) was also detected in INRs compared with IRs and HC, respectively (55.7 ± 23.1% vs 37.3% ± 28.4% and 21.9% ± 19.7%, $P = 0.0267$ and $P < 0.0001$) while memory β7+ B cells ($CD19^+CD27^+IgD^-IgM^-$) were decreased in INRs compared with IRs and HC respectively (23.6% ± 15.7% vs 40.4% ± 21.6%, $P = 0.0026$ 23.6% ± 15.7% vs 43.1% ± 16.3%, $P = 0.002$), (Fig. 1D).

GALT β7+ CD4 T Cells Are Highly Depleted in INRs

GALT lymphocyte depletion is severe in HIV-infected individuals and occurs at early stages of infection. HAART is not sufficiently efficient to correct this depletion. Apparently, GALT lymphocyte depletion of IRs and INRs was equivalent since rectal CD4/CD8 T-cell ratio was equivalent[1] (Fig. 2B). In INRs, α4β7 expression was analysed at the same time on peripheral and rectal lymphocytes. Frequencies of GALT CD4+ and CD8+ T cells (Fig. 2B) of IRs and INRs seem similar in the rectum of the patients (27.88% ± 11.89% vs 37.02% ± 15.59%, $P = 0.4394$ and 55.84% ± 8.68% and 51.57% ± 11.325%, $P = 0.3840$). Surprisingly, frequencies of β7+ CD4+ but not β7+ CD8+ T cells appeared significantly lower in INRs than in IRs, respectively (35.54% ± 14.34% vs 52.90% ± 8.5%, $P = 0.0414$ and 65.07% ± 32.72% vs 86.75% ± 6.48%, $P = 0.01807$) (Fig. 2B). Downregulation of α4β7 after T-cell migration has been documented only in mice.[20] In INRs, we observed a significant negative correlation between quantity of GALT proviral DNA integration and blood frequency of β7+ CD8+ ($r = 0.95$, $P = 0.0045$). Conversely, a significant positive correlative between blood β7+ CD4+ T cells and GALT proviral DNA integration was also observed ($r = 0.72$, $P = 0.007$). Negative and positive correlations between rectal β7+ CD8+ and β7+ CD4+ vs proviral DNA integration, respectively, were also observed (Fig. 2C). Most of the peripheral lymphocytes in INRs are able to migrate into GALT and are subsequently depleted by HIV-1 potentially due to affinity maturation of the ability of gp120 to bind to α4β7.

DISCUSSION

To correct deep intestinal CD4+ depletion, we first observed that INRs have a high peripheral α4β7+ CCR9+

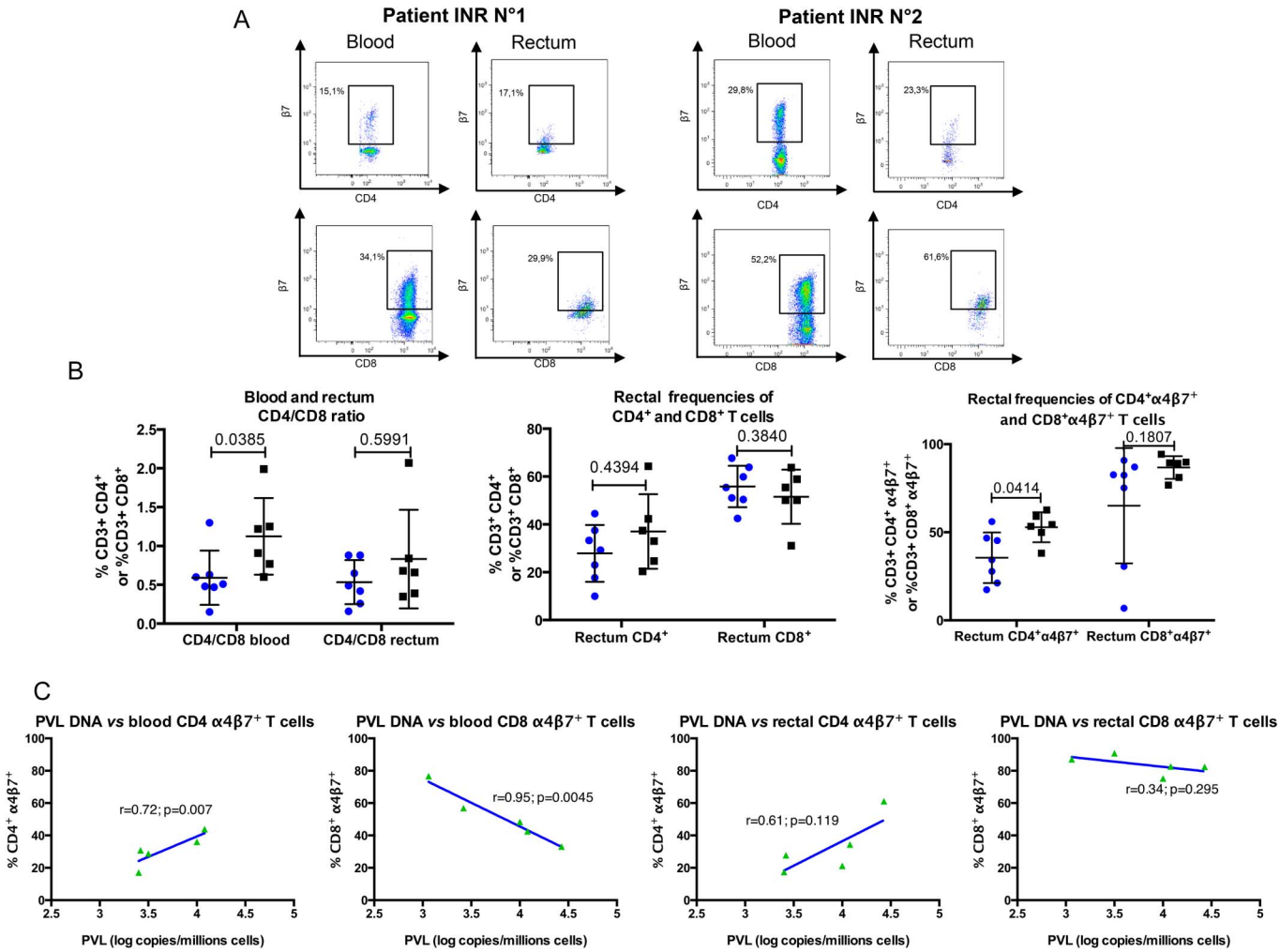

FIGURE 2. Depletion of β7+ T cells in the rectum of INRs. A, Representative figure showing the frequencies of both β7+ CD4+ T cells and β7+ CD8+ T cells in the gut and blood at the same time from 2 representative INRs of the 7 studied. B, *Right panel* CD4/CD8 ratio in the peripheral blood and rectum from 7 INRs (circles) and 6 IRs (squares). *Middle panel* frequency of CD4+ T cells and CD8+ T cells in the rectum from 7 INRs (circles) and 6 IRs (squares). *Left panel* rectal proportion of both β7+ CD4+ and β7+ CD8+ T-cell subsets from 7 INRs (circles) and 5 IRs (squares). *P* value is indicated (Mann–Whitney test). C, Correlation between proviral load (PVL) in the rectal cells and frequencies of β7+ CD4+ T cells and β7+ CD8+ T cells in the gut and blood. Each dot represents a single individual. *P* value is indicated (Mann–Whitney test). Each comparison of the peripheral and rectal compartments was performed at the same time.

CD4+ T-cell levels. Once in the gut, these cells are probably quickly depleted and/or α4β7 expression could be reduced on the surface of intestinal T cells. Chronic HIV+ individuals seem to have a reduction of CCL25 secretion in the small intestine resulting in an increase of α4β7+ CCR9+ CD4+ T cells in the blood and a default of lymphocyte gut homing compared with that of healthy individuals.[21] However, mucosal lymphocyte homing is not only driven by CCR9-CCL25 interactions. More investigations are needed to evaluate the real impact for the lack of CCL25 and its role in homing alterations during infection. Our result could explain in part that GALT recruitment of β7+ CCR9+ CD4 T cells could be involved in perpetual viral stimulation which results in rapid depletion of these cells, low reconstitution, increase of intestinal permeability resulting in microbial translocation, and in the constitution of HIV reservoir observed in INRs.[13,22]

We also show that α4β7 integrin is upregulated on different lymphocytes subsets involved in GALT homeostasis in INRs. Th17 depletion in the gut mucosa after HIV-1 and pathogenic SIV infections could compromise the integrity of the gut mucosal barrier and increasing immune activation, microbial translocation, and disease progression. Th17 CD4+ T cells are highly permissive to HIV/SIV infection, and the β7 CD4+ T cell subset is mainly composed of Th17 cells.[23] CCR6 expression imprints on the Th17 phenotype a great capacity to migrate to Peyer patches.[24] Mucosal homing recetpors on Th17 and Treg cells are stimulate in INRs, but once into GALT

CCR6+ β7+ Th17 are more sensitive to infection and more depleted than Treg,[23] thus disturbing the mucosal Th17/Treg ratio and intestinal homeostasis.

The cytotoxic activity of HIV-specific CD8+ T cells helps in the control of infection in HIV controllers.[25] CD8+ T-cell migration in the proximity of β7+ CD4+ target T cells has been described as being crucial in the control of viral replication via cytotoxic and noncytotoxic mechanisms.[26] Upregulation of β7 on CD8+ T cells in INRs may suggest an increase in trafficking of these cells to GALT which may block HIV replication in CD4+ T cells. Recruitment of HIV-specific CD8+ T cells in the proximity of CD4+ T cells into the *lamina propria* seems to be α4β7+ dependent but not CCR6+ dependent.[25] As gp120-HIV binds to α4β7+ CD8+ T cells, the recruitment of β7+ CD8+ T cells in the GALT could participate to HIV transmission to CD4+ T cells after α4β7/viral particle interaction and could promote infection in INR patients.

The absence or low reconstitution in INRs seems to be associated with a central origin with a defect in thymopoiesis and peripheral phenomena, including excessive destruction of mature CD4+ T cells.[14] Previous study shows that INRs possess less naive and more memory cells without any modification for EM T-cell subset than do IRs.[18] This is consistent with our hypothesis that β7+ CD4+ T cells are depleted after their migration in the GALT. As for CD4, the frequency of naive, CM and EM β7+ CD8+ T cells was higher in INRs than in IRs. In the natural host sooty mangabeys, CD8+ T_{SCM} were preferentially conserved during SIV infection. The CD8+ memory stem T-cell (T_{SCM}) subset were associated with improved prognosis in chronic infection. Their frequency in the blood and the rate of T_{SCM} are inversely correlated with EM CD8+ T-cell and lymphocyte activation, respectively.[27] Here, we show an increase of EM β7+ CD8+ T frequencies in INRs which could result in T_{SCM} loss and potentially explain in part CD8+ T-cell activation.

During the early stages of infection, it has been reported that the decrease of B-cell proliferation in GALT by HIV is mediated by α4β7.[28] In INRs, the majority of B cells expresses a high level of α4β7 integrin and could interact with HIV particles. Decreasing of β7+ memory B cells could be due to downregulation of integrin or α4β7+ mature B-cell recruitment into GALT or the bone marrow. Further investigations will be necessary to clarify this point.

The imprinting of α4β7 may concern all maturation CD4 stages. The mechanisms of aberrant α4β7 expression on CD4, CD8, and B cells in INRs are certainly common. However, a previous study shows that intravenous administration of r-huIL7 in treated HIV-infected individuals with low rates of CD4+ T cells increase circulating α4β7+ CD4+ and α4β7+ CD8+ with a significant recruitment of CD4+ into GALT.[29] At baseline, higher levels of endogenous IL-7 and a lower expression of IL-7 receptor were observed in INRs than in IRs.[18] Previous studies have shown that retinoic acid (RA) produced by CD103+ dendritics cells (DCs) can upregulate α4β7 expression in vitro on T and B cells. In vivo, mucosal CD103+ DCs release RA which imprint α4β7 on the same cells. Moreover, RA regulates expression of MAdCAM-1 on monocytes-derived DC and could promote HIV infection by increasing interactions with α4β7+ CD4+ T cells.[30] Recently, a study also showed that the release of TGF-β by pDC amplifies the differentiation of α4β7+ CCR9+ Treg producing more IL-10 which is involved in immunological tolerance and T-cell suppression.[31] It remains essential to study if the production of RA in INR patients could be involved in the maintenance of a high proportion of α4β7+ memory CD8 and B cells into the GALT. Eradication of *Helicobacter pylori* in INRs seems to facilitate immune reconstitution by reducing T-cell activation, probably also α4β7+ expression on lymphocytes and mucosal RA levels.[32] Mucosal activation of α4β7+ expression in INRs could be involved in a pernicious cycle which promotes the emergence of HIV reservoir. Several anti-α4β7 mAbs have been approved for the treatment of inflammatory bowel disease to reduce recruitment of T cells and inflammation. These antibodies could be useful to block homing of T and B cells into GALT and also block interaction between HIV particles and target α4β7+ CD4+ T cells thus promoting a better reconstitution in INRs. The use of a α4β7+-specific antibody has been recently shown to reduce significantly plasma and tissue viral loads in vivo in a SIV infection.[33] The treatment of monkeys with α4β7 antibody reduces the trafficking of mucosal DC-producing RA in the rectum.[34] According to our results, it will be interesting to reduce gathering of target CD4+ T cells into GALT with α4β7 antibody to prevent proviral DNA integration. An interventional human study in INRs aiming to block α4β7+ lymphocytes homing in GALT should be of interest to increase immune reconstitution.

MATERIALS AND METHODS

Patients

All individuals included in this study had signed their consent. Thirty-one INRs and 19 IRs were enrolled in this study from the Saint-Etienne University Hospital, France. INR patients were defined by an undetectable plasma viral load RNA less than 40 copies per milliliter and CD4+ T-cell count <500 cells per cubic milliliter in at least 3 years as described previously.[35] Samples of healthy controls were obtained from EFS Auvergne Loire, France, and all had been tested negative for HIV infection by reverse transcriptase–polymerase chain reaction. Characteristics of our cohorts are summarized in Table S2 (see Supplemental Digital Content, http://links.lww.com/QAI/A787).

Tissue and Cell Preparation

Patients had an indication for anal cancer screening by rectoscopy. For each patient, 2 EDTA-blood samples and 4 rectal biopsies were collected at the Gastroenterology Unit of the University Hospital of Saint-Etienne. Two biopsies were dedicated to immunophenotypical analysis and were first incubated in RPMI 1640 (PAA, Pasching, Austria), supplemented with 1% of antibiotic (penicillin, 100 U/mL and streptomycin, 100 μg per milliliter, PAA) and 10% of fetal bovine serum (Sigma Aldrich, Saint-Louis). Biopsies were then crushed immediately with a sterile scalpel and applied to

a 50-μm filter (Medico; BD Biosciences, San Jose). Then 1 mL of EDTA/Versene solution (Gibco Life Technologies, Paisley, United Kingdom) was added to the cellular suspension to avoid aggregates. Mechanical disruption and filtration of the suspension were performed with a syringe equipped with a 30-gauge blunt-end needle (Nipro Europe, Zaventem, Belgium) and a sterile filter of 30 μm (BD Biosciences). Then, rectal cells were washed with RPMI fetal calf serum and resuspended in this medium. The quantity of cells was determined by the automated cell counter TC10 (Biorad, Hercules, CA), and the viability was estimated by trypan blue labeling.

Total Cell-Associated HIV-1 DNA Quantification

Total DNA was extracted from peripheral blood mononuclear cells and biopsies using the QIAamp DNA Mini Kit (Qiagen, Courtaboeuf, France), then cell-associated HIV-1 DNA was quantified by using Generic HIV DNA cell (Biocentric, Bandol, France) according to the manufacturer's instructions. The quantification of the glyceraldehyde-3-phosphate dehydrogenase gene was undertaken to estimate the amount of cells tested. Results of total cell-associated HIV-1 DNA were expressed in log of copies per one million of cells.

Flow Cytometer Analysis

The following antibodies were obtained from BD Biosciences (San Diego, CA) CCR9-AF647, CCR6 (CD196)-AF647, CD27-APC-H7, CD38-PeCY5, CD197 (CCR7)-FITC, CD195 (CCR5)-APC-Cy7, Fib 504 (β7)-Pe, IgM-APC, and CXCR4 (CD184)-PE-Cy7. The following antibodies were purchased from Beckman Coulter (TCR PAN γ/δ-PC7, CD25-PC5, CD45RA-PC7, CD5-PC7, IgD-FITC, and IgM-APC), Dako (CD19-pacific blue), or Miltenyi Biotec [CD3-Vioblue, CD4 (VIT4)-VioGreen, CD127-FITC, CD8-APC-Vio770]. Cells were stained using an anti-β7 mAb as a surrogate for α4β7 cells.[21,23] Flow cytometry and data analysis were, respectively, performed on a BD CANTO II (BD Biosciences, San Diego, CA) and FlowJo software (FlowJo LLC, Ashland, OR). Peripheral blood mononuclear cells were stained using antibodies cocktail in 25 minutes at 4°C, and then, red cells were lysed in 10 minutes with Versalyse (Beckman Coulter, Brea, CA). All statistical analyses were performed using the InStat version 5.02 software from GraphPad Software (San Diego, CA).

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REFERENCES

1. Rueda CM, Velilla PA, Chougnet CA, et al. Incomplete normalization of regulatory t-cell frequency in the gut mucosa of Colombian HIV-infected patients receiving long-term antiretroviral treatment. *PLoS One*. 2013;8:e71062.
2. Brenchley JM, Schacker TW, Ruff LE, et al. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med.* 2004;200:749–759.
3. Mehandru S, Poles MA, Tenner-Racz K, et al. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. *J Exp Med.* 2004;200:761–770.
4. Guadalupe M, Reay E, Sankaran S, et al. Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. *J Virol.* 2003;77:11708–11717.
5. Mavigner M, Delobel P, Cazabat M, et al. HIV-1 residual viremia correlates with persistent T-cell activation in poor immunological responders to combination antiretroviral therapy. *PLoS One*. 2009;4:e7658.
6. Kaufmann GR, Furrer H, Ledergerber B, et al. Characteristics, determinants, and clinical relevance of CD4 T cell recovery to <500 cells/microL in HIV type 1-infected individuals receiving potent antiretroviral therapy. *Clin Infect Dis.* 2005;41:361–372.
7. Dandekar S, George MD, Baumler AJ. Th17 cells, HIV and the gut mucosal barrier. *Curr Opin HIV AIDS.* 2010;5:173–178.
8. Suy F, Botelho-Nevers E, Gagneux-Brunon A, et al. Immunologic nonresponders and T-regulatory cells in HIV-1 infection. *AIDS*. 2013;27:2968–2971.
9. Kader M, Wang X, Piatak M, et al. Alpha4(+)beta7(hi)CD4(+) memory T cells harbor most Th-17 cells and are preferentially infected during acute SIV infection. *Mucosal Immunol.* 2009;2:439–449.
10. Arthos J, Cicala C, Martinelli E, et al. HIV-1 envelope protein binds to and signals through integrin alpha4beta7, the gut mucosal homing receptor for peripheral T cells. *Nat Immunol.* 2008;9:301–309.
11. Nawaz F, Cicala C, Van Ryk D, et al. The genotype of early-transmitting HIV gp120s promotes alpha(4)beta(7) -reactivity, revealing alpha(4)beta(7)/CD4 T cells as key targets in mucosal transmission. *PLoS Pathog.* 2011;7:e1001301.
12. Wang X, Xu H, Gill AF, et al. Monitoring alpha4beta7 integrin expression on circulating CD4+ T cells as a surrogate marker for tracking intestinal CD4+ T-cell loss in SIV infection. *Mucosal Immunol.* 2009;2:518–526.
13. Kok A, Hocqueloux L, Hocini H, et al. Early initiation of combined antiretroviral therapy preserves immune function in the gut of HIV-infected patients. *Mucosal Immunol.* 2015;8:127–140.
14. Delobel P, Nugeyre MT, Cazabat M, et al. Naive T-cell depletion related to infection by X4 human immunodeficiency virus type 1 in poor immunological responders to highly active antiretroviral therapy. *J Virol.* 2006;80:10229–10236.
15. Gazzola L, Tincati C, Bellistri GM, et al. The absence of CD4+ T cell count recovery despite receipt of virologically suppressive highly active antiretroviral therapy: clinical risk, immunological gaps, and therapeutic options. *Clin Infect Dis.* 2009;48:328–337.
16. Li T, Wu N, Dai Y, et al. Reduced thymic output is a major mechanism of immune reconstitution failure in HIV-infected patients after long-term antiretroviral therapy. *Clin Infect Dis.* 2011;53:944–951.
17. Massanella M, Negredo E, Perez-Alvarez N, et al. CD4 T-cell hyperactivation and susceptibility to cell death determine poor CD4 T-cell recovery during suppressive HAART. *AIDS*. 2010;24:959–968.
18. Marziali M, De Santis W, Carello R, et al. T-cell homeostasis alteration in HIV-1 infected subjects with low CD4 T-cell count despite undetectable virus load during HAART. *AIDS*. 2006;20:2033–2041.
19. de Kivit S, Lempsink LJ, Plants J, et al. Modulation of TIM-3 expression on NK and T cell subsets in HIV immunological non-responders. *Clin Immunol.* 2015;156:28–35.
20. Gorfu G, Rivera-Nieves J, Ley K. Role of beta7 integrins in intestinal lymphocyte homing and retention. *Curr Mol Med.* 2009;9:836–850.
21. Mavigner M, Cazabat M, Dubois M, et al. Altered CD4+ T cell homing to the gut impairs mucosal immune reconstitution in treated HIV-infected individuals. *J Clin Invest.* 2012;122:62–69.
22. Ciccone EJ, Read SW, Mannon PJ, et al. Cycling of gut mucosal CD4+ T cells decreases after prolonged anti-retroviral therapy and is associated with plasma LPS levels. *Mucosal Immunol.* 2010;3:172–181.
23. Monteiro P, Gosselin A, Wacleche VS, et al. Memory CCR6+CD4+ T cells are preferential targets for productive HIV type 1 infection regardless of their expression of integrin β7. *J Immunol.* 2011;186:4618–4630.
24. Wang C, Kang SG, Lee J, et al. The roles of CCR6 in migration of Th17 cells and regulation of effector T-cell balance in the gut. *Mucosal Immunol.* 2009;2:173–183.

25. Wacleche VS, Chomont N, Gosselin A, et al. The colocalization potential of HIV-specific CD8(+) and CD4(+) t-cells is mediated by integrin beta7 but not CCR6 and regulated by retinoic acid. *PLoS One*. 2012;7:e32964.
26. Migueles SA, Osborne CM, Royce C, et al. Lytic granule loading of CD8+ T cells is required for HIV-infected cell elimination associated with immune control. *Immunity*. 2008;29:1009–1021.
27. Ribeiro SP, Milush JM, Cunha-Neto E, et al. The CD8(+) memory stem T cell (T(SCM)) subset is associated with improved prognosis in chronic HIV-1 infection. *J Virol.* 2014;88:13836–13844.
28. Jelicic K, Cimbro R, Nawaz F, et al. The HIV-1 envelope protein gp120 impairs B cell proliferation by inducing TGF-beta1 production and FcRL4 expression. *Nat Immunol.* 2013;14:1256–1265.
29. Sereti I, Estes JD, Thompson WL, et al. Decreases in colonic and systemic inflammation in chronic HIV infection after IL-7 administration. *PLoS Pathog.* 2014;10:e1003890.
30. Guerra-Perez N, Frank I, Veglia F, et al. Retinoic acid imprints a mucosal-like phenotype on dendritic cells with an increased ability to fuel HIV-1 infection. *J Immunol.* 2015;19:2415–2423.
31. Bakdash G, Vogelpoel LT, van Capel TM, et al. Retinoic acid primes human dendritic cells to induce gut-homing, IL-10-producing regulatory T cells. *Mucosal Immunol.* 2014;8:265–278.
32. Magen E, Elbirt D, Agmon-Levin N, et al. Eradication of Helicobacter pylori can facilitate immune reconstitution in HIV-1-infected immunological non-responders. *Int J Infect Dis.* 2010;14: e322–e327.
33. Byrareddy SN, Kallam B, Arthos J, et al. Targeting alpha4beta7 integrin reduces mucosal transmission of simian immunodeficiency virus and protects gut-associated lymphoid tissue from infection. *Nat Med.* 2014; 20:1397–1400.
34. Kwa S, Kannanganat S, Nigam P, et al. Plasmacytoid dendritic cells are recruited to the colorectum and contribute to immune activation during pathogenic SIV infection in rhesus macaques. *Blood*. 2011;118: 2763–2773.
35. Horta A, Nobrega C, Amorim-Machado P, et al. Poor immune reconstitution in HIV-infected patients associates with high percentage of regulatory CD4+ T cells. *PLoS One*. 2013;8:e57336.