



Etude de la biologie des lymphocytes B et du mécanisme d'action du vedolizumab dans la rectocolite hémorragique et au cours de l'infection chronique à VIH

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UMR1149, « Inflammation Intestinale »

Mehandru Lab, Immunology Institute at Mount Sinai

**Etude de la biologie des lymphocytes B et du
mécanisme d'action du vedolizumab dans la
rectocolite hémorragique et au cours de l'infection
chronique à VIH**

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Thèse de doctorat d'Immunologie

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Titre : Etude de la biologie des lymphocytes B et du mécanisme d'action du vedolizumab dans la rectocolite hémorragique et au cours de l'infection chronique à VIH

Résumé : La rectocolite hémorragique (RCH) est une maladie inflammatoire chronique intestinale (MICI) qui est caractérisée par l'inflammation chronique du rectum et/ou du côlon. Bien que l'idée qu'il existe une réponse immunitaire exagérée contre le microbiote intestinal soit communément acceptée, la physiopathologie est encore insuffisamment connue. Parmi les dysfonctions immunitaires, il existe des arguments en faveur d'un dérèglement de la biologie des lymphocytes B. A l'aide de plusieurs techniques (dont la cytométrie en flux, le *single-cell RNA sequencing* et le *single-cell BCR sequencing*), nous avons mis en évidence des modifications marquées des populations plasmocytaires coliques chez des patients avec RCH, dont une augmentation de l'expression des IgG et IgA1 et une diminution en IgA2 associées à l'accroissement de la proportion des plasmocytes à durée de vie courte. Ce *turnover* accru se reflétait dans la circulation sanguine par l'élévation des plasmablastes $\beta 7^+$ à tropisme intestinal, associé à l'activité de la maladie et à la survenue de complications. Notre second travail s'intéressait au mécanisme d'action du vedolizumab (anti- $\alpha 4\beta 7$), traitement couramment utilisé dans les MICI et prometteur pour le VIH. Dans une cohorte de patients atteints à la fois de MICI et du VIH, nous avons mis en évidence un impact majeur sur les follicules lymphoïdes intestinaux avec une absence d'effet significatif sur la fréquence des lymphocytes T effecteurs de la lamina propria. Cette découverte, décrite pour la première fois chez l'homme, ouvre de nouvelles perspectives quant au mécanisme d'action du vedolizumab dans les MICI et le VIH.

Mots clés : Maladie inflammatoire chronique intestinale, Rectocolite hémorragique, VIH, lymphocytes B

Title: Characterizing the B cell response and understanding the mechanism of action of vedolizumab in ulcerative colitis and chronic HIV-1 infection

Abstract: Ulcerative Colitis (UC) is an inflammatory bowel disease (IBD) that leads to chronic inflammation of the rectum and the colon. Even though it is commonly accepted that it results from an exaggerated immune response toward the gut microbiota, the pathophysiology is still not fully understood. Among immune defects, many evidences exist supporting a disturbed B cell system, including the presence of (auto-)antibodies and the infiltration of the lamina propria by plasma cells. Using multiple advanced techniques including single-cell RNA sequencing and single-cell BCR sequencing we extensively characterized the B cell compartment in the blood and the intestinal mucosa of UC patients. We found that the colonic plasma cells phenotype was skewed toward an increased expression of IgG and IgA1 and an increased proportion of short-lived plasma cells. This increased turnover was reflected in the blood by the expansion of $\beta 7^+$ plasmablasts, which correlated with disease activity and predicted disease course. Our second work focused on the mechanism of action of vedolizumab, a monoclonal antibody targeting the $\alpha 4\beta 7$ integrin, for both IBD and HIV. In a unique cohort of patients with concomitant IBD and HIV, we unexpectedly found that memory T cells within the lamina propria were not significantly affected. Conversely, lymphoid aggregates, mostly in the terminal ileum were massively impacted. These findings are being further explored and may change the paradigm regarding the mechanism of action of vedolizumab.

Keywords: Inflammatory bowel disease, Ulcerative colitis, HIV, B cells

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LISTE DES ABBREVIATIONS

ASCA - *Anti-saccharomyces cerevisiae antibody*
ANCA - *Anti-neutrophil cytoplasmic antibody*
BAFF - *B-cell activating factor*
BCR - *B-cell receptor*
CARD9 - *Caspase recruitment domain-containing protein 9*
CMH - *Complexe majeur d'histocompatibilité*
CRP - *C-reactive protein*
CXCR4 - *CXC chemokine receptor 4*
FoxP3 - *Forkhead box P3*
GALT - *Gut-associated lymphoid tissue*
GM-CSF - *Granulocyte macrophage colony-stimulating factor*
GWAS - *Genome-wide association study*
HEV - *High endothelial venule*
Hp - *Helicobacter pylori*
ICAM-1 - *Intercellular adhesion molecule-1*
IFN γ - *Interferon γ*
Ig - *Immunoglobuline*
IL - *Interleukine*
ILC - *Innate lymphoid cell*
KO - *Knock out*
MAdCAM-1 - *Mucosal addressin cellular adhesion molecule-1*
MAMP - *Microbe-associated molecular pattern*
MICI - *Maladie inflammatoire chronique intestinale*
NLR - *Nod-like receptor*
NOD2 - *Nucleotide-binding oligomerization domain-containing protein 2*
RCH - *Recto-colite hémorragique*
ROR γ - *RAR-related orphan receptor γ*
SIV - *Simian immunodeficiency virus*
TGF β - *Transforming growth factor β*
TLR - *Toll-like receptor*
TNF α - *Tumor necrosis factor α*
VIH - *Virus de l'immunodéficience humaine*

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INTRODUCTION

Au cours de ces dernières décennies, de nombreux travaux de recherches ont permis d'identifier des facteurs impliqués dans la physiopathologie des maladies inflammatoires chroniques intestinales (MICI). Parmi ces facteurs, on peut citer : l'environnement au sens large avec le microbiote, des susceptibilités génétiques et une réponse immunitaire défectueuse. La découverte de facteurs mis en cause dans les MICI a été réalisée grâce à différentes modalités allant de la recherche épidémiologique à la recherche fondamentale en biologie cellulaire et/ou moléculaire.

Du fait de leur complexité et de leur présentation sous des formes cliniques variées, aucune étiologie uniciste ne peut être avancée pour expliquer la survenue de l'ensemble des MICI. De nombreux dysfonctionnements ont été décrits et certains ne concernent que des sous groupes de patients. La recherche tend par ailleurs de plus en plus à décortiquer la physiopathologie des patients avec MICI à l'échelle individuelle en ayant pour objectif d'aboutir à un traitement ciblé adapté à chaque patient.

La majorité des lymphocytes de l'organisme se situe au niveau du tractus gastro-intestinal. Ils se trouvent au niveau des sites effecteurs (comme la lamina propria) et/ou au niveau des sites inductifs (comme les plaques de Peyer) (1, 2). Ainsi, en plus d'être à un carrefour clé dans le dialogue entre l'hôte et le microbiote via entre autres le système immunitaire, les intestins constituent une cible privilégiée de l'infection à VIH. Outre la présence importante de lymphocytes étant la cible du virus, il s'y trouve des conditions favorables au développement et à la prolifération du virus comme la concentration élevée d'acide rétinoïque.

Au cours de cette introduction seront tout d'abord exposées les connaissances actuelles quant à la compréhension de la physiopathologie des MICI, ayant trait principalement à l'immunologie. Nous aborderons ensuite les thématiques en lien avec le projet de thèse que sont : 1) la biologie des lymphocytes B au cours des MICI ; 2) le *trafficking* intestinal et son implication dans les stratégies thérapeutiques mises en place dans les MICI et au cours de l'infection à VIH.

LA PHYSIOPATHOLOGIE DES MICI : ETAT DES LIEUX

Les pistes immunologiques et environnementales fournies par l'épidémiologie

Les MICI sont devenues des maladies à forte prévalence dans le monde occidental au cours du 20^e siècle. L'incidence des MICI augmente drastiquement en parallèle avec le développement industriel et l'occidentalisation, ce que l'on constate aujourd'hui dans des pays émergents comme l'Inde. Ainsi, le mode de vie moderne a été incriminé de manière empirique pour expliquer cet accroissement de l'incidence des MICI (3, 4). Des facteurs liés au mode de vie occidental, comme l'altération du microbiote intestinal, le régime alimentaire, le tabac, les antibiotiques ou encore la vitamine D, ont un impact démontré sur l'immunité systémique et intestinale suggérant fortement l'implication d'une réponse immunitaire défectueuse dans la physiopathologie des MICI.

Le risque de MICI augmente en cas d'exposition précoce aux antibiotiques (5). Le tabagisme et la faible exposition solaire sont également associés à la maladie de Crohn (6). Des données épidémiologiques montrent une relation claire entre la diminution des maladies infectieuses, d'un environnement pauvre en parasite, de l'usage des antibiotiques et d'une amélioration globale des conditions sanitaires avec l'augmentation de l'incidence des maladies auto-immunes et inflammatoires chroniques (7). Ces constats soutiennent la théorie hygiéniste qui se justifie également par le rôle primordial qu'a le microbiote à éduquer le système immunitaire dès la naissance. Allant dans le sens de la théorie hygiéniste, des études ont montré une corrélation inverse entre la colonisation par *Helicobacter pylori* (*Hp*) et le risque d'asthme dans l'enfance (8). Oertli et al. ont étudié plus précisément le mécanisme dans un modèle murin d'asthme allergique. Ils ont

constaté que l'infection par *Hp* durant la période néonatale entraînait la différenciation de cellules dendritiques vers un profil tolérogéniques au niveau de la muqueuse gastrique. Ceux-ci produisent de l'IL-18 induisant un phénotype T régulateur (Treg) et ont *in fine* un effet protecteur sur la survenue de l'asthme (9). Cette théorie hygiéniste peut aussi s'appliquer aux MICI et notamment à travers le lien bien établi entre un microbiote perturbé et leur survenue (10). L'ensemble de ces données brièvement exposées pointent ainsi vers un rôle central du système immunitaire, de sa base génétique et de son interaction avec l'environnement, dans la physiopathologie des MICI.

Les susceptibilités génétiques et l'immunité dans les MICI

En plus d'une association entre certains polymorphismes et la survenue d'une MICI, des variants sont associés à certains phénotypes de la maladie. Par exemple, des variants dans le gène codant pour NOD2 (*nucleotide-binding oligomerization domain-containing protein 2*) sont associés à une atteinte iléale de la maladie de Crohn et à un âge plus précoce de diagnostic (11). La localisation colique et particulièrement les formes sévères et étendues de RCH sont associées à des variants du gène du complexe majeur d'histocompatibilité (CMH) (11–13).

De manière intéressante, l'association NOD2-maladie de Crohn iléale se retrouve uniquement chez des patients d'origine européenne ou Juive mais pas chez des patients d'ascendance Japonaise ou chinoise (14, 15). Cette observation souligne la complexité des facteurs génétiques prédisposant aux MICI et aux multiples voies de l'inflammation potentiellement impliquées. Ces dernières années, les *genome-wide association studies* (GWAS) ont permis de révéler de nombreux (>200) variants génétiques rares associés

aux MICI (16). La plupart sont communs entre la RCH et la maladie de Crohn. Nombre d'entre eux soulignent le rôle clé du système immunitaire dans la physiopathologie des MICI. Des variants associés aux MICI existent entre autres pour les gènes IL23R et IL10R. Ces loci sont notamment enrichis pour des gènes impliqués dans les déficits immunitaires primitifs, la fonction des lymphocytes T, la production cytokinique ou encore les infections par mycobactéries (17).

Le rôle du microbiote intestinal

Le microbiote et le système immunitaire

Le tractus gastro-intestinal contient la plus grande communauté microbienne de l'organisme. Des études de métagénomiques ont révélé qu'un nombre important de gènes microbiens modulaient de manière significative l'expression génétique de l'hôte (18). Des événements survenant précocement après la naissance, comme le mode de délivrance, le type d'allaitement, la nourriture ou l'hygiène, ont un impact majeur sur la constitution du microbiote dans l'enfance (19) alors que celui-ci est plus stable à l'âge adulte. La colonisation intestinale initiale par le microbiote est essentielle au bon développement du système immunitaire (20) et permet d'établir à la fois une tolérance immunitaire et une immunité protectrice. Les MAMP (*microbe-associated molecular patterns*) sont reconnus par les cellules de l'immunité innée via des récepteurs comme les TLR (*Toll-like receptors*) ou les NLR (*Nod-like receptor*). Ces interactions constituent un processus primordial de l'homéostasie intestinale (21). Le maintien de cette homéostasie est garanti par un contrôle bilatéral entre l'hôte et le microbiote. Le microbiote est contrôlé par la barrière intestinale constituée de produits des cellules épithéliales et/ou

des cellules immunitaires, comme le mucus, les défensines et d'autres peptides antimicrobiens comme RegIII γ ainsi que les immunoglobulines sécrétées (principalement les IgA). Inversement, l'immunité intestinale est stimulée et influencée par le microbiote. Par exemple, les *segmentous filamentous bacteria*, les *Clostridia* et les *Bacteroides fragilis* promeuvent respectivement l'induction d'une réponse Th17, T régulatrice et Th1 (22).

Les altérations du microbiote dans les MICI

Il est communément admis que les patients atteints de MICI présentent une réponse immunitaire exagérée contre des antigènes du microbiote intestinal. La présence d'anticorps sériques circulants peut en être le témoin. Ils peuvent être dirigés contre divers éléments dont *Saccharomyces cerevisiae*, OmpC (*outer membrane protein C*) d'*Escherichia coli*, des antigènes de flagellines ou encore *Pseudomonas fluorescens* (23, 24). Il est également trouvé des anticorps dirigés contre les glycanes, qui sont des constituants communs des cellules de microorganismes (25). Il a également été montré *ex vivo* que les lymphocytes T CD4 périphériques prélevés chez des patients MICI et activés par des antigènes bactériens du microbiote s'orientaient exagérément vers le phénotype Th17 (26).

Chez les patients souffrant de RCH et de maladie de Crohn, de nombreuses altérations quantitatives et qualitatives du microbiote ont été décrites (27, 28). Les principales modifications décrites sont une réduction de la diversité, une augmentation de l'abondance des *Bacteroides* et des *Proteobacteria* et une réduction des *Firmicutes* (29, 30). Par ailleurs certains germes ont été incriminés comme pouvant avoir un rôle potentiellement pathogénique dans la maladie de Crohn comme des mycobactéries

atypiques, des *Campylobacter*, des *Helicobacter* ainsi que l'*E. Coli* adhérent et invasif (29).

Inversement, la diminution de microorganismes ayant des propriétés « anti-inflammatoires » a également été décrite. Bien qu'initialement décrite dans la maladie de Crohn (31), une réduction de *Faecalibacterium prausnitzii* est également noté dans la RCH (32). Son effet anti inflammatoire est médié par une molécule du quorum sensing, une homosérine lactone, la 3-oxo-C12 :2 (33) et son abondance prédit le risque de rechute post-résection iléocæcale ou après arrêt d'un traitement par anti-TNF dans la maladie de Crohn (31, 34). Il a été démontré chez d'autres bactéries un effet protecteur. Par exemple, le polysaccharide A, un produit de *Bacteroides fragilis*, peut limiter la production d'IL-17 et avoir un effet bénéfique sur des modèles murins de colite (29).

Par ailleurs, il a été rapporté que la composition globale du microbiote pouvait être prédictive dans des situation particulières au cours des MICI, comme la survenue de pouchite après colectomie totale dans la RCH (35) ou la récurrence post-opératoire après résection iléocæcale dans la maladie de Crohn (36). Le microbiote pourrait également constitué un outil pour la prédiction de la réponse au traitement (37).

En plus de perturbations du microbiote bactérien, des études se sont récemment penchées sur la composition des microbiotes fongique et viral et ont mis en évidence des altérations chez les patients ayant une MICI par rapport aux contrôles sains (38, 39).

Il a également était décrit un impact des mutations génétiques de l'hôte sur la composition du microbiote. Ainsi, Lamas et al. ont décrit que les souris CARD9 KO avaient des altérations des microbiotes bactérien et fongique. Celui-ci avait alors des propriétés pro-inflammatoires. Ainsi des souris *germ-free*, transplanté avec le microbiote

CARD9 KO, présentaient une susceptibilité à la colite pouvant s'expliquer par l'altération de la métabolisation du tryptophane (40).

Le microbiote et la physiopathologie des MICI

Une question est récurrente dans l'étude du microbiote dans les MICI : les perturbations constatées peuvent-elles induire/aggraver l'inflammation intestinale ou sont-elles seulement consécutives à l'inflammation intestinale?

De plus en plus d'études établissent un lien potentiel de causalité. Ainsi, une composition particulière du microbiote pourrait induire un profil immunologique plus inflammatoire au niveau de la muqueuse intestinale favorisant la survenue d'une MICI. Une étude récente de Britton et al. a analysé l'induction d'une réponse immunitaire au niveau de la muqueuse intestinale de souris *germ-free* après transplantation de bactéries issues de selles de patients ayant une MICI ou de personnes saines. Les auteurs ont constaté que les bactéries du microbiote de MICI induisaient une réponse davantage « pro-inflammatoire » avec une fréquence accrue de Th17 et une diminution des Treg *antigen-specific* double positifs pour les facteurs de transcription ROR γ et FOXP3, au niveau de la muqueuse intestinale. Cela se traduisait par un effet délétère de la flore de MICI dans un modèle de souris gnotobiotique avec induction de colite (*adoptive T-cell transfer model*) (10) (figure 1).

De manière similaire la MECONIUM study a étudié le microbiote de nouveau-nés et de leurs mères ayant (ou non) une MICI. Une différence a été constatée au niveau de la composition du microbiote ainsi que dans sa capacité à induire une réponse immunitaire B mémoire et IgA au niveau de la muqueuse colique chez des souris *germ-free* transplantées (41) .

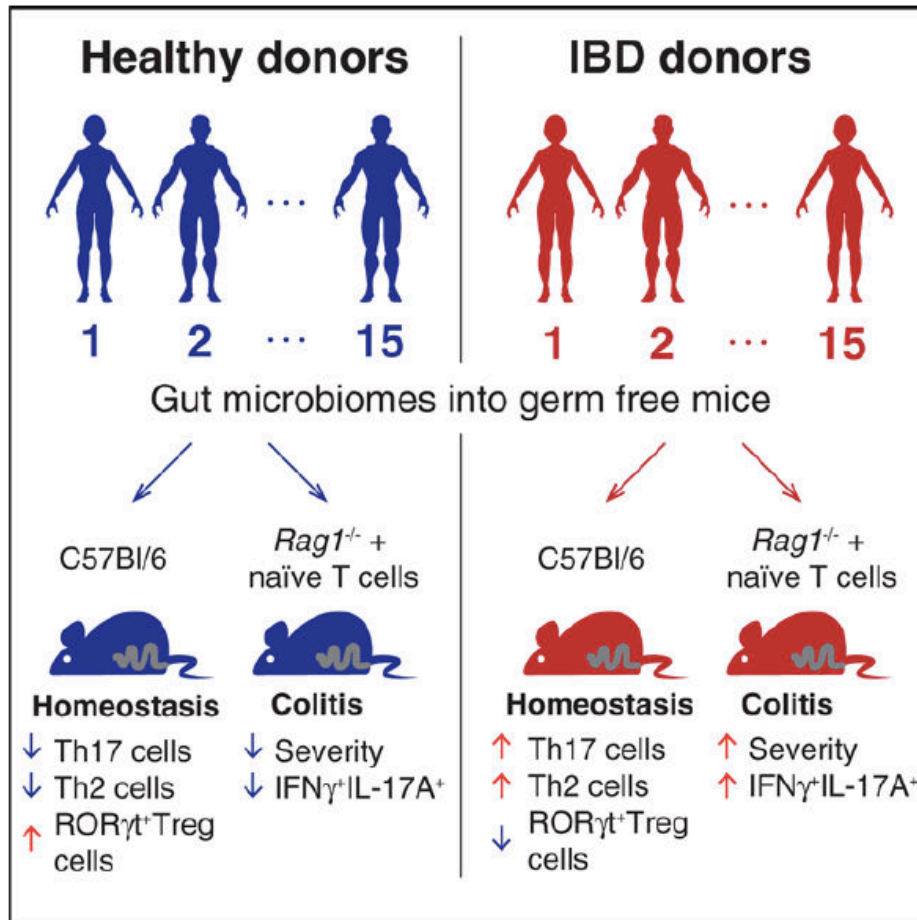


Figure 1 - Effet de la transplantation de microbiote provenant de sujets sains ou de sujets avec MICI chez des souris *germ-free* avec et sans modèle de colite (D'après Britton et al. (10))

Historiquement, il existe un argument physiologique fort incriminant le microbiote intestinal comme facteur causal. En cas de dérivation du flux fécal dans la maladie de Crohn, les segments distaux sont indemnes d'inflammation, qui réapparaît après ré-institution des selles du segment d'amont (42).

Finalement, les stratégies thérapeutiques par transplantation de microbiote servent également d'arguments en faveur d'une causalité. A ce jour, il existe des résultats contradictoires mais prometteurs dans la RCH. L'efficacité semble notamment liée à

l'intensité et à la fréquence des transplantations ainsi qu'à la qualité et à la composition des selles transplantées, dépendant du donneur (43).

Ainsi, le microbiote et le système immunitaire de l'hôte ont une relation complexe et clé dans les MICI. Ils s'influencent mutuellement. Mieux appréhender cette complexité pourrait aboutir au développement de nouvelles stratégies thérapeutiques.

Les dysfonctions des cellules immunitaires dans les MICI

Le système immunitaire est particulièrement influencé par le fond génétique de l'hôte et par le microbiote et se situe à leur intersection. Ses dysfonctions dans les MICI font l'objet de nombreuses recherches et sont la cible des principaux traitements jusqu'à présent utilisés.

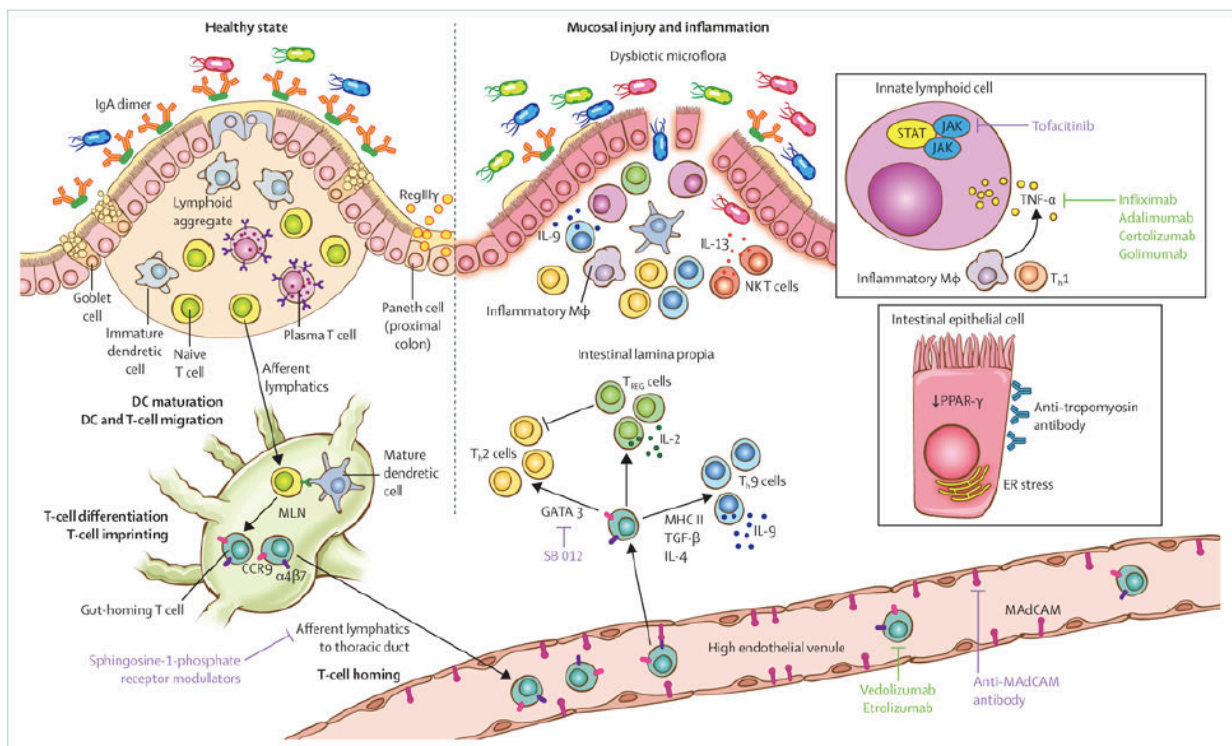


Figure 2 - Immunité intestinale en situation physiologique et au cours de l'inflammation intestinale dans la RCH avec potentielles cibles thérapeutiques (D'après Ungaro et al. (30)).

L'immunité innée

Bien que l'attention principale se focalise sur les lymphocytes T auxiliaires, de nombreuses dysfonctions des cellules myéloïdes ont également été décrites. L'immunité innée constitue la première ligne de défense contre les agressions et est médiée par divers types de cellules dont les polynucléaires neutrophiles, les cellules dendritiques, les monocytes et les macrophages mais aussi par des cellules non immunes comme les cellules épithéliales ou mésenchymateuses. L'infiltration par les polynucléaires neutrophiles constitue notamment le signe évocateur de la présence d'une inflammation active. Les neutrophiles participe à l'inflammation dans les MICI à travers de nombreux mécanismes dont la destruction tissulaire via des dégâts oxydatifs ou protéolytiques, et le largage de nombreux médiateurs de l'inflammation (44). Soulignant le rôle complexe des polynucléaires neutrophiles dans l'homéostasie intestinale, un défaut de leur fonction dans la granulomatose chronique aseptique conduit à des atteintes digestives inflammatoires chroniques dont le tableau clinique peut se confondre avec celui des MICI (45).

Les macrophages sont des phagocytes et des cellules présentatrices d'antigènes extrêmement adaptés au tissu dans lequel ils résident (46). Au niveau de la muqueuse intestinale, ce sont des cellules hautement tolérogènes, évitant une réponse exagérée envers le microbiote (47). Les connaissances actuelles concernant les macrophages (intestinaux) et leur ontogénie provient essentiellement d'études murines. Ces études ont notamment révélé que les macrophages de tissus autres que l'intestin, proviennent typiquement de précurseurs fœtaux issus de la vésicule vitelline et/ou du foie. Les macrophages intestinaux sont quant à eux constamment renouvelés à partir du pool de

monocytes Ly6C^{high} circulant et rejoignent la muqueuse intestinale grâce à CCR2 (48). Les monocytes ainsi fraîchement arrivés sont influencés par le microenvironnement tissulaire local pour se différencier en macrophages résidents tolérogènes (49). Les macrophages matures maintiennent ainsi l'homéostasie locale via une hyporéactivité face aux stimulations microbiennes, à la prise en charge de débris tissulaires et microbiens mais aussi via leur production de cytokines anti-inflammatoires comme l'IL-10 (49). Chez la souris, il a été noté un accroissement du recrutement de monocytes circulants ainsi qu'une abrogation de leur différenciation vers un profil tolérogène au cours de modèles de colites (50). De manière similaire chez l'homme, Bernardo et al. ont constaté à la fois dans la RCH et la maladie de Crohn actives, qu'il y avait une augmentation de l'infiltration intestinale par des monocytes-macrophages (CD11c^{high}CCR2⁺CX3CR1⁺) ayant un phénotype proche des monocytes circulants et produisant de l'IL-1 β . A contrario, les macrophages résidents intestinaux matures (CD11c⁻CCR2⁻CX3CR1⁻) au profil tolérogène et produisant de l'IL-10 étaient diminués au cours des MICI (51).

Les cellules dendritiques sont des cellules spécialisées dans la présentation antigénique et ont un rôle clé dans l'initiation des réponses immunes adaptatives ainsi que dans l'induction de la tolérance périphérique. Classiquement, elles captent les antigènes de leur microenvironnement local, qu'elles présentent aux lymphocytes T naïfs et fournissent des signaux orientant leur différenciation et le type de réponse immunitaire (52). Leurs fonctions les placent à une position clé à l'interface entre immunité innée et adaptative. Les cellules dendritiques de la muqueuse intestinale ont la capacité unique d'interagir avec une multitude de types cellulaires dont les lymphocytes T et B, les cellules épithéliales et stromales et ont ainsi un rôle central dans le maintien de

l'homéostasie intestinale (53). En situation physiologique, le dialogue entre l'épithélium et les cellules dendritiques promeut un environnement non inflammatoire (54). Il a été montré dans les MICI que les cellules dendritiques intestinales avaient un profil plus inflammatoire avec notamment une augmentation de l'expression des TLR2 et TLR4. De plus Hart et al. ont montré que les cellules dendritiques intestinales de patients avec MICI exprimaient plus fortement CD40 et produisaient davantage d'IL-12 et d'IL-6 que les contrôles sains (55).

Ainsi, les données existantes témoignent d'une infiltration accrue de la muqueuse intestinale inflammée au cours des MICI par des cellules myéloïdes dont les macrophages et les cellules dendritiques ayant un profil inflammatoire *a contrario* de la situation physiologique où leur rôle est essentiel dans le maintien de l'homéostasie intestinale.

Le rôle des lymphocytes

Les lymphocytes T effecteurs

Les lymphocytes T auxiliaires sont intrinsèquement plastiques et s'adaptent à leur environnement (56). Pour maintenir un état d'équilibre face à un contexte changeant et modifié par de nombreux facteurs, comme l'âge, le régime alimentaire ou encore la rencontre de nouveaux microorganismes, l'adaptation à l'hôte et à son environnement est une caractéristique essentielle. Un défaut d'adaptation - une « rigidité » biologique - peut ainsi être à l'origine d'une réponse immunitaire inadaptée et d'une inflammation chronique (57). Historiquement, les dysfonctions immunitaires de la maladie de Crohn et de la RCH ont été définies par le paradigme Th1 / Th2. Ainsi, la maladie de Crohn était considérée comme Th1-médiée avec une prépondérance de cytokines comme l'IL-12 et

l'IFN- γ , alors que la RCH était vue comme une maladie Th2 atypique avec une élévations de l'IL-5 et de l'IL-13 sans élévation de l'IL-4 (17). Ce concept s'est par la suite complexifié depuis la découverte de la forte plasticité des populations de lymphocytes T CD4 auxiliaires. Il a notamment été découvert le rôle prépondérant des Th17 dans le maintien de l'intégrité intestinale mais également dans la physiopathologie des MICI, ainsi que leur proximité non seulement avec les autres types de lymphocytes T auxiliaires mais aussi avec les lymphocytes T régulateurs (58). Ce qui était vu comme des modèles typiquement Th1 de MICI, correspondait certainement à des modèles reposant principalement sur les lymphocyte T produisant l'IL-17 et régulés par l'IL-23 (59). De plus, ajoutant encore de la complexité, Becker et al. ont montré à partir de modèles murins, que l'IL-23 pouvait réguler l'IL-12, au cours de colites à acide trinitrobenzène sulfonique (TNBS) (60).

L'IL-22 a été décrite comme augmentée dans les lésions de maladie de Crohn mais diminuée au cours de la RCH (61). L'IL-22 semble jouer un rôle protecteur contre l'inflammation intestinale au niveau colique (62).

Par ailleurs, les Th17 et l'IL-17 n'ont pas qu'un impact néfaste dans les MICI. Ils jouent un rôle important pour éliminer les microorganismes pathogéniques au niveau de la muqueuse intestinale et limite ainsi l'inflammation médiée par ces germes. L'essai clinique testant un anticorps monoclonal ciblant l'IL-17 n'a pas montré d'efficacité dans la maladie de Crohn (63). De plus, certains cas de MICI pourraient se révéler sous ce traitement lorsqu'il est utilisé dans le cadre d'un psoriasis (64).

Des études continuent de décrire des nouvelles fonctions et/ou des nouveaux types de lymphocytes T auxiliaires. Ainsi, en 2014, Gerlach et al. ont décrit les Th9, produisant

l'IL-9 et pouvant jouer un rôle dans l'exacerbation de la colite murine via l'IL9R exprimé par les cellules épithéliales coliques (65).

Les lymphocytes T régulateurs

Les cellules immunitaires régulatrices sont essentielles au développement de la tolérance immunitaire vis-à-vis des antigènes du soi et des antigènes étrangers. Les lymphocytes T CD4 qui expriment le facteur de transcription FoxP3 et le récepteur pour l'IL-2 sont nommés T régulateurs. Leur importance dans la tolérance immunitaire est soulignée par le fait qu'un défaut dans leur fonction induit des syndromes poly-autoimmuns et inflammatoires avec des atteintes digestives similaires aux MICI (66). Des Treg se trouvent au sein de la muqueuse intestinale et peuvent être largement influencés par le microbiote (67). Cependant leur implication dans les MICI est mal définie. Le nombre de Treg circulant est réduit au cours des MICI et ils sont augmentés dans la muqueuse intestinale. Il a même été montré qu'ils conservaient une capacité immunosuppressive (68). Il apparaît donc que leur fonction régulatrice est insuffisante pour contrecarrer le déséquilibre pro-inflammatoire dans les MICI. Des stratégies thérapeutiques sont par ailleurs à l'étude pour stimuler leur nombre et leur capacité régulatrice, notamment par des injections d'IL-2 à faible dose (69).

Les cellules NK et les cellules lymphoïdes innées

D'autres types cellulaires comme les lymphocytes NK (natural killer) et les cellules lymphoïdes innées (ILC) ont un rôle clé dans le maintien de l'homéostasie intestinale. Un déséquilibre peut s'observer dans les MICI. Il a été décrit une modification du phénotype des cellules NK avec une infiltration des CD16⁺ au cours de la RCH (70). Par ailleurs, il a été récemment décrit chez la souris que l'IL-2 produite par les ILC3 (et pas

celle produite par les lymphocytes T) était un puissant facteur permettant le maintien des Treg intestinaux et de la tolérance immunitaire. Dans cette étude, la production d'IL-2 par les ILC3 était réduite dans l'intestin grêle chez des patients atteints de la maladie de Crohn et corrélait avec la présence des Treg (71).

LA BIOLOGIE DES LYMPHOCYTES B DANS LES MICI

La dysfonction du système humorale

L'immunité humorale est perturbée dans les MICI. Il a été suggéré chez la souris qu'au cours de l'inflammation intestinale, la production des IgA polymériques, aux propriétés anti-inflammatoires en se liant aux bactéries commensales après translocation transépithéliale, était affectée (72). Concernant également la production d'immunoglobulines sécrétoires, des études historiques ont montré une modification de la production au niveau intestinal. La sécrétion d'IgA2 - une sous classe d'IgA peu étudiée chez l'homme et isotype principal au niveau de la muqueuse intestinale - serait diminuée au cours des MCI, en faveur de l'augmentation de la production d'IgA1 et d'IgG (73).

Une infiltration de la lamina propria par les plasmocytes est un élément caractéristique de la RCH. Le plasmocyte est la principale cellule sécrétrice d'immunoglobulines. Il peut donc être la source des IgG et des IgA monomérique dont le niveau est augmenté au cours des MICI (74). Cette infiltration lympho-plasmocytaire, évocatrice d'un processus inflammatoire chronique, s'organise parfois en follicules lymphoïdes nouvellement formés. Ceux-ci comprennent alors une importante quantité de cellules productrices d'IgG (75–77).

Au cours des MICI les IgG produits au niveau de la muqueuse intestinale et/ou du GALT (*Gut-associated lymphoid tissue*) sont dirigés contre des antigènes microbiens (78). Parmi les anticorps antimicrobiens circulants retrouvés chez les patients avec MICI et soulignant la dysfonction du système humorale, on peut citer les anti-I2, les anti-anti-OmpC, les anti-flagellines comme les anti-CBir1 et les anti-*saccharomyces-cerevisiae*

(ASCA) (79). Il est aussi identifié des auto-anticorps chez les patients avec MICI dont les *antineutrophil cytoplasmic antibodies* (ANCA) (30) et des anticorps dirigés contre une protéine de l'épithélium, la Tropomyosine 5 (80) .

De plus, dans la maladie de Crohn, bien que leur rôle pathogénique soit débattu, les anticorps *anti-granulocyte macrophage colony-stimulating factor* (GM-CSF) sont associés à un phénotype iléal et à une évolution compliquée de la maladie (81). Par ailleurs, les souris NOD2 KO traitées par anti-GM-CSF développent une iléite transmurale après prise d'AINS, soulignant l'implication majeure de cet auto-anticorps dans la physiopathologie de la maladie de Crohn iléale (81).

Cependant, il reste incertain que les anticorps antimicrobiens trouvés dans le sang des patients avec MICI soient à l'origine de la maladie ou bien soient simplement une conséquence de la perméabilité de la barrière intestinale. Malgré tout, ces anticorps peuvent être détectés des années avant le diagnostic et peuvent être associés à une évolution compliquée de la MICI (79, 82–85).

Enfin, en plus de la présence dans la circulation de certains anticorps, les patients ayant une MICI présente une glycosilation modifiée de leurs IgG circulants par rapport aux contrôles sains. Ainsi, les patients ayant une RCH ont une galactolysation et une fucolysation de leurs IgG diminuée et la diminution de la galactolysation est associée à un recours accru à la chirurgie (86).

Les thérapies ciblant les lymphocytes B

Le Rituximab, un anticorps monoclonal dirigé contre le CD20 est utilisé avec succès dans certaines pathologies inflammatoires et/ou auto-immunes comme la polyarthrite rhumatoïde ou les vascularites à ANCA (87, 88). Cependant, chez certains patients, des

manifestations inflammatoires paradoxales peuvent survenir (89, 90). Ces effets contradictoires mettent en lumière le rôle complexe joué par les lymphocytes B. Par exemple, alors que certains plasmocytes à longue durée de vie peuvent induire des pathologies autoimmunes par la production d'auto-anticorps (91), les lymphocytes B régulateurs ont un effet anti-inflammatoire. Ainsi, dans les modèles murins d'encéphalomyélites autoimmunes, les souris déficientes en lymphocytes B (après traitement par anti-CD20) n'ont pas une guérison spontanée mais évoluent vers la chronicisation de leur maladie (92, 93). Cet effet anti-inflammatoire peut être médié par l'IL10 (94) mais aussi par d'autres mécanismes (95). De plus, les lymphocytes B régulateurs peuvent promouvoir l'effet anti-inflammatoire des lymphocytes T. Il a été montré *in vitro* et *in vivo* que les lymphocytes B peuvent induire l'expression de FoxP3 via la production d'IL-10 et de TGF β (96–98).

S'ajoutant à cette complexité du rôle des lymphocytes B dans les maladies inflammatoires, les plasmocytes résidents, n'expriment pas CD20 et leur nombre n'est pas affecté par un traitement par Rituximab (99, 100).

Concernant les MICI, il a été décrit à la fois que le Rituximab pouvait améliorer l'inflammation colique (101, 102) et que dans certains cas il pouvait aggraver voire déclencher des cas de colites (103–105). Dans la RCH, un essai contrôlé randomisé de phase II a été mené chez 24 patients cortico-dépendants et/ou cortico-résistants et n'a pas montré d'amélioration significative après traitement par Rituximab (101).

Ainsi, devant les arguments incriminant la lignée lymphocytaire B dans les MICI et l'échec du Rituximab, des stratégies plus nuancées visant les lymphocytes B pourraient être envisagées.

L'exemple de la cytokine BAFF

BAFF (*B-cell activating factor*) est une cytokine ciblant principalement les lymphocytes B et les plasmocytes et ayant un impact sur leur activation, leur survie et leur prolifération (106). Les souris surexprimant BAFF développe des atteintes ressemblant à celles de maladies inflammatoires et/ou autoimmunes telles que le lupus érythémateux disséminé (107). Cela pourrait être la conséquence de signaux de survie transmis aux lymphocytes B autoréactifs. Le niveau de BAFF est ainsi élevé dans le sérum et le tissu de nombreuses maladies immunologiques comme la polyarthrite rhumatoïde, la maladie de Sjogren et le lupus (108–111). Dans la polyarthrite rhumatoïde, la production locale de neutrophiles et de fibroblastes activés augmente le niveau de BAFF dans le liquide synovial (112, 113).

Dans les MICI, il a été noté des niveaux de BAFF augmentés dans le sérum, les selles et les biopsies de patient atteints de MICI par rapport à des patients avec troubles fonctionnels intestinaux (114). Le niveau de BAFF corrélait notamment avec celui d'autres cytokines pro-inflammatoires comme le TNF α ou l'IL1 β mais aussi avec la CRP et l'activité clinique (114).

Ainsi des stratégies thérapeutiques ciblant BAFF aurait un impact sur la biologie des lymphocytes B et notamment sur les plasmocytes, dont certains sous-types pourraient avoir un rôle néfaste dans les MICI. Des traitements anti-BAFF sont par ailleurs utilisés aujourd'hui avec succès en pratique courante dans le lupus érythémateux disséminé.

Les plasmocytes dans la RCH

Les plasmocytes constituent un type cellulaire clé dans l'homéostasie intestinale. Ils sont la source principale des immunoglobulines sécrétoires - quasi exclusivement des IgA en

situation saine. Il existe par ailleurs des sous type de plasmocytes intestinaux ayant une durée de vie très longues dépassant parfois la décennie suggérant un apport majeur dans le maintien d'une immunité spécifique contre des pathogènes comme le rotavirus (115).

Dans les MICI, il semble que la biologie des ces plasmocytes soit perturbée pouvant même avoir un impact pro-inflammatoire au niveau de la muqueuse intestinale. Ainsi, Uo et al. ont décrit un sous-type de plasmocyte exprimant l'IgG mais aussi le récepteur de chimiokine CXCR4 infiltrant la muqueuse colique de patients avec RCH (116). Ces plasmocytes IgG⁺ pourraient exacerber l'inflammation via l'activation de cellules myéloïdes par la voie de signalisation du récepteur FCγ.

De manière intéressante, Castro-Dopico et al. ont montré récemment qu'il existait un déséquilibre entre les récepteurs FCγ activateurs (FCγRIIA, FCγRIIIA, FCγRIIIB) et inhibiteurs (FCγRIIB) dans la RCH (117). Cette activation excessive des récepteurs activateurs de FCγ entraînait une augmentation de la production d'IL1β par les macrophages, promouvant ainsi l'immunité Th17 et le recrutement de polynucléaires neutrophiles (117) (Figure 3). Il est par ailleurs à noter qu'un polymorphisme de FCGR2A (codant pour un récepteur FCγ activateur) réduisant l'affinité pour les IgG, est protecteur pour la RCH (118).

Enfin, Cupi et al. ont décrit l'accumulation de certains plasmocytes pathogéniques dans la muqueuse intestinale au cours des MICI. Ces derniers exprimaient Granzyme B et pouvaient avoir une activité cytotoxique. Ces plasmocytes étaient résistants à l'apoptose induite par le Rituximab (119).

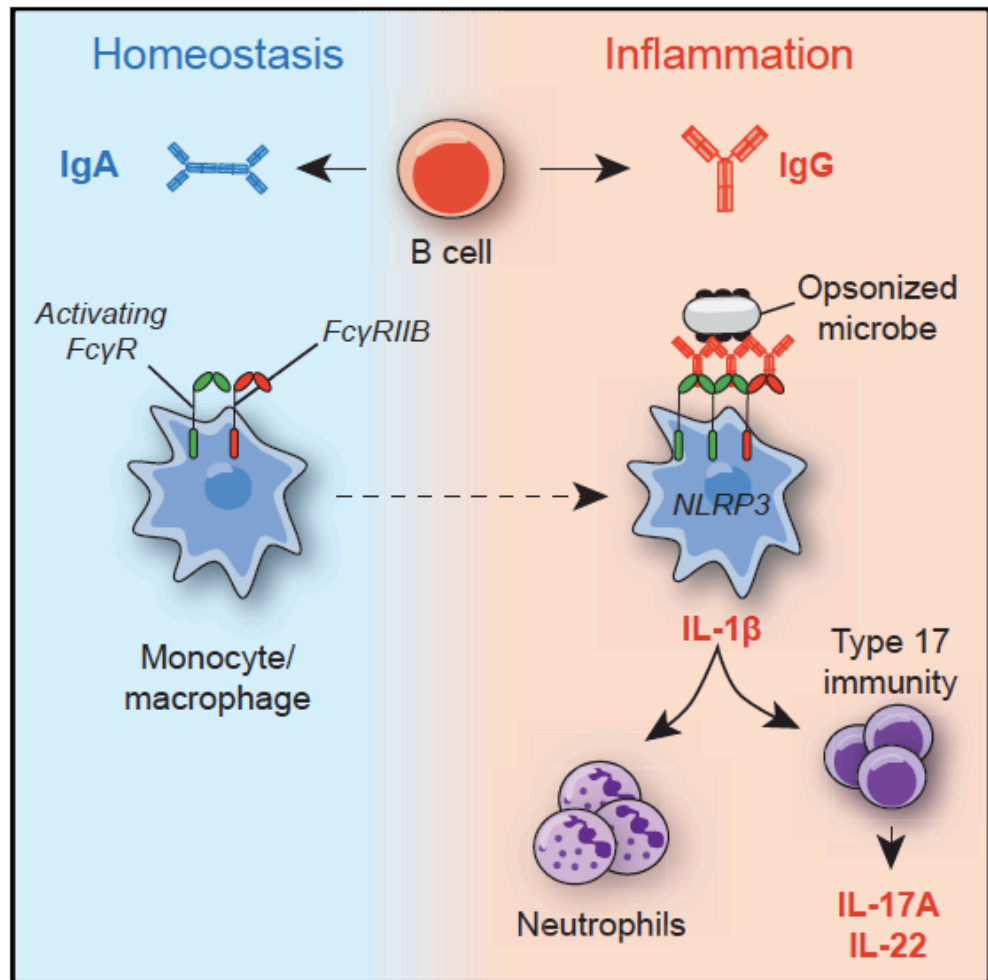


Figure 3 - Rôle des IgG au cours de l'inflammation intestinale (D'après Castro-Dopico et al. (117))

LES STRATEGIES THERAPEUTIQUES CIBLANT LE *TRAFFICKING* INTESTINAL ET LES INTEGRINES DANS LES MICI

Généralités sur les intégrines

Les intégrines sont des glycoprotéines transmembranaires longues de plus de 1500 acides aminés. Elles sont impliquées dans les interactions cellule-cellule et cellule-matrice extracellulaire. A ce jour, 24 hétérodimères ont été décrits et sont constituées de sous-unités α et β (respectivement 18 et 8 existantes) (120). L'intégrine $\beta 7$ est une intégrine clé du *trafficking* intestinal. Elle peut se lier avec les sous-unités $\alpha 4$ ou αE (CD103). L'hétérodimère $\alpha 4\beta 7$ se lie à MAdCAM-1 (*mucosal addressin cellular adhesion molecule-1*), qui est exprimé quasi exclusivement sur les cellules endothéliales du GALT (*gut-associated lymphoid tissue*) et de la lamina propria au niveau intestinal. MAdCAM-1 se lie également à CD62L (L-selectin) et est particulièrement exprimé au niveau des veinules à endothélium épais (*high endothelial venules* (HEV)) des organes lymphoïdes du GALT (dont les plaques de Peyer et les ganglions mésentériques). A ce niveau les interactions $\alpha 4\beta 7$:MAdCAM-1 et CD62L:MAdCAM-1 sont indispensables pour permettre le recrutement des lymphocytes T naïfs au niveau notamment des plaques de Peyer. Les lymphocytes T naïfs exprimant CCR7 et faiblement $\alpha 4\beta 7$ rentrent en contact avec les HEV via CD62L puis CCR7 est activé par ses ligands CCL19 et CCL21. Cela induit un « *inside-out-signalling* » entraînant l'activation et l'augmentation de l'affinité des intégrines de surface conduisant aux interactions $\alpha 4\beta 7$:MAdCAM-1 et $\alpha L\beta 2$: ICAM-1 (*intercellular adhesion molecule-1*) et à l'arrêt de la cellule (121) (figure 4).

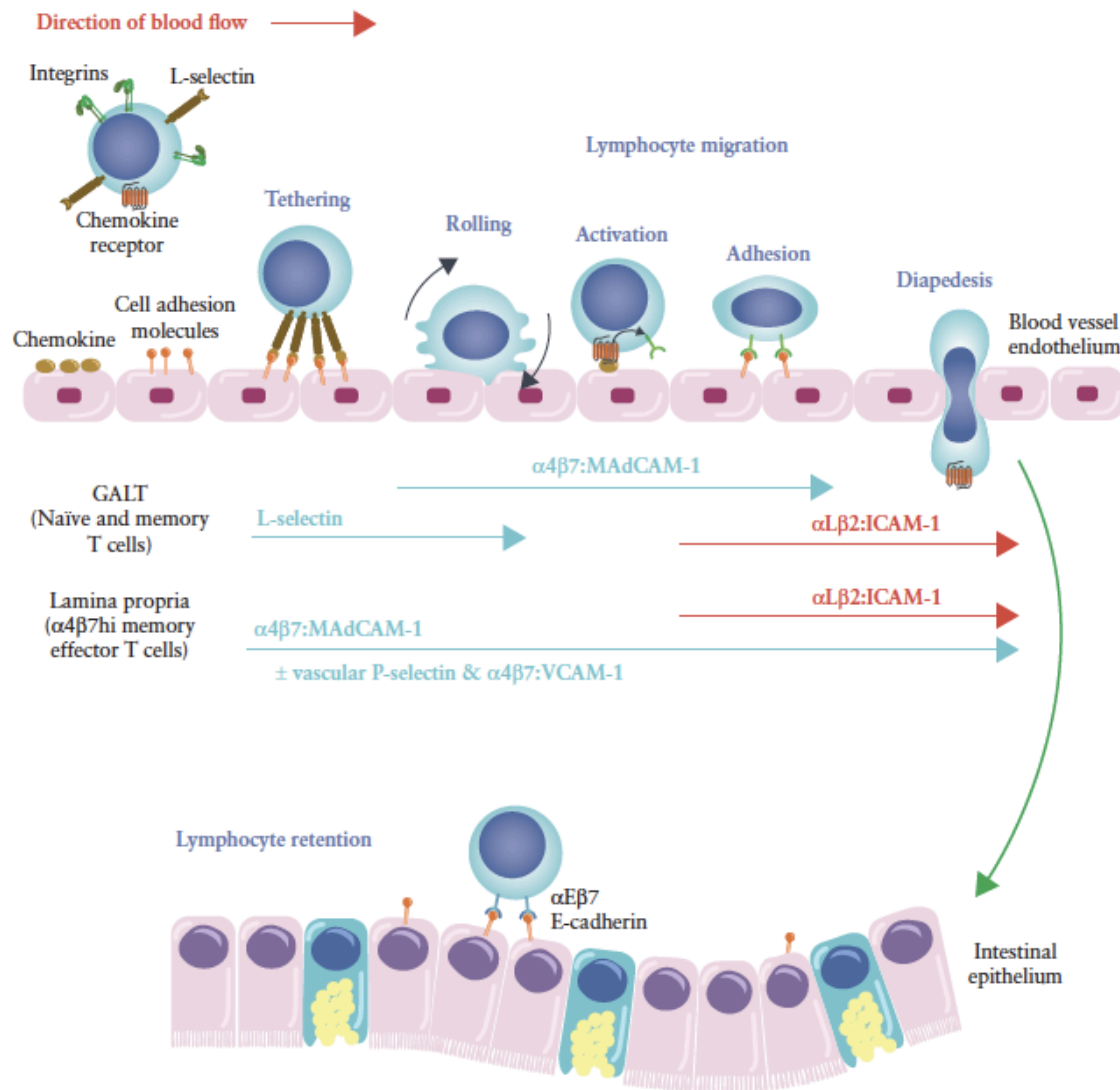


Figure 4 - Rôle des intégrines dans la migration et la localisation des lymphocytes T au niveau du GALT, de la lamina propria et de l'épithélium intestinal (d'après Lamb et al. (120)).

Cibler $\alpha 4\beta 7$ et $\alpha 4\beta 1$

Les traitements actuellement utilisés en pratique courante ou testés dans les MICI ciblent soit des sous-unités ($\alpha 4$, $\beta 7$) ou se lient spécifiquement à l'hétérodimère $\alpha 4\beta 7$ (Figure 5).

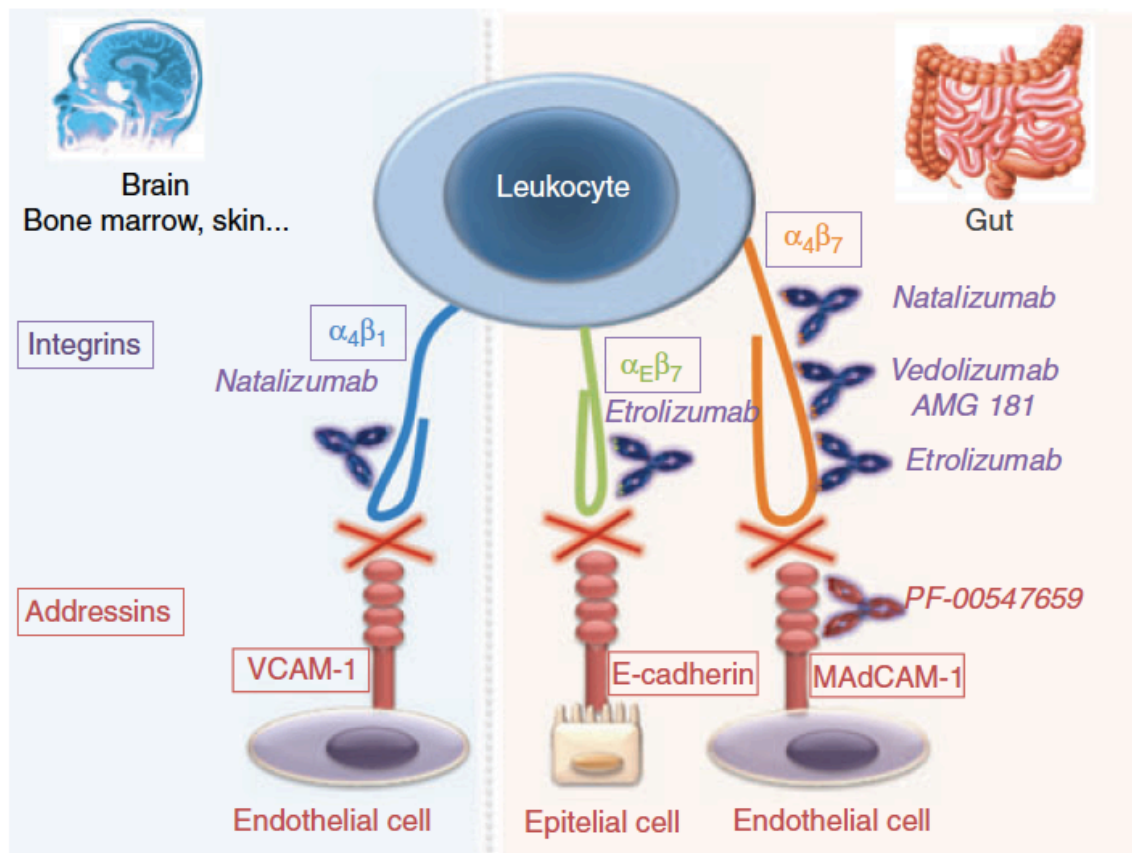


Figure 5 - Effet des traitements ciblant MadCAM et les intégrines $\alpha_4\beta_1$, $\alpha_4\beta_7$ et $\alpha_E\beta_7$ (D'après Lobaton et al. (122))

En partant de la constatation qu'à la fois VCAM-1 (ligand de $\alpha_4\beta_1$) et MadCAM-1 (ligand de $\alpha_4\beta_7$) ont une expression augmentée dans les MICI, l'intégrine α_4 a été la première intégrine ciblée. Après l'obtention de données positives dans un modèle préclinique de colite, un essai contrôlé randomisé a démontré l'efficacité du Natalizumab (anticorps monoclonal humanisé ciblant l'intégrine α_4) dans l'induction et le maintien d'une réponse clinique dans la maladie de Crohn modérée à sévère (123, 124).

Cependant, le manque de spécificité intestinale et l'altération de la migration des cellules immunitaires - positive pour l'intégrine $\alpha 4\beta 1$ - vers le système nerveux central où s'exprime VCAM-1, entraînait un risque accru de leucoencéphalopathie multifocale (124). Cet effet secondaire particulièrement sévère a entraîné l'arrêt en Europe de son utilisation dans les MICI. Cependant, l'intérêt d'un ciblage double des 2 hétérodimères persiste et des solutions sont étudiées pour accroître la spécificité intestinale et éviter les complications neurologiques. Ainsi, des petites molécules ciblant $\alpha 4$, prise oralement et à action locale sont actuellement développées. Notamment, la molécule AJM300 a été examinée pour la RCH, dans un essai contrôlé-randomisé de phase 2a avec une efficacité rapportée sur les taux de rémissions clinique et histologique (*mucosal healing*) (125).

Cibler spécifiquement l'intégrine à tropisme intestinal $\alpha 4\beta 7$

Le vedolizumab est un anticorps monoclonal qui se lie spécifiquement à $\alpha 4\beta 7$ inhibant son interaction avec MadCAM-1. Etant donné que l'expression de MadCAM-1 est quasi exclusivement limitée à la muqueuse intestinale et au GALT, le vedolizumab est considéré comme un traitement spécifique intestinal, sans risque d'effets secondaires à distance à l'inverse du natalizumab. Après des études précliniques chez le primate démontrant son efficacité sur la colite et sa spécificité intestinale (126, 127), le vedolizumab a été testé chez l'homme avec succès pour la RCH et la maladie de Crohn réfractaires (128, 129). Les études chez le singe ont montré l'impact sur la migration des cellules immunitaires vers les intestins avec une diminution de la présence de cellules mononuclées $\beta 7^+$ au niveau de la lamina propria colique des animaux traités et une accumulation des cellules $\beta 7^+$ dans le sang (126). Des données générées à partir de

modèles *in vivo* dans des souris humanisées ont également suggéré un impact du vedolizumab sur la migration intestinale (130).

Cependant, le mécanisme d'action du vedolizumab semble plus complexe qu'initialement pensé. En effet, aucune étude menée chez l'homme n'a aujourd'hui montré une diminution de la migration des lymphocytes T effecteurs vers la lamina propria. Zeissig et al. ont même constaté l'absence d'effet sur la fréquence et la composition des lymphocytes T coliques avant ou après traitement par vedolizumab (131). Ils ont par ailleurs réalisé des études fonctionnelles *in vivo* en injectant de manière autologue des leucocytes marqués par fluorescéine puis en détectant leur migration colique lors d'une coloscopie. Cette analyse a été réalisée chez 3 patients avant et à 2 et 6 semaines après traitement par vedolizumab. De manière inattendue, il n'a pas été noté de différence dans le taux de migration vers la muqueuse colique des leucocytes marqués après traitement par vedolizumab (131). Dans cette même étude, les analyses par *RNA sequencing* de la muqueuse colique ont quant à elles suggérées des modifications plus marquées du compartiment myéloïdes plutôt que des lymphocytes T (131). Il reste cependant indéterminé si il s'agit d'un effet direct du vedolizumab ou simplement le reflet de la diminution de l'inflammation intestinale.

Ces résultats contraires à l'hypothèse initiale et ces études contrastés soulignent le caractère complexe du mécanisme d'action du vedolizumab et du *trafficking* intestinal. En effet des voies redondantes existent et de nouvelles voies ont été et restent à découvrir. On peut citer notamment le rôle de GPR15 dans la migration colique des lymphocytes T régulateurs (132). De plus, bien que les études ont jusqu'à présent été

principalement focalisées sur les lymphocytes T, l'intégrine $\alpha 4\beta 7$ est exprimée par de nombreux types de cellules immunitaires et notamment les lymphocytes B.

Des essais cliniques ont également été mené pour bloquer le ligand d' $\alpha 4\beta 7$, MadCAM-1 dans la RCH, avec des résultats encourageants (133). L'intérêt serait d'ajouter à l'inhibition de l'interaction $\alpha 4\beta 7$:MadCAM-1, le blocage CD62L:MadCAM-1, ce qui pourrait potentiellement accroître l'inhibition de migration des lymphocytes T et B naïfs vers le GALT et l'efficacité thérapeutiques.

L'impact d'un traitement ciblant l'intégrine $\beta 7$

Une stratégie consistant à bloquer l'intégrine $\beta 7$ permet d'avoir un impact sur les 2 hétérodimères $\alpha 4\beta 7$ et $\alpha E\beta 7$ et leurs interactions avec leurs ligands respectifs. Cette stratégie peut se révéler intéressante car il a été suggéré que les lymphocytes T αE^+ pouvait favoriser l'inflammation intestinale dans la RCH (134). L'etrolizumab est un anticorps monoclonal IgG1 ciblant $\beta 7$, inhibant ainsi les interactions $\alpha 4\beta 7$:MadCAM-1 et $\alpha E\beta 7$:E-cadherine. Dans un modèle de souris humanisé l'etrolizumab avait un impact plus important sur la migration intestinale par rapport au vedolizumab (135). Des données murines ont également montré qu'un blocage ciblé de l'intégrine $\alpha E\beta 7$ avait un effet bénéfique sur un modèle de colite induite par immunisation chez des souris déficiente pour l'IL2 (136). De plus, une étude récente a montré que les lymphocytes intraépithéliaux $\beta 7^+$ pouvait avoir une action néfaste sur le métabolisme en se liant avec GLP-1, limitant ainsi sa biodisponibilité systémique (137).

Chez l'homme, plusieurs essais cliniques ont montré l'efficacité de l'etrolizumab dans les MICI et notamment dans la RCH (138). De manière intéressante, le taux de réponse

variait selon le niveau d'expression colique initiale de l'intégrine αE , mesuré par *RNA sequencing*.

VIH, INTESTINS ET INTEGRINE $\alpha 4\beta 7$

Le rôle central du tractus digestif au cours de l'infection par VIH

A tous les stades de l'infection par VIH, la muqueuse intestinale est un lieu clé ciblé par le virus. Tout d'abord le VIH est transmissible par la muqueuse digestive et quelque soit le mode de contamination, le virus trouve dans le GALT un environnement idéal pour se répliquer à très haut niveau (139). Il a été montré que ce haut niveau de réplication était associé à une déplétion profonde et préférentielle en CD4 aux stades précoces de l'infection par le VIH (140). Certaines caractéristiques du tractus digestif (et notamment du GALT) comme les fortes concentrations de TGF β et d'acide rétinoïque, le rende particulièrement permissif au virus du VIH (141). De plus, par rapport aux CD4 du sang et des ganglions périphériques, les lymphocytes T CD4 intestinaux expriment CCR5 à un niveau plus important (142). La plupart sont activés, bien différenciés et avec un phénotype mémoire (CD45RA⁻) faisant d'eux des cibles privilégiées pour le virus. Dans une étude menée chez le primate, Veazy et al. ont montré qu'après infection intraveineuse par le SIV (*simian immunodeficiency virus*) les lymphocytes T CD4 mémoires exprimant CCR5 étaient déplétés de manière sélective des organes lymphoïdes. A J14 post-infection, les CD4 CCR5⁺ étaient virtuellement absents de tous les organes lymphoïdes ainsi que du tractus intestinal (143).

Ce tropisme particulier du virus lors de la phase aiguë de l'infection entraîne de nombreuses dysfonctions de la barrière intestinale. Ces événements se produisant à la phase initiale jouent un rôle pivot dans la physiopathologie de l'infection et le développement ultérieur de défauts immunitaires.

En plus d'une déplétion profonde et prépondérante des CD4, l'immunité intestinale est impactée de nombreuses autres façons lors de la phase aiguë de l'infection par le VIH (144). Les lymphocytes T sont atteints non seulement au niveau de leur nombre mais aussi de leur fonction. Le ratio des lymphocytes T auxiliaires est ainsi modifié. Les lymphocytes T CD4 produisant IL-17/IL-22 sont atteints de manière préférentielle (145). Ces cytokines sont particulièrement importantes dans le maintien de l'homéostasie de la barrière intestinal en participant entre autres à la lutte antimicrobienne ainsi qu'en promouvant la croissance et la régénération épithéliale. Ainsi ce défaut en IL-17/IL-22 est une des raisons principales des altérations structurelles et profondes du tractus intestinal au cours de l'infection par le VIH, pouvant même aboutir à une entité cliniquement symptomatique nommée entéropathie chronique associée au VIH (144).

Bien que souvent ignorés, les lymphocytes B intestinaux sont aussi largement impactés par l'infection à VIH (146). Dans un travail phare sur le sujet, Levesque et al. ont montré que les lymphocytes B sanguins mais aussi intestinaux étaient affectés à la phase aiguë du VIH. Chez 6 patients, ils ont noté l'accroissement initial des lymphocytes B mémoires (définis par CD19⁺IgD⁻CD38^{+/+}CD27⁺) et des plasmocytes (CD38^{hi}IgD⁻CD27⁺) au niveau de la muqueuse iléale, associé à une perte des lymphocytes B naïfs (CD19⁺IgD⁺CD27⁻). Une des constations majeures de cette étude était la perturbation du réseau des cellules dendritiques folliculaires et l'infiltration des follicules lymphoïdes B ainsi que des centres germinatifs par les cellules inflammatoires dès le stade initial de l'infection (146). Par ailleurs, les auteurs ont analysé la spécificité antigénique des lymphocytes B mémoires activés qui n'étaient pas uniquement dirigés contre le VIH mais également contre une

grande variété d'autre antigènes dont des antigènes de la grippe et des auto-antigènes (146).

Un nombre croissant d'études démontre par ailleurs l'association de l'infection par le VIH avec une modification du microbiote intestinal (147–149). A l'inverse des patients avec une MICI, les patients infectés par le VIH et non traités ont une diversité globale augmentée (147). Les modifications du microbiotes sont corrélées à des marqueurs de l'inflammation et de progression du VIH, comme l'IL-6 plasmatique (148). Guillen et al. ont quant à eux montré que la dysbiose corrélait fortement au nadir de CD4 plutôt qu'à d'autres critères virologiques (149).

Une des caractéristiques emblématiques du tractus intestinal au cours de l'infection par le VIH est la récupération immunitaire incomplète après traitements antirétroviraux. De nombreuses modifications survenant au cours de la phase aiguë ne récupèrent que partiellement au contraire de ce qui se passe au niveau systémique et des autres organes. C'est notamment le cas pour le taux de CD4 qui même après retour au niveau basal dans la circulation sanguine, reste bas dans la muqueuse intestinale (140, 150).

L'intégrine $\alpha 4\beta 7$ et la physiopathologie de l'infection par VIH

Pour infecter les cellules de l'hôte, les virus interagissent tout d'abord avec la surface cellulaire suivi d'un processus d'entrée dans la cellule. Dans de nombreuses infections, les intégrines peuvent se lier au virus, servant de point d'ancrage pour infecter la cellule (151). Bien que l'intégrine $\alpha 4\beta 7$ ne soit pas requise de manière indispensable pour l'entrée du VIH, il est bien établi que le virus peut se lier à elle (152, 153). Cependant, son implication véritable dans la physiopathologie de l'infection reste débattue. L'interaction entre le virus et l'intégrine $\alpha 4\beta 7$ est médiée par la glycoprotéine de

l'enveloppe, gp120 (154). La sous-unité gp120 du VIH est faite de 5 régions constantes alternant avec 5 régions variables selon l'ordre suivant : C1, V1, V2, C2, V3, C3, V4, C4, V5, C5. Certaines données montrent que c'est la boucle V2 de gp120 qui se lie à l'intégrine $\alpha 4\beta 7$. En plus de se lier à l'intégrine, la boucle V2 est une région clé pour le développement du virus. Elle joue un rôle dans la liaison avec le CD4 et contribue à la formation du trimère env (155–157).

Plus qu'une simple interaction Arthos et al. ont montré que le complexe gp120- $\alpha 4\beta 7$ influençait l'activation de $\alpha L\beta 2$ sur les lymphocytes T (158). $\alpha L\beta 2$ en plus de médier en coordination avec $\alpha 4\beta 7$ et CD62L la migrations des lymphocytes vers le GALT (121), promeut la formation de synapses immunologiques (159). Des expériences *in vitro* ont révélé que la liaison de $\alpha 4\beta 7$ avec gp120 activait $\alpha L\beta 2$. CD4 et l'intégrine activée $\alpha L\beta 2$ se regroupait à la surface cellulaire avec $\alpha 4$ et gp120 dans des zones de contacts cellule:cellule, suggérant un rôle dans la formation des synapses virologiques et la propagation du virus (158).

Récemment, une étude menée à partir d'une population de femme Sud-Africaines à risque d'infection par le VIH a montré que les personnes avec une fréquence élevée de lymphocytes T CD4 $\alpha 4\beta 7^+$ sanguins avaient un risque accru d'infection, indépendamment du phénotype global des lymphocytes T et de l'inflammation génitale. De plus, dans leur étude, Siro et al. ont montré que le taux pré-infection de lymphocytes T CD4 $\alpha 4\beta 7^+$ prédisait une progression plus rapide et sévère de la maladie, définie par l'augmentation de la charge virale plasmatique et la chute des CD4 (160) (figure 6). De manière intéressante, les auteurs ont ensuite analysé les caractéristiques virales des patients infectés. Ils ont noté que les patients infectés par des virus avec la boucle V2

contenant des motifs P/SDI/V avaient un taux pré-infection de lymphocytes T CD4 $\alpha 4\beta 7^+$ plus élevé que ceux infectés par des virus avec une boucle V2 ayant les motifs P/SDI/V (160).

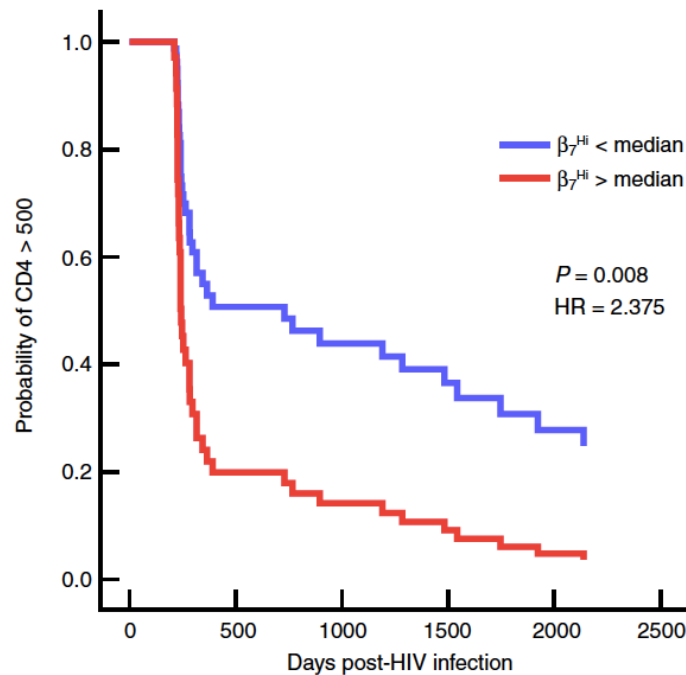


Figure 6 - Prédiction de la chute des lymphocytes T CD4 sous 500/ μl en fonction du taux initial des lymphocytes T $\beta 7^+$ (D'après Siro et al. (160))

Les études menées chez les primates infectés par le SIV testant un traitement par anti- $\alpha 4\beta 7$

Du fait de ces multiples preuves de l'implication de $\alpha 4\beta 7$ dans la physiopathologie du VIH, des recherches ont été menées analysant l'effet d'un traitement ciblant $\alpha 4\beta 7$. De nombreuses études menées chez les primates ont notamment abouti à des résultats très

prometteurs. Un traitement anti- $\alpha 4\beta 7$ a montré des effets bénéfiques dans de nombreuses situations dans l'infection à SIV chez le primate. Tout d'abord, l'intérêt a été décrit pour prévenir l'infection après instillation vaginale du virus (161). De plus, dans cette étude, chez les animaux finalement infectés, le niveau d'ADN viral dans la muqueuse intestinale était bien inférieur chez ceux ayant reçu le traitement anti- $\alpha 4\beta 7$ (161).

Une étude a évalué l'effet de 2 perfusions de l'anticorps monoclonal anti- $\alpha 4\beta 7$ durant la phase aiguë de l'infection par le SIV, transmis par voie intraveineuse. Ansari et al. ont ainsi montré que bien que l'effet sur la charge viral plasmatique était modeste, il y avait une forte diminution de l'ADN proviral mesuré dans la muqueuse intestinale. Un impact sur la mortalité a également été noté (162). D'autres études ont trouvé un bénéfice à des stades plus tardifs de l'infection par le SIV (163, 164). Un traitement par anti- $\alpha 4\beta 7$ a notamment été investigué en combinaison avec un traitement précoce par antirétroviral. Byraredy et al. ont ainsi traité par antirétroviraux des primates infectés par le SIV de manière intraveineuse 5 semaines post-infection puis 4 semaines plus tard ont démarré un traitement par anti- $\alpha 4\beta 7$ (ou IgG pour le group contrôle). Les primates ont reçu 9 semaines de traitement antiviral qui a été cessé et le traitement par anti- $\alpha 4\beta 7$ (ou IgG) a été poursuivi. De manière frappante, les 8 animaux traités ont eu une virémie contrôlée (basse ou indétectable) après arrêt des traitements antirétroviraux alors que les 7 primates du groupe contrôle ont tous eu un rebond viral. Le contrôle virémique a persisté durant les 3 ans de suivi avec un recouvrement complet du taux de CD4 sanguins et intestinaux (164). Malgré l'effet spectaculaire dans cette étude, le mécanisme sous-jacent reste à identifier. Une des différences majeures entre les 2 groupes était l'augmentation des CD4 intestinaux chez les primates recevant l'anti- $\alpha 4\beta 7$, et notamment l'accroissement des

lymphocytes Th17/22 évoquant une amélioration de l'intégrité de la barrière intestinale tout comme la diminution des taux plasmatiques de I-FABP (164). A partir des mêmes animaux et en utilisant une technique par PET/CT, Santangelo et al. ont ensuite établi que le nombre de CD4 infectés était diminué précocement au niveau intestinal chez les animaux traités par anti- $\alpha 4\beta 7$ (163).

En conclusion, ces études menées chez le primate ont montré un impact favorable d'un traitement par anti- $\alpha 4\beta 7$ pour prévenir la transmission du SIV ainsi que sur les phases précoces et tardives de l'infection en combinaison avec les antirétroviraux. Cela ouvre des perspectives pour l'utilisation du vedolizumab au cours de l'infection par VIH.

OBJECTIFS

1 - Déterminer l'impact du vedolizumab sur le système immunitaire intestinal et sur le réservoir viral chez des patients atteints de maladie inflammatoire chronique intestinale et infectés par le VIH

2 - Caractériser la réponse immunitaire B dans la recto-colite hémorragique et la corrélér au phénotype clinique

PARTIE 1 : EFFET DU VEDOLIZUMAB SUR L'IMMUNITE INTESTINALE ET LE RESERVOIR VIRAL.

Contexte

Les résultats spectaculaires de l'étude de Byraredy et al. mené chez les primates (164) ainsi que l'ensemble des éléments présentés précédemment dans l'introduction ont contribué à un intérêt majeur pour l'utilisation du vedolizumab dans le contexte de l'infection à VIH. Malgré l'efficacité des antirétroviraux, le virus persiste et la charge virale rebondit après leur arrêt. Ainsi, un des aspects majeurs de la recherche actuel sur le VIH et de cibler le réservoir viral. L'intestin en est un des principaux sanctuaires. Le vedolizumab pourrait donc être utilisé en combinaison avec les antirétroviraux pour mieux contrôler l'infection et le réservoir viral mais aussi pour améliorer un système immunitaire compromis.

Quatre essais cliniques dont 1 mené en France sont actuellement en cours évaluant l'efficacité d'une stratégie incluant le vedolizumab après arrêt des antirétroviraux chez des patients avec un VIH chronique.

Cependant, en plus des interrogations quant à son efficacité, il existe également des préoccupations quant à l'innocuité de ce traitement chez des patients immunocompromis.

Ainsi, en profitant du fait que le vedolizumab soit couramment utilisé dans les MICI, nous avons généré les premières données humaines évaluant l'impact du vedolizumab sur les risques d'effets secondaires, sur l'immunité intestinale et le réservoir viral chez des patients atteints de MICI et infectés par le VIH.

PARTIE 2 : CARACTERISATION DE LA REPONSE IMMUNITAIRE B ET CORRELATION AVEC LE PHENOTYPE CLINIQUE DANS LA RCH.

Contexte

L'échec de l'essai clinique testant le rituximab dans la RCH a éteint l'enthousiasme pour la recherche sur les lymphocytes B dans les MICI (101). Cependant, plusieurs études récentes démontrent une dysfonction humorale au niveau de la muqueuse intestinale. Certaines ont notamment révélé la présence de sous-types de plasmocytes pathogéniques (116). Castro-Dopico et al. ont montré que les IgG présents au niveau intestinal lors des MICI pouvaient accroître les phénomènes inflammatoires via leur portion FC (117).

Le but de notre étude était de décrire de manière extensive les modifications du compartiment lymphocytaire B aux niveaux sanguin et de la muqueuse intestinale. Nous avons également appliqué des techniques à l'échelle unicellulaire (séquençage du gène de la chaîne lourde de l'immunoglobuline et *single-cell RNA sequencing*) pour mieux caractériser ce compartiment. Enfin, à partir de 3 cohortes indépendantes de patients nous avons corrélié les modifications constatées au niveau des lymphocytes B avec des caractéristiques cliniques.

DISCUSSION

PARTIE 1

Dans cette étude, nous avons fourni les premières données humaines sur l'utilisation d'un traitement par anti- $\alpha 4\beta 7$ au cours du VIH. Tous les patients avaient une activité modérée de leur MICI avec seulement 1 patient ayant une CRP supérieure à 5 mg/ml (16 mg/ml) avant initiation du traitement par vedolizumab.

Nous avons ainsi mis en évidence un effet majeur sur les follicules lymphoïdes intestinaux et sur les cellules qu'ils contiennent. Cet effet est intéressant dans l'optique de cibler le réservoir viral car les follicules B peuvent être un sanctuaire pour les CD4 infectés qui échappent ainsi aux CD8 n'exprimant pas CXCR5 (*165*). Cependant, nous n'avons pas constaté d'impact sur le réservoir viral tant au niveau sanguin qu'au niveau de la muqueuse intestinale. Cela pourrait être dû au fait que les patients étaient traités depuis une longue période par antirétroviraux avec un réservoir viral déjà faible. L'impact du vedolizumab sur le développement du virus pourra mieux être évalué dans des études ayant un design incluant l'arrêt des antirétroviraux.

Des études complémentaires sont également nécessaires dans le cadre des MICI pour mieux comprendre le mécanisme sous-jacent ainsi que les conséquences de cet affaïssement des sites inductifs intestinaux. De manière intéressante et à l'instar de Zeissig et al., nous n'avons pas constaté de diminution de la fréquence des lymphocytes T au niveau de la lamina propria de l'iléon terminal et du côlon (*131*).

PARTIE 2

Nous avons ici montré que le phénotype de la lignée cellulaire B était profondément modifié au cours de la RCH active, à la fois au niveau sanguin et au sein de la muqueuse colique inflammée. Notamment, le phénotype des plasmocytes est perturbé au cours de l'inflammation intestinale chronique. Il existe un accroissement de ceux exprimant IgG et IgA1 au détriment de IgA2. De plus, la proportion de plasmocytes à durée de vie courte est augmentée et celle à longue durée de vie - ayant un rôle majeur dans le maintien de l'homéostasie intestinale - est diminuée. Cela souligne une augmentation du *turnover* des plasmocytes colique au cours de la RCH. Au niveau de la circulation sanguine, ceci est reflété par l'élévation des plasmablastes exprimant l'intégrine $\beta 7$ à tropisme intestinal. Nous avons ensuite constaté que cette réponse immunitaire B à potentiel pathogénique était corrélée et pouvait être influencée par un accroissement de lymphocytes TFH-*like* pouvant produire CXCL13. Enfin, nous avons montré que ces modifications notées au cours de la RCH reflétaient l'activité clinique et pouvaient prédire la survenue de complications et la réponse aux anti TNF.

Cette étude ouvre la voie au développement de stratégie thérapeutique ciblant cette réponse B intense et pathologique dans la RCH.

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**PARTIE 1 : EFFET DU VEDOLIZUMAB SUR
L'IMMUNITE INTESTINALE ET LE
RESERVOIR VIRAL DANS LES MICI ET
L'INFECTION A VIH**

HIV

Anti- $\alpha 4\beta 7$ therapy targets lymphoid aggregates in the gastrointestinal tract of HIV-1–infected individuals

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Gut homing CD4⁺ T cells expressing the integrin $\alpha 4\beta 7$ are early viral targets and contribute to HIV-1 pathogenesis, likely by seeding the gastrointestinal (GI) tract with HIV. Although simianized anti- $\alpha 4\beta 7$ monoclonal antibodies have shown promise in preventing or attenuating the disease course of simian immunodeficiency virus in nonhuman primate studies, the mechanisms of drug action remain elusive. We present a cohort of individuals with mild inflammatory bowel disease and concomitant HIV-1 infection receiving anti- $\alpha 4\beta 7$ treatment. By sampling the immune inductive and effector sites of the GI tract, we have discovered that anti- $\alpha 4\beta 7$ therapy led to a significant and unexpected attenuation of lymphoid aggregates, most notably in the terminal ileum. Given that lymphoid aggregates serve as important sanctuary sites for maintaining viral reservoirs, their attrition by anti- $\alpha 4\beta 7$ therapy has important implications for HIV-1 therapeutics and eradication efforts and defines a rational basis for the use of anti- $\alpha 4\beta 7$ therapy in HIV-1 infection.

INTRODUCTION

Lentiviruses such as human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) are uniquely adapted to infect activated, memory CD4⁺ T cells that are specifically enriched at mucosal surfaces (1). Consequently, mucosal tissues including those of the gastrointestinal (GI) tract play a critical role in disease pathogenesis during acute (2, 3) and chronic HIV-1 infection (4).

The GI tract can be immunologically subclassified into inductive and effector sites (5). Aggregates of lymphoid tissue, including Peyer's patches (PPs) and isolated lymphoid follicles (intrinsic to the bowel wall) and mesenteric lymph nodes (extrinsic to the bowel wall), serve as the major immune inductive sites. Naïve T and B cells express integrin $\alpha 4\beta 7$ ($\alpha 4\beta 7$), which mediates their migration into the inductive sites through specific interactions with mucosal addressin cell adhesion molecule-1 (MAdCAM-1) (6). Notably, the expression of $\alpha 4\beta 7$ on naïve T and B cells is significantly lower than its expression on memory cells (6). PP-resident dendritic cells (DCs) prime naïve

T and B cells and simultaneously induce the expression of $\alpha 4\beta 7$ in a retinoic acid and transforming growth factor- β -dependent fashion (7). These $\alpha 4\beta 7^{\text{hi}}$, gut-primed, antigen-experienced memory cells egress into the draining lymph and subsequently into circulation and home to immune effector sites such as intestinal lamina propria, again via specific interactions between MAdCAM-1 and $\alpha 4\beta 7$ (8). Although the putative mechanism of action (MOA) of anti- $\alpha 4\beta 7$ therapy is to prevent the entry of $\alpha 4\beta 7^{\text{hi}}$ memory T cells into the intestinal lamina propria, to date, the published reports show no change in the frequency of lamina propria CD4⁺ T cells after anti- $\alpha 4\beta 7$ therapy, either in SIV-infected macaques (9) or in humans with inflammatory bowel disease (IBD) (10). The effects of anti- $\alpha 4\beta 7$ therapy on lymphoid aggregates, where cellular entry is also $\alpha 4\beta 7$ -MAdCAM-dependent (6), remain unappreciated.

The pathogenesis of HIV-1 infection intersects with intestinal homing pathways at multiple levels that are yet poorly understood. GI-resident CD4⁺ T cells are preferentially targeted during acute HIV and SIV. Regardless of the route of infection and mode of virus delivery, intestinal CD4⁺ T cells are profoundly depleted during the earliest stages of HIV-1 and SIV infection (11). This strongly suggests that HIV-1, either cell-free or cell-associated, has evolved specific mechanisms to localize to GI tract during acute infection to infect CCR5-expressing (12) physiologically activated memory T cells (13, 14) that are exceptionally HIV-1 susceptible (2). In this regard, studies have reported a direct interaction between $\alpha 4\beta 7$ and the viral envelope (15–17). Thus, HIV-1–susceptible $\alpha 4\beta 7^+$ CD4⁺ T cells may serve to deliver the virus into the gut tissues.

Multiple lines of evidence demonstrate that $\alpha 4\beta 7$ -expressing cells represent early targets for the virus (18–22). This was highlighted in a recent report, demonstrating that preinfection frequencies of $\alpha 4\beta 7$ on circulating CD4⁺ T cells may predict the risk of HIV-1 acquisition and disease progression independent of other T cell phenotypes and genital inflammation in a large cohort of at-risk South African women

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(23). Supporting this finding, sexually transmitted diseases that have been linked with increased risk of HIV-1 acquisition increase the frequency of $\alpha 4\beta 7^{+}CD4^{+}$ memory T cells in both the mucosa and blood (24, 25).

Because of the important role of $\alpha 4\beta 7^{+}CD4^{+}$ T cells in viral pathogenesis, anti- $\alpha 4\beta 7$ therapy has been considered in the management of HIV-1 infection. However, no human studies are available to date. In nonhuman primate (NHP) models, using simianized anti- $\alpha 4\beta 7$ antibodies has shown promising results. Salient among these studies is the demonstration of disease prevention or an attenuated disease course when anti- $\alpha 4\beta 7$ antibodies preceded low-dose repeated intravaginal SIV challenge (26). In addition, a recent report found that SIV-infected macaques that were treated during acute infection with combination antiretroviral therapy (cART) and anti- $\alpha 4\beta 7$ therapy (or isotype control) achieved long-term viremic control after cART and antibody interruption, whereas isotype-treated animals became viremic (27). Despite multiple NHP studies, clear mechanisms underlying the potential efficacy of anti- $\alpha 4\beta 7$ therapy in HIV (SIV) infection remain elusive.

Although no HIV-related studies have been reported to our knowledge, anti- $\alpha 4\beta 7$ therapy [vedolizumab (VDZ)] has become a frontline strategy in the management of patients with IBD (28, 29), where it has demonstrated strong efficacy and an excellent safety profile (30). To determine VDZ's role in HIV-1 infection, we have assembled a cohort of IBD patients with concomitant HIV-1 infection. Here, we provide data describing the safety and the immunological and virological effects of anti- $\alpha 4\beta 7$ therapy in HIV-1-infected patients receiving VDZ therapy over 30 weeks, with detailed analyses in the GI tissue and in peripheral blood.

RESULTS

VDZ was administered safely and without any serious adverse events to patients with HIV-1 infection

Six patients (five males and one female) with a median age of 51.7 years (interquartile range, 36.8 to 62.2) were followed prospectively for 30 weeks after VDZ treatment. Five were receiving cART for a minimum of 5.6 years and had an undetectable plasma viral load at VDZ start (threshold of 20 viral copies/ml). One patient (583-016)

was cART-treated for 9 months and had a plasma viral load of 156 copies/ml at the time of VDZ initiation. In one subject (583-004), colonoscopy could not be performed before treatment due to logistical reasons (the patient had already received the first dose of VDZ as the colonoscopy was being scheduled). Therefore, immunological analyses before and after treatment are being reported on five of six patients. Detailed HIV characteristics are shown in Table 1.

All patients included in the study had very mild IBD activity (table S1), characterized by mild proctitis and endoscopically normal appearing colonic and ileal mucosa with the exception of subject 583-016 where endoscopic inflammation was observed up to 25 cm from the anal verge. However, proximal parts of the colon and the terminal ileum (TI), where study-related biopsies were obtained, were normal in 583-016. None of the study subjects had pancolitis, history of bowel surgery, or previous use of biologic medications, all signifying severe disease (31, 32). In addition, and as further evidence of mild IBD, five of six patients had normal levels of complement-reactive protein (CRP) at the time of starting VDZ (583-016 had a CRP of 16.5 mg/liter at the time of recruitment). Finally, histology, arguably the gold standard for assessment of mucosal inflammation, showed only a mild increase in inflammatory cells limited to the rectum of five of six patients. The TI and left colon (LC), sites where immunological and virological analyses were performed, were histologically normal in each of the study subjects (fig. S1).

Patients were monitored with serial laboratory and clinical assessments. One of the six patients reported mild, self-limited nasopharyngitis, a previously reported adverse effect of VDZ (33). Two of six patients reported mild, self-limited headache, and one patient had intravenous infiltration during infusion. No other adverse events (AEs) or serious AEs related to VDZ were reported during the course of 30 weeks of follow-up, highlighting the safety of this drug in our cohort of HIV-1-infected subjects. These data mirror previous, extensive data in IBD (33).

Anti- $\alpha 4\beta 7$ therapy results in a significant reduction in B cell subsets within the GI tract

In previous studies in subjects with acute HIV-1 infection, we have observed a profound reduction in CD4⁺ T cells in the GI lamina propria

Table 1. HIV-related clinical characteristics. F, female; M, male. ATV, atazanavir; COBI, cobicistat; DTG, dolutegravir; EVG, elvitegravir; FPV, fosamprenavir; FTC, emtricitabine; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.							
ID	Sex	Age at VDZ (years)	CD4 count at VDZ initiation (cells/ μ l) (%)	HIV viral load* at VDZ initiation (copies/ml)	Duration of HIV infection† at VDZ initiation (years)	Duration of ART at VDZ initiation (years)	ART regimen
583-004‡	F	64.1	634 (34%)	<20	28.2	>20	DTG FTC TDF
583-012	M	46.8	834 (39%)	<20	14.2	14	DTG FTC TDF
583-013	M	56.6	632 (37%)	<20	21.9	21.8	FPV FTC TDF
583-016	M	24.1	709 (25%)	156	0.9	0.8	EVG COBI FTC TDF
583-017	M	66.7	683 (44%)	<20	28	17	ATV COBI FTC TDF
583-024	M	33.5	1021 (49%)	<20	5.7	5.6	EVG COBI FTC TAF
*This assay is performed with the Roche COBAS AmpliPrep/COBAS TaqMan HIV Test. Immunological intestinal analyses were not performed.				†From the date of diagnosis		‡Complete virological and	

(2, 34). Although CD4⁺ T cell depletion was less marked in the lymphoid aggregates, HIV-1 RNA (as measured by in situ hybridization) was mainly detected in the lymphoid aggregates (2, 34). These data suggested to us that the intestinal lamina propria and lymphoid aggregates have distinct immunological and virological readouts during HIV-1 infection. Therefore, in addition to the LC, we decided to perform full colonoscopies to biopsy the TI enriched for lymphoid aggregates and comprehensively defined B and T cell subsets as detailed below.

We first defined a flow cytometric strategy to identify the known B cell subsets and plasma cells in intestinal mucosa and in circulation, identifying plasma cells as live CD45⁺CD38^{hi}CD27⁺ cells and nonplasma cell B cells as live CD45⁺CD38[−]CD19⁺ cells. Among nonplasma cell B cells, naïve B cells were defined as CD45⁺CD38[−]CD19⁺IgD⁺IgM⁺ cells, whereas switched memory (SM) B cells were defined as CD45⁺CD38[−]CD19⁺IgD[−]IgM[−] cells (fig. S2).

The TI contains more lymphoid aggregates and therefore more nonplasma cell B cells, whereas the LC harbors more lamina propria lymphocytes and therefore mostly differentiated plasma cells (5). In our patients, there was a clear dichotomy in the effects of anti- α 4 β 7 therapy between the TI and LC, reflecting distinct cellular composition of these two intestinal sites. For example, we observed a marked decrease in nonplasma cell B cells (CD19⁺CD38[−]) in the TI of all five patients by flow cytometry. In contrast, in the LC that contains fewer nonplasma B cells, the decrease was less pronounced (Fig. 1, A and B). Among B cell subsets, both naïve and SM B cells were reduced in the TI after therapy. Again, in the LC with fewer total B cells, decrease in B cell subsets was less pronounced. (Fig. 1C). Among GI plasma cells, no decrease was noted in either the TI or LC (Fig. 1, A and B).

In circulation, although there was interindividual variability, we observed an early increase in all nonplasma cell B cells and in circulating plasmablasts (defined as Ki67⁺CD38⁺CD27⁺IgD[−]CD19⁺/_{int} cells; fig. S2) at week 2 after VDZ initiation. No statistically significant changes were observed over the course of 30 weeks of VDZ treatment ($P = 0.43$; Fig. 1D).

Next, we compared B cell composition between a cohort of healthy volunteers, patients with HIV-1 alone (without IBD), and the VDZ-treated HIV-1⁺ patients. Compared to healthy volunteers, a significant decrease was noted in total nonplasma cell B cells in the TI after VDZ treatment ($P < 0.05$; Fig. 1E). This was associated with a reduced frequency of naïve B cells, although changes in SM B cells and plasma cells in the TI were not significantly different between normal volunteers and VDZ-treated patients ($P = 0.19$ and $P = 0.07$, respectively). In contrast to the TI, we did not observe significant changes in total nonplasma cell B cells ($P = 0.12$), B cell subsets (naïve B cells, $P = 0.15$; SM B cells, $P = 0.11$), or plasma cells ($P = 0.17$) in the LC after VDZ treatment, when compared to healthy volunteers (Fig. 1E). All B cell subsets were comparable in the TI and LC between HIV-infected controls and HIV-infected IBD patients before VDZ (Fig. 1E). Finally, no significant changes in circulating B cell subsets were noted after VDZ treatment when compared to healthy volunteers or HIV controls (Fig. 1F). Overall, all five patients presented a major decrease in nonplasma cell B cells (including both naïve and memory subsets) in the TI with anti- α 4 β 7 therapy.

Anti- α 4 β 7 therapy results in attrition of lymphoid aggregates within the GI tract

Next, we quantified GI B cells by immunohistochemistry (IHC) to confirm and further define the anatomical compartments showing changes in B cells after VDZ. Because CD19 also identifies a subset

of plasma cells, we used CD20 staining to quantify nonplasma cell B cells per unit area in lymphoid aggregates and lamina propria in the TI and LC. Lymphoid aggregates were noted after treatment in four of five subjects in the TI and two of five subjects in LC. In the TI, there was a pronounced CD20⁺ B cell reduction in TI-associated lymphoid aggregates after VDZ in all four of five subjects, where lymphoid aggregates were detectable before treatment. In the LC, marked reduction in lymphoid aggregate-associated B cells was noted in one subject, whereas in the other, the reduction was more modest (Fig. 2B). VDZ treatment induced a reduction in lamina propria B cells in the TI and had a variable effect on lamina propria B cells in the LC (Fig. 2, A and B, and fig. S3).

Having observed a significant decrease in nonplasma cell B cells in the TI, we hypothesized that anti- α 4 β 7 therapy has a pronounced effect on lymphoid aggregates. To define lymphoid aggregates within tissue, we used IHC and quantified the surface area covered by lymphoid aggregates in each of the tissue sections before and after VDZ (pathologists were blinded to the identity of the samples). In every subject, we observed a decrease in the percentage of total tissue surface covered by lymphoid aggregates after VDZ in the TI (from $24.1 \pm 19.3\%$ on average to $4.1 \pm 2.9\%$; Fig. 2, C and D). Again, more variability was observed in the LC (from $3.8 \pm 3.6\%$ on average to $3.9 \pm 4.5\%$), likely because lymphoid aggregates are less pronounced in the LC compared to the TI (5). To validate the IHC data, we performed immunofluorescence microscopy to examine B and T cell populations in the lymphoid aggregates versus the lamina propria. Consistent with the other findings, in this study, we found a significant decrease in number and size of lymphoid aggregates after VDZ ($P < 0.001$; Fig. 2, E and F).

To better understand the attrition of lymphoid aggregates, we examined for cellular proliferation using Ki67. As described previously, Ki67⁺ cells were predominantly found in the lymphoid aggregates (35). We did not observe a significant decrease in Ki67⁺ cells after VDZ (TI, $P = 0.64$; LC, $P = 0.65$), suggesting that lack of cell proliferation was likely not responsible for the attrition of lymphoid aggregates (fig. S4).

Next, we asked whether cellular apoptosis was responsible for the attrition of lymphoid aggregates by examining for apoptotic bodies by histology. As a result of negative selection of low-affinity and autoreactive B cells, apoptotic cells are present physiologically within the germinal centers of lymphoid aggregates (36). Accordingly, we found apoptotic cells within the lymphoid aggregates before VDZ, as shown in fig. S5. We did not observe an increase in apoptotic cells after VDZ (fig. S5).

Overall, we found profound and consistent changes of the lymphoid aggregates of the TI after VDZ therapy. They were substantially decreased in number and size, and they contained fewer CD20⁺ B cells.

Anti- α 4 β 7 therapy results in a decrease in naïve CD4⁺ T cells in the TI

SIV macaque studies have demonstrated that anti- α 4 β 7 therapy in combination with early cART enables better reconstitution of CD4⁺ T cells in the colonic mucosa compared to cART alone (27). We therefore investigated the impact of VDZ on T cell subsets, including CD4⁺ and CD8⁺ T cells in the GI tract and in circulation. In the TI, there was a trend toward an increased frequency of total CD3⁺ T cells after VDZ treatment, whereas CD3⁺ T cell changes in the LC after VDZ were variable (Fig. 3A). Among T cell subsets, there was no significant change in the CD4/CD8 ratio in the TI ($P = 0.24$) or the LC ($P = 0.19$) in each of the patients after VDZ (Fig. 3B). When compared to healthy

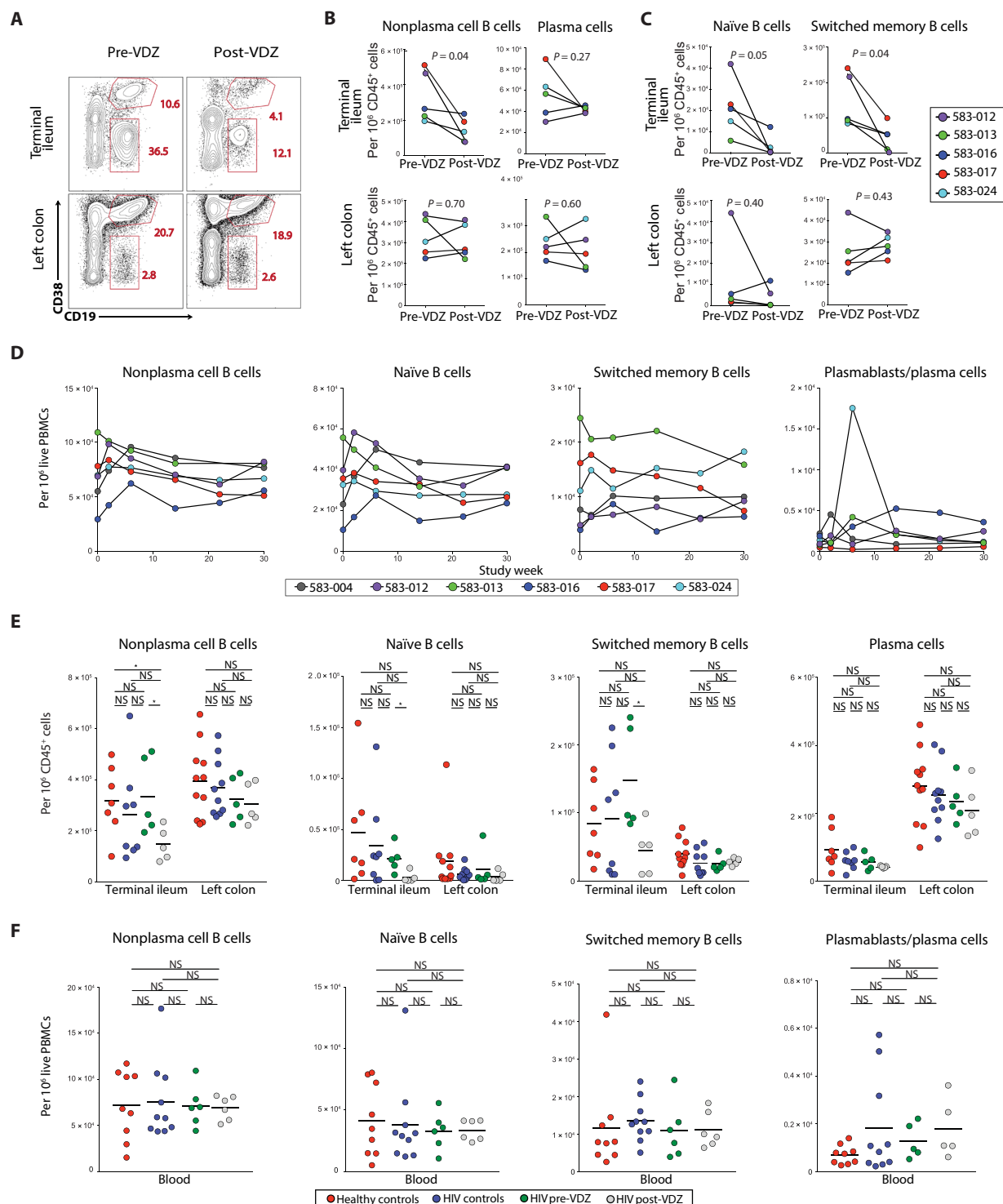


Fig. 1. Anti- $\alpha 4\beta 7$ therapy decreases the frequency of B cell subsets in the TI. (A to C) Frequency of B cells and plasma cells in the GI tract before and after VDZ therapy. (A) Representative flow cytometry plots showing the expression of CD19 and CD38 among live, CD45⁺ cells derived from the TI (top panels) and LC (bottom panels) of subject 583-017, before VDZ and at week 30 after VDZ. (B) Plots comparing the change in frequency of nonplasma cell (CD19⁺CD38⁻) B cells and plasma cells (CD45⁺CD38⁺⁺CD27⁺) in the TI (top) and LC (bottom) between before and after VDZ treatment. (C) Plots demonstrating changes in the frequency of naïve (CD45⁺CD19⁺CD10⁻CD38⁻IgM⁺IgD⁺) and SM B cells (CD45⁺CD19⁺CD10⁻CD38⁻IgM⁺IgD⁻) in TI (top) and LC (bottom). (D) Change in the frequency of nonplasma cell B cells, B cell subsets, and plasma cells in the blood after VDZ therapy. In (A) to (D), each of the patients is represented with a unique color code. (E) Group comparisons in the frequency of B cell subsets and plasma cells within the TI and the LC. (F) Group comparisons in the frequency of B cell subsets and plasma cells in the peripheral blood. In (E) and (F), healthy volunteers ($n = 11$) are shown in red, HIV controls ($n = 10$) in blue, HIV-IBD subjects ($n = 5$) before VDZ in green, and HIV-IBD subjects after VDZ in gray. Two-tailed t test was performed to compare the different groups, and two-tailed paired t test was used to compare pre- and post-VDZ time points in the HIV-IBD patients. Statistical values are as indicated. * $P < 0.05$. NS, not significant.

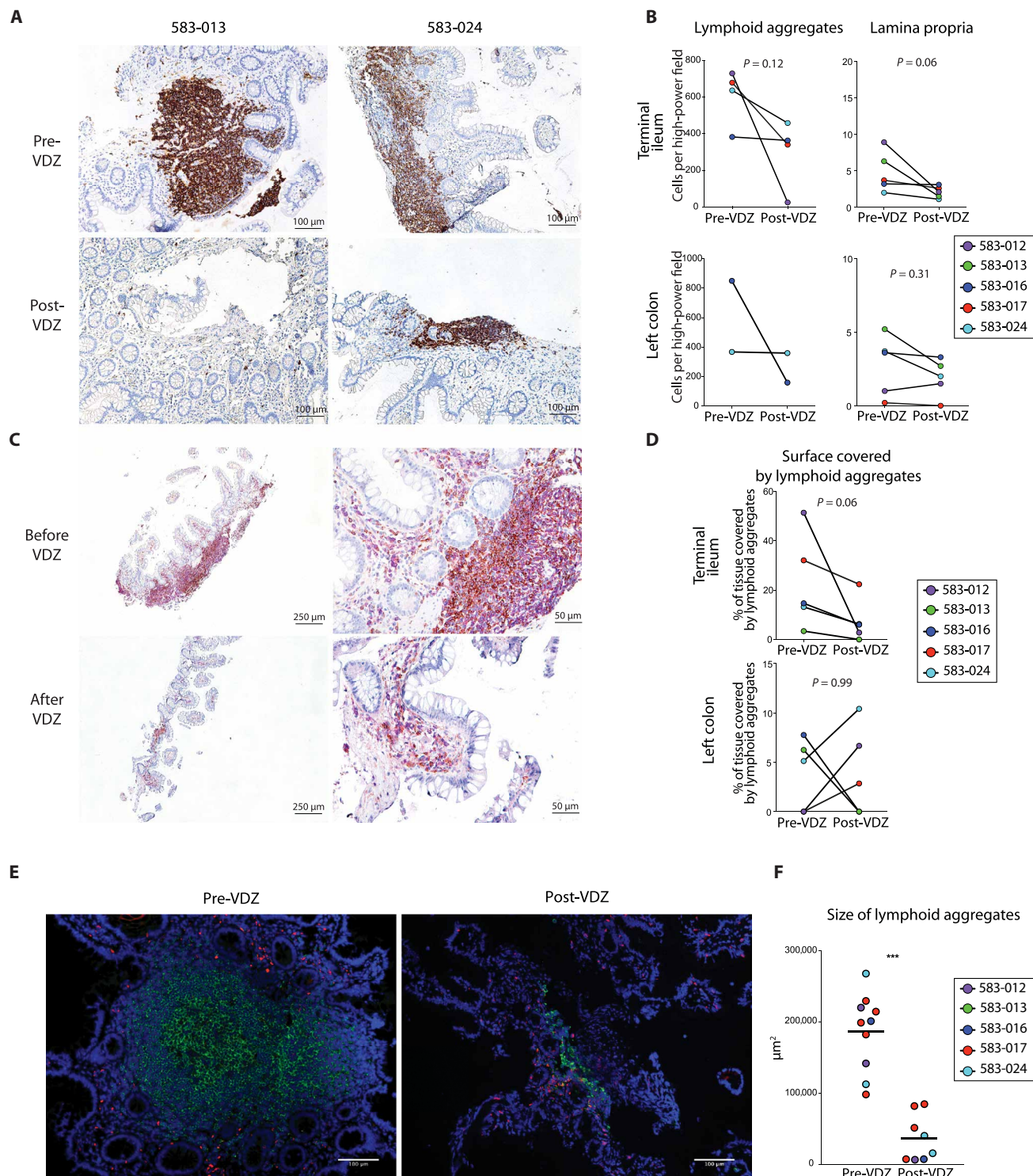


Fig. 2. Anti- $\alpha 4\beta 7$ therapy results in a significant attenuation of lymphoid aggregates, most pronounced in the TI. (A) Representative $\times 10$ magnification images of TI-derived biopsies immunostained for CD20 expression (brown) in two subjects (583-013 and 583-024) before (top panels) and after (bottom panels) VDZ. **(B)** Quantitative analyses of CD20⁺ B cells in the TI (top) and LC (bottom). Cell frequency was determined separately in lymphoid aggregates (left) and in lamina propria (right). **(C)** Representative images from subject 583-017 showing dual immunohistochemical staining with CD19 (pink) and CD4 (brown) before (top) and after (bottom) VDZ. Original magnification, $\times 4$ (left panel) and $\times 20$ (right panel). **(D)** Percentage of tissue covered by lymphoid aggregates in the TI (top) and LC (bottom) in each of the subjects. **(E)** Representative immunofluorescence image showing the expression of CD3 (red), CD20 (green), and 4',6-diamidino-2-phenylindole (blue) from the TI of subject 583-024, before (left panel) and after VDZ (right panel). Original magnification, $\times 10$. **(F)** Cumulative data showing size of lymphoid aggregates between before and after VDZ treatment. In (B), (D), and (F), each of the patients is represented with a unique color code. Statistical values are as indicated. Wilcoxon matched-pairs signed-rank test and two-tailed t test (F) were used for statistical comparisons. Statistical values are as indicated. *** $P < 0.0005$.

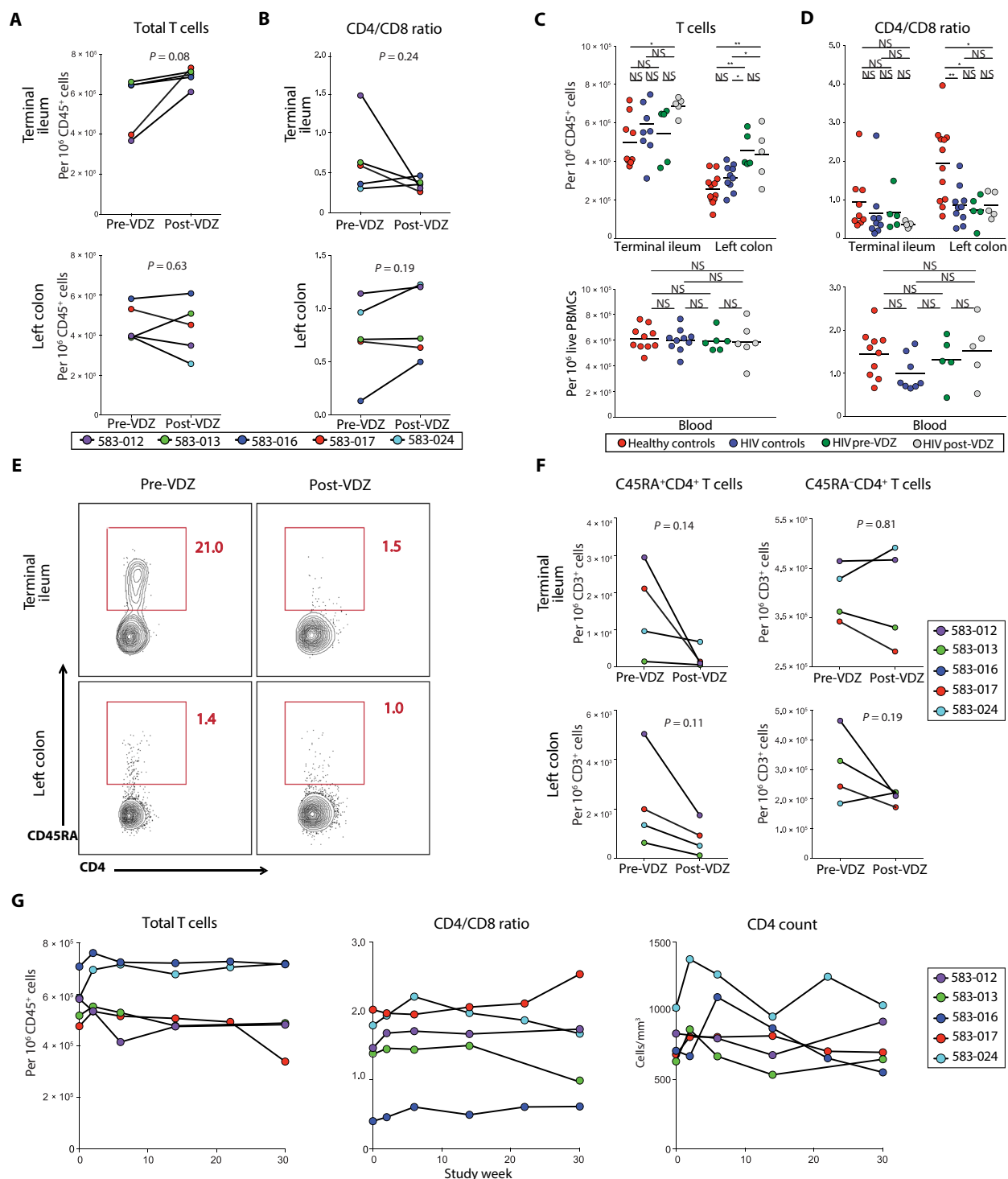


Fig. 3. Anti- $\alpha 4 \beta 7$ therapy results in a decrease in naïve CD4⁺ T cells in the TI. (A and B) Frequency of T cells in the GI tract before and after VZD therapy. (A) Frequency of total CD3⁺ T cells and (B) CD4/CD8 T cell ratio in the TI (top panels) and LC (bottom panels). Each of the patients is represented with a unique color code, and statistical values are indicated. (C and D) Group comparisons between the frequency of T cells (C) and CD4/CD8 ratio (D) in the GI tract (top panels) and peripheral blood (bottom panels). Healthy volunteers ($n = 12$) are shown in red, HIV alone controls ($n = 10$) in blue, HIV-IBD subjects before VZD ($n = 5$) in green, and HIV-IBD subjects after VZD ($n = 5$) in gray. (E and F) Frequency of naïve and memory CD4⁺ T cells in the GI tract before and after VZD. (E) Representative flow cytometry plots showing the expression of CD45RA on CD45⁺CD3⁺CD4⁺ T cells derived from the TI (top panels) and LC (bottom panels) of subject 583-024 before VZD and at week 26 after VZD. (F) Cumulative data showing changes in CD45RA⁺ (left) and CD45RA⁻CD4⁺ T cell subsets (right) within the TI (top panels) and LC (bottom panels). Notably, CD45RA⁺ staining on CD4⁺ T cells was available on four of five patients. (G) Cumulative data showing changes in the frequency of circulating total CD3⁺ T cells, CD4/CD8 ratio, and total CD4⁺ T cells during VZD therapy for each patient. In (F) and (G), each of the patients is represented with a unique color code, and statistical values are indicated. * $P < 0.05$, ** $P < 0.005$. Two-tailed t test was performed to compare the different groups, and two-tailed paired t test was used to compare pre- and post-VZD time points in the HIV-IBD patients.

volunteers, there was a significant increase in CD3⁺ T cells in the TI ($P < 0.05$) and the LC ($P < 0.01$) after VDZ (Fig. 3C, top panel). In contrast to total T cells, there was no significant change in the CD4/CD8 ratios in the TI after VDZ when compared to healthy volunteers ($P = 0.06$). HIV-1-infected subjects had significantly reduced CD4/CD8 ratio in the LC when compared to healthy volunteers ($P < 0.01$), consistent with the published literature (37). However, CD4/CD8 ratio in the LC did not change significantly after VDZ treatment ($P = 0.19$; Fig. 3D, top panel). In the LC, the HIV-IBD patients (before and after VDZ) had higher frequencies of CD3⁺ T cells (Fig. 3C). Although LC biopsies were obtained from histologically uninfamed areas (fig. S1), we cannot exclude IBD as a cause of increased frequencies of CD3⁺ T cells in the LC. Finally, among circulating T cells, there were no significant differences in the total T cells or CD4/CD8 ratios between healthy volunteers, HIV controls, or the study subjects before or after VDZ treatment (Fig. 3, C and D, bottom panels).

Within T cells, there was no significant change in the total numbers of CD4⁺ T cells as quantified by IHC (TI lamina propria, $P = 0.46$; LC lamina propria, $P = 0.17$; fig. S6). Among CD4⁺ T cell subsets, there were no significant changes in the memory CD4⁺ T cells (CD3⁺CD4⁺CD45RA⁻) after VDZ in the TI or LC ($P = 0.81$ and $P = 0.19$, respectively; Fig. 3, E and F). In contrast to memory CD4⁺ T cells, we noticed a decrease in naïve CD4⁺ T cells (CD3⁺CD4⁺CD45RA⁺) in the TI and LC after VDZ, although the changes were not statistically significant (TI, $P = 0.14$; LC, $P = 0.11$; Fig. 3, E and F). As shown in fig. S7, naïve T cells express $\alpha 4\beta 7$ at an intermediate level which potentially enables naïve T cell entry into the GI tract as has been documented in seminal studies by Butcher *et al.* (6, 8, 38). Thus, an intermediate level of expression of $\alpha 4\beta 7$ may underlie the impact of VDZ on this population. Naïve T cells reside within the lymphoid aggregates, whereas memory T cells are largely distributed in the intestinal lamina propria (5). These data again demonstrate a previously unappreciated effect of anti- $\alpha 4\beta 7$ therapy on lymphoid aggregates in the GI tract. In contrast, in circulation, although there was a short-term rise in total CD3⁺ T cells, CD4/CD8 ratios, and absolute CD4⁺ T cell counts at week 2 after treatment, over the course of 30 weeks, these counts tended to return to baseline (Fig. 3G).

In circulation, because $\alpha 4\beta 7$ was almost completely saturated by VDZ, $\beta 7$ integrin was used as a surrogate marker of $\alpha 4\beta 7$ (fig. S8). There was a significant rise in $\beta 7^{\text{hi}}$ CD45RA⁻CD4⁺ T cells, shown to be extremely susceptible to HIV-1 infection (23) at week 2, sustained over the duration of therapy (Fig. 4, A and B).

To further define the impact of VDZ on T cell subsets, we performed mass cytometry (CyTOF), allowing resolution of dozens of markers on a single cell (39, 40). Cryopreserved peripheral blood mononuclear cell (PBMC) samples from each of the patients were studied at baseline (week 0), week 2, and week 30. The data generated were analyzed using a combination of manual gating based on the expression of canonical markers and dimensionality reducing data visualization using visualization of *t*-distributed stochastic neighbor embedding (viSNE) (41) based on all phenotypic markers (Fig. 4C). To define major CD4⁺ T cell subsets including naïve, central memory (CM), effector memory (EM), terminally differentiated effector memory (EMRA) cells, regulatory T (T_{reg}) cells, and αE^+ (CD103) populations, we manually gated cells based on the expression of canonical markers as shown in the heat map in Fig. 4C. We then used viSNE analyses to examine the expression of $\beta 7$ integrin on each of the major CD4⁺ T cell subsets and further defined the expression of multiple phenotypic markers including CD29 ($\beta 1$ integrin), CD38

(activation), and CD161 (T helper 17 cells; Fig. 4D). Although we did not observe population-level differences among naïve, EMRA, and T_{reg} cells, there was a significant increase in the frequency of $\beta 7^+$ CM CD4⁺ T cells ($P < 0.05$ at week 2) and $\beta 7^+$ EM CD4⁺ T cells ($P = 0.01$ at week 30) in circulation after VDZ (Fig. 4E). Lack of population-level changes in T cell subsets was also observed using flow cytometry (fig. S9). Because of limited sample availability, CyTOF analyses could not be performed on GI-resident T cells in the present study.

Because the $\beta 7$ integrin can dimerize with either $\alpha 4$ or αE (fig. S10), we examined the contribution of $\alpha E\beta 7^+$ cells in the expansion of peripheral $\beta 7^+$ cells. $\alpha E\beta 7^+$ cells had high expression of $\beta 7$ integrin (Fig. 4D). In every patient, and most notably in 583-012 and 583-017, there was an increase in $\alpha E\beta 7^+$ cells in circulation after VDZ (Fig. 4E). In confirmation of the CyTOF data, conventional flow cytometry also revealed an expansion of CD45RA⁻ $\alpha E\beta 7^+$ cells in circulation (Fig. 4, F and G). That said, because $\alpha E\beta 7^+$ cells account for a minority (~3%) of the $\beta 7^+$ cells (fig. S10), we posit that the overall expansion of $\beta 7^+$ cells in circulation was due to $\alpha 4\beta 7$.

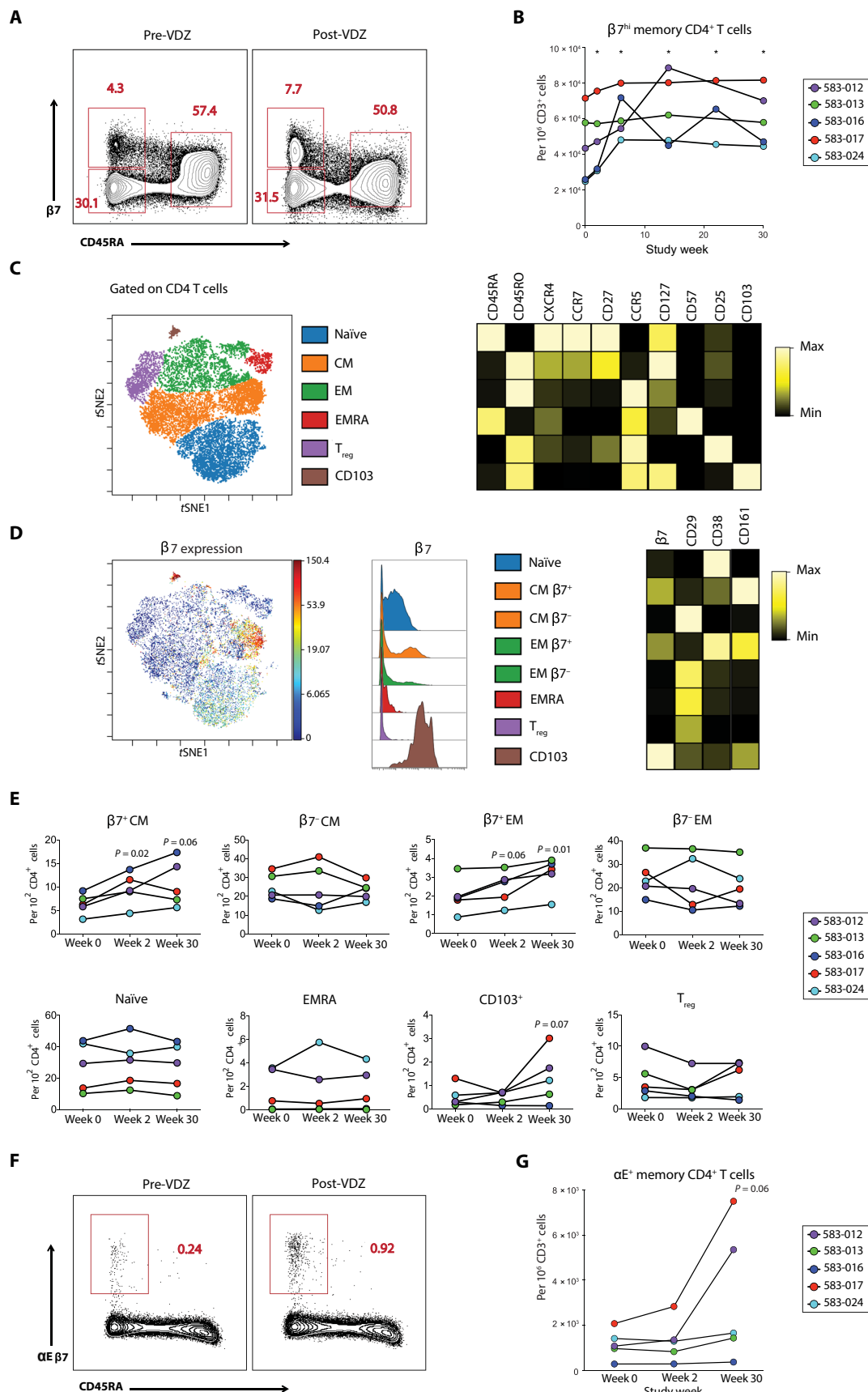
Anti- $\alpha 4\beta 7$ therapy is associated with a decrease in activated CD4⁺ T cells in the TI

Cellular activation, causally related to susceptibility to HIV-1 infection and HIV-1 latency (42) and an independent marker of disease progression (43, 44), is significantly greater in the GI tract than that in the peripheral blood of patients with HIV-1 infection (34). To determine the impact of anti- $\alpha 4\beta 7$ therapy on immunological activation in the GI tissue and peripheral blood, we assessed for the expression of CD38 on CD4⁺ and CD8⁺ T cells (defined in fig. S11). A significant reduction in the frequency of CD4⁺CD38⁺ T cells was seen in the TI in all five subjects after VDZ treatment ($P = 0.01$; Fig. 5, A and B). In the LC, CD4⁺CD38⁺ cells decreased in four of five subjects. Among CD8⁺ T cells, changes in CD8⁺CD38⁺ cells were not significant in the TI ($P = 0.17$) or the LC ($P = 0.65$; Fig. 5, A and B). In the peripheral blood, although individual subjects showed a reduction in CD4⁺CD38⁺ cells and CD8⁺CD38⁺ cells, there was variability as a group, and the results did not reach significance ($P = 0.88$ CD4⁺CD38⁺ cells and 0.68 for CD8⁺CD38⁺ cells at week 30; Fig. 5C). The change of the frequency of circulating CD38⁺HLA-DR⁺ double-positive CD4⁺ and CD8⁺ T cells was also variable between individuals, and no significant differences were noted after 30 weeks of treatment ($P = 0.25$ for CD4⁺ T cells and 0.68 for CD8⁺ T cells at week 30; fig. S12). To obtain a better resolution on the major CD4⁺ T cell subsets, we defined the expression of CD38 on memory and naïve cell subsets using CyTOF as detailed in Fig. 4 and segregated cells by $\beta 7$ expression. As shown in Fig. 5D, $\beta 7^+$ CM CD4⁺ T cells and naïve CD4⁺ T cells had a significant reduction in CD38 expression after VDZ, seen at week 2 ($\beta 7^+$ CM CD4⁺ T cells, $P = 0.02$; naïve CD4⁺ T cells, $P = 0.04$) and week 30 ($\beta 7^+$ CM CD4⁺ T cells, $P = 0.02$; naïve CD4⁺ T cells, $P = 0.02$). When compared with healthy volunteers and HIV controls, there was a nonstatistically significant reduction in CD4⁺CD38⁺ cells in the TI after VDZ treatment. In contrast, among circulating T cells, the frequency of bulk CD4⁺CD38⁺ and CD8⁺CD38⁺ cells was remarkably similar between healthy volunteers, HIV controls, and VDZ-treated subjects (Fig. 5D).

Anti- $\alpha 4\beta 7$ therapy results in early changes in natural killer cell composition and activation that equilibrates over time

Natural killer (NK) cells play a critical role in viral immunity including HIV with multiple lines of evidence supporting a role for both

Fig. 4. Anti- $\alpha 4\beta 7$ therapy is associated with alterations in the number and phenotype of $\beta 7^+$ cells in circulation. (A and B) Frequency of $\beta 7^{\text{hi}}$ memory T cell subsets in the peripheral blood before and after VDZ as measured by flow cytometry. (A) Representative flow cytometry plots from subject 583-017 comparing the expression of $\beta 7$ integrin and CD45RA on circulating CD4 $^+$ T cells before VDZ and at week 30 after VDZ. Three distinct populations are defined: $\beta 7^{\text{hi}}$ CD45RA $^-$, $\beta 7^+$ CD45RA $^-$, and $\beta 7^{\text{int}}$ CD45RA $^+$. (B) Cumulative data showing changes in circulating $\beta 7^{\text{hi}}$ CD45RA $^-$ CD4 $^+$ T cells during VDZ therapy for each patient. (C to E) CyTOF analyses to define alterations in the frequency of immune cell subsets after VDZ. (C) *t*-distributed stochastic neighbor embedding (tSNE) analyses showing the major T cell subsets including naïve, CM, EM, EMRA, T $_{\text{reg}}$ cells, and αE^+ (CD103) cells. These populations were manually gated on the basis of the expression of canonical markers as shown in the heat map on the right. (D) $\beta 7$ integrin expression on each of the immune populations defined in (C). The expression of CD29, CD38, and CD161 on each of the cellular subsets is shown by a heat map. (E) Frequency of the indicated cell populations by CyTOF for each of the patients at baseline, week 2, and week 30. (F and G) Flow cytometric evaluation of αE^+ (CD103) cells after VDZ. (F) Representative flow cytometry plots from subject 583-017 comparing the expression of αE (CD103) and CD45RA on circulating CD4 $^+$ T cells before VDZ and at week 30 after VDZ. (G) Cumulative data showing changes in circulating $\alpha E\beta 7^+$ CD45RA $^-$ CD4 $^+$ T cells during VDZ therapy for each patient. In (E) and (G), each of the patients is represented with a unique color code. Significance values are as indicated in the figure. Two-tailed *t* test was performed to compare the different groups, and two-tailed paired *t* test was used to compare pre- and post-VDZ time points in the HIV-IBD patients.



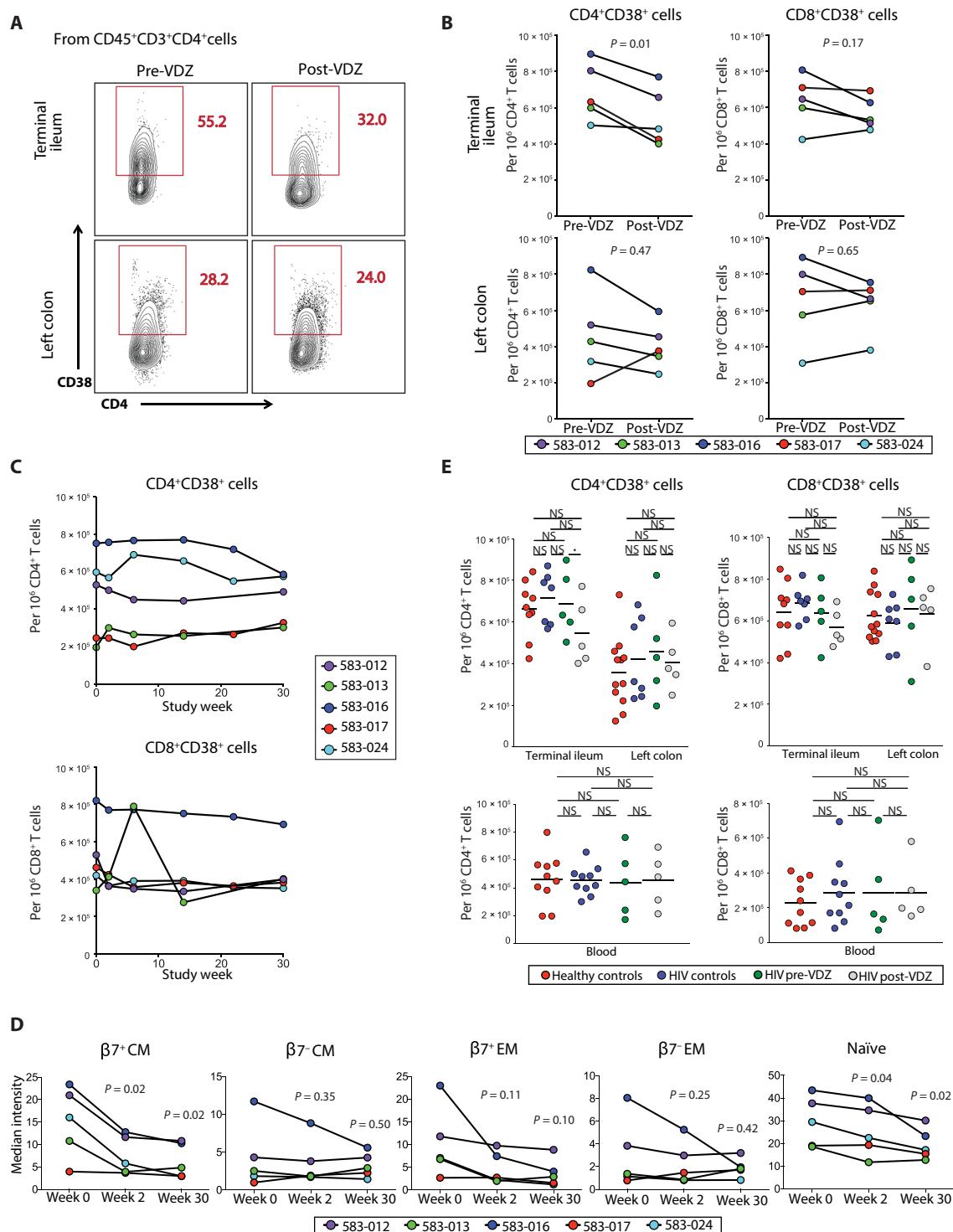


Fig. 5. Anti- $\alpha 4\beta 7$ therapy is associated with a decrease in activated CD4⁺ T cells in the TI. (A and B) Frequency of activated T cells in the GI tract before and after VDZ. (A) Representative flow cytometry plots showing the expression of CD38 on live CD45⁺CD3⁺CD4⁺ T cells derived from TI (top) and LC (bottom) in subject 583-013 before VDZ and at week 26 after VDZ. (B) Cumulative data showing changes in the number of CD4⁺CD38⁺ T cells (left) and CD8⁺CD38⁺ T cells (right) in the TI (top panels) and LC (bottom panels) in each of the study subject after VDZ. (C) Frequency of CD4⁺CD38⁺ T cells (top) and of CD8⁺CD38⁺ T cells (bottom) in the peripheral blood throughout the study. (D) CyTOF analyses showing the median intensity of expression of CD38, compared for the indicated cell population on each of the patients at baseline, week 2, and week 30. In (B) to (D), each of the patients is represented with a unique color code. Significance values are as indicated in the figure. (E) Group comparisons between the frequency of CD4⁺CD38⁺ T cells (top left) and CD8⁺CD38⁺ cells (top right) in the GI tract and in the peripheral blood (bottom panels). Healthy volunteers ($n = 12$) are shown in red, HIV controls ($n = 10$) in blue, HIV-IBD subjects before VDZ ($n = 5$) in green, and HIV-IBD subjects after VDZ ($n = 5$) in gray. Two-tailed paired t test was performed to compare the different groups, and two-tailed paired t test was used to compare pre- and post-VDZ time points in the HIV-IBD patients.

cytotoxic and regulatory functions in HIV-1 infection (45). Therefore, we analyzed the composition and the activation of NK cells at baseline, week 2, and week 30 after VDZ. We defined NK cells in three distinct subsets as follows: Cytolytic NK cells were defined as $CD56^{dim}CD16^{high}$ NK cells, cytokine-secreting NK cells as $CD56^{bright}CD16^{low}$ NK cells, and $CD56^{null}$ cells as $CD56^{low}CD16^{high}$ NK cells (Fig. 6A and fig. S13). We found that, in all patients, the frequency of circulating $CD56^{bright}CD16^{low}$ cytokine-secreting NK cells decreased at week 2 and increased back to a level similar to baseline at week 30 (Fig. 6B). No clear differences were seen in the two other subsets (Fig. 6, C and D) or in the subset of $CD56^{+}$ NK cells coexpressing CD8 (Fig. 6E).

We further looked at the expression of human leukocyte antigen-DR isotype (HLA-DR) and programmed cell death-1 (PD-1) on $CD56^{dim}$ cytolytic NK cells. An increase in the expression of HLA-DR was noted in four of five subjects at week 2, suggesting the activation of cytolytic NK cells after VDZ (Fig. 6F and fig. S14). Finally, the expression of PD-1 on $CD56^{dim}$ cytolytic NK cells also increased in four of five subjects at week 2 (Fig. 6G). Because of paucity of GI-derived cells, we were unable to assess for NK cell changes in the GI tract after VDZ. In summary, our data demonstrate changes in NK cell frequency and increased NK cell activation in circulation, early after anti- $\alpha 4\beta 7$ therapy.

Anti- $\alpha 4\beta 7$ therapy results in a variable effect on stimulated and unstimulated multiply spliced HIV-1 transcripts in blood-derived $CD4^{+}$ T cells

To study the impact of VDZ on the HIV reservoir, we used a combination of approaches assessing HIV-related measurement. All the patients continued on fully suppressive antiretroviral therapy for the course of this study. We observed a decrease in total HIV-1 DNA levels in sorted $CD4^{+}$ T cells derived from PBMCs in two of five patients. In addition, changes in proviral DNA were minimal in two of five patients and increased in one of five patients (total DNA, $P = 0.63$; integrated DNA, $P = 0.13$; Fig. 7A). In the TI, although there was a decrease in total and integrated HIV-1 DNA in three of five subjects, there was an increase in total and integrated HIV-1 DNA in two of five subjects and the changes were not statistically significant (total DNA, $P = 0.31$; integrated DNA, $P = 0.44$; Fig. 7B, top panels). Similarly, the effect of VDZ treatment on HIV-1 DNA levels in the LC was variable and not significant (total DNA, $P = 0.52$; integrated DNA, $P = 0.60$; Fig. 7B, bottom panels). We did not detect HIV-1 RNA levels in unstimulated GI-derived $CD4^{+}$ T cells, and paucity of cell numbers made performance of quantitative viral outgrowth assays not feasible in this initial study.

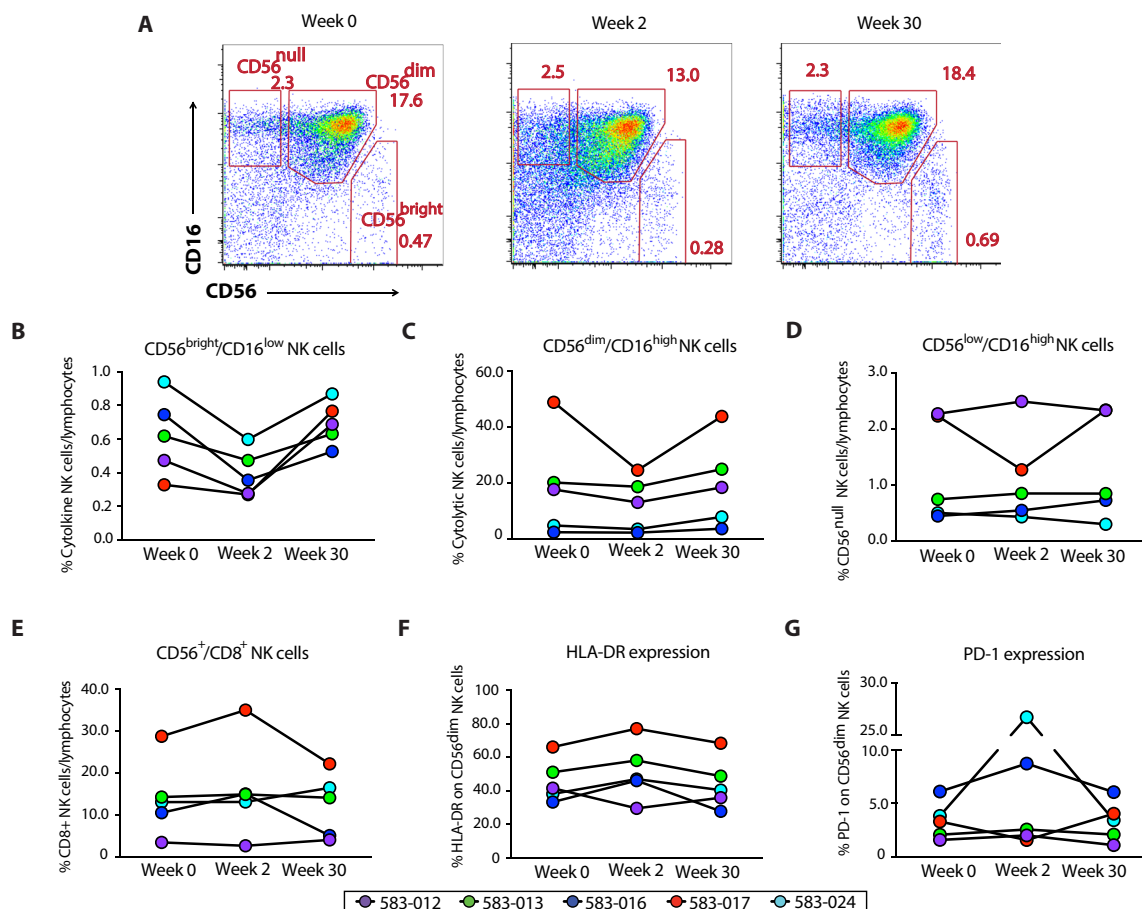


Fig. 6. Anti- $\alpha 4\beta 7$ therapy results in the activation of circulating NK cell subsets. (A) NK cell phenotype at weeks 0, 2, and 30 of therapy with VDZ. After exclusion of dead cells, monocytes, B cells, and T cells, cytolytic NK cells were gated as $CD56^{dim}CD16^{high}$ NK cells, cytokine-secreting NK cells were gated as $CD56^{bright}CD16^{low}$ NK cells, and $CD56^{null}$ cells were gated as $CD56^{low}CD16^{high}$ NK cells. (B to E) Composite graphs representing frequency of cytokine-producing (B), cytolytic (C), and $CD56^{null}$ NK cells (D), as well as $CD56^{+}CD8^{+}$ NK cells (E) from each of the five subjects (color-coded) at weeks 0, 2, and 30 of therapy with VDZ. (F and G) Change in the expression of HLA-DR (F) and PD-1 (G) on cytolytic $CD56^{dim}CD16^{high}$ NK cells from five subjects (color-coded) at weeks 0, 2, and 30 of therapy with VDZ.

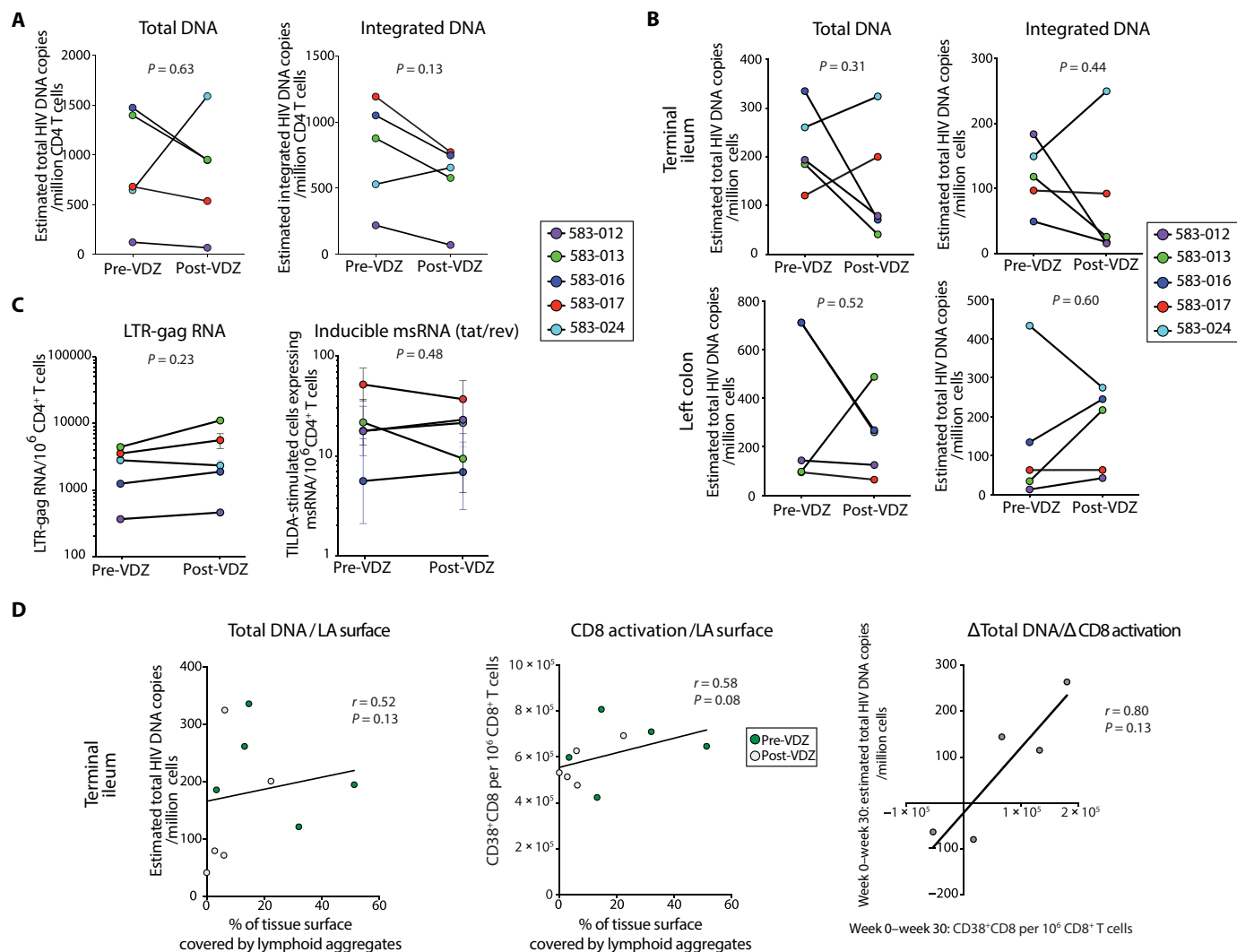


Fig. 7. Impact of anti- $\alpha 4\beta 7$ therapy on HIV-1 levels in the peripheral blood and in the GI tract. (A) Estimated copies per million cells of total and integrated HIV DNA in sorted CD4⁺ T cells derived from PBMCs before and after VZD. (B) Total and integrated HIV DNA in whole biopsies derived from the TI (top panels) and LC (bottom panels) before and after VZD. DNA copy number was normalized per housekeeping gene (CD3) copy number. (C) HIV-1 long terminal repeat (LTR)-gag RNA in unstimulated bead-selected, circulating CD4 cells before VZD and at week 30 after VZD (left panel). In the right panel, frequency of cells with inducible multiply spliced RNA (msRNA; tat/rev), measured with the TILDA assay, is compared before and after VZD. (D) Plots showing the linear regression line between various parameters in the TI mucosa. Left panel shows correlation between HIV total DNA within the TI and the percentage of tissue covered by lymphoid aggregates (LA). Middle panel shows the correlation between the frequency of CD8⁺CD38⁺ activated cells and the surface covered by lymphoid aggregates in the TI. The right panel represents the correlation between the magnitude of week 0 to week 30 changes in total HIV-1 DNA levels and the frequency of CD8⁺CD38⁺ cells. A positive number is representative of a decrease from week 0 to week 30. Correlation factors and *P* values were estimated with the Spearman correlation test. Two-tailed paired *t* test was used to compare before and after VZD values. Statistical values are as indicated.

Tat/rev-induced limiting dilution assay (TILDA) was performed on peripheral blood-derived, bead-sorted CD4⁺ T cells before VZD and at week 30 after treatment. In subjects 583-013 and 583-017, lower frequencies of cells with multiply spliced HIV RNA (tat/rev) were seen after treatment. In the absence of stimulation, LTR-gag transcripts were rare, and did not change significantly after VZD ($P = 0.23$; Fig. 7C, left panel). Overall, in the face of ongoing cART, spliced and unspliced HIV-1 RNA in the blood showed variability and inconclusive overall effect of anti- $\alpha 4\beta 7$ on markers of HIV-1 persistence ($P = 0.48$; Fig. 7C, right panel).

Further, we correlated the total DNA level in the TI with immunological parameters. We found a positive correlation ($r = 0.52$, $P = 0.13$) between the lymphoid aggregates and the DNA level, suggesting a po-

tential role of the TI lymphoid structures as viral reservoirs (Fig. 7D, left panel). In addition, the percentage of the GI surface covered by lymphoid aggregates in the TI correlated positively with CD8⁺ T cell activation ($r = 0.58$, $P = 0.08$; Fig. 7D, middle panel). Finally, we found a strong correlation ($r = 0.80$) between the magnitude of CD8⁺ T cell activation decrease and decrease in total DNA levels in the TI after VZD (Fig. 7D, right panel).

DISCUSSION

A growing body of evidence has demonstrated that $\alpha 4\beta 7$ ⁺CD4⁺ T cells represent an early target for HIV-1 (20, 21, 23). Accordingly, NHP studies using an anti- $\alpha 4\beta 7$ monoclonal antibody (mAb), that is a close

analog of VDZ, either before infection or in the context of SIV-infected animals receiving cART, have yielded promising and provocative results (26, 27). This has prompted two ongoing phase 1 clinical trials of VDZ in HIV-infected subjects. However, the underlying mechanism(s) by which anti- $\alpha 4\beta 7$ mAb therapy affected SIV pathogenesis remains unknown. Subjects coinfected with HIV and IBD represent a unique opportunity to investigate the clinical utility of VDZ therapy in HIV disease. The goals of the present report were to determine the safety and tolerability of anti- $\alpha 4\beta 7$ therapy in humans with HIV-1 infection. In addition, we performed detailed immunological analyses in the intestinal tissue and peripheral blood to provide insight into the mechanism(s) of action of this drug.

Data reported herein from six patients with chronic HIV-1 infection (five patients with detailed intestinal immunophenotyping) demonstrated that VDZ can be safely administered over extended periods of time. The only AEs recorded in our study were minor and self-limited, including episodes of upper respiratory infection as described previously (28).

To determine the immunological effects of VDZ on the GI tract, we examined both immune inductive sites (represented by lymphoid aggregates, concentrated in the TI) and effector sites (represented by lamina propria-associated lymphocytes). Notably, inductive sites such as the PPs harbor antigen-naïve T and B cells, whereas effector sites such as colonic lamina propria contain antigen-experienced memory T cells and plasma cells (5). Significantly reduced frequency of nonplasma cell B cell subsets (but not plasma cells) and naïve T cells (but not memory T cells) as assessed by flow cytometry after VDZ treatment drew our attention to the effect of anti- $\alpha 4\beta 7$ therapy on lymphoid aggregates. In confirmation of our hypothesis that anti- $\alpha 4\beta 7$ therapy targets lymphoid aggregates, we observed a marked attenuation of lymphoid aggregates in the course of treatment. These data, represent a previously unreported MOA of anti- $\alpha 4\beta 7$ therapy and can be explained, in part, by the fact that lymphocyte homing to both inductive and effector sites, are $\alpha 4\beta 7$ -dependent (6, 38). Although the existing literature has focused on drug therapeutic effects on memory cells homing to the effector compartment, we observed an even greater effect on the homing of naïve T and B cells to the inductive sites. Consistent with our observations, previous studies have reported variable effects of anti- $\alpha 4\beta 7$ treatment on lamina propria lymphocytes (10). This may reflect redundant cellular homing pathways to the lamina propria and a more exclusive dependence on $\alpha 4\beta 7$ -MAdCAM interactions in homing to inductive sites (6, 8, 38). In support, we have observed a significant increase in $\beta 7^{\text{hi}}$ memory $\text{CD}4^+$ T cells in circulation after VDZ therapy. Furthermore, NHP studies do not demonstrate hypocellularity in the lamina propria after VDZ treatment, which would be expected in the face of blocking cellular ingress (46). Finally, in one preclinical study where anti- $\alpha 4\beta 7$ therapy was administered to cynomolgus monkeys and as many as 48 different sites were examined for drug effect, atrophy of PPs was observed after treatment, whereas no changes in the intestinal lamina propria were discernable (47). Viewed collectively, no significant change in $\text{CD}4^+$ T cell frequencies in the lamina propria concurrent with an increase in $\beta 7^{\text{hi}}$ memory $\text{CD}4^+$ T in circulation implies that $\text{CD}4^+$ T cells are using redundant and perhaps $\alpha 4\beta 7$ -independent pathways of localizing to the GI lamina propria during VDZ therapy. Although $\alpha 4\beta 7$ is best described as a “pan-GI” homing marker, it is likely that alternative and more specialized pathways exist. As an example, a G protein-coupled receptor, GPR-15, was recently described to mediate homing of regulatory T cells to the colon (48). A better understanding

of $\alpha 4\beta 7$ -independent pathways of gut homing has important implications for IBD therapeutics and for HIV.

In addition to its effect on lymphoid aggregates, anti- $\alpha 4\beta 7$ was associated with a decrease in the frequency of activated $\text{CD}4^+$ T cell in the TI, which could be the consequence of either decreased activation or in reduced homing of activated cells. These data may also suggest an effect of immune stimulation by viral reservoirs in the TI. However, an obvious limitation here is the low number of patients—such observations would need to be validated in a larger data set. An additional point of interest and perhaps the subject of future investigation is the impact of therapy on the activation of $\text{CD}56^{\text{dim}}$ cytolytic NK cells at week 2 after treatment. Unfortunately, we do not have tissue assessments of NK cell phenotype/function to better understand the impact of therapy on this important innate immune subset.

Virological effects of therapy, modest at best in the present study, were not unexpected in the face of ongoing, fully suppressive cART. In addition, in contrast to a SIV-macaque study (9), our data do not show clear differences between reduction of viral loads in the small and large intestines.

The patients described herein had concomitant IBD which could potentially confound the interpretation of our results. Given that anti- $\alpha 4\beta 7$ therapy is not yet licensed for clinical use in HIV alone, this was a practical limitation for this study. That said, we believe that the impact of IBD on our analysis was minimal. The patients had very mild IBD. Furthermore, histological examination of tissues, arguably the gold standard for defining tissue inflammation, confirmed that the biopsied areas within the TI or the LC did not have mucosal inflammation.

Attenuation of lymphoid aggregates by anti- $\alpha 4\beta 7$ therapy may have important implications for HIV-1 infection. It is well established that B cell follicles are a source of viral replication in the context of chronic infection (49). Notably, emerging data suggest that B cell follicles are a major source of replication-competent virus during cART (50) and may represent one barrier to viral eradication. Follicular DCs, physiological long-term stores of antigen for the development of high-affinity antibodies (51), accumulate a large reservoir of infectious extracellular HIV virions within the B cell follicles and serve as a major source of infectious virions in vivo (52–54). Virus can then be passed to follicular $\text{CD}4^+$ T cells (including T follicular helper cells) that are highly susceptible to HIV-1 infection (55, 56) through a variety of mechanisms including B cell lymphoma 6 (BCL-6)-mediated diminished expression of interferon-stimulated genes (57). Moreover, B cell follicles may be semi-immune privileged sites due to the inability of cytotoxic T cells lacking follicular homing molecule CXCR5 (58) to enter them (59). In accordance with this concept, it has been hypothesized that overcoming the immune privilege of lymphoid follicles may be the key to HIV cure efforts (60). Among the strategies being considered are the development of HIV-specific, CXCR5-expressing chimeric antigen receptor T cells (61), the use of bispecific antibodies (62), or treatment with latency reversal agents (LRAs) (63). Although significant attention has been paid to LRAs such as histone deacetylase inhibitors, protein kinase C agonists, and recombinant interleukin-15, to date, no LRA agent is known to specifically target the lymphoid aggregates. Here, unexpectedly, we have identified that a gut-specific immunotherapeutic agent can lead to the attrition of B cell follicles in the GI tract. This represents a potentially important tool in the HIV-1 cure effort. Given that the impact of anti- $\alpha 4\beta 7$ therapy is primarily GI specific, it would possibly need to be combined with other agents to affect HIV-1 reservoirs in

In summary, the present report provides evidence of safety and tolerability of anti- $\alpha 4\beta 7$ therapy in HIV-infected subjects and provides insights into its effect on lymphoid aggregates. This activity may represent a new approach toward affecting persistent viral replication in gut tissues, which play a central role in HIV pathogenesis. In the ongoing efforts to develop an HIV cure, we believe that treatment with anti- $\alpha 4\beta 7$ agents in combination with other LRAs may represent a new therapeutic approach.

MATERIALS AND METHODS

Study design

Patients were recruited from within a cohort of HIV-1-infected patients with concomitant IBD (64) being followed at the Icahn School of Medicine at Mount Sinai and its affiliated hospitals and clinics. Informed consent was obtained from all the participants. The study protocol was approved by the Mount Sinai institutional review board.

Six subjects with IBD and concomitant HIV-1 infection were prospectively enrolled into the study. Of these, ileocolonoscopy was performed before VDZ initiation in five subjects, and biopsies were obtained from the TI and LC. In one subject (583-004), colonoscopy could not be performed before treatment due to logistical reasons (the patient had already received the first dose of VDZ as the colonoscopy was being scheduled). Before VDZ, blood from this patient was stored, and she signed on to the study to be followed prospectively. Thus, although we have detailed safety data and immunological and virological data in circulation before and after VDZ on this patient, we have not included her in the analyses, lacking the pre-VDZ gut data. Full immunological and virological analyses are being reported on five subjects. VDZ infusions were administered following the U.S. Food and Drug Administration–approved protocol for IBD, including an induction phase with a 300 mg intravenous (iv) infusion at weeks 0, 2, and 6, subsequently followed by a maintenance phase with a 300 mg iv infusion every 8 weeks. Before each infusion, blood was collected for analysis. A repeat colonoscopy was performed on each of the five subjects between week 22 and week 30 after treatment. Although the patients continue to be on long-term follow-up and on VDZ therapy, for the present report, we are describing the effects of 30 weeks of VDZ therapy. All the patients continued stable and uninterrupted cART for the length of the study.

In addition, we recruited a cohort of healthy volunteers ($n = 12$) and a cohort of non-IBD patients identified during chronic HIV-infection who were all cART-treated and well controlled ($n = 10$; clinical details in tables S2 and S3). All healthy volunteers and chronic HIV-1 subjects underwent colonoscopy and phlebotomy for immunological analyses in the GI tract and peripheral blood, respectively. In addition to existing protocols in the lab, we studied the impact of collagenase digestion on the expression of CD4 receptor (fig. S15). Primary data are located in table S6.

Statistical analysis

Plots were drawn using GraphPad Prism software. Statistical significance of immunophenotyping and viral data was assessed using the

two-sample paired Wilcoxon signed-rank test and two-tailed (paired) Student's *t* test when appropriate. Correlations were assessed using the Spearman test.

SUPPLEMENTARY MATERIALS

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Methodological details

Fig. S1. Histological examination of TI and LC before and after VDZ.

Fig. S2. B cell gating strategy.

Fig. S3. CD20 immunostaining of terminal ileal biopsies before and after VDZ.

Fig. S4. Change in the frequency of Ki67⁺ cells in GI tissue.

Fig. S5. Representative image of apoptotic bodies.

Fig. S6. CD4 quantification in biopsies of the TI and LC.

Fig. S7. Representative flow cytometry plots of showing the expression of $\alpha 4\beta 7$ on naïve and memory CD4⁺T cells in the peripheral blood of a healthy volunteer.

Fig. S8. Masking of $\alpha 4\beta 7$ by VDZ and $\beta 7$ as a surrogate marker of $\alpha 4\beta 7^+$ cells.

Fig. S9. Change in the frequency of CD4⁺ T cells subsets in circulation after VDZ.

Fig. S10. Expression of integrin $\alpha 4$ and αE on $B7^+$ memory $CD4^+$ T cells.

Fig. S11. Gating strategy to define the frequency of CD38⁺ "activated" CD4⁺ and CD8⁺ T cells in the GI tract.

Fig. S12. Change in the frequency of CD4⁺ and CD8⁺HLA-DR⁺CD38⁺ cells in circulation.

Fig. S13. Gating strategy for identifying NK cells.

Fig. S14. HLA-DR expression on cytolytic CD56^{dim}CD16^{high} NK cells.

Fig. S15. Impact of collagenase digestion on the frequency of intestinal CD4⁺ T cells.

Table S1. IBD-related clinical characteristics.

Table S2. Clinical characteristics of the healthy volunteers.

Table S3. HIV control cohort: Clinical characteristics.

Table S4. Flow cytometry antibodies

Table S5. List of CyTOF reagents.

Table S6. Primary data.

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Supplementary Materials for

Anti- $\alpha 4\beta 7$ therapy targets lymphoid aggregates in the gastrointestinal tract of HIV-1–infected individuals

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The PDF file includes:

Methodological details

Fig. S1. Histological examination of TI and LC before and after VDZ.

Fig. S2. B cell gating strategy.

Fig. S3. CD20 immunostaining of terminal ileal biopsies before and after VDZ.

Fig. S4. Change in the frequency of Ki67⁺ cells in GI tissue.

Fig. S5. Representative image of apoptotic bodies.

Fig. S6. CD4 quantification in biopsies of the TI and LC.

Fig. S7. Representative flow cytometry plots of showing the expression of $\alpha 4\beta 7$ on naïve and memory CD4⁺ T cells in the peripheral blood of a healthy volunteer.

Fig. S8. Masking of $\alpha 4\beta 7$ by VDZ and $\beta 7$ as a surrogate marker of $\alpha 4\beta 7$ ⁺ cells.

Fig. S9. Change in the frequency of CD4⁺ T cells subsets in circulation after VDZ.

Fig. S10. Expression of integrin $\alpha 4$ and αE on $\beta 7$ ⁺ memory CD4⁺ T cells.

Fig. S11. Gating strategy to define the frequency of CD38⁺ “activated” CD4⁺ and CD8⁺ T cells in the GI tract.

Fig. S12. Change in the frequency of CD4⁺ and CD8⁺HLA-DR⁺CD38⁺ cells in circulation.

Fig. S13. Gating strategy for identifying NK cells.

Fig. S14. HLA-DR expression on cytolytic CD56^{dim}CD16^{high} NK cells.

Fig. S15. Impact of collagenase digestion on the frequency of intestinal CD4⁺ T cells.

Table S1. IBD-related clinical characteristics.

Table S2. Clinical characteristics of the healthy volunteers.

Table S3. HIV control cohort: Clinical characteristics.

Table S4. Flow cytometry antibodies.

Table S5. List of CyTOF reagents.

References (65, 66)

Other Supplementary Material for this manuscript includes the following:

(available at www.sciencetranslationalmedicine.org/cgi/content/full/10/461/eaau4711/DC1)

Table S6 (Microsoft Excel format). Primary data.

METHODOLOGICAL DETAILS

Peripheral blood mononuclear cell (PBMC) isolation

50 ml of blood were drawn in EDTA tubes at baseline and preceding each VDZ infusion. Blood was processed, under sterile condition, within 2 hours after collection. Plasma was collected and stored immediately at -80°C. PBMCs were obtained using standard Ficoll Hypaque centrifugation gradient. Briefly, whole blood was diluted 1:1 with phosphate buffered Saline (PBS, Gibco) and gently overlaid on lymphocyte separation medium (MP Biomedical). After centrifugation, the phase containing PBMCs was harvested and washed 2 times with sterile PBS. A subset of the PBMCs was directly used for staining while the remaining was cryopreserved at -150°C using standard techniques.

Isolation of GI mononuclear cells

During the ileocolonoscopy procedure, mucosal biopsies were obtained using standard large cup biopsy forceps. We harvested biopsies from the TI as well as the uninflamed LC (n=30, limiting the effect of variability in sampling). Fresh biopsies were transported in RPMI (GIBCO) and processed immediately using standard techniques in the lab (2). Briefly, the intestinal biopsies were transferred to 10 ml of RPMI containing 0.005 g of Type IV collagenase (Sigma-Aldrich). Since exposure to collagenase has the potential to down-regulate cell surface receptors including CD4, we further optimized existing protocols in our lab to minimize confounders. As shown in fig. S15, there was no difference in the frequency of CD4/CD8 ratio between mechanical digestion only, a 22-minute incubation, and a 45-minute incubation with collagenase solution. Therefore, all the biopsies were incubated for a total of 44 min (2 rounds of 22 min for optimized yield) with gentle agitation (215 rpm) at 37°C. After the first 22-min round of incubation with

collagenase solution, biopsies were physically disrupted by chopping with scissors until obtaining a “porridge-like” consistency. The chopped tissue was transferred into a fresh RPMI-collagenase solution for a second round of 22-minute incubation at 37°C with gentle agitation. After incubation, the single-cell suspension was obtained by filtering through a 100-µm and a 40-µm cell strainer and washed with RPMI twice.

Flow cytometry analysis

Isolated cells were incubated with respective antibodies for 20 min at 4°C in the dark, washed 2 times with PBS and then fixed using Cytofix/Cytoperm buffer (BD) and subsequently washed with Perm/wash buffer (BD) and stored in PBS containing FBS (2%) until acquisition. Samples were acquired using LSR Fortessa (BD), and data were analyzed using FlowJo v10 (Tree Star). Dead cells and doublets were excluded from all analyses. The list of used Abs is shown in table S4.

Mass Cytometry (CyTOF) analyses

All mass cytometry reagents were purchased from Fluidigm Inc. (formerly DVS), unless otherwise noted. Cryopreserved samples from each of the patients were analyzed at baseline (wk0), wk2 and wk30. After thawing and washing twice with RPMI, PBMCs were incubated with Rh103 nucleic acid intercalator for 20 minutes to facilitate dead cell exclusion. The matched timepoint samples from patients were first barcoded using a cocktail of antibodies against CD45, B2m, and CD298 conjugated to distinct platinum isotopes. The barcoded timepoints from each patient were then pooled prior to surface staining to minimize any potential technical variation and facilitate accurate measurement of longitudinal changes in marker expression. The barcoded samples were then stained with a panel of metal-labeled antibodies against 33 cell surface markers (Table S5) for

30 min on ice and then washed. All antibodies were either purchased pre-conjugated to metal tags or conjugated in-house using MaxPar X8 Conjugation Kits according to the manufacturer's instructions. After antibody staining, the cells were washed and fixed with freshly diluted 1.6% formaldehyde in PBS containing 0.2% saponin and 1:4000 dilution of Ir nucleic acid intercalator to label all nucleated cells and stored until acquisition. Immediately prior to acquisition, the cells were washed in PBS, and CAS solution (Fluidigm) and resuspended in CAS containing a 1/10 dilution of EQ 4 Element Calibration beads. After routine instrument tuning and optimization, the samples were acquired on a Helios Mass Cytometer modified with a wide bore sample injector at an acquisition rate of <300 events/s. The resulting FCS files were concatenated and normalized using a bead-based normalization algorithm in the CyTOF acquisition software and analyzed with Cytobank. FCS files were manually pre-gated on Ir193 DNA⁺ events, excluding dead cells, doublets, and DNA-negative debris, and the samples associated with the corresponding timepoints were manually debarcoded using Boolean gating. The samples were analyzed using a combination of manual gating based on the expression of canonical markers and dimensionality reducing data visualization using viSNE (41) based on all phenotypic markers. Putative cell populations on the resulting viSNE maps were manually gated based on the expression of canonical T markers, while allowing for visualization of additional phenotypic heterogeneity within and outside the labeled populations. Aggregate statistics for defined cell populations were exported and analyzed using Prism (Graphpad Software).

Routine microscopy and Immunohistochemistry

Biopsies were collected directly in formalin and fixed for 24-48 hours. Fixed tissue was then embedded in paraffin and cut into 4 µm sections. Tissue sections were

immunohistochemically (IHC) stained with anti-CD20 and anti-CD4 antibodies. 4 µm paraffin sections were deparaffinized, and this was followed by antigen retrieval in EDTA buffer (pH 9.0) (Er2, Leica) for CD4 and in low pH (pH 6.0) citrate solution (Er1, Leica) for detection of CD20 and Ki67. The rest of the procedure was done in a Leica bond machine for CD20 and CD4 and in a Dako bond 3 automated instrument for Ki67. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide. The slides were incubated with pre-diluted antibodies against CD20 (clone L26, Bond RTU Primary, Leica), CD4 (clone 4B12, Bond RTU Primary, Leica) or Ki67 (clone MIB-1, unconjugated, Flex RTU, Agilent) at room temperature for 1 hour. The detection was done using the Bond PolymerRefine Detection system (Leica) including a hematoxylin counter stain. Frequency of Ki67, CD20 and CD4 within the lamina propria and the lymphoid aggregates was then counted in a blinded fashion by HMK and MT and expressed as positive cells per high power field.

CD19 - CD4 double staining was performed using the Ventana Discovery ULTRA platform. Antigen retrieval was done at a slightly basic pH with CC1 reagent (Ventana). A mouse anti-human CD4 (clone 4B12, Agilent) and a rabbit anti-human CD19 (clone EPR5906, abcam) were used as primary antibodies. For 3, 3'-diaminobenzidine (DAB), anti-mouse HRP pre-diluted secondary antibody (Ventana) was used and then Discovery ChromoMap DAB was applied (Ventana). For purple stain, we used anti-Rabbit 3 hydroxy-quinoxaline (HQ) (Ventana) secondary, then applied anti-HQ HRP (Ventana) followed by the application of Discovery purple color (Ventana).

Lymphoid aggregates were determined by an experienced gastrointestinal pathologist (HMK), who was blinded to the identity of the samples. A “lymphoid

aggregate” was defined as an aggregate of lymphocytes with or without identifiable germinal centers within the basal aspect of the lamina propria (background lymphocytes in the lamina propria tend to be evenly distributed). Lymphoid aggregates were quantified per mm².

Immunofluorescence assay

Tissues were fixed in 10% neutral buffered formalin, and immunofluorescence was performed on 5µm paraffin-embedded sections. Deparaffinization and antigen retrieval were performed by heating sections 55°C for 20 minutes, followed by a series of hydration washes as follows: three 100% Xylene for 5 minutes each, two 90% EtOH for 3 minutes each, 70% EtOH for 3 minutes, 30% EtOH for 3 minutes, diH₂O for 3 minutes. Antigen retrieval was achieved by boiling sections in citrate buffer (pH=6.5) for 10 minutes. Tris Buffered Saline (TBS) supplemented with 0.05% Tween 20 was used as a wash solution and TBS supplemented with 0.1% Tween 20 with 2.5% bovine serum albumin (BSA) was used as an antibody diluent. Primary antibodies for CD20 (polyclonal Goat Ab, Sigma Aldrich) and CD3 (clone SP7, Rabbit mAb, Abcam) were diluted with diluent to 1:200 and 1:100 respectively and incubated at room temperature for 1 hour. Secondary antibodies for CD20 (Alexafluor 488 Donkey anti Goat 705) and CD3 (Alexafluor 594 Donkey anti Rabbit) were diluted with diluent to 1:200 each and incubated at room temperature for 1 hour. Slides were mounted with Vectashield Hardset with DAPI and allowed to cure for 1 hour before storing slides at 4°C. Slides were analyzed using an AxioImager Z2 microscope (Zeiss) and Zen 2012 software (Zeiss).

To quantify the size of the lymphoid aggregates, original images were produced on a Zeiss AxioImager Z2 using 10X objective with air immersion and 0.75 numerical

aperture. Tiff images were exported with a scale-bar using Zen2 Blue Edition from original .czi files. Multiple sections were stained to ensure that the lymphoid aggregates were represented. For each section where a lymphoid aggregate was present, the area of the aggregate was measured and the average area was reported for each patient using ImageJ version 2.0.0-rc-67/1.52d. The pixel/micron scale was verified prior to data collection. First, .tif images were used to verify pixels/micron scale using the scale bar burned into the image. These values were compared to the pre-populated pixel/micron scale automatically generated by Image J when .czi files were uploaded.

Cell-associated HIV viral DNA measurements

For the peripheral blood derived CD4 T cell DNA/RNA isolation cryopreserved PBMCs were rapidly thawed and enriched for CD4⁺ T cells to purities >95% with an EasySep Human CD4⁺ T cell enrichment kit (Stemcell Technologies). Both DNA and RNA were extracted from enriched CD4 T cells using AllPrep DNA/RNA/miRNA Universal Kit (Qiagen). DNA and RNA purity and quantity were measured using the ND-2000 spectrophotometer (NanoDrop Technologies).

For the gastrointestinal tissue (Ileal and sigmoid colonic) DNA/RNA isolation, 4 to 6 biopsies were placed in RNALater (Ambion) and stored at -80°C until nucleic acid isolation. Tissue biopsies were homogenized using the Qiagen TissueRuptor II in Qiagen RLT Buffer. DNA and RNA were isolated from homogenized tissue biopsies using the Qiagen AllPrep DNA/RNA/miRNA Universal kit according to manufacturer's protocol for tissue specimens.

Determination of total cellular and integrated HIV-1 DNA was conducted according to previously published methods (65). In brief, pre-amplification using LTR-gag-specific

primers ULF1 (5' ATG CCA CGT AAG CGA AAC TCT GGG TCT CTC TDG TTA GAC 3'; HXB2 452-471) and UR1 (5' CCA TCT CTC TCC TTC TAG C 3'; HXB2 775-793) for total HIV-1 DNA, and using LTR-Alu specific primers ULF1, Alu1 (5' TCC CAG CTA CTG GGG AGG CTG AGG 3') and Alu2 (5' GCC TCC CAA AGT GCT GGG ATT ACA G 3') for integrated HIV-1 DNA was conducted. Pre-amplification process was carried out using a reaction volume of 50 µl with 5 µl Taq Buffer, 3 µl of MgCl₂, 0.6 µl of each dNTP, 1.5 µl of each primer, 0.5 µl of Taq polymerase and 5 µl of DNA template. Cycling conditions are 95°C for 8 min, then 12 cycles of 95°C for 1 min, 55°C for 40s (total DNA) and 1 min (integrated DNA) and 72°C for 1 min (total DNA) and 10 min (integrated DNA), followed by elongation at 72°C for 15 min. The pre-amplification process was performed on a GeneAmp PCR System 9700 (Applied Biosystems Inc). For the subsequent total and integrated HIV-1 DNA quantification, samples were quantified with a qPCR TaqMan assay using Lambda T (5' ATG CCA CGT AAG CGA AAC T 3') and UR2 (5' CTG AGG GAT CTC TAG TTA CC 3'; HXB2 583-602) coupled with a FAM-BHQ probe (5' CAC TCA AGG CAA GCT TTA TTG AGG 3) on a StepOne Plus Real-time PCR System (Applied Biosystems Inc). Cell-associated total and HIV DNA quantification was measured using a reaction volume of 20 µl with 10 µl of 2x TaqMan Universal Master Mix II including UNG (Life technologies), 0.25 µl of 100 µM each primer, 0.4 µl of 10 µM probe, and 5 µl of 1:10 diluted pre-amplified PCR product as template. Cycling conditions were 50°C for 2 min, 95°C for 10 min, then 60 cycles of 95°C for 15s and 60°C for 1 min. Cells-equivalent DNA copy number was determined using the CD3 gene, and CD3 DNA copy number was quantified by direct extrapolation against a standard curve.

For the quantification of cell associated total HIV-1 RNA, qPCR TaqMan assay using LTR-specific primers F522-43 (5' GCC TCA ATA AAG CTT GCC TTG A 3'; HXB2 522–543) and R626-43 (5' GGG CGC CAC TGC TAG AGA 3'; 626–643) coupled with a FAM-BQ probe (5' CCA GAG TCA CAC AAC AGA CGG GCA CA 3) on a StepOne Plus Real-time PCR System (Applied Biosystems Inc) was conducted. Cell associated HIV-1 RNA copy number was determined in a reaction volume of 20 µl with 10 µl of 2x TaqMan RNA to Ct 1 Step kit (Life Technologies), 4 pmol of each primer, 4pmol of probe, 0.5 µl reverse transcriptase, and 5µl of RNA under identical cycling conditions. Cells-equivalent RNA copy number was determined. Patient specimens were assayed with up to 800 ng total cellular RNA or DNA in replicate reaction wells and copy number determined by extrapolation against a 7-point standard curve (1–10,000 cps) performed in triplicate.

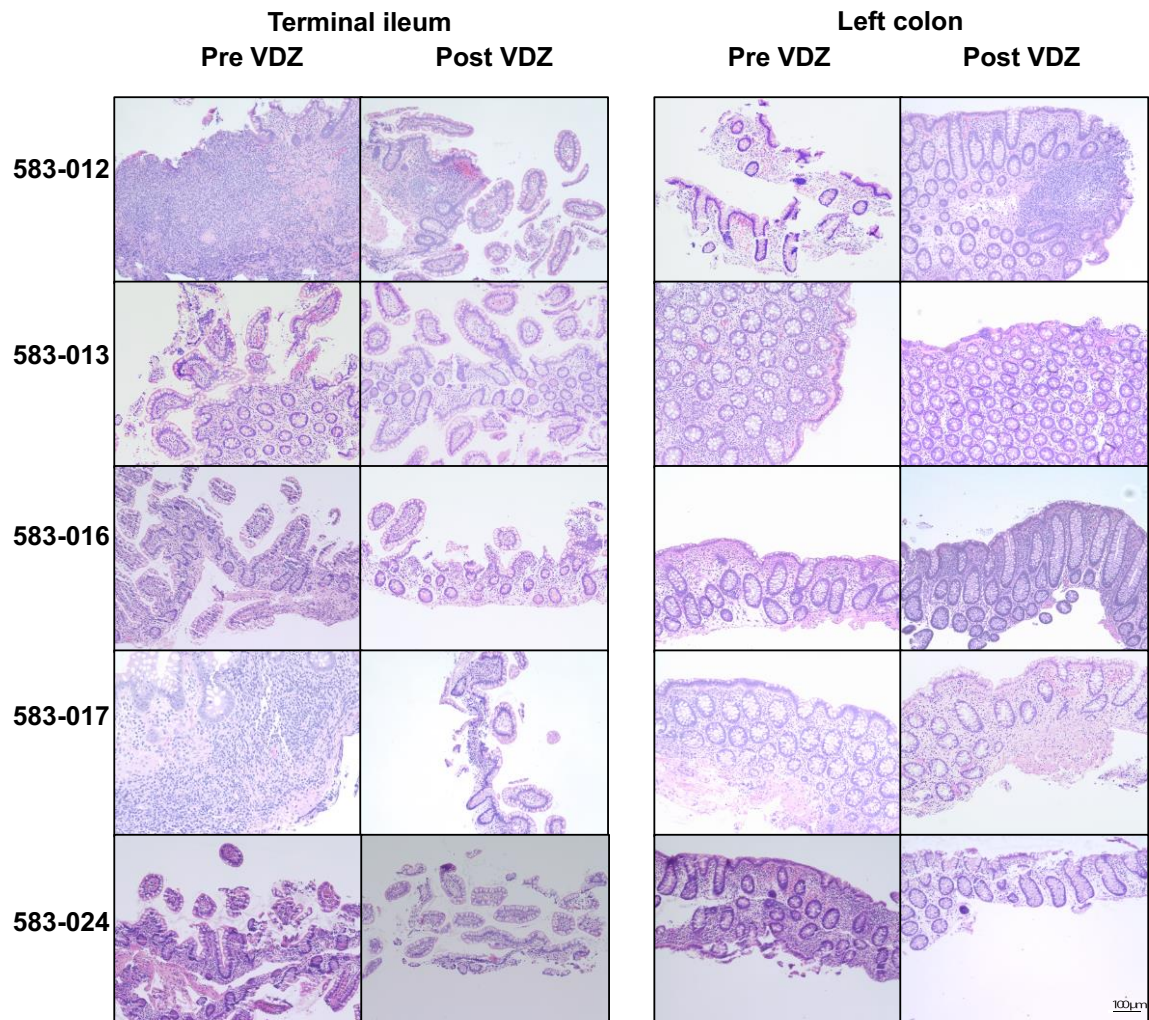
Quantification of cell-associated LTR-Gag RNA

Total CD4 T cells were isolated from frozen PBMCs using negative magnetic selection (Stem Cell technologies). RNA was extracted from isolated CD4 T cells with the Qiagen RNeasy Mini kit adding the Dnase step. Cell-associated LTR-gag transcripts were measured by nested real-time PCR. The primers used were previously described in Vandergeeten et al. (65). Results were expressed as HIV RNA copy numbers per million CD4⁺ T cells.

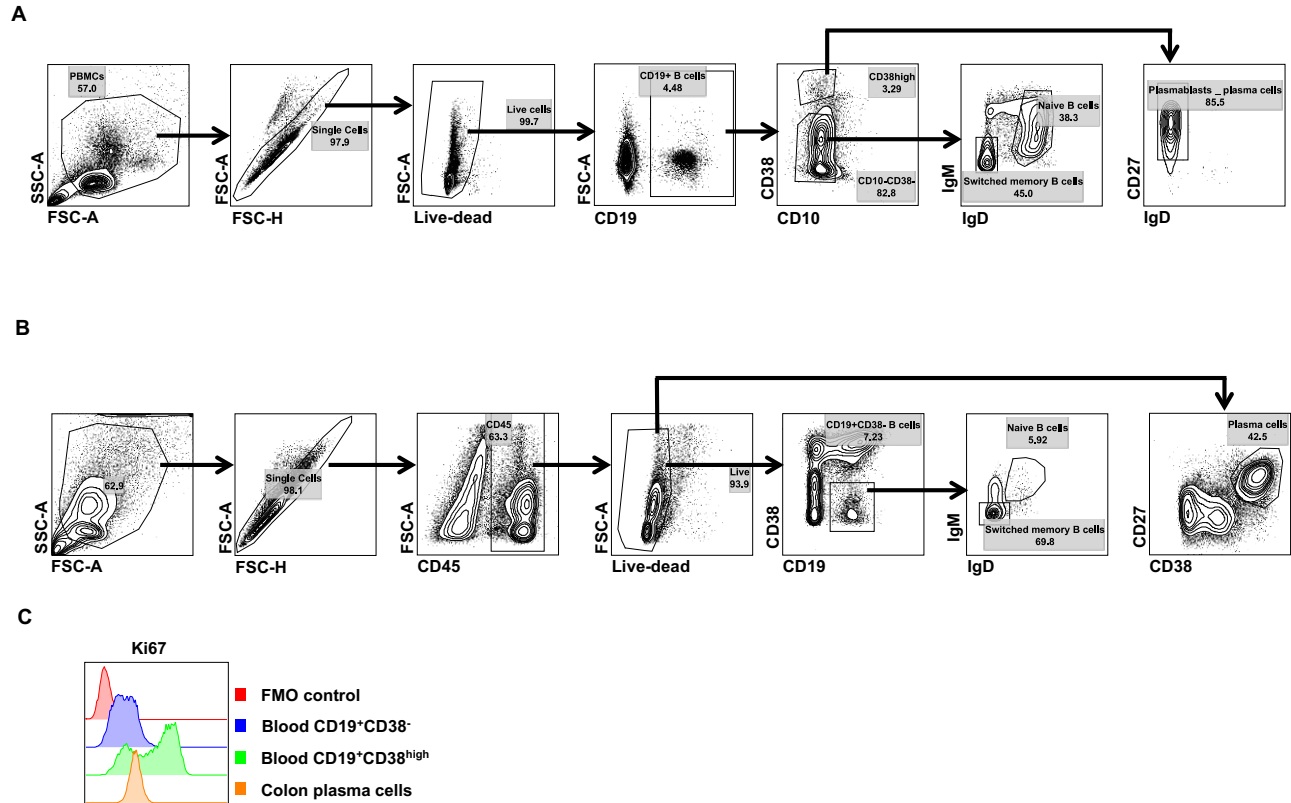
Tat/rev induced Limiting dilution Assay (TILDA)

The frequency of CD4⁺ T cells expressing multiply-spliced HIV RNA upon stimulation was measured using the tat/rev Induced Limiting Dilution Assay (TILDA) as previously described (66). Briefly, CD4 T cells were isolated from cryopreserved PBMCs

using negative magnetic selection (Stem Cell technologies) and stimulated with PMA (100ng/mL) and ionomycin (1µg/mL) for 18 hours. Stimulated cells were serially diluted and the wells in which cells producing tat/rev RNA were identified by semi-nested real-time PCR.



Supplementary Figure 1. Histological examination of TI and LC before and after VDZ. Hematoxylin and eosin (H&E) staining for GI biopsy samples where immunological or virological analyses were performed. Original Magnification= 10x. magnitude).

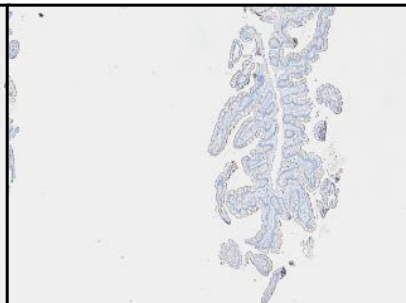
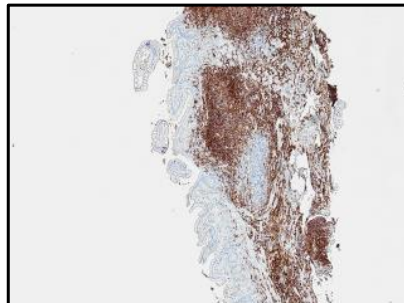


Supplementary Figure 2. B cell gating strategy. (A) Representative gating strategy to define B cell circulating subsets in blood including plasmablasts/plasma cells subset ($CD19^{+/\text{int}}CD38^{\text{high}}CD10^{-}CD27^{+}IgD^{-}$), switched memory B cells ($CD19^{+}CD38^{-}CD10^{-}IgM^{-}IgD^{-}$), and naive B cells ($CD19^{+}CD38^{-}CD10^{-}IgM^{+}IgD^{+}$). (B) Corresponding gating strategy in intestinal mucosa (here a colonic sample is displayed). Total plasma cells are defined as live $CD45^{+}CD38^{\text{high}}CD27^{+}$, while switched memory B cells and naive B cells are defined as $CD19^{+}CD38^{-}CD10^{-}IgM^{-}IgD^{-}$ and $CD19^{+}CD38^{-}CD10^{-}IgM^{+}IgD^{+}$, respectively, from live $CD45^{+}$ cells. (C) Representative expression of Ki67 on various blood and intestinal cell subsets.

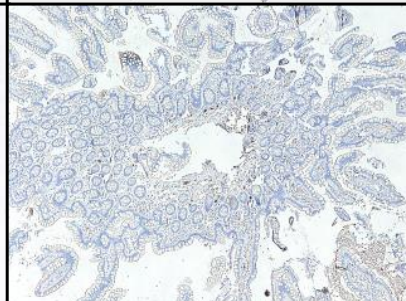
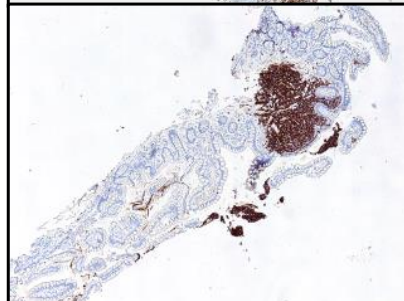
Pre VDZ

Post VDZ

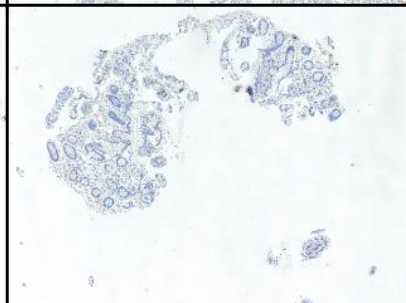
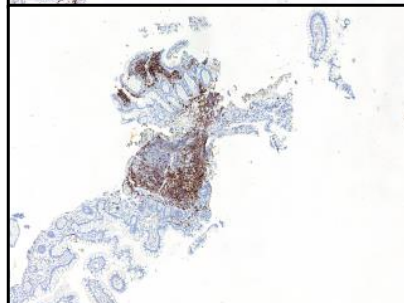
583-012



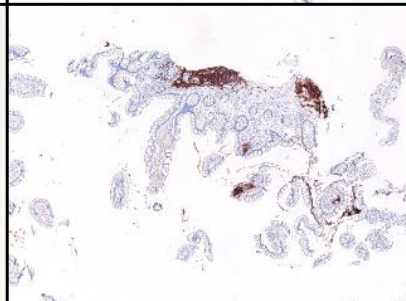
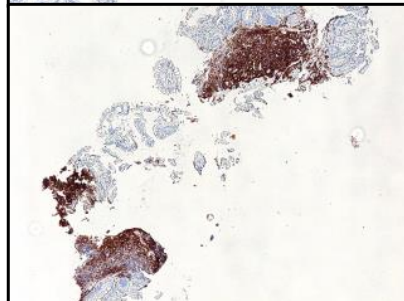
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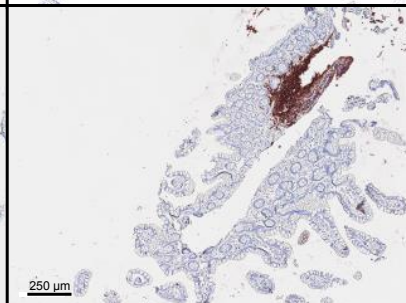
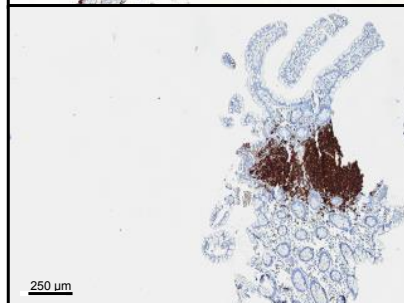
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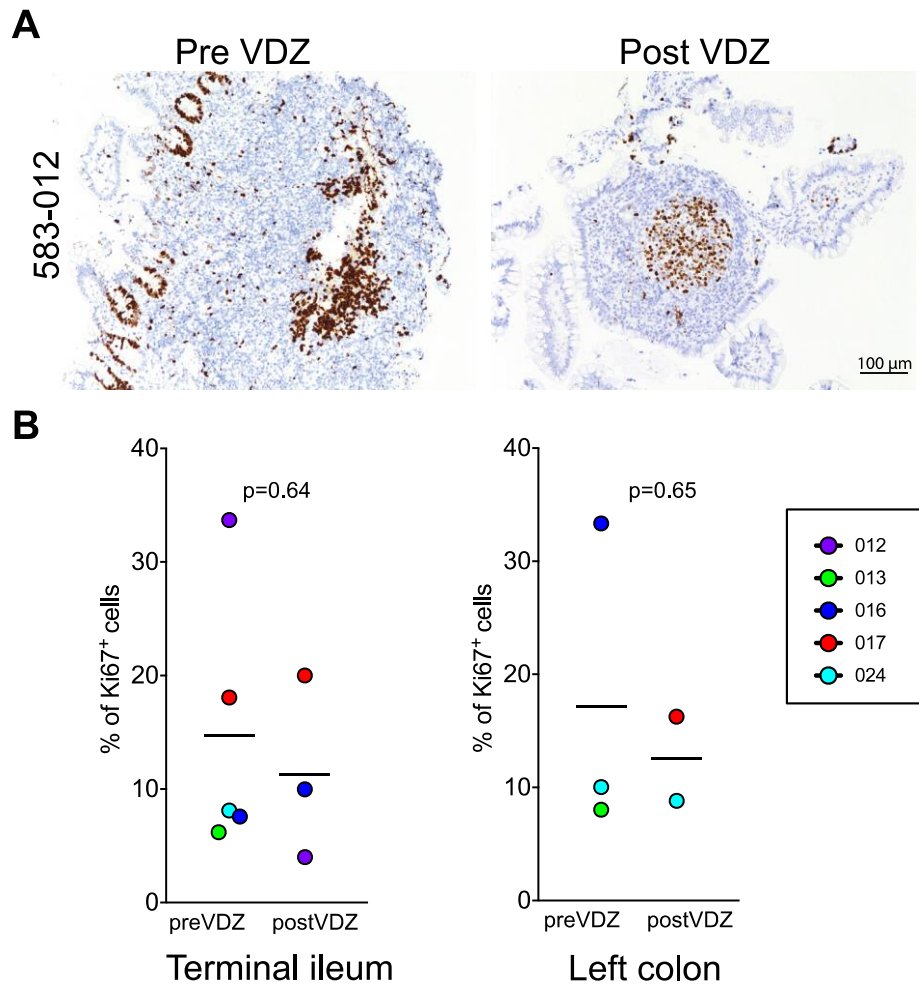
583-017



583-024



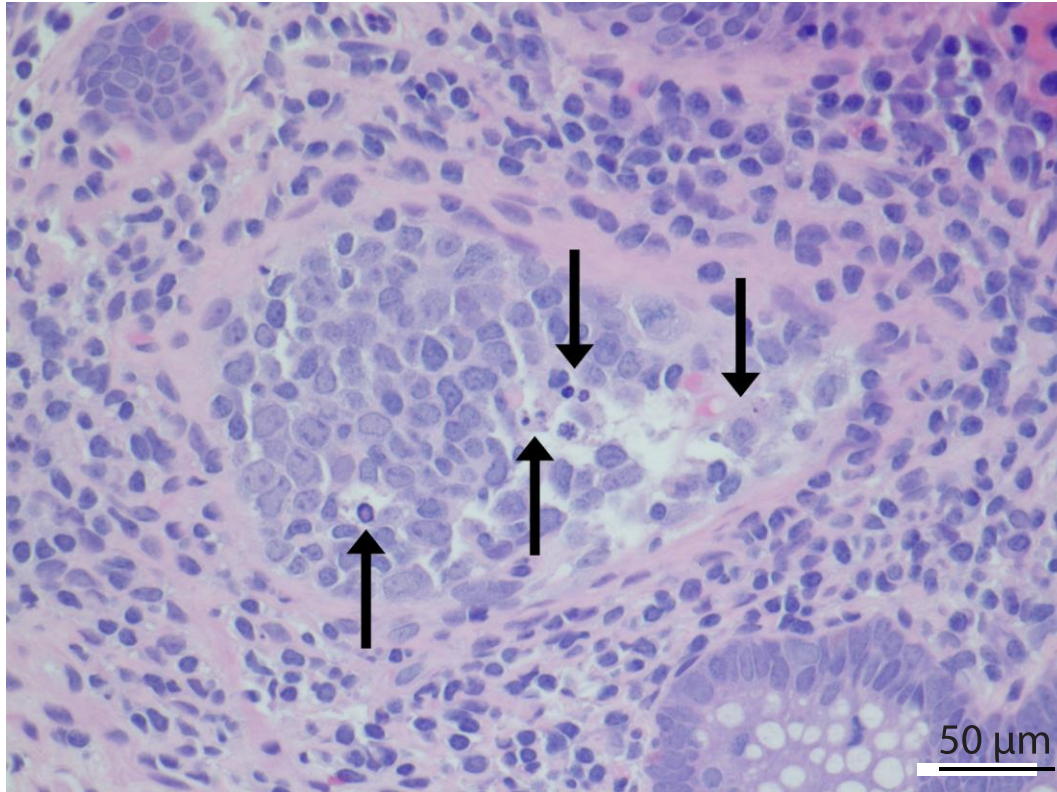
Supplementary Figure 3. CD20 immunostaining of terminal ileal biopsies before and after VDZ. Representative images of CD20 staining (Original Magnification=40x).



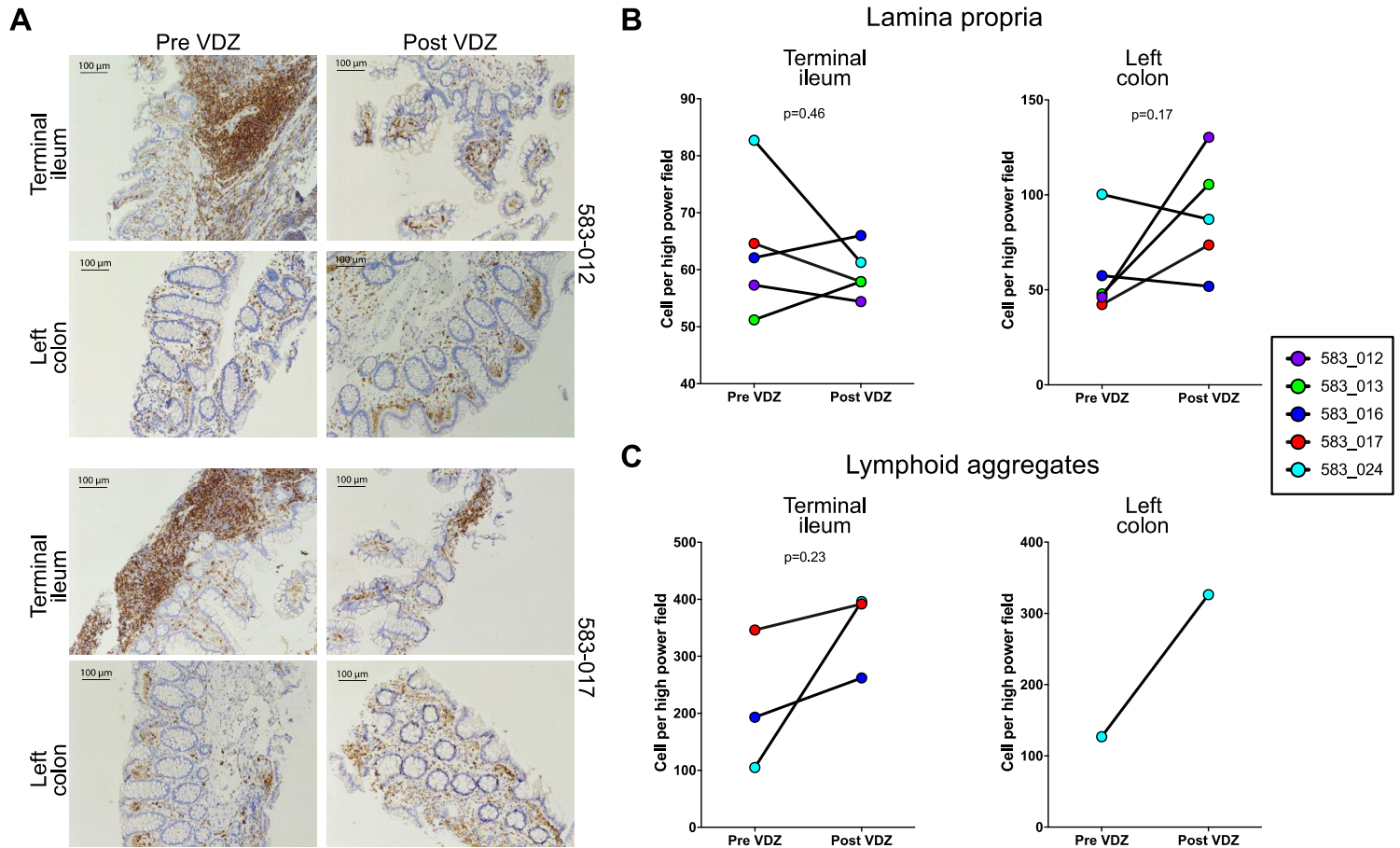
Supplementary Figure S4. Change in the frequency of Ki67⁺ cells in GI tissue.

Proliferating (Ki67⁺) cells were quantified in the terminal ileum and left colon post-VDZ.

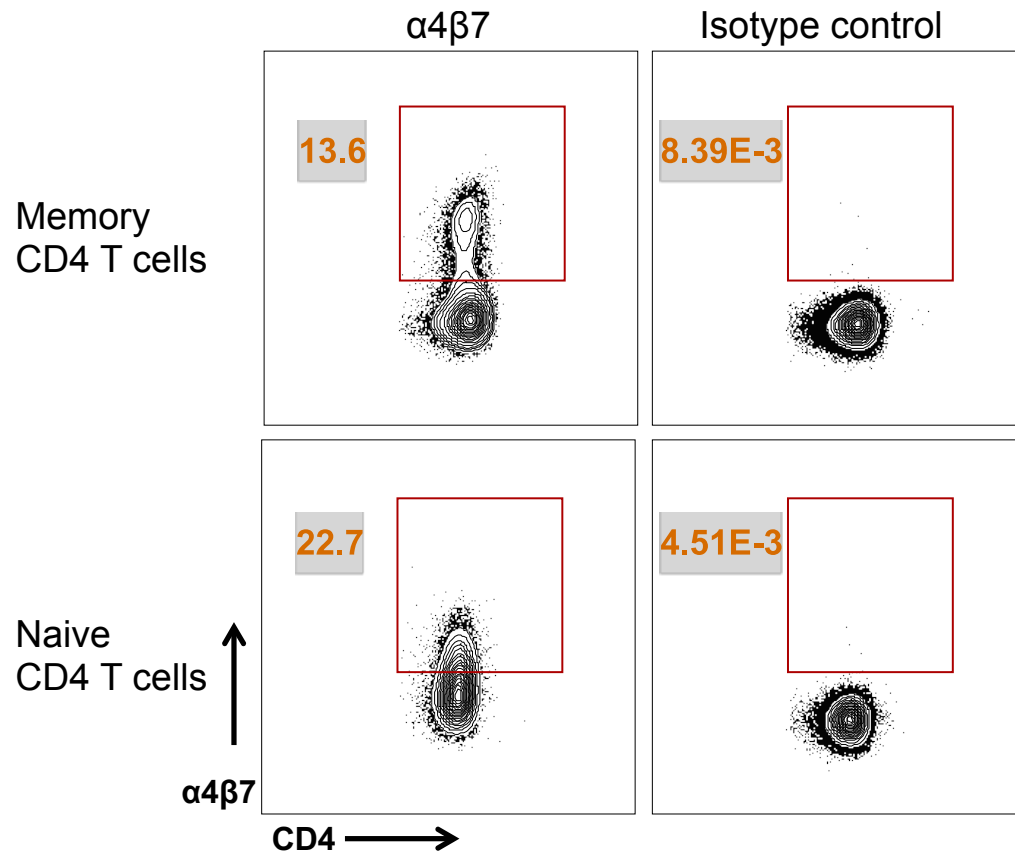
(A) Representative staining of TI biopsies. (B) Quantification of the percentage of Ki67⁺ cells (of total cells) within lymphoid aggregates in the terminal ileum and left colon.



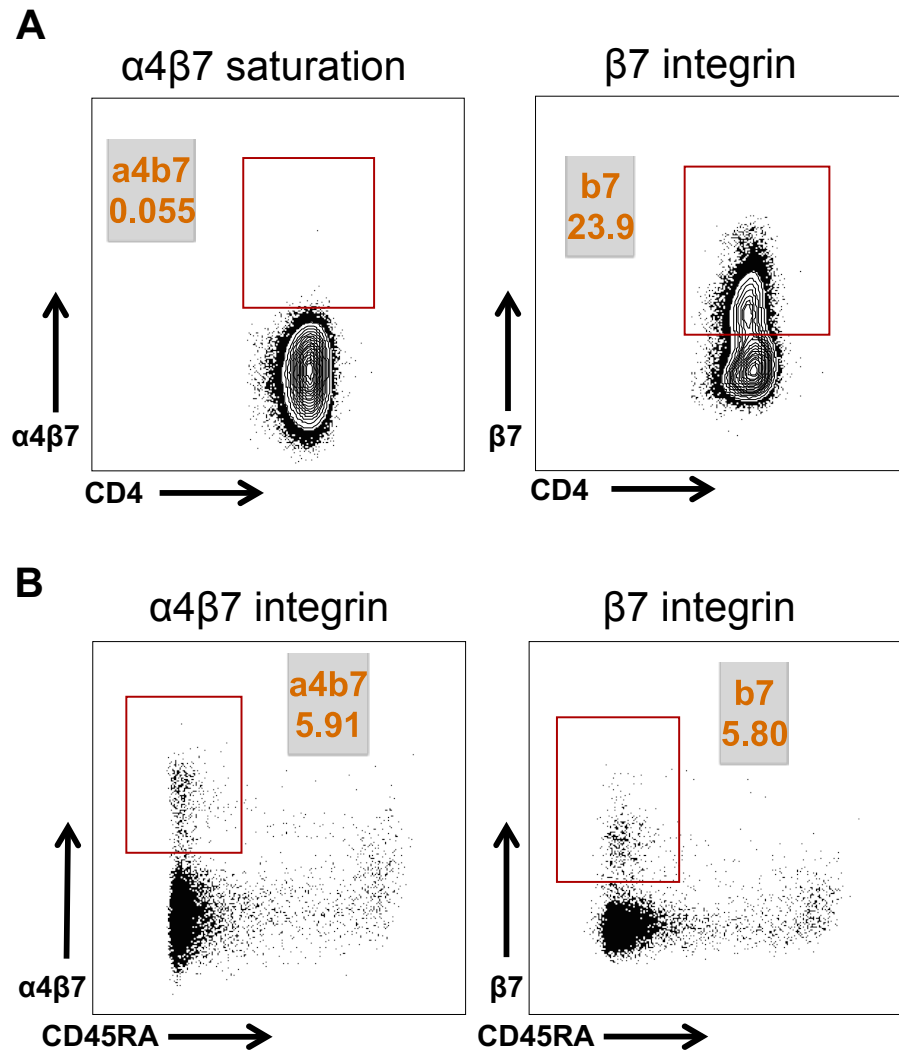
Supplementary Figure S5. Representative image of apoptotic bodies. Apoptotic bodies indicated by black arrows in the germinal center of a terminal ileal biopsy from patient 583-012 post-VDZ (Original magnification = 400x, hematoxylin and eosin staining).



Supplementary Figure S6. CD4 quantification in biopsies of the TI and LC. (A) Representative immunohistochemical images showing CD4⁺ cells (brown) in the terminal ileum (TI) and left colon (LC) from two representative subjects (583-012 and 583-017) pre- and post-VDZ (Original magnification= 100X). (B) Change in the frequency of CD4⁺ cells within the lamina propria of the TI and LC for each patient. (C) Change in the frequency of CD4⁺ cells within lymphoid aggregates of the TI and LC for each patient (Trend lines are displayed when at least one lymphoid aggregate was identified both pre and post treatment).



Supplementary Figure S7. Representative flow cytometry plots showing the expression of $\alpha 4 \beta 7$ on naïve and memory CD4⁺ T cells in the peripheral blood of a healthy volunteer.

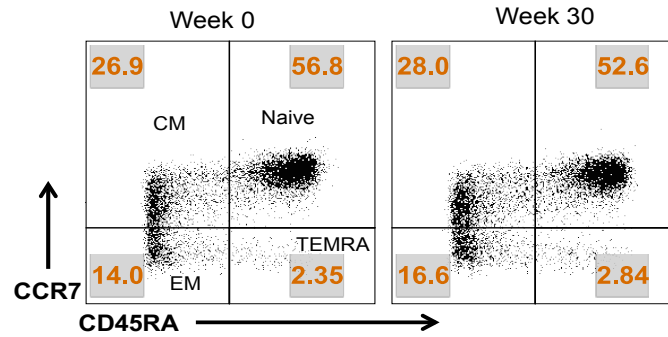
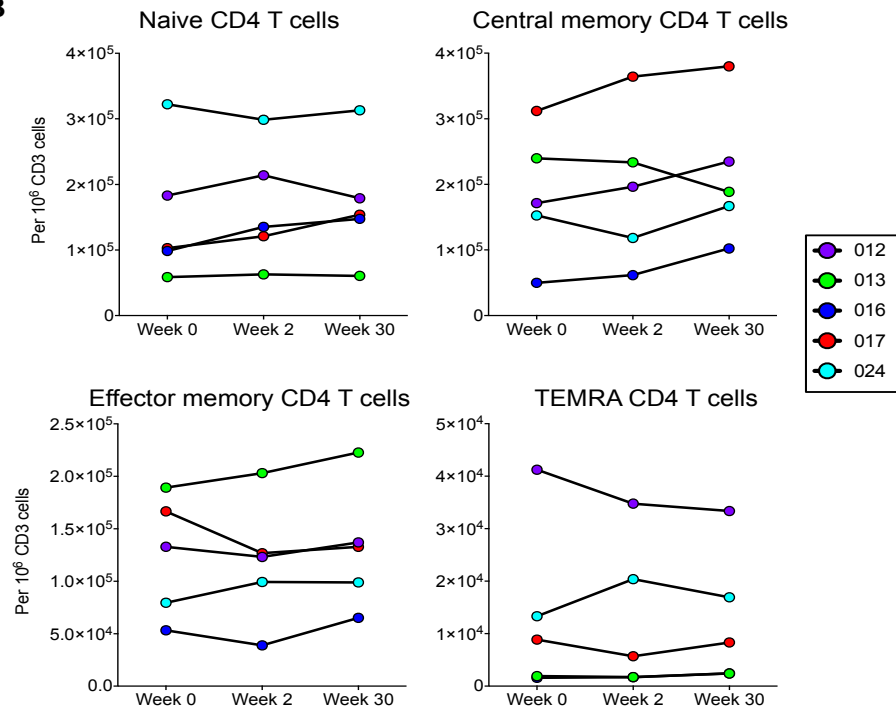


Supplementary Figure S8. Masking of $\alpha 4\beta 7$ by VDZ and $\beta 7$ as a surrogate

marker of $\alpha 4\beta 7^+$ cells. (A) Flow cytometry plot showing complete masking of $\alpha 4\beta 7$ in circulating memory $CD4^+$ T cells of a patient treated with VDZ, while $\beta 7$ expression is still detectable. (B) Flow cytometry plots comparing the expression of $\alpha 4\beta 7$ and $\beta 7$ separately on circulating $CD4^+$ T cells of a healthy volunteer.

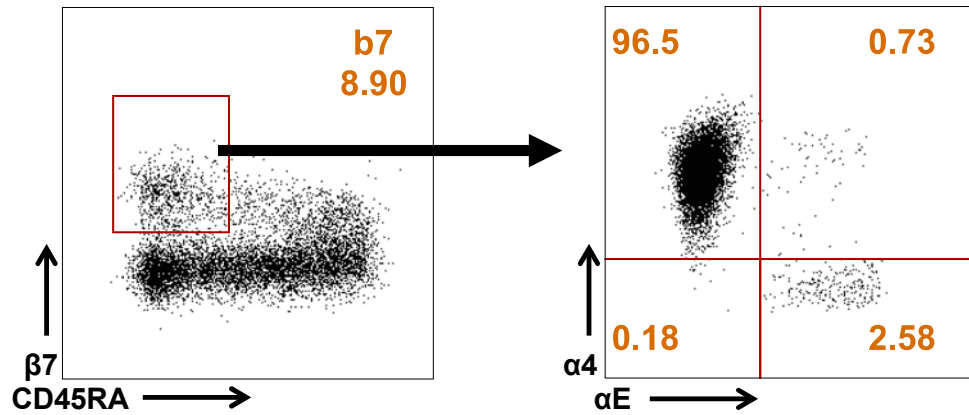
A

Gated out of CD4 T cells

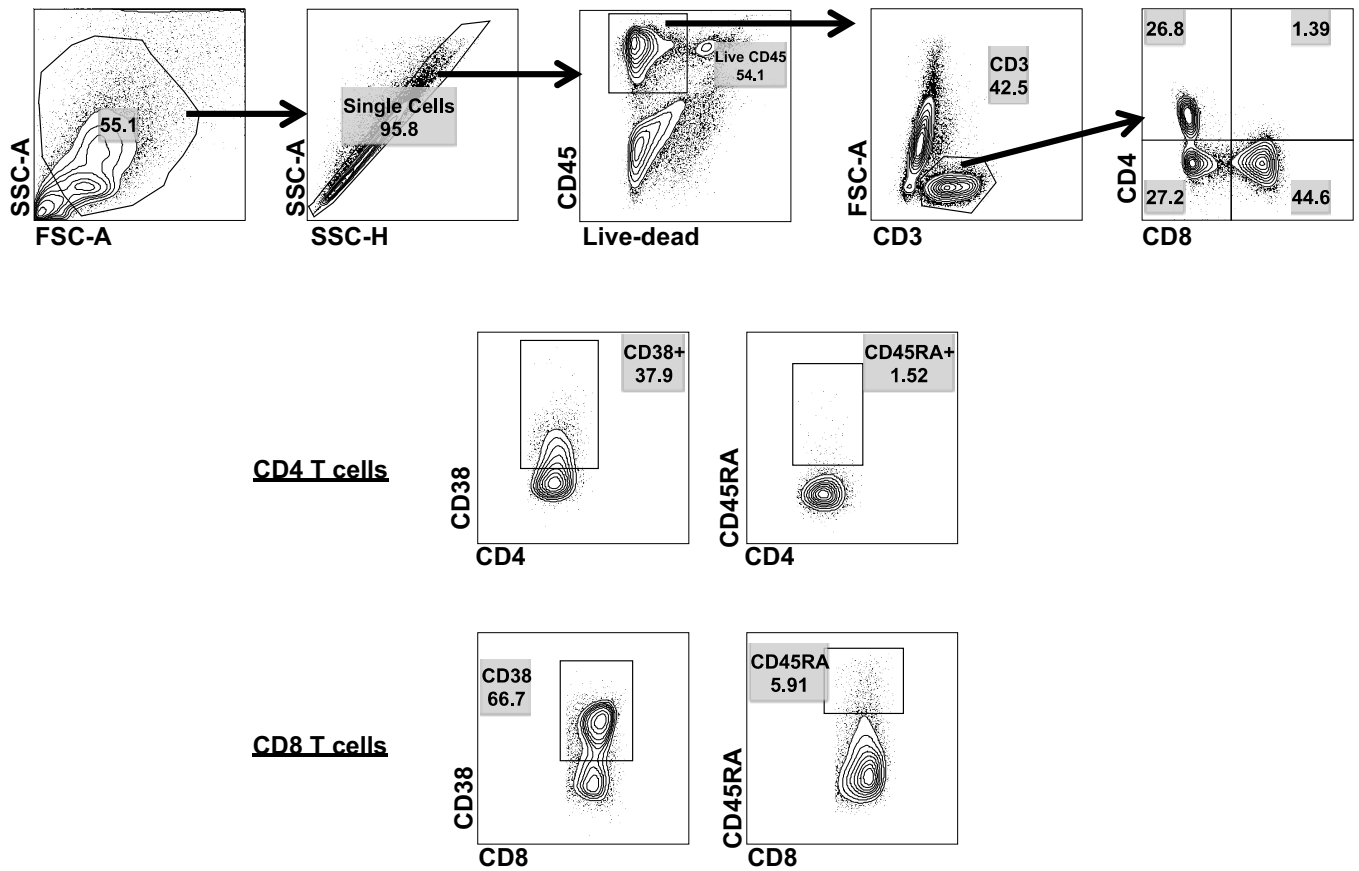
**B**

Supplementary Figure S9. Change in the frequency of CD4⁺ T cells subsets in circulation after VDZ. (A) Immune memory subsets are classified based on the expression of CCR7 and CD45RA as follows: CCR7⁺CD45RA⁻ = central memory (CM); CCR7⁻CD45RA⁻ = effector memory (EM), CD45RA⁺CCR7⁻ = terminally differentiated effector memory cells (TEMRA) and the CCR7⁺CD45RA⁺ = naive. (B) Change in the frequency of each subset for individual patients over time.

A



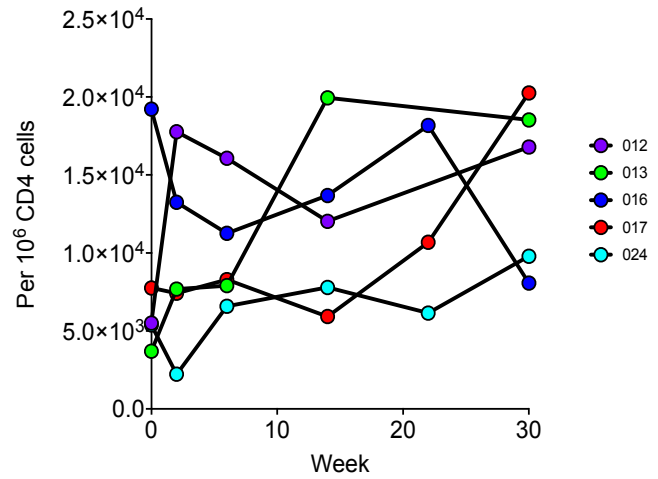
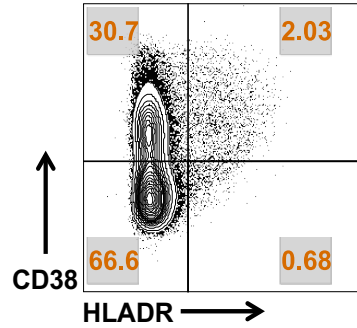
Supplementary Figure S10. Expression of integrin $\alpha 4$ and αE on $\beta 7^+$ memory $CD4^+$ T cells. (A) Flow plot showing the expression of αE and $\alpha 4$ among $\beta 7^+$ memory $CD4^+$ T cells isolated from the peripheral blood of a healthy volunteer.



Supplementary Figure S11. Gating strategy to define the frequency of CD38⁺ “activated” CD4⁺ and CD8⁺ T cells in the GI tract. Live cells were gated for the expression of CD3. CD4⁺ and CD8⁺ T cells were defined and expression of CD38 was determined on the respective cell subsets.

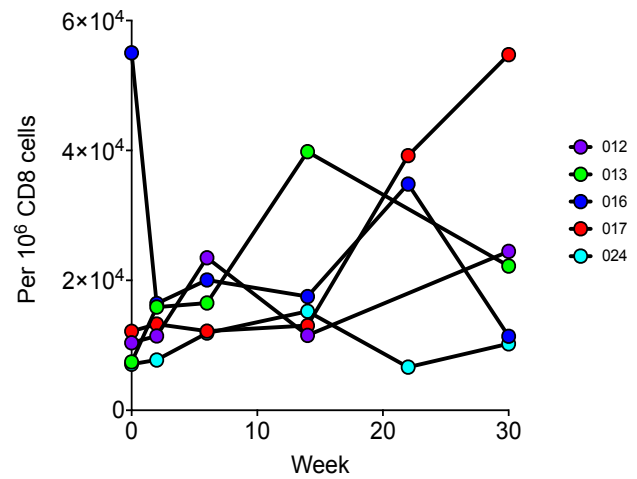
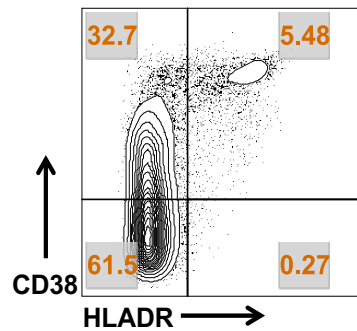
A

CD4⁺ T cells CD38⁺HLA-DR⁺



B

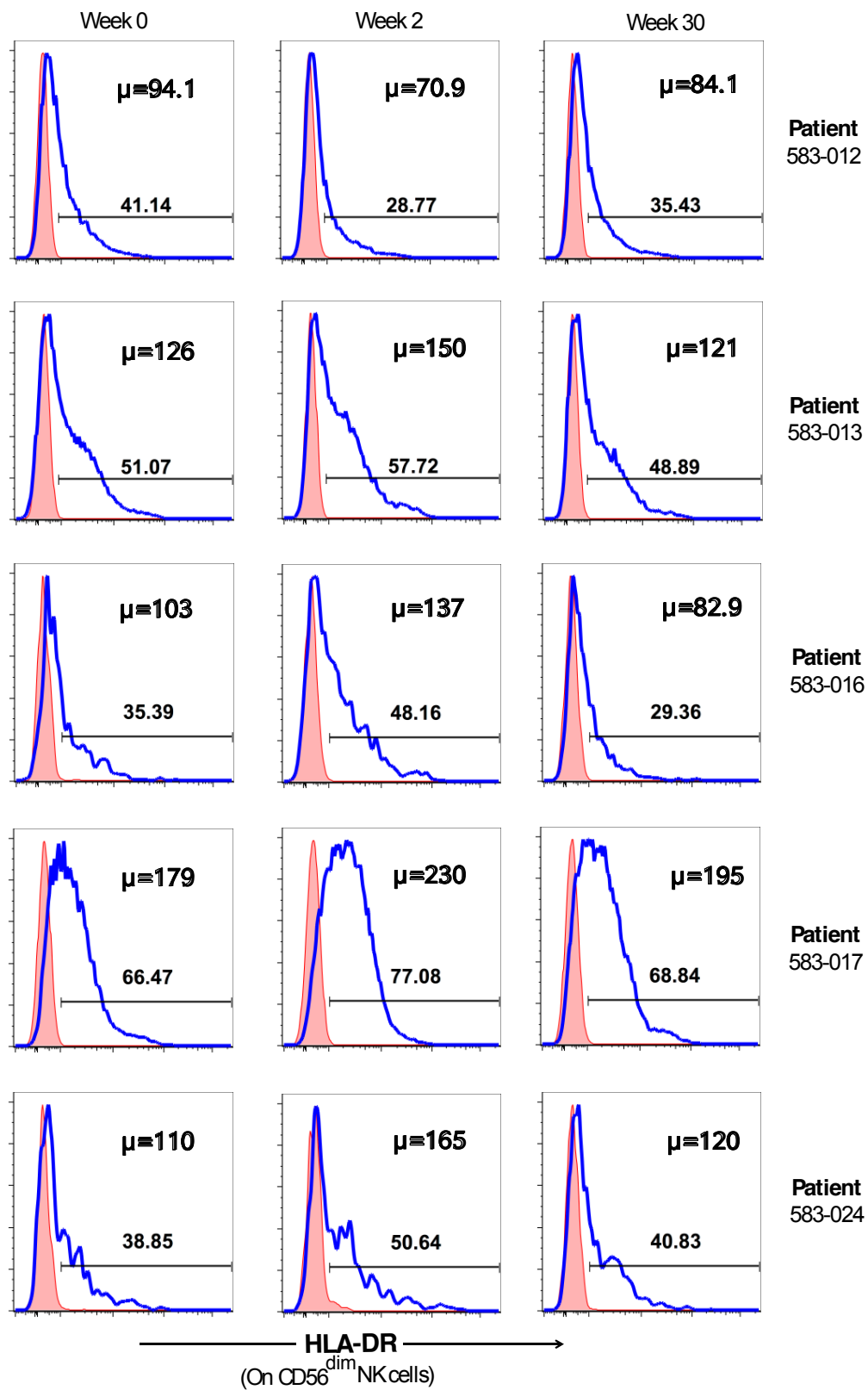
CD8⁺ T cells CD38⁺HLA-DR⁺



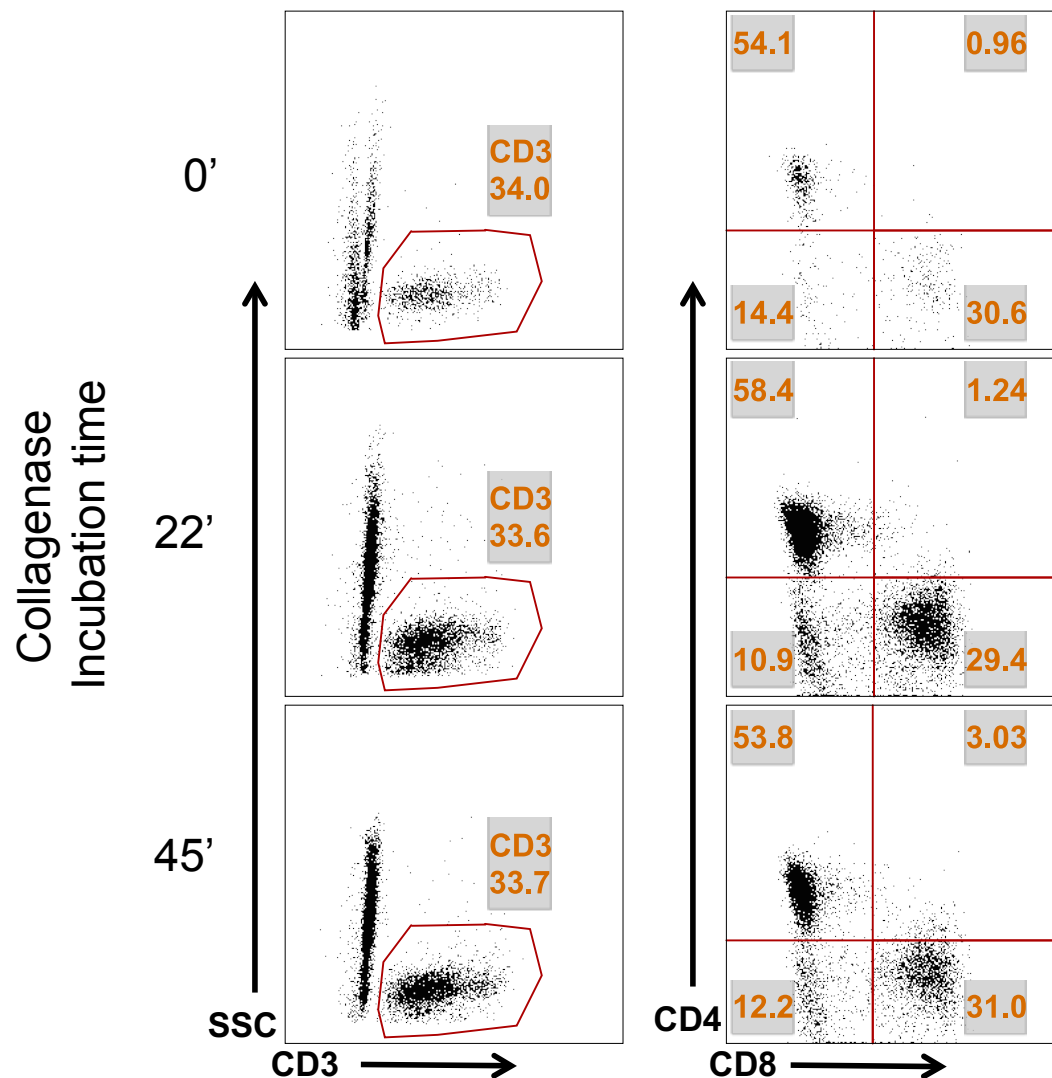
Supplementary Figure S12. Change in the frequency of CD4⁺ and CD8⁺ HLA-DR⁺CD38⁺ cells in circulation. (A, left panel) Representative flow plot showing the expression of CD38 and HLA-DR on CD4⁺ T cells (A, left panel) and CD8⁺ T cells (B, left panel). Cumulative data over time are shown in the respective panels on the right.



Supplementary Figure S13. Gating strategy for identifying NK cells. NK cells from thawed PBMC identified through the exclusion of doublets (Singlet gate), granular cells (Lymphocyte gate), dead cells and monocytes/B cells (Dump gate), and T cells (CD3 Exclusion gate) followed by gating for CD56 and CD16. “Cytolytic NK cells” were further defined as CD56^{dim}/CD16^{high} NK cells, “Cytokine secreting” NK cells were gated as CD56^{high}/CD16^{low} NK cells and CD56^{null} cells were gated as CD56^{low}/CD16^{high} NK cells.



Supplementary Figure S14. HLA-DR expression on cytolytic CD56^{dim}CD16^{high} NK cells. Histograms showing the percentage of cells positive for HLA-DR activation marker expression (dark, blue line) on cytolytic CD56^{dim}CD16^{high} NK cells from 5 subjects at weeks 0, 2 and 30 of therapy with VDZ. Gates for activation marker expression were set according to fluorescence minus one control staining tube, which contained NK phenotypic markers, but not activation stains (shaded, red line). Red numbers in upper right corner represent the Geometric Mean Fluorescence Intensity (GMFI) of HLA-DR in each subject.



Supplementary Figure S15. Impact of collagenase digestion on the frequency of intestinal CD4⁺ T cells. Biopsies from the left colon of a healthy volunteer were mechanically disrupted and split into three parts: 1) no collagenase; 2) a 22 minute collagenase incubation; or 3) standard protocol with 45 min collagenase incubation. CD3 gated T cells were examined for the expression of CD4 and CD8.

Table S1. IBD-related clinical characteristics.

IBD characteristics						
ID	IBD type	Involved GI tract segments	Duration of IBD at VDZ initiation (years)	Age at IBD diag. (years)	Concomitant IBD therapies	CRP (mg/l) before VDZ initiation
583-004	UC	Recto-sigmoid	5.2	58.9	5-ASA	1.6
583-012	CD	Right colon, Rectum	1.3	45.5	None	1.4
583-013	CD	Recto-sigmoid with history of rectal abscess and perianal fistula	14.9	41.7	5-ASA	2.3
583-016	UC	Recto-sigmoid	1	23.1	5-ASA	16.5
583-017	CD	Rectum with history of para-rectal abscess	10	56.6	None	2.4
583-024	UC	Recto-sigmoid	12	21.5	5-ASA	0.2
Pre and post VDZ clinical and endoscopic assessments						
ID	Clinical assessment pre VDZ	Endoscopic findings pre VDZ	Clinical assessment post VDZ	Endoscopic findings post VDZ		
583-004	- 3-4 bm / 24 hours - 50% with blood	Mild proctitis with aphthoid ulcerations	- 1-2 normal bm - Non bloody	Improved mild proctitis		
583-012	- 6-8 bm - 25% with blood	Mild right sided colitis	- 2 bm - Non bloody	No inflammation		
583-013	- 4-5 bm - Non bloody	Mild proctitis	- 1-2 normal bm - Non bloody	Improved mild proctitis		
583-016	- 6 bm - 100% with blood	Ulcerated proto-sigmoiditis up to 25 cm from the anal verge	- 2-3 normal bm - Non bloody	Healed inflammation		
583-017	- 2 semi formed bm - Non bloody	No inflammation	- 2 semi formed bm - Non bloody	No inflammation		
583-024	- 4-5 bm - 25% with blood	Mild proctitis	- 1-2 normal bm - Non bloody	No inflammation		

bm: bowel movements; CD: Crohn's disease; GI: Gastrointestinal; IBD: Inflammatory bowel disease; UC: Ulcerative colitis; VDZ:

Vedolizumab; 5-ASA: 5-aminosalicylic acid

Table S2. Clinical characteristics of the healthy volunteers.

ID	Sex	Age (years)	Indication for colonoscopy	Endoscopic findings	Blood*	TI*	Left colon*
1	F	50	Colorectal cancer screening	Unremarkable, normal mucosa	1	0	1
2	F	35	Unspecific abdominal pain	Unremarkable, normal mucosa	1	0	1
3	F	27	Unspecific abdominal pain	Unremarkable, normal mucosa	1	1	1
4	M	45	Unspecific abdominal pain	Uncomplicated diverticulas, normal mucosa	1	1	1
5	F	74	Gastrointestinal bleeding	Uncomplicated diverticulas, 8 mm polyp in the transverse colon, normal mucosa elsewhere	1	1	1
6	M	45	Colorectal cancer screening	Unremarkable, normal mucosa	1	1	1
7	M	23	Unspecific diarrhea	Unremarkable, normal mucosa	1	0	1
8	M	69	Colorectal cancer screening	Uncomplicated diverticulas, 6 mm polyp in the ascending colon, normal mucosa elsewhere	1	1	1
9	M	53	Colorectal cancer screening	5 mm polyp in the sigmoid colon, normal mucosa elsewhere	1	1	1
10	M	42	Unspecific abdominal pain	Unremarkable, normal mucosa	0	1	1
11	M	67	Colorectal cancer screening	3 infracentrimetric polyps of the transverse en descending colon, normal mucosa elsewhere	0	1	1
12	M	34	Prior history of perianal fistula, suspected IBD	Unremarkable, normal mucosa	1	1	1

* Sampling site, no=0, yes=1.

F: Female; M: Male; TI: Terminal ileum.

Table S3. HIV control cohort: Clinical characteristics.

ID	Sex	Age (years)	HIV viral load* (copies/ml)	Duration of ART (years)	ART regimen	Indication for colonoscopy	Endoscopy findings	Blood**	TI**	Left colon**
583-028	M	37	<20	6	DTG FTC TAF	Unspecific abdominal pain	Unremarkable, normal mucosa	1	0	1
583-029	M	28	53	2.2	3TC ABC DTG	Unspecific diarrhea	Unremarkable, normal mucosa	1	0	1
583-033	M	52	<20	12	COBI EVG FTC TDF	Colorectal cancer screening	10 mm polyp in the transvers colon, normal mucosa elsewhere	1	1	1
583-034	M	58	<20	10	3TC ABC ATV RAL	Colorectal cancer screening	5 mm polyp in the sigmoid colon, normal mucosa elsewhere	1	1	1
583-035	F	59	<20	20	FTC RPV TDF DTG	Colorectal cancer screening	Two 5-mm polyp in the sigmoid and transverse colon, normal mucosa elsewhere	1	1	1
583-037	M	36	<20	3	COBI EVG TAF FTC	Unspecific abdominal pain	Unremarkable, normal mucosa	1	1	1
583-115	M	69	<20	1.8	DRV DTG RTV	Colorectal cancer screening	Unremarkable, normal mucosa	1	1	1
583-117	F	51	<20	10	DTG FTC TDF	Unspecific abdominal pain	Unremarkable, normal mucosa	1	1	1
583-130	M	63	<20	17	COBI DRV FTC TAF	Unspecific abdominal pain	Uncomplicated diverticulas, normal mucosa	1	1	1
583-133	M	51	<20	12	BIC FTC TAF	Colorectal cancer screening	Unremarkable, normal mucosa	1	1	1

ART: Antiretroviral therapy; F: Female; HIV: Human Immunodeficiency Virus; M: Male; TI: Terminal ileum.

3TC: Lamivudine; ABC: Abacavir; ATV: atazanavir; BIC: Bictegravir; COBI: cobicistat; DRV: Darunavir; DTG: dolutegravir; EVG: elvitegravir; FPV: fosamprenavir; FTC: emtricitabine; RAL: Raltegravir; RPV: Rilpivirine; RTV: Ritonavir; TAF: tenofovir alafenamide; TDF: tenofovir disoproxil fumarate;.

*This assay is performed with the ROCHE COBAS AmpliPrep/COBAS TaqMan HIV Test.** Sampling site, no=0, yes=1.

Table S4. Flow cytometry antibodies.

Antibody	Fluorochrome	Clone	Isotype	Company
CD45	PE	HI30	Mouse IgG1, κ	Biolegend
CD3	PB	UCHT1	Mouse IgG1, κ	Biolegend
CD4	A700	RPA-T4	Mouse IgG1, κ	Biolegend
CD8	APC.Cy7	SK1	Mouse IgG1, κ	Biolegend
CD45RA	PE.Cy7	HI100	Mouse IgG2b, κ	Biolegend
CD38	APC	HB-7	Mouse IgG1, κ	Biolegend
CD19	PE.Cy7	SJ25C1	Mouse IgG1, κ	Biolegend
CD27	Percp.Cy5.5	O323	Mouse IgG1, κ	Biolegend
IgD	PB	IA6-2	Mouse IgG2a, κ	Biolegend
IgM	A700	MHM-88	Mouse IgG1, κ	Biolegend
CD10	APC.Cy7	HI10a	Mouse IgG1, κ	Biolegend
Ki67	A488	Ag Ki-67	Mouse IgG1, κ	Biolegend
β 7	FITC	FIB27	Rat IgG2a, κ	Biolegend
β 7	PE	FIB27	Rat IgG2a, κ	Biolegend
α E	APC	Ber-ACT8	Mouse IgG1, κ	Biolegend
CCR7	A488	G043H7	Mouse IgG2a, κ	Biolegend
CD3	A700	UCHT1	Mouse IgG1, κ	BD Biosciences
CD56	Percp.Cy5.5	B159	Mouse IgG1, κ	BD Biosciences
CD16	APC-H7	3G8	Mouse IgG1, κ	BD Biosciences
HLA-DR	PE	G46-6	Mouse IgG2a, κ	BD Biosciences
PD-1	PE	MIH4	Mouse IgG1, κ	BD Biosciences
CD14	PE-CF594	MqP9	Mouse IgG2b, κ	BD Biosciences
CD19	PE-CF594	HIB19	Mouse IgG1, κ	BD Biosciences

List of used flow cytometry antibody including their clone, isotype and company from which they were purchased.

Table S5. List of CyTOF reagents.

Antibody panel			
Isotope	Target	Clone	Source
89 Y	CD45	HI30	Fluidigm
113 In	CD57	HCD57	Biolegend
115 In	CD11c	Bu15	Biolegend
142 Nd	CD19	HIB19	Biolegend
143 Nd	CD45RA	HI100	Fluidigm
144 Nd	CD62L	DREG-56	Biolegend
145 Nd	CD4	RPA-T4	Biolegend
146 Nd	CD8	RPA-T8	Biolegend
148 Nd	CD16	3G8	Biolegend
149 Sm	CD127	A019D5	Biolegend
150 Nd	CD1c	L161	Biolegend
151 Eu	CD123	6H6	Biolegend
152 Sm	CD66b	G10F5	Biolegend
153 Eu	PD-1	EH12.2H7	Biolegend
154 Sm	CXCR4	12G8	Biolegend
155 Gd	CD27	O323	Biolegend
156 Gd	CCR5	J418F1	Biolegend
159 Tb	CD103	Ber-Act8	Biolegend
160 Gd	CD14	M5E2	Biolegend
161 Dy	CD56	B159	BD Biosciences
162 Dy	gdTCR	REA591	Miltenyi
163 Dy	CXCR5	J252D4	R&D Systems
164 Dy	CD45RO	CCR10	Fluidigm
165 Ho	CCR6	G034E3	Biolegend
166 Er	CD25	M-A251	Biolegend
167 Er	CCR7	G043H7	Biolegend
168 Er	CD3	UCHT1	Biolegend
169 Tm	Beta7	FIB504	Biolegend
170 Er	CD38	HB-7	Biolegend
171 Yb	CD161	HP-3G10	Biolegend
174 Yb	HLADR	L243	Biolegend
175 Lu	CD29	TS2/16	Biolegend
176 Yb	CCR4	205410	R&D Systems
209 Bi	CD11b	ICRF44	Fluidigm
Barcoding			
195 Pt	CD45	HI30	Biolegend
196 Pt	CD45	HI30	Biolegend
198 Pt	CD45	HI30	Biolegend
195 Pt	B2m	2M2	Biolegend
196 Pt	B2m	2M2	Biolegend
198 Pt	B2m	2M2	Biolegend
195 Pt	CD298	REA217	Miltenyi
196 Pt	CD298	REA217	Miltenyi
198 Pt	CD298	REA217	Miltenyi
Cell identification/viability			
103 Rh	Nucleic acid		Fluidigm
191/193 Ir	Nucleic acid		Fluidigm

**PARTIE 2 : CARACTERISATION DE LA
REPONSE IMMUNITAIRE B ET CORRELATION
AVEC LE PHENOTYPE CLINIQUE DANS LA
RCH.**

Pathogenic B cell response in Ulcerative Colitis that associates with treatment response and disease complications

Abstract

B cells, critical for intestinal homeostasis, remain under-studied in Ulcerative Colitis (UC). Using single cell RNA sequencing and single cell IgH gene sequencing we mapped the cellular and clonotypic landscape of B cells in UC. In doing so, we have identified dynamic and major perturbations within mucosal B cells including a dramatic expansion of IgG⁺ plasma cells with curtailed diversity and maturation. Further, we have identified a novel subset of CXCL13-expressing T follicular helper cells, that likely drive the pathogenic B cell response in UC. Finally, using three distinct cohorts, we confirmed that tissue changes are reflected in circulation by the expansion of gut-homing plasmablasts that associate with disease activity and predict disease complications. Our data identify the origins and consequences of a dysregulated B response in UC with novel therapeutic implications.

Key words: inflammatory bowel disease, ulcerative colitis, B cells, plasma cells, single cell RNA sequencing, clonotypic analysis

Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) characterized by relapsing episodes of mucosal inflammation, starting in the rectum and extending to proximal segments of the colon[1]. The current view of disease pathogenesis implicates a dysregulated mucosal immune response directed primarily towards the intestinal microflora in genetically predisposed individuals[1]. Genome-wide association studies have revealed over 200 IBD-risk loci with genes that incriminate both innate and adaptive immune pathways[2, 3].

Among the adaptive immune cells, B cells, dominated by IgA producing plasma cells (PC) induce a tolerizing phenotype at mucosal surfaces through pleotropic effects that include noninflammatory sequestration of luminal microbes[1, 4, 5], neutralization of toxins[6] down-regulation of proinflammatory epitopes on commensal bacteria[7], secretion of a biofilm that favors the growth of commensals[8], direction of luminal bacteria to M cells[9], maturation of DCs[10], production of IL-10[11], and Fc α RI-mediated suppression of immune responses[12].

T cell help to B cells is a fundamental mechanism for the generation of humoral immunity. A subset of CD4⁺ T cells, termed T follicular helper cells (TFH), are specialized regulators of T cell help to B cells via the expression of a network of molecules that promote T-B cell interactions as well as elaboration of soluble factors including IL21 and IL4[13, 14]. While the humoral immune system of human intestines is well studied at steady state, much less is known about the B cell response during intestinal inflammation. In particular, events inciting B cell immunopathology in bowel inflammation are unknown.

Here, utilizing single cell sequencing of cellular RNA and of the Immunoglobulin heavy (IgH) chain gene, we sought to map the cellular and clonotypic architecture of B cells in patients

with UC. Further, we sought to understand the mechanisms underlying B cell dysregulation in UC by studying TFH cells at the advancing edge of colonic inflammation. Finally, we aimed to define B cell-associated markers in circulation that would be indicative of mucosal inflammation and their association with clinical outcomes.

Results

Description of patient cohorts.

Three distinct cohorts of patients were studied. In the principal cohort (PCo), we enrolled a total of 145 subjects which included 88 UC patients with active disease, 14 UC patients with quiescent disease and 43 healthy controls (Table 1). UC patients with active disease had pancolitis (57%), left sided colitis (35%) or proctitis (8%) with a clinical Mayo score of 5.3 ± 2.0 , rectal bleeding score of 1.8 ± 0.8 and partial Mayo score of 2 (58.3%) or 3 (20.8%). Further clinical details are provided in supplementary table 1. Additionally, two distinct cohorts of patients were enrolled in order to validate the findings of our PCo and to define a circulating B cell-associated biomarker that relates to disease activity. In validation cohort 1 (VCo1), 177 subjects were enrolled which included 53 patients with active UC, 59 patients with quiescent UC and 65 normal volunteers from whom 73 UC patients had follow-up clinical data available (Supplementary table 2). In validation cohort 2 (VCo2), 141 subjects were studied which included 65 patients with active UC, 42 patients with quiescent UC and 36 normal volunteers as previously detailed[15].

Single-cell RNA sequencing analysis reveals a major expansion of IgG+ plasma cells and cycling plasmablasts in the colon

Lamina propria cells were isolated from identical segments of the colon of 5 healthy controls and 4 active UC patients respectively (clinical characteristics in Supplementary Table 3). Single-cell transcriptomes were isolated and Unique Molecular Identifier (UMIs) count matrices were generated [16]. Using the same approach as we recently described (Martin JC et al., Cell, in press), lamina propria cells were clustered jointly. The clustering analysis revealed xxx clusters

with variable number of cells (xxx-xxx cells) and UMIs counts per cell. Hierarchical clustering grouped the xxx clusters by main cellular compartments of stromal/glia cells and immune cell lineages of T cells (*CD3D*, *CD2*, *CD7*), innate lymphoid cells (ILCs) (*CD7*, no *CD3D*), mononuclear phagocytes (MNP) (*LYZ*, HLA-DR genes), plasmacytoid dendritic cells (pDCs) (*IL3RA*, *IRF7*, *GZMB*, *LILRA4*, *CLEC4C*), mast cells (*TPSAB1*, *CMA1*, *KIT*) and B cells (*CD79A*). B cell clusters comprised both B lymphocytes (*CD19*, *MS4A1*, *BANK1*, *HLA-DRA*) and plasma cells (PC) (*SDC1*, *XBPI*, *TNFRSF17*, *PRDM1*) (Figure 1A). To explore the diversity encompassed within the B cell subsets in healthy and UC colon, we analyzed gene modules defined by strong gene-to-gene correlation of expression (Figure 1B). The gene module analysis revealed transcriptomic programs that uniquely defined clusters reminiscent of naïve (*IGHD*, *FCER2*, *CD72*, *TCL1A*), memory (*CD27*, *TNFRSF13B*, no expression of *IGHD*) and germinal center (GC) (cell cycling genes, *AICDA*) B lymphocytes (Figures 1C). Similarly, PC clusters were referred as PC expressing IgA (*IGHA1*, *IGHA2*), IgM (*IGHM*) and IgG PC (*IGHG1*, *IGHG3*, *IGHG2*, *IGHG4*). In agreement with the polymeric nature of secretory SIgA and SIgM produced in the intestinal lamina propria, PC in both IgA and IgM PC clusters expressed high levels of the J-chain encoding gene (*JCHAIN*), necessary for multimeric IgA and IgM assembly and epithelial transcytosis (Figure 1D). Aside from the PC clusters, xx clusters were defined by cells sharing strong expression of PC genes such as *SDC1*, *PRDM1*, *XBPI*, *MZB1* and *SEC11C*, together with a cell cycling gene program (Figures 1A). Genes encoding for heavy chain constant regions were detected at lower levels as compared to PC and we referred these cells as plasmablasts (Figure 1D). PB clusters and PC clusters with shared annotations were grouped together, leading to the definition of a total of 7 different cellular subtypes of B cells in the colonic lamina propria of healthy controls and UC patients (Figure 1E). Quite strikingly,

examination of B cell subtype proportions within the PC compartment revealed a massive enrichment of IgG PC and plasmablasts in the inflamed colonic lamina propria of UC patients as compared to healthy controls, for whom plasmablasts were barely detected (Figures 1E&F). Analysis of the B lymphocyte compartment revealed the concomitant enrichment of naïve and GC B cells in inflamed UC colons (Figures 1E&F).

Together, results from the scRNAseq analysis of colonic lamina propria cells identified a dramatic remodeling of the B lymphocyte and PC compartments in inflamed UC colons as compared to healthy controls, which was characterized by a significant plasmablasts and IgG PC enrichment.

Major disruption in homeostatic B cell populations in the inflamed colon of patients with Ulcerative Colitis.

To validate and extend the B cell findings determined by unbiased scRNA-seq analyses, we examined colonic B cell populations by flow cytometry on 21 biopsies from healthy controls, 18 noninflamed and 24 inflamed biopsies from UC patients of our principal cohort (PCo) (Supplementary Figure 1). Confirming the scRNAseq results, colonic CD38⁺⁺CD27⁺ PC from active UC patients were marked by a significant increase in IgG⁺ PC and IgG:IgA ratio (Figure 2D&E). Furthermore, a significant frequency of colonic PC were cycling (Figure 2F&G), thus validating the existence of a strong PB response. To further describe colonic PC in active UC, using the criteria proposed by Landsverk et al. [17] we defined short lived (CD19⁺CD45⁺), long-lived (CD19⁻CD45⁺) and ultra long-lived (CD19⁻CD45⁻) CD38⁺⁺CD27⁺ PC. Concordant with the massive increase of PB suggesting an acute B cell response, we observed a significant increase in the frequency of short-lived PC and the ratio of short lived PC:long lived PC in UC patients with

active disease (Figure 2H&I). Furthermore, short-lived PC correlated positively with IgM⁺IgD⁺CD38⁻CD10⁻ naïve B cells in the inflamed colon, reflecting either co-recruitment or inter-dependence between these two populations (Figure 2J). As observed in the scRNAseq analysis, naïve B cells were significantly increased in the inflamed UC colon as compared to HC despite similar frequency of total CD19⁺ B cells (Figure 2A). No difference existed in CD38⁻CD10⁻CD27⁺IgM⁺IgD⁻ switch memory (SM) B cell numbers (Figure 2A) but we observed a significant increase in the percentage of IgG⁺ SM and the IgG:IgA SM B cell ratio in UC patients (Figure 2B&C). Overall, these data confirm major changes in the B cell repertoire identified by scRNAseq using a large cohort of patients, and suggest the presence of an acute B cell response skewed toward the production of pro-inflammatory IgG in the inflamed colon.

Plasma cells of patients with ulcerative colitis have reduced somatic hypermutations and reduced diversity

To define the clonotypic architecture of PC in patients with UC, we single-cell sorted short-lived (CD38⁺⁺CD27⁺IgD⁻CD19⁺CD45⁺) PCs from the colon and gut-homing plasmablasts (β 7⁺CD19^{+/int}CD38⁺⁺CD27⁺IgD⁻) from the peripheral blood of 4 subjects with active UC and 4 healthy volunteers (serving as controls) (Supplementary Table 5). Immunoglobulin heavy (IgH) chain genes were sequenced at the resolution of a single cell and a total of 862 clones from the colon and 1129 clones from the blood were analyzed.

In healthy controls, the dominant Ig isotype among colonic PCs and blood β 7⁺plasmablasts was IgA (90.9% $\pm$ 6.6 of the clones and 62.2% $\pm$ 5.7 of the clones for colonic PCs and circulating β 7⁺plasmablasts, respectively). Among IgA⁺ PC, IgA2 represented 71.6% $\pm$ 5.6 clones of total IgA⁺ clones in the colon and 44.3% $\pm$ 15.1 of IgA⁺ clones in the blood in

healthy controls. A significant reduction in the frequency of IgA2 clones was seen in the colon and blood of patients with UC ($40.8\% \pm 9.9$ and $22.9\% \pm 3.3$ of total IgA, respectively) (Figure 3A&B). Consistent with the scRNA-seq and flow cytometric data, IgG clone frequency was dramatically increased in the colon of UC patients ($5.8\% \pm 5.2$ of total clones in healthy controls vs $39.6\% \pm 12.9$ of total PC in UC) (Figure 3A). VH3 and JH4 genes were dominant and expressed in more than 50% of both healthy control and active UC. An increased usage of JH6 was noted in colonic PC of active UC patients when compared with healthy controls (Supplementary Figure 2A&B). CDR3 length was significantly greater in colonic PCs of UC patients, when compared to healthy controls, with a trend for a longer CDR3 among IgA⁺ clones in the colon and blood both (Figure 3C&D).

All the IgH genes showed high numbers of somatic mutations as previously reported[18]. In healthy controls, V genes of gut-homing plasmablasts carried on average 20.1 ± 4.6 mutations while colonic PC had 25.4 ± 3.4 mutations. Notably, in patients with UC, the number of somatic mutations was reduced among IgA⁺ and IgG⁺ clones in circulation and in the colon with the most dramatic reduction noted in IgG PCs derived from the inflamed colon (Figure 3E&F). Among the short-lived PCs, clonally related sequences with identical IGHV and J, with the same CDR3 length and at least 80% of homology in the amino acid sequence were found in 3 of 4 healthy controls and in all UC patients. 6.5% of all colonic PC clones belonged (found at least 2 times) to expanded clones in the healthy controls whereas this number was at 11.2% in UC. Accordingly, the clonal diversity assessed by the Shannon index was decreased in colonic short-lived PC of UC patients (Figure 3G). Additionally, shared clones between the blood and the mucosal compartments were found in all UC patients, yet were present in only 1 of 4 of the healthy controls (Supplementary Figure 2C). Together, these data draw attention to dramatically

increased IgG PC clones in the inflamed colon with reduced diversity and fewer somatic hypermutations.

To understand the biological significance of an IgG-dominated B cell response in UC, we generated a genetic signature of IgG-mediated colonic macrophage activation (Figure 3H), the dominant immune cell expressing UC-associated FcγRIIA, and determined the ability of this signature to predict clinical disease course in transcriptomics datasets from UC colonic biopsies. In a publicly available UC transcriptomics dataset, the macrophage immune complex signature could predict infliximab non-response prior to treatment initiation. Furthermore, this signature was more effective than an LPS-associated macrophage response, despite an equal ability between signatures to discriminate between inflamed and non-inflamed tissue, as well as infliximab responders and non-responders post-treatment. To extend these observations, we analyzed genetic signature expression in our own colonic UC transcriptomics datasets. In agreement with public datasets, we observed an increase in IC-associated signatures in inflamed UC biopsies compared to healthy controls and non-inflamed colonic tissue (Figure 3I), as well as an ability to discriminate anti-TNF treatment response (Figure 3K). Further profiling of colonic macrophage IgG signatures was performed by analyzing the top 200 IgG-induced colonic macrophage genes without prior refinement. Once more, this signature was significantly associated with colonic inflammation (Figure 3J) and expression further increased in UC patients non-responsive to anti-TNF therapy (Figure 3J, lower panel). Therefore, an IgG-dominated B cell response is associated with a pathogenic IgG immune complex-macrophage signature capable of defining local inflammation and a priori anti-TNF response.

PD1^{hi} CD4 T cells with the ability to produce CXCL13 are expanded in the inflamed mucosa of patients with ulcerative colitis

TFH cells play a fundamental role in extrafollicular and germinal center antibody responses, where they drive affinity maturation as well as maintenance of humoral memory[13, 14]. Deeper analysis of the T cell scRNAseq clusters revealed the presence of cells sharing a transcriptomic program indicative of TFH cells. TFH-like cells expressed *PDCD1* (PD-1), *TIGIT*, *BATF*, *ICAI*, *CTLA-4* and *TNFRSF4* (OX-40). These genes were also detected in Tregs but cells in the TFH-like cluster lacked the expression of key Treg-defining transcripts such as *FOXP3*, *IL2RA* and *IKZF2* (HELIOS). In turn TFH-like cells expressed *CD40LG* and the gene encoding for the follicular B-cell attracting chemokine *CXCL13* while no transcripts for *CXCR5* were detected, thus evocative of the recently described “peripheral helper cells” (TPH) population [19]. Within the T cell compartment, the proportion of TFH-like cells was increased in inflamed UC colon when compared to controls (Figure 4A). Quite strikingly, however, the expression of *CXCL13* in TFH-like cells was detected at significantly much higher levels in UC patients (Figure 4B). In addition, UC patients were significantly enriched for activated cycling T cells, a large proportion of which also expressed the TFH-like program, including high levels of the chemokine *CXCL13*. These data thus suggested the presence of an acute T cell response in UC inflamed colon, which comprised a significant increase in B cell-helping, *CXCL13*-expressing, TFH-like cells.

Using multiparameter flow cytometry, we confirmed the expansion of $PD1^{hi}TIGIT^{+}$ T cells in the inflamed colon of UC patients (Figure 4C&D). Concordant with a TFH phenotype, $PD1^{hi}TIGIT^{+}$ T cells were $ICOS^{+}$ and $CD45RA^{-}$. Ex-vivo stimulation experiments confirmed the production of *CXCL13* by $PD1^{hi}TIGIT^{+}$ CD4 T cells (Figure 4J) as well as the increased production in UC inflamed colon as compared to HC (Figure 4H&I). Because, virtually all $PD1^{hi}$ cells coexpressed *TIGIT* and *ICOS* (Supplementary Figure 3), we used $PD1^{hi}$ as a surrogate of the TFH-like subset in larger number of patients. Notably, $PD1^{hi}$ cells were increased in UC

patients while PD1^{int} were not significantly altered (Figure 4E). Moreover, the frequency of PD1^{hi} CD4 T cells correlated with the frequency of both short-lived PC and naïve B cells in the colonic mucosa (Figure 4F&G) suggesting an interdependence between these 3 populations.

Gut homing plasma cells are significantly increased in circulation of patients with ulcerative colitis

We next investigated how tissue changes in the intestinal B cell populations are reflected in the peripheral blood of patients with UC. Again, we first defined naïve B cells, switched memory B cells and PCs based on the expression of canonical markers (Supplementary Figure 4). Total, CD19⁺ B cells, CD19⁺CD38⁻CD10⁺IgM⁺IgD⁺ naïve B cells, CD19⁺CD38⁻CD27⁺IgM⁻IgD⁻ SM B cells (both IgA⁺ SM B cells and IgG⁺ SM B cells) were comparable between healthy volunteers, patients with quiescent UC and patients with active UC (figure 5A). In contrast, there was a significant increase in CD19^{+/int}CD38⁺⁺CD27⁺IgD⁻ PCs of patients with active UC (figure 5B&C). Among PCs, both IgG⁺ and IgA⁺ subsets were dramatically increased while the increase in IgM⁺ PCs was not significant (figure 5C). A majority of the circulating CD19^{+/int}CD38⁺⁺CD27⁺IgD⁻ cells were Ki67⁺ and had no or low expression of CD138 (Supplementary Figure 5) defining them as plasmablasts. Notably, there was a significant increase in the expression of the gut-homing integrin, β 7 on total plasmablasts as well as the IgA⁺ and IgG⁺ plasmablast subsets (Figure 5D&E) suggesting that their origin and destination to be the GI tract[20]. On a subset of patients with active UC, we defined the expression of putative homing molecules (including CCR9, GPR15, CXCR3, CXCR4 β 7 and β 1 integrin) and found a significant increase in CXCR3 with a decrease in the expression of β 1 integrin on circulating plasmablasts in patients with UC (Supplementary Figure 6).

To relate the circulating plasmablasts to inflammatory burden and to predict disease complications, we performed consensus clustering in order to cluster patients with similarities in the immune landscape into different groups. A total of 96 patients from the PCo where complete T cell and B cell phenotypic data from the peripheral blood was available were included in the analyses. Clinical variables including sex, extent of disease, disease duration, rectal bleeding score, clinical Mayo index and C-reactive protein level were considered. Upon clustering, markers differentially expressed across different groups were identified (Figure 5F). Notably, patients with the highest frequency of gut homing plasmablasts and PCs, both within the IgG and IgA isotypes in circulation clustered together (cluster 1) and had the highest clinical Mayo index (figure 5G, T test p-value < 0.05). Furthermore, the frequency of circulating gut-homing plasmablasts directly correlated with disease extent (Figure 5H) and disease activity as measured by rectal bleeding score (figure 5I) and C-reactive protein (figure 5J). Remarkably, the association between partial Mayo index and cluster groups remained significant after adjusting for CRP levels via a multivariate linear regression (p-value = 0.01).

Two distinct validation cohorts confirm a significant increase in gut homing plasma cells in circulation of patients with ulcerative colitis that relate to disease activity and complications.

To confirm that gut homing plasmablasts define a circulating cellular biomarker of active disease, we examined two distinct validation cohorts. In Vco1, a total of 177 subjects were enrolled which included 53 patients with active UC, 59 patients with quiescent UC and 65 normal volunteers. Mass cytometry (CyTOF) was performed on whole blood. The plasmablast cluster was defined as the cells expressing CD19, CD38 and CD27. A significant increase in

plasmablasts was seen in patients with active UC when compared to patients with quiescent UC or normal volunteers (Figure 6A&B). Interrogation of other markers associated with migratory behavior of cells revealed a significant increase in the expression of $\beta 7$ (Figure 6C) and CXCR3 (Figure 6D) and a decrease in $\beta 1$ expression in patients with active UC when compared to patients with quiescent UC or normal volunteers (Figure 6D).

Next we wanted to address whether circulating plasmablasts predicted disease complications. Since the duration of follow up for the patients recruited in our PCo was short (less than one year for the majority of patients), we focused on the VCo1 where patients had been followed for up to 4 years after the index blood draw. Clinical follow-up information was available for 73 UC patients of VCo1 (Supplementary Table 2). Complicated disease was defined as the occurrence of one of the following event: change in biologic treatment (identifying non-responsive disease), UC-related hospitalizations or UC-related surgery. 30 patients (41%) had an event over the time of follow-up. Patients were segregated into two groups based on the median frequency of plasmablasts in circulation and Kaplan-Meier curves were generated to estimate the incidence of disease complications over time. Patients with plasmablasts above the median had a significantly higher probability of disease complications over time (figure 6E, median follow-up of 1.6 years, Interquartile range [0.4-2.8], log-rank test p-value = 0.02). Plasmablasts were associated with disease complications after adjusting for age, sex, disease extent and disease duration in cox-regression analysis (p-value = 0.004).

A second validation cohort (VCo2) further helped strengthen this finding. Whole blood from 41 patients with active UC, 66 patients with quiescent UC and 36 normal volunteers (as previously detailed[15]) were used to profile expression of a gene strongly linked with plasmablast abundance, IgJ[21, 22]. A significant increase in the relative expression of IgJ was

noted in patients with active UC (define as XX) when compared to quiescent UC and healthy volunteers (Figure 6F). Finally, for patients including in VCo2, increased IgJ expression was associated with rectal bleeding score (Figure 6G) and fecal lactoferrin (Figure 6H). Thus, from three independent cohorts, we could establish that increased frequency of gut homing plasmablasts in circulation identified patients with higher disease activity and predicted an increased risk of longer term disease complications.

Discussion

B cells are pivotal to mucosal homeostasis with critical roles that include GALT organogenesis[23], antigen presentation[24], orchestration of secretory immunity[25], maintenance of immunological tolerance [26] and microbial regulation[27, 28]. Failure of CD20-directed Rituximab[29] to provide clinical benefit in patients with UC[30] illustrates that disruption of the entire B cell compartment, while sparing gut-resident PCs[31] may be counterproductive in IBD therapeutics. Thus, it is of considerable importance to understand the spectrum of IBD-associated B cell immunopathology and to distinguish ‘homeostatic’ from ‘pathogenic’ B cell signatures in order to define novel therapeutic targets. With these as our guiding principles, we dissected B cell dynamics during chronic mucosal inflammation using high-resolution techniques such as scRNA sequencing and single cell IgH sequencing. To identify the origins of B cell dysfunction, given the obvious difficulty in capturing patients prior to the onset of disease, we studied intestinal tissue at the advancing edge of mucosal inflammation. Finally, we defined B cell-associated markers in circulation that are reflective of mucosal inflammation and confirmed their validity using two additional and distinct cohorts of patients with UC.

Our data demonstrate profound alterations in mucosal B cells in UC that span immune inductive and effector sites[32]. Among the inductive compartments, there is a significant influx of naïve B cells and generation of de novo lymphoid aggregates reflecting increased B cell priming. Indicative of B cell alterations within immune effector sites, there is a dramatic increase in cycling plasmablasts and recently recruited PCs suggestive of the participation of B cell effectors in the inflammatory response. Akin to healthy volunteers[33], in our patients with UC, IgA⁺ PCs continued to be largest populations within the PC compartment. However, we found a

dramatic increase in IgG⁺ PCs in the inflamed mucosa, in agreement with previous reports [34-37]. This is highly significant as it indicates a shift in the intestinal immune milieu from homeostatic, non-complement fixing, IgA dominant responses in health[34] to inflammasome recruiting, inflammatory[37], IgG-predominant response in UC. This IgG-enriched B cell response likely amplifies the inflammatory cascade due to Fc receptor-dependent recruitment of inflammatory monocytes and TH₁₇ cells[37] and further sensitizes the mucosal immune system to microbial[35] and perhaps dietary[38] antigens.

In addition to the above quantitative changes in B cell populations, significant qualitative responses were noted on clonotype analyses as well. While on the one hand there was a consistent increase in IgG clonotypes in the mucosa, there was a striking reduction in IgA2 clones and an increase in protease sensitive[39] IgA1 clones on the other, again illustrating a de-emphasis of the homeostatic IgA environment in UC. We detected an increase VH6 usage in colonic PCs of UC patients compared to normal volunteers as previously reported[40]. However, we did not observe the reported differences in VH3 usage between normal volunteers and patients with UC[41] possibly due to important methodological differences (single-cell sorting of colonic PCs in the present study vs bulk sequencing of EBV transformed colonic B cells of a sparse number of clones done previously). Colonic PCs from healthy volunteers were highly mutated as previously reported[18, 40]. However, there was a striking reduction in the number of somatic mutations in the colonic IgG⁺ PC clonotypes with a similar but less pronounced reduction of mutations in colonic IgA⁺ PCs (as well as circulating plasmablasts). These data suggest that lack of germinal center maturation of IgG⁺ (and IgA⁺) PCs either due to antigenic over stimulation or due to extrafollicular development, resulting perhaps in retained auto-reactive B cells[42, 43] .

Next, we sought to determine the origins of the dysregulated B cell response in UC. TFH cells represent the major TH subset involved in the induction of high affinity B cell responses through cellular (CD40L and ICOS) and secreted (IL-21) molecules[13]. GC TFH cells express CXCR5 which guides them into B cell follicles along a CXCL13 gradient[44], while the recently described CXCR5-negative, TPH cells promote B cell responses and antibody production within inflamed, non-lymphoid tissues[19]. The ‘TFH family’ of cells (comprising of GC TFH cells, TPH cells, as well as circulating TFH cells) have been implicated in the pathogenesis of a number of immunological diseases including seropositive rheumatoid arthritis[19, 45], celiac disease[46], systemic lupus erythematosus[46, 47], systemic sclerosis[46], Sjogren’s syndrome[47], myasthenia gravis[48], autoimmune thyroid disease[49] and type 1 diabetes[50]. Two studies have reported an increase in circulating TFH cells in patients with IBD[51, 52]. However, despite the possibility of a significant role in disease pathogenesis, tissue TFH cells have not been well studied in IBD. Herein, we provide the first evidence of tissue resident TFH cells with aberrant expression of B cell attractant, CXCL13 in the inflamed intestines. This provides the first evidence regarding TFH-directed assembly of inflammation associated lymphoid aggregates in patients with UC. The origins of CXCL13⁺ TFH cells were not investigated in our study but we hypothesize that they arise from Treg cells due to shared development programs [53]. The CXCL13⁺TFH-driven neo-lymphoid aggregates lacking an organized GC help explain the “immature”, pauci-mutated B cell response seen on single cell Ig sequencing. The significance of this finding is further enhanced by the fact that multiple genes implicated in TFH differentiation (IL-12B, IL-23R, IL-21, IRF5, STAT3[54-56]), function (CXCR5[57]) and negative regulation (PTPN22[58]) are strongly implicated in the susceptibility to IBD[2]. Further exploration of the TFH cells creates avenues for novel therapeutic targets as

well as a better understanding of the mechanisms of existing drugs including IL-12/IL-23-targeted therapies.

Given the profound B cell alterations that were observed, next we aimed to define a circulating B cell-associated marker that would be a dynamic reflection of events in the inflamed mucosa. Sequence analysis of human IgVH genes indicates that intestinal lamina propria PCs are derived from Peyer's patches[40]. Therefore, we hypothesized that circulating plasmablasts with high expression of integrin $\alpha 4\beta 7$ (indicating their origin within gut associated lymphoid tissues[59]) could serve as sensitive indicators of intestinal B cell changes during inflammation. In confirmation, we noted that while the total B cells, naïve B cells and SM B cells were unchanged in circulation during active UC, $\alpha 4\beta 7^{\text{hi}}$ plasmablasts were highly correlated with the inflamed colon. Further, $\alpha 4\beta 7^{\text{hi}}$ plasmablasts were predictive of complicated disease over a median of 1.6 years of follow up, perhaps reflecting the severity of mucosal inflammation. Alternatively, this identifies an adverse disease phenotype with prognostic significance that needs to be explored further. The occurrence of circulating $\alpha 4\beta 7^{\text{hi}}$ plasmablasts during active inflammation was reproducible across two independent validation cohorts demonstrating the robustness of this finding during active inflammation, defining $\alpha 4\beta 7^{\text{hi}}$ plasmablasts as novel cellular circulating biomarkers of mucosal inflammation.

Together, our data, entirely human, provides detailed insights into the magnitude and breadth of B cell perturbations during active colitis, allowing for B cell associated events to be integrated into the current paradigms of IBD pathogenesis. Our data provides the first evidence of CXCL13⁺TFH cells that are associated with aberrant, B-cell dominated lymphoid aggregates and a dysregulated, IgG-dominated inflammatory B cell response. Finally, our study identifies $\alpha 4\beta 7^{\text{hi}}$ plasmablasts as circulating surrogates of mucosal inflammation.

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Figure legends

Figure 1 - Single-cell RNA sequencing analysis on B cell subsets reveals an expansion of highly proliferative plasmablasts along with an increase in naïve B cells in the inflamed mucosa of UC patients

a, B cells include transcriptomically well-defined B lymphocytes (*CD19*, *MS4A1*, *BANK1*, *HLA-DRA*) and plasmablasts (Pbs) / plasma cells (PC) (*SDC1*, *XBPI*, *TNFRSF17*, *PRDMI*). **b**, Analysis of gene modules defined by strong gene-to-gene correlation of expression. **c**, Within B cells, gene module analysis shows transcriptomic programs defining naïve (*IGHD*, *FCER2*, *CD72*, *TCL1A*), memory (*CD27*, *TNFRSF13B*, no expression of *IGHD*) and germinal center (GC) (cell cycling genes, *AICDA*) B lymphocytes. **d**, Pbs, PC clusters were referred as PC expressing IgA (*IGHA1*, *IGHA2*), IgM (*IGHM*) and IgG PC (*IGHG1*, *IGHG3*, *IGHG2*, *IGHG4*) or highly proliferative Pbs. **e**, Proportion of B lymphocytes and Pbs / PC clusters of individual healthy controls and UC patients. **f**, IgG and IgA expression of GC cells of healthy controls and UC patients.

Figure 2 - Colonic plasma cells and memory B cells have a skewed isotype usage toward less IgA and more IgG

a, Frequency of total CD19⁺ B cells, Naïve (CD45⁺CD19⁺CD38⁻CD10⁻IgD⁺IgM⁺) and SM (CD45⁺CD19⁺CD38⁻CD10⁻IgD⁻IgM⁻) B cells out of live CD45⁺ lamina propria mononuclear cells in healthy controls, inflamed and noninflamed biopsies from UC patients. Data are shown as individual data and mean, **p<0.01, Mann-Whitney test, two-tailed. **b**, Representative flow plots showing the frequency of IgA⁺ and IgG (IgA⁻) expressed on live SM B cells in biopsies of a healthy control and an active UC patient. **c**,

Frequency of IgG, IgA and IgG:IgA ratio on SM B cells in healthy controls, inflamed and noninflamed biopsies from UC patients. Data are shown as individual data and mean, *** $p < 0.001$, Mann-Whitney test, two-tailed. **d**, Representative flow plots showing the frequency of IgA and IgM expressed on CD27⁺CD38⁺⁺ plasma cells in biopsies of a healthy control and an active UC patient. **e**, Frequency of IgG (IgA⁻IgM⁺), IgA and IgG:IgA ratio on colonic plasma cells in healthy controls, inflamed and noninflamed biopsies from UC patients. Data are shown as individual data and mean, *** $p < 0.001$, **** $p < 0.0001$, Mann-Whitney test, two-tailed. **f**, Representative images of colonic biopsies from a healthy control (left panel) and an active UC patient (right panel) after immunofluorescence staining with CD79a (green), Ki67 (blue) and IgA (red). **g**, Quantification of lamina propria IgA⁺ (IgA, CD79A, Ki67 triple positive cells) and IgA-plasmablasts (CD79A, Ki67 double positive cells) in 5 healthy controls and 5 active UC patients. Data are shown as individual data and mean, ** $p < 0.01$, Mann-Whitney test, two-tailed. **h**, Representative flow plots showing the frequency of short-lived (CD27⁺CD38⁺⁺CD19⁺CD45⁺), long-lived (CD27⁺CD38⁺⁺CD19⁻CD45⁺) and very long-lived (CD27⁺CD38⁺⁺CD19⁻CD45⁻) plasma cells in colonic biopsies of a healthy control and an active UC patient. **i**, Frequency of each subset among total plasma cells and ratio of short-lived to long-lived plasma cells in healthy controls, inflamed and noninflamed biopsies from UC patients. Data are shown as individual data and mean, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$, Mann-Whitney test, two-tailed. **j**, Linear regression showing the relationship between the frequencies of short-lived plasma cells and Naive B cells. Data are shown as individual data. The correlation was assessed using a Pearson test.

Figure 3 - IgH gene sequencing of plasma cells reveals a reduced diversity, a longer CDR3 region and fewer somatic mutations in patients with ulcerative colitis

a,b, Isotype frequencies in short-lived colonic plasma cells (CD27⁺CD38⁺⁺CD19⁺CD45⁺) (a) and circulating gut-homing plasmablasts (β 7-integrin⁺CD19⁺CD38^{hi}CD10⁻CD27⁺IgD) (b) in 4 healthy controls and 4 UC patients. Data are shown as individual data, mean and standard deviation, *p<0.05, **p<0.01, ****p<0.0001. Mann-Whitney test, two-tailed.

c,d, Mean IgH amino acid CDR3 length per patient (c) and for each IgA and IgG clone (d) of short-lived colonic plasma cells and gut-homing plasmablasts from 4 healthy controls and 4 active UC patients. Data are shown as individual data, mean and standard deviation, *p<0.05. Mann-Whitney test, two-tailed.

e,f, Mean (e) V gene somatic mutations and for each IgA and IgG clone (f) of short-lived colonic plasma cells and gut-homing plasmablasts from 4 healthy controls and 4 active UC patients. Data are shown as individual data, mean and standard deviation, **p<0.01. Mann-Whitney test, two-tailed.

g, Shannon index diversity of gut-homing plasmablasts and short-lived colonic plasma cells. Data are shown as individual data, mean and standard deviation, *p<0.05. Mann-Whitney test, two-tailed.

h, genetic signature of IgG-mediated colonic macrophage activation **i,j,k** macrophage immune complex and LPS signatures could predict inflamed status (i) of the tissue and the infliximab non-response prior to treatment initiation (k). Top 200 IgG-induced colonic macrophage genes generated in mice without prior refinement identify inflamed and non-inflamed intestinal tissue (j).

Figure 4 - PD1^{high} CD4 T cells expressing CXCL13 are expanded in the inflamed mucosa of UC patients

a, T cell subset frequencies as defined by scRNA-seq analysis representative for each individual control and UC patient. **b**, Level of CXCL13 RNA expression within T cells for each individual. Wilcoxon test, two-tailed. **c**, Representative flow plots showing the frequency of PD1^{hi}TIGIT⁺ cells among live CD45⁺CD3⁺CD4⁺CD45RA⁻ cells in a single UC patient in paired noninflamed and inflamed areas. **d**, Frequency of PD1^{hi}TIGIT⁺, PD1^{hi}ICOS⁺ and PD1^{hi}TIGIT⁺ICOS⁺ among memory (CD45RA⁻) CD4 T cells in healthy controls, inflamed and noninflamed biopsies from UC patients. Data are shown as individual data and mean, **p<0.01. Mann-Whitney test, two-tailed. **e**, Frequency of PD1^{hi} and PD1^{int} among memory (CD45RA⁻) CD4 T cells in healthy controls, inflamed and noninflamed biopsies from UC patients. Data are shown as individual data and mean, **p<0.01. Mann-Whitney test, two-tailed. **f,g**, Linear regression showing the relationship between the frequencies of short-lived plasma cells and PD1^{hi} CD4 cells (f) and Naive B cells and PD1^{hi} CD4 cells (g) in colonic biopsies of UC patients. Data are shown as individual data. The correlation was assessed using a Pearson test. **h**, Representative flow plots showing the expression of CXCL13 in CD4 T cells with or without stimulation of lamina propria mononuclear cells with CD3CD28 beads from biopsies of a healthy control and an active UC patient. **i**, Frequency of CXCL13 expression within lamina propria CD4 T cells with or without CD3CD28 stimulation. Data are shown as individual data, mean and standard deviation, *p<0.05. Mann-Whitney test, two-tailed. **j**, Representative flow plots showing PD1 and TIGIT expressions of CXCL13⁺ (red) and CXCL13⁻ (grey) CD4 T cells after CD3CD28 stimulation.

Figure 5 - β 7-integrin expressing plasmablasts are expanded in the circulation of active UC patients and relate to disease activity and extent

a, Frequency of total B cells (CD19⁺), naive (CD19⁺CD38⁻CD10⁻IgM⁺IgD⁺), CD27⁺ SM B cells (CD19⁺CD38⁻CD10⁻IgM⁻IgD⁻) as well as IgA⁺ and IgG (IgA⁻) SM in healthy controls, quiescent and active UC of PCo. Data are shown as individual data and mean, Mann-Whitney test, two-tailed, **b**, Representative flow plots showing the frequency of CD38^{hi}CD10⁻ and CD38⁻CD10⁻ among live CD19⁺ PBMCs in a healthy control and in an active UC patient. **c**, Frequency of plasmablasts (CD19⁺CD38^{hi}CD10⁻CD27⁺IgD⁻) and IgA⁺, IgG⁺ (IgA⁻IgM⁻) and IgM⁺ out of live PBMCs in healthy controls, quiescent and active UC patients of PCo. Data are shown as individual data and mean, *p<0.05, **p<0.01, ***p<0.001, Mann-Whitney test, two-tailed. **d**, Representative flow plots of the expression of β 7-integrin on 3 main B cell subsets. **e**, Level of expression of β 7-integrin on total, IgA⁺ and IgG⁺ plasmablasts in healthy controls and active UC of PCo. Data are shown as individual data and mean, ***p<0.001, ****p<0.0001, Mann-Whitney test, two-tailed. **f**, Consensus clustering on PCo defined 3 clusters of patients and controls according to their B cell blood phenotyping. Cluster 1 only comprised active UC patients. **g**, Comparison of clinical mayo scores across the three consensus clusters. Data are shown as median and standard deviation, Mann-Whitney test, two-tailed. **h**, Frequency of β 7⁺ gut-homing plasmablasts (CD19⁺CD38^{hi}CD10⁻CD27⁺IgD⁻) according to their disease extent defined by the Montreal classification i.e. E1 defines proctitis, E2 left-sided colitis and E3 pancolitis. Data are shown as individual data and mean, *p<0.05, **p<0.01, ****p<0.0001, Mann-Whitney test, two-tailed. **i**, Frequency of β 7⁺ gut-homing plasmablasts according to the rectal bleeding score (0: no blood, 1: blood in less than

50% of bowel movements, 2: blood in more 50% of bowel movements, 3: each bowel movement contains blood). Data are shown as individual data and mean, ** $p < 0.01$, Mann-Whitney test, two-tailed. **j**, Frequency of $\beta 7^+$ gut-homing plasmablasts in UC patients with a C-reactive protein level of less or more than 5mg/l. Data are shown as individual data and mean, ** $p < 0.01$, Mann-Whitney test, two-tailed.

Figure 6 – Two distinct validation cohorts confirm the increase in gut-homing plasmablasts in active UC that identify a complicated disease course.

a-d, Mass Cytometry (CyTOF) derived data from validation cohort 1 (VCo1) comparing plasma cell parameters between healthy controls, patients with quiescent and active UC. **a**, Representative tSNE plots (top panel) of demonstrating cellular subsets in the peripheral blood of 1 healthy control, 1 patient with quiescent UC and 1 patient with active UC. The bottom panels compare the expression of $\beta 7$ -integrin on $CD27^+CD38^{high}$ plasmablasts. **b**, Cumulative data from the entire VCo1 comparing the frequency of plasmablasts between healthy controls, quiescent UC and active UC. Data are shown as individual data, mean and standard deviation, *** $p < 0.001$, **** $p < 0.0001$, Mann-Whitney test, two-tailed. **c,d**, Mean level of expression of $\beta 7$ -integrin (c), $\beta 1$ -integrin, and CXCR3 (d) on circulating plasmablasts of healthy controls and UC patients. Data are shown as individual data, mean and standard deviation, * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$, Mann-Whitney test, two-tailed. **e**, Kaplan-Meier estimates showing survival free of occurrence of complications (i.e. intestinal surgery, UC-related hospitalization or introduction of a new biologic treatment) for patients of VCo1 with plasmablast frequency below the median (red curve) or above the median (blue curve).

Survival curves were compared using a log-rank test. **f-h**, IgJ gene signatures, representing circulating plasma cells in validation cohort 2 (VCo2). **f**, Relative expression of IgJ gene from whole blood of healthy controls, quiescent UC and active UC patients. **g,h**, Relative expression of IgJ compared with rectal bleeding scores (g) and fecal lactoferrin (h). Data are shown as individual data, median and inter-quartile range, **p<0.01, ***p<0.001, t-test, two-tailed. Correlation coefficient (rho) was calculated using Speraman's test.

Figure 1

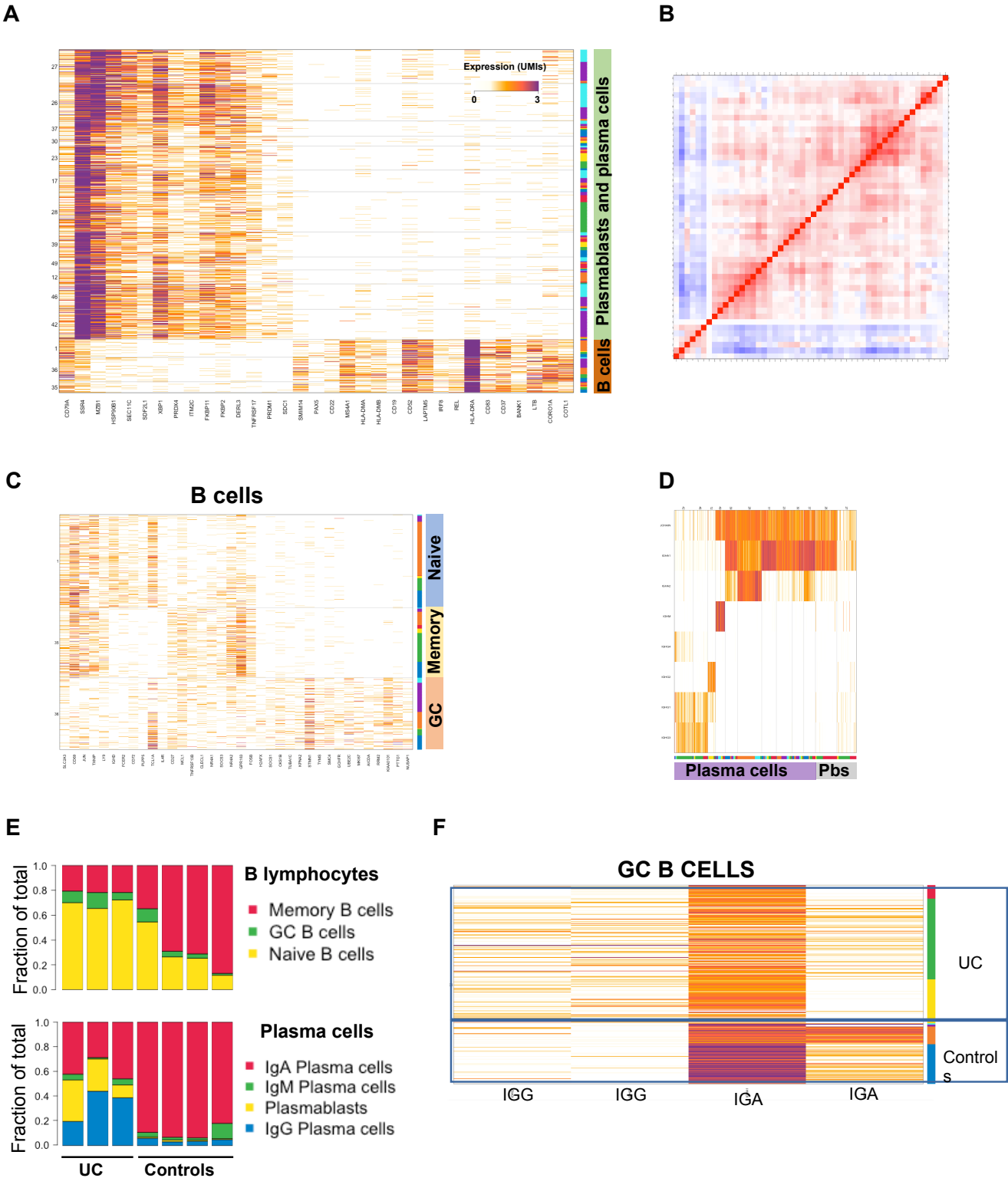


Figure 2

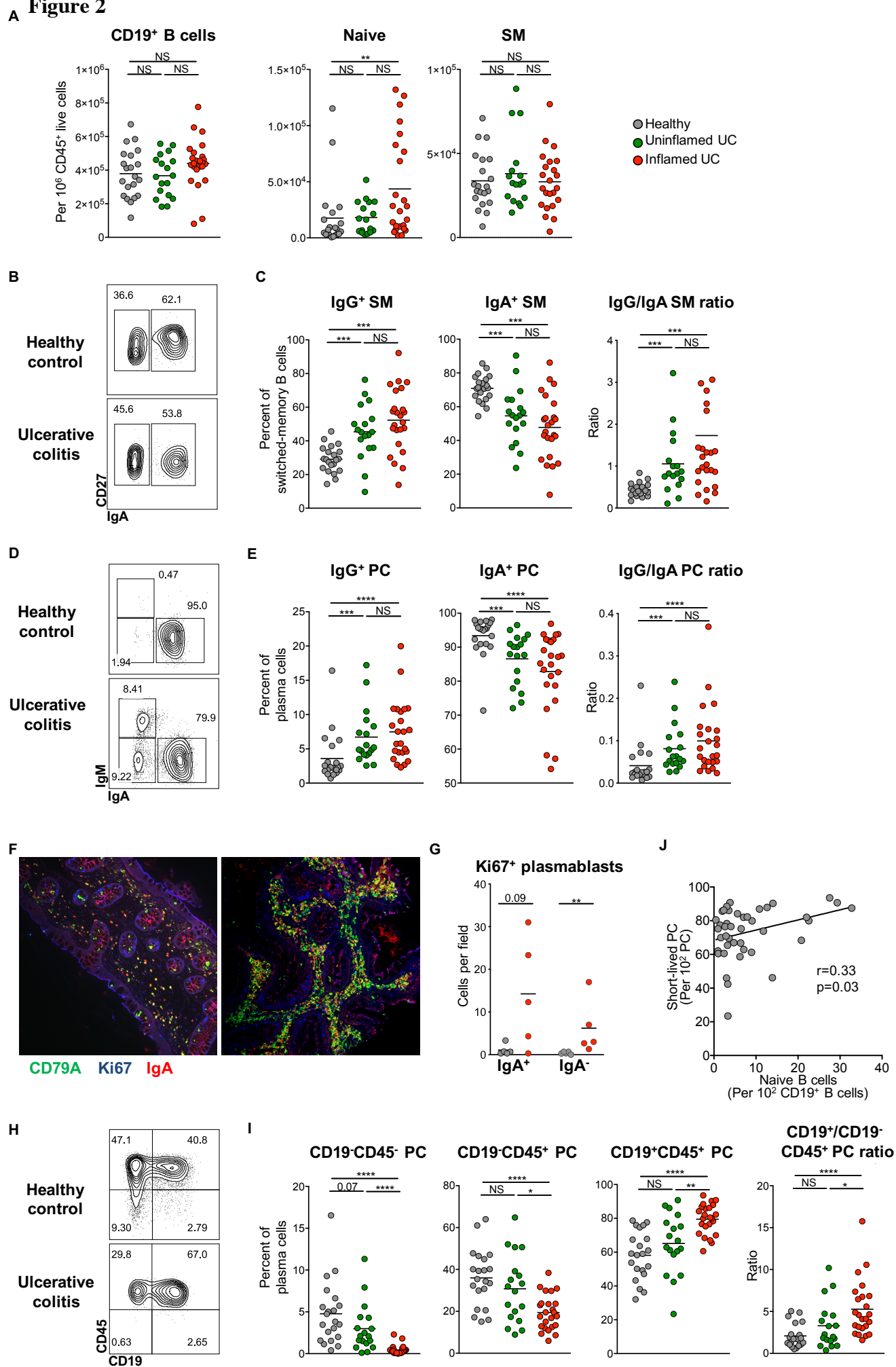


Figure 3

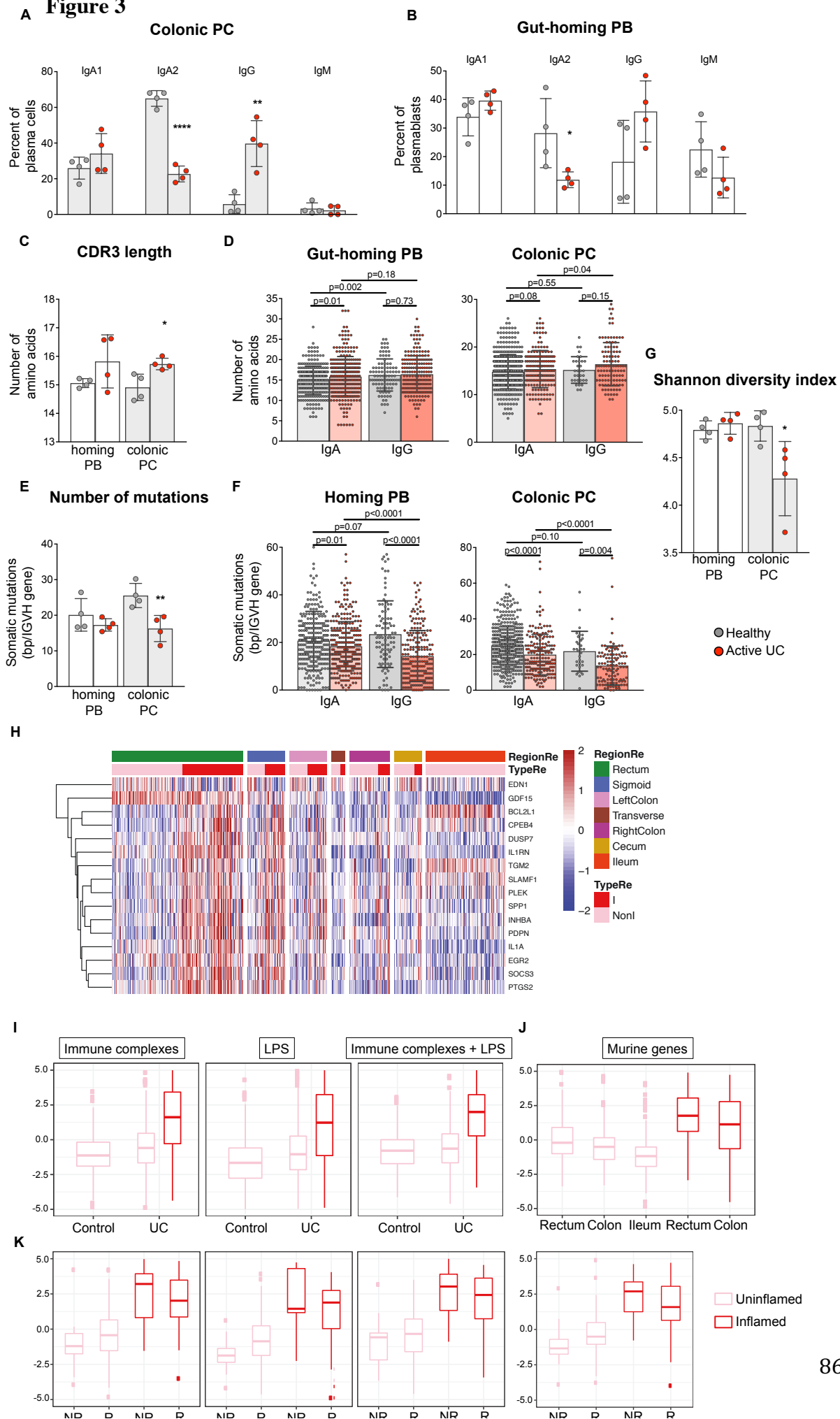
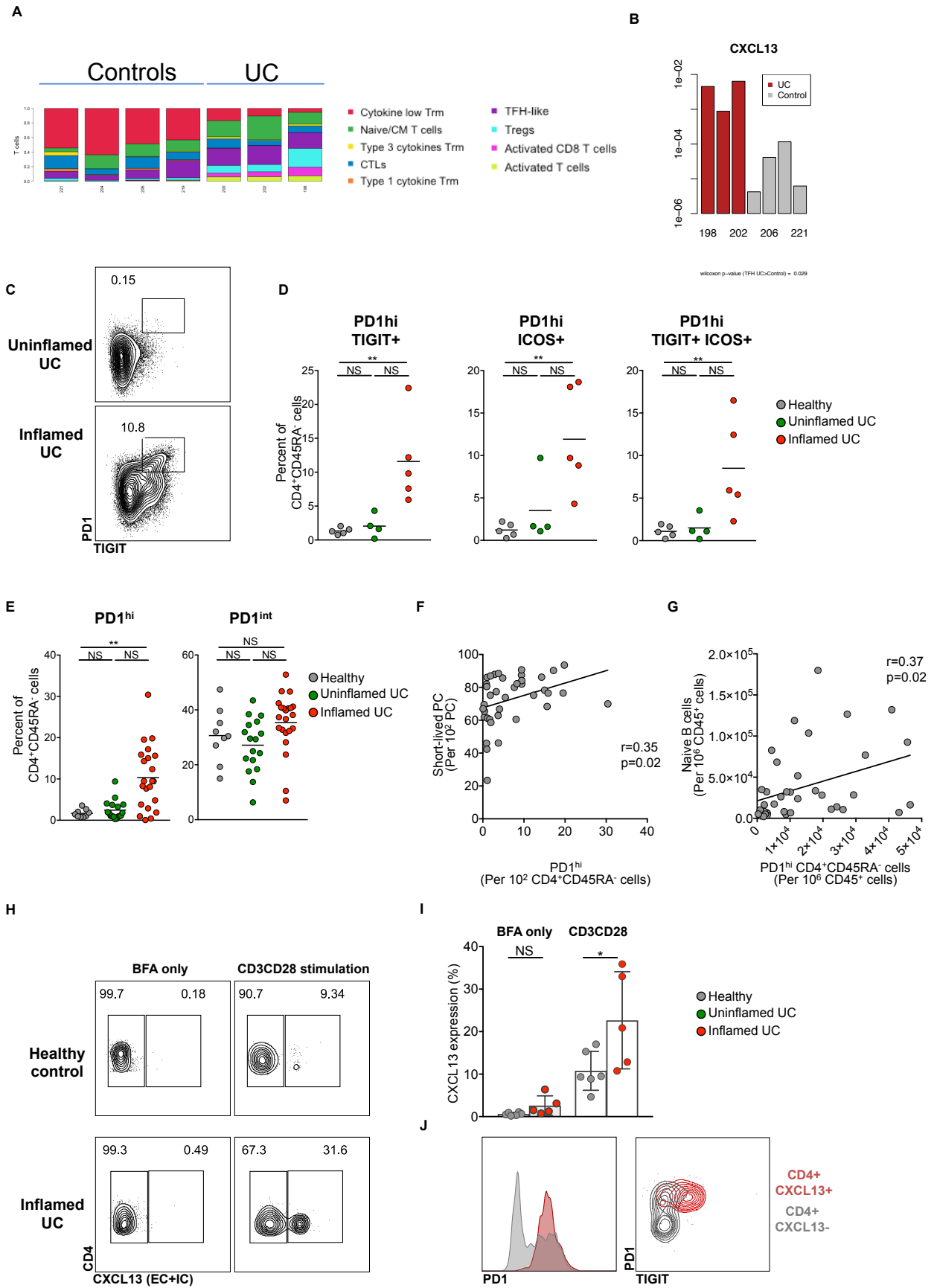


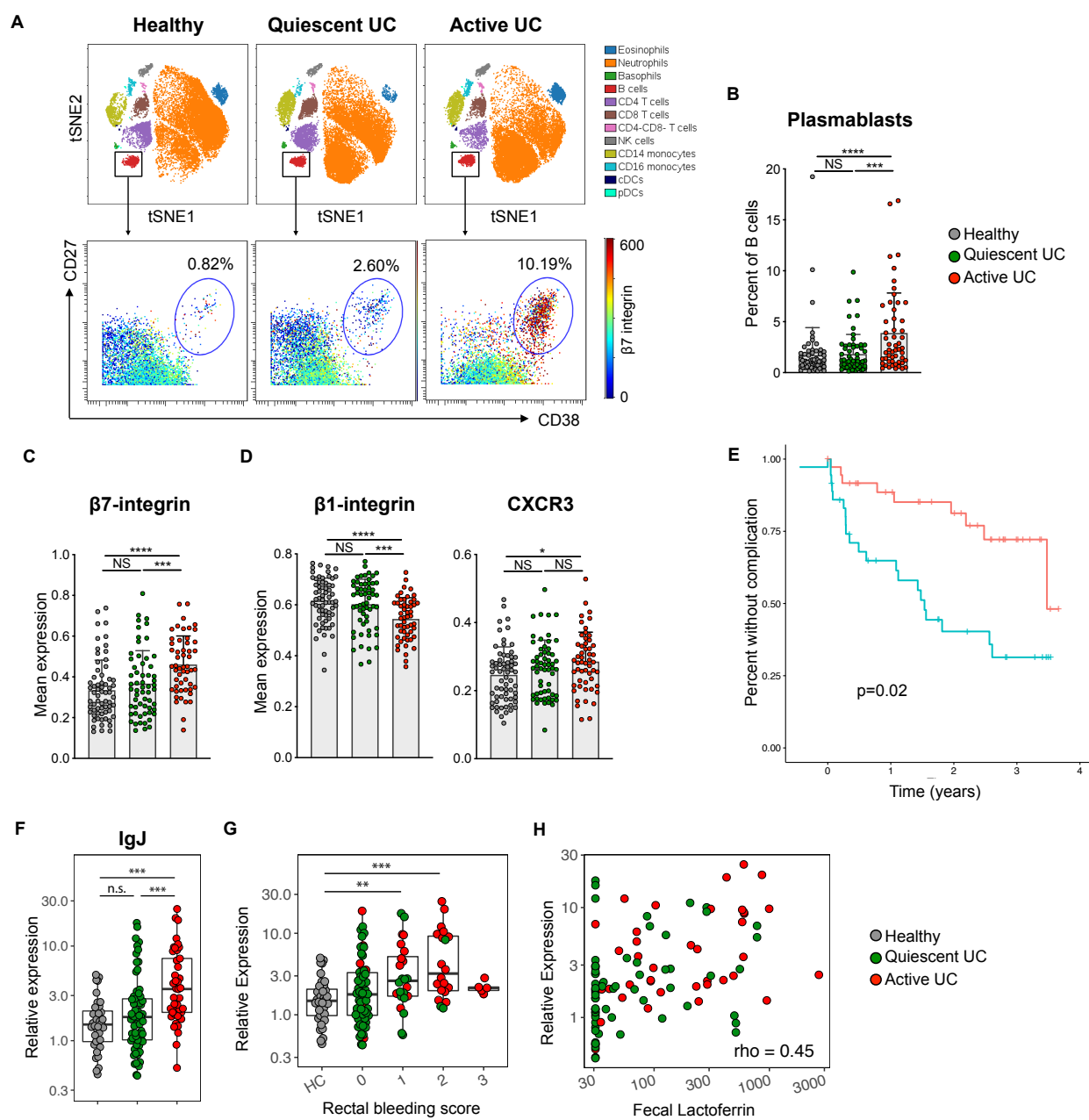
Figure 4



A



Figure 6



Methods

Patients

Patients and healthy controls were recruited from the Inflammatory Bowel Disease (IBD) Center, the gastroenterology department and the digestive endoscopy unit at Mount Sinai Hospital. The protocol was approved and informed consents were obtained from all participants in accordance with the institutional review board. Patients who were enrolled in the study were asked to provide blood and/or biopsies. The latter were collected during a colonoscopy planned for regular care. Clinical characteristics of patients of the PCo are displayed in supplementary Table 1.

Blood collection and PBMCs isolation

Ten to 30 ml of blood were drawn in EDTA tubes. Blood was freshly processed, under sterile condition, within 2 hours after collection. Plasma was collected and stored immediately at -80°C. The blood was then diluted with PBS 1X (Gibco) and gently overlaid on lymphocyte separation medium® (MP Biomedical). After centrifugation, the phase containing PBMCs was harvested and washed 2 times with sterile PBS. All flow cytometry data presented in the study were generated from freshly stained PBMCs.

Intestinal lamina propria cells isolation

During the colonoscopy procedure, 8 to 12 biopsies from the right and/or left colon were collected with forceps directly in RPMI, on wet ice. Biopsies were processed fresh within 2 hours of collection. They were first transferred in 10 ml HBSS (free of calcium and magnesium) containing EDTA (0.5M, pH 8, Invitrogen) and Hepes (1M, Lonza) and incubated for 20 minutes at 37°C. The biopsies were then vortexed, washed with HBSS and transferred in a digestion media (10 ml of RPMI containing 0.005 mg of collagenase

(Sigma-Aldrich) and DNase I (Sigma-Aldrich). After an incubation of 40 minutes at 37°C in a rotating incubator (215 rpm), biopsies were physically disrupted by pipetting up and down 10 times through a 25-gauge needle, filtered through a 100- μ m and a 40- μ m cell strainer and washed with RPMI twice.

Flow cytometry

Isolated cells were incubated with PBS containing the flow Abs cocktail for 20 min at 4°C in the dark. Cells were washed 2 times with PBS, then fixed using Cytofix/Cytoperm buffer (BD) and washed 2 times with Perm/wash buffer (BD). Finally, cells were harvested in FACS buffer (PBS w/o Ca²⁺ Mg²⁺ with 5mM EDTA) before acquisition. In case of intracellular staining, cells were permeabilized for 15 minutes in Cytofix/Cytoperm buffer (BD), washed with Perm/wash buffer (BD) and incubated with the intracellular Ab for 30 minutes. Samples were acquired using LSR Fortessa (BD Bioscience), and data were analyzed using FlowJo v10 (FlowJo). Dead cells and doublets were excluded from all analyses. The list of used Abs is displayed in supplementary Table 7.

Mass cytometry (CyTOF) analyses

Mass cytometry was performed on whole blood samples by the Human Immune Monitoring Core (HIMC). Full description of the methods is provided elsewhere (Uzzan et al. STM). List of antibodies used is shown in supplementary Table 8.

Stimulation assay

Cells obtained from the lamina propria were resuspended at a concentration of 1 million cells per 1 ml of R10 solution (RPMI with 10% heat-inactivated fetal bovine serum and antifungal and antibiotic solutions (1X, Gibco). Cells were then cultured in a U-bottomed

96-well plate with each well containing 200,000 cells. The cells were incubated overnight for 18 hours with 2.5 μ l of CD3CD28 beads (gibco) or 2.5 μ l of PBS in 200 μ l as control. Brefeldin A (1000X, Biolegend) was added in all wells at a final concentration of 1X, 4 hours before harvesting the cells. Cells were then stained extra and intracellularly and analyzed by flow cytometry as previously described.

Immunofluorescence staining

Immunofluorescence staining was performed on 5 patients with untreated active colitis and 5 healthy controls. Formalin-fixed paraffin-embedded human tissue sections 3- μ m in thickness were treated in xylene, a decreasing alcohol gradient and distilled water to achieve de-waxing and rehydration of the tissue. Heat induced epitope retrieval was performed for 15 min in Tris-EDTA buffer (pH 9). After epitope retrieval, tissue sections were permeabilized with 0.2% Triton X-100 in PBS, blocked with 5% bovine serum albumin and 5% Fc receptor blocking (Miltenyi Biotec) and stained with polyclonal Rabbit anti-human IgA (A0262, DAKO at 1:1000 dilution), monoclonal mouse anti-human CD79alpha (clone JCB117, DAKO at 1:25 dilution) and monoclonal rat anti-human Ki67 (SolA15, Invitrogen at 1:50 dilution). Staining with primary antibodies was followed by detection with polyclonal donkey Alexa Fluor 546-conjugated anti-mouse IgG antibody (Invitrogen; 1:200), Alexa Fluor 647-conjugated anti-rabbit IgG antibody (Invitrogen; 1:500) and Alexa Fluor 488-conjugated anti rat IgG antibody (invitrogen; 1:500). Nuclear DNA was visualized with DAPI and coverslips applied with FluorSave reagent (Merck Millipore). Images were acquired either with a Leica TCS SP5 Upright confocal microscope (Leica) or a Nikon Eclipse Ni-E microscope (Nikon) and were further analyzed with ImageJ software.

IgH gene sequencing

The IgH gene of cells of interest was sequenced from 4 patients with active UC and 4 healthy volunteers (Clinical characteristics in supplementary Table 5) using a method previously described[60]. Briefly, we single-cell sorted short-lived plasma cells (live CD45⁺CD38^{hi}CD27⁺CD19⁺) and gut-homing plasmablasts (CD19^{+/int}CD38^{hi}CD27⁺IgD⁻β7⁺) in a 96-well PCR plates in a lysis solution containing Dithiothreitol DTT (100mM, Thermofisher scientific) and RNase inhibitor (RNAsin, Promega). PCR plates were immediately frozen on dry ice and stored at -80°C until further processing. Single cell cDNA synthesis was performed, in the 96-well plates initially used for the sort, using random hexamer primer, dNTP mix (Denville), NP-40 (2%), RNase inhibitors (RNAsin, Promega) and Maxima Reverse Transcriptase (RT Max, Thermofisher scientific). IgH genes were then amplified with 2 rounds of nested PCRs (Primers list in supplementary Table 8). All PCR products were then sequenced (Genewiz) and analyzed for VH and JH usage, CDR3 mutations, V gene mutations and isotype. Sequences were analyzed by IMGT (www.imgt.org) to identify germline VDJ gene segments with the highest homology. Two clones were considered as the same clonotype when they had the same VH and JH families as well as the same CDR3 length and a least 80% homology in the amino acid sequence of the CDR3.

Single-cell RNA sequencing

Single-cell RNA sequencing analysis was performed from single-cell suspension of lamina propria mononuclear cells after using a dead cell depletion kit (Miltenyi Biotec).

Data were generated using the 10X genomics technology. A comprehensive methods description was provided previously (Martin JC et al., Cell, in press).

Macrophage activation signature analysis

Macrophage activation signatures were created using a previously generated RNAseq dataset of murine colonic macrophages stimulated *ex vivo* with IgG immune complexes (GEO dataset: GSE109040). Geneset enrichment analysis of the top 200 up- and down-regulated macrophage genes by IgG stimulation and/or DSS administration was performed on pre-ranked RNAseq dataset of human monocytes stimulated with complexed IgG and/or LPS (GEO dataset: GSE102728) respectively, to further select highly conserved relevant genes that are enriched in the leading edge of the analysis. Genetic signatures were refined to obtained better predictors for disease-relevant networks using elastic-net regularized logistic regression implemented in the *glmnet* R package with the alpha parameter set as 0.5 and leave-one-out cross-validation procedure applied [61] by training it on a publicly available microarray dataset of colonic mucosal biopsies from human ulcerative colitis patients and controls (GSE38713). The fitted model was tested internally with the training set and achieved 96.29 % classification accuracy. This signature was then applied to validation datasets to determine the association between immune complex signaling and various IBD parameters.

Statistical analyses

Consensus Clustering based on CD19_CD4_CD8 Blood Data

To remove the skewness in the data, marker abundance was $\log(x+1)$ transformed. For this analysis, a total number of 96 samples with T cells and B cells measurements was considered. T cells and B cells markers were considered in order to cluster samples into different groups with similar immune infiltration landscape. For this purpose, package ConsensusClusterPlus[62] from Bioconductor[63] was utilized. Specifically, at each iteration, 80% of the features were randomly sub-sampled without replacement and samples were clustered via K-means clustering. This procedure was repeated for 1,000 iterations and the number of clusters resulting in less variability across iterations was selected. Once the consensus clusters were derived, markers differentially expressed across clusters were identified. Upregulation/downregulation of each marker in different consensus groups was assessed using a linear regression where the marker abundance was modeled as a function of consensus clusters. First, every marker was normalized to z-score across samples. Then, the abundance of the j -th marker for sample i was modeled as:

$$x_{i,j} = \sum_{k=1}^3 \beta_{k,j} 1(i \in I_k) + \epsilon_{i,j}$$

with $\epsilon_{i,j} \sim N(0, \sigma_j)$, I_k being the set of samples belonging to the k -th consensus cluster, $1(A)$ being an indicator function equal to 1 if the event A occurs and 0 otherwise, and $\beta_{k,j}$ being the coefficient capturing the association between marker j and the k -th group. P-values were derived via t-test. Plots were drawn using GraphPad Prism software. Statistical significance of data between groups was assessed using the Mann-Whitney test and the two-sample paired Wilcoxon signed-rank test when appropriate. Correlations were assessed using the Spearman test.

Supplementary material

Supplementary Table 1 - Primary cohort

Supplementary Table 2 - Validation Cohort 1 (73 UC patients with clinical follow-up)

Supplementary Table 3 - Clinical characteristics single-cell RNA seq patients

Supplementary Table 4 - Clinical characteristics IgH seq patients

Supplementary Table 5 - Flow Cytometry Abs

Supplementary Table 6 - CyTOF Abs

Supplementary Table 7 - IgH seq primers

Supplementary Figure 1 - Intestinal B cells gating strategy

Supplementary Figure 2- IgH sequencing

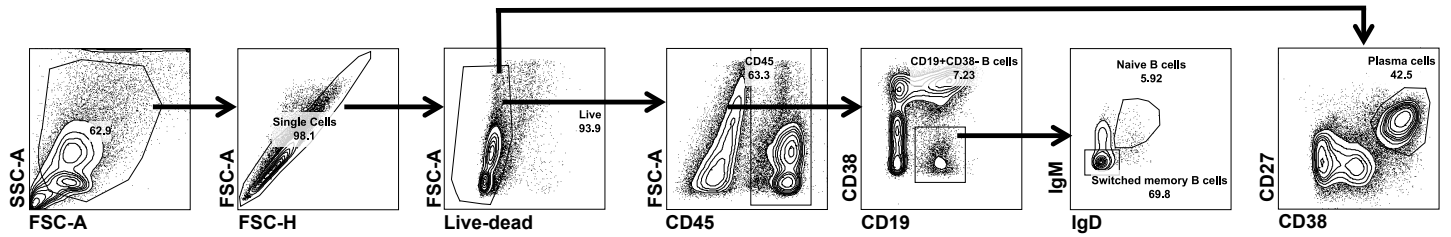
Supplementary Figure 3 - TIGIT ICOS expression

Supplementary Figure 4 - Blood B cells gating strategy

Supplementary Figure 5 - Ki67 and CD138 expression on plasmablasts

Supplementary Figure 86- Homing markers PCo

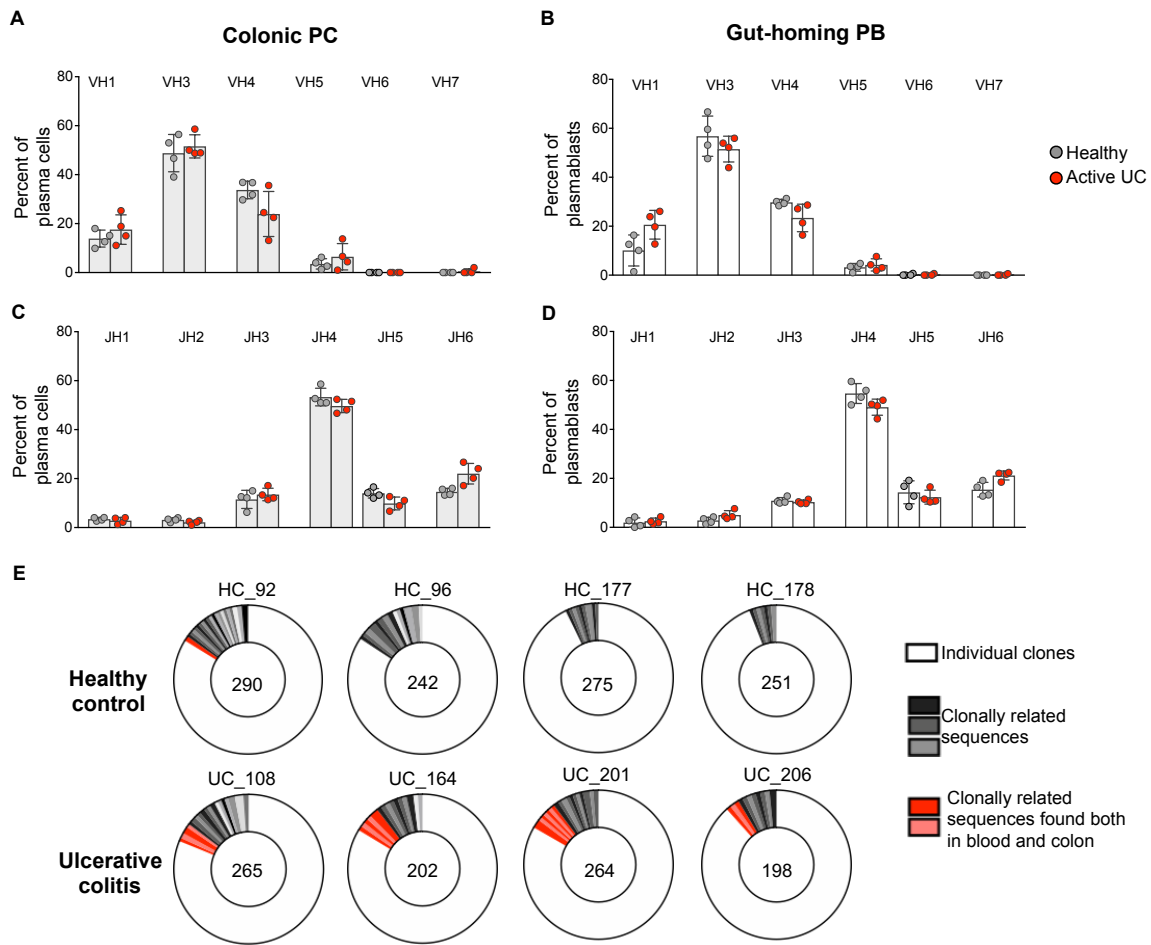
Supplementary figures



Supplementary Figure 1 - Colonic B cells flow cytometry gating strategy

Representative gating strategy to define B cells and plasma cells within lamina propria mononuclear cells.

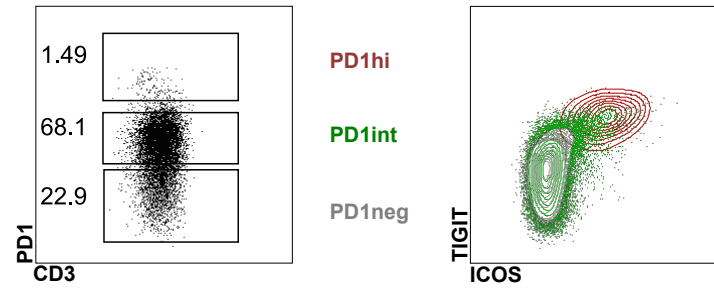
Total plasma cells are defined as live CD38^{hi}CD27⁺ and other B cell subsets include switched memory (live CD45⁺CD19⁺CD38⁻IgD⁻IgM⁻) and naive (live CD45⁺CD19⁺CD38⁻IgD⁺IgM⁺) B cells.



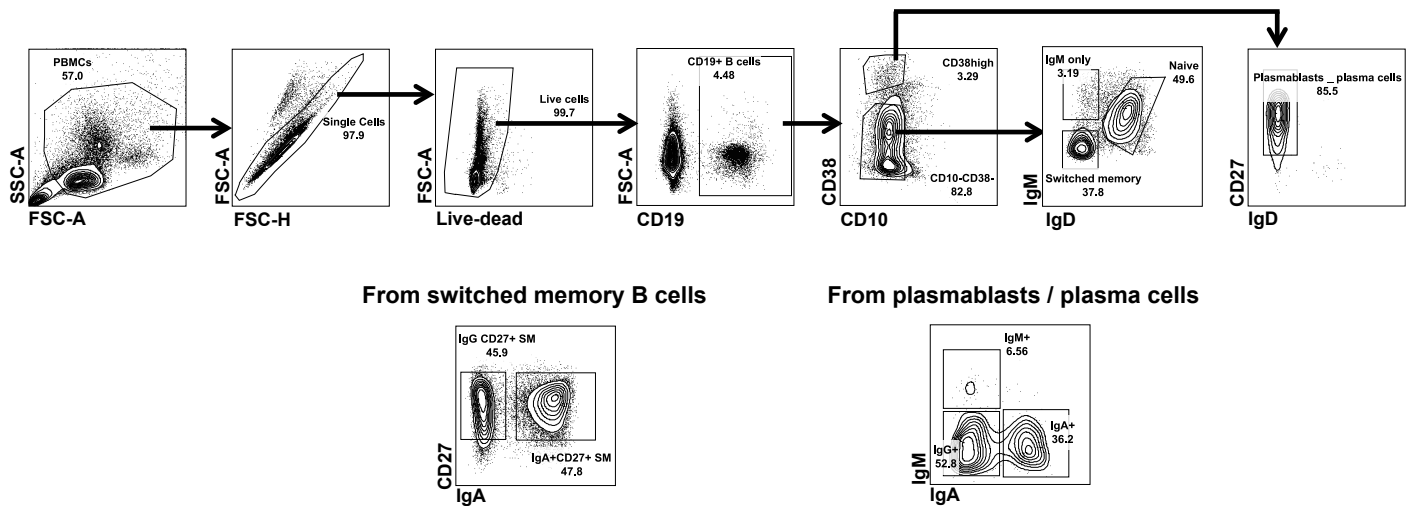
Supplementary figure 2 - JH, VH family usages and clonal relationships

a-d, IgH V and J gene family usages in short-lived colonic plasma cells (a,c) and in circulating gut-homing plasmablasts (b,d). Data are shown as individual data, mean and standard deviation. **e**, Clonal relationships of all clones analyzed for each control and patient.

Gated on live CD45⁺CD3⁺CD4⁺CD45RA⁻

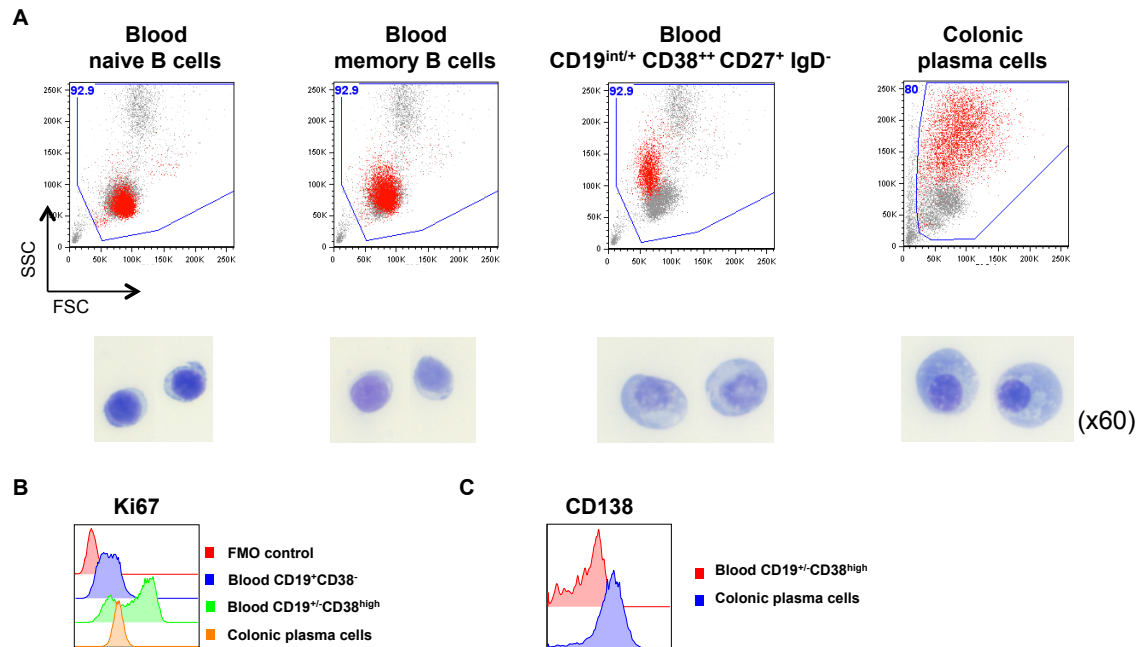


Supplementary figure 3 - Representative flowplots showing the expression of TIGIT and ICOS in PD1^{hi}, PD1^{int} and PD1^{neg} CD4 colonic T cells



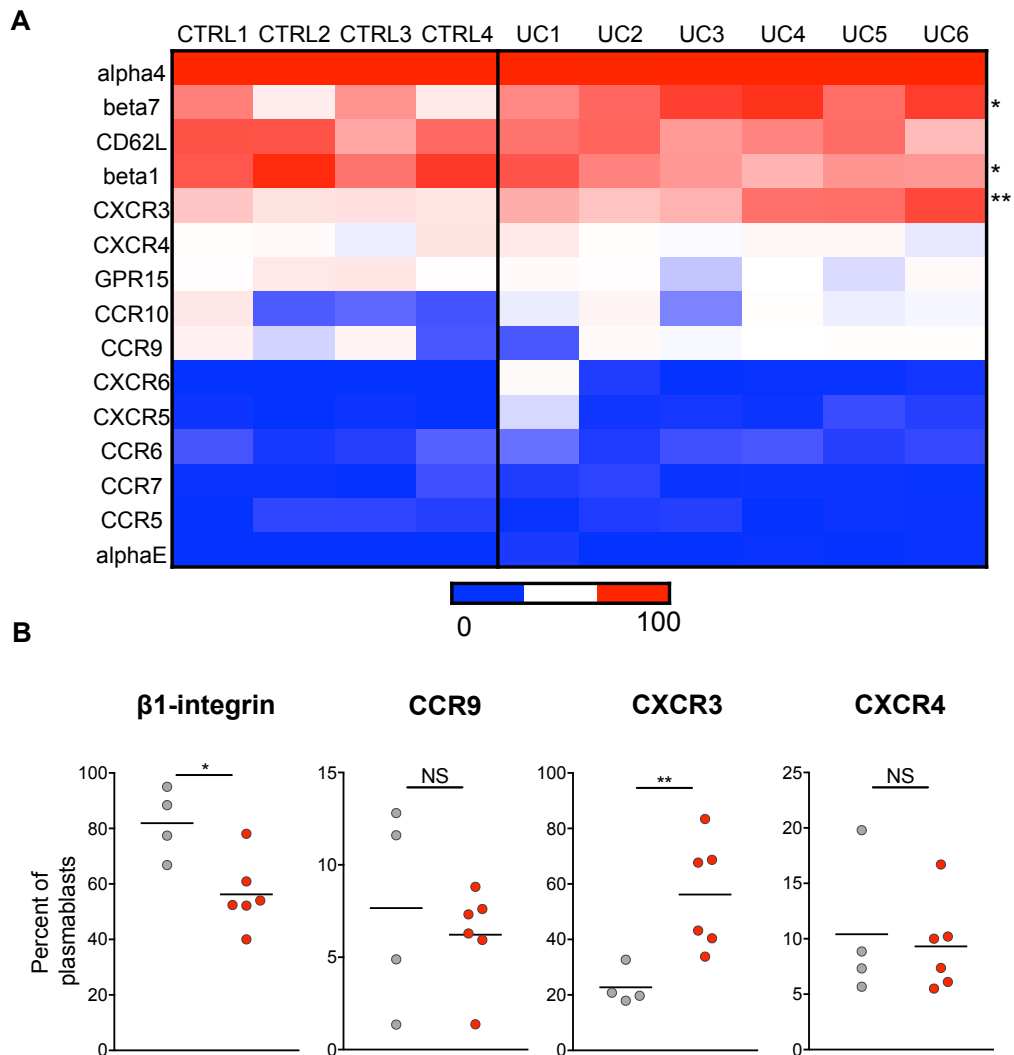
Supplementary Figure 4 - Blood B cells flow cytometry gating strategy

Representative gating strategy to define B cells and plasmablasts / plasma cells within peripheral blood mononuclear cells. Circulating plasmablasts were defined as live $CD19^{+}/int^{+}CD38^{hi}CD10^{-}CD27^{+}IgD^{-}$ and other B cell subsets include switched memory (live $CD19^{+}CD38^{-}IgD^{-}IgM^{+}$) and naive (live $CD19^{+}CD38^{-}IgD^{+}IgM^{+}$) B cells.



Supplementary figure 5 - Morphology, Ki67 and CD138 expression of blood CD19⁺CD27⁺CD38^{hi}IgD⁻ cells define them as plasmablasts

a, Side versus forward scatter representative flow cytometry plots of 3 circulating blood subsets: ⁻ switched memory (live CD19⁺CD38⁻IgD⁻IgM⁻), naive (live CD19⁺CD38⁻IgD⁺IgM⁺) B cells and CD19^{+/int}CD38^{hi}CD27⁺IgD cells and of colonic plasma cells (live CD38^{hi}CD27⁺). Pictures of corresponding sorted cell subsets stained with giemsa are shown below. **b**, Representative expression of Ki67 on 3 subsets including blood CD19^{+/int}CD38^{hi}. **c**, Representative comparative expression of CD138 on blood plasmablasts and colonic plasma cells.



Supplementary figure 6 - Level of expression of homing markers on circulating plasmablasts of patients from the PCo

a, Heatmap showing the level of expression of several homing molecules on circulating plasmablasts of 4 healthy controls and 6 active UC of PCo **b**, Level of expression of β 1-integrin, CCR9, CXCR3 and CXCR4 on circulating plasmablasts of 4 healthy controls and 6 active UC of PCo. Data are shown as individual data and mean, * $p < 0.05$, ** $p < 0.01$, Mann-Whitney test, two-tailed.

Supplementary Tables

Primary Cohort		Controls (n=43)	Quiescent UC (n=14)	Active UC (n=88)
Sex	female, n (%)	20 (46.5%)	9 (64.3%)	45 (51.1%)
Age	years, average $\pm$ SD	44.9 $\pm$ 12.2	46.6 $\pm$ 15.7	36.3 $\pm$ 13.3
Age at UC diagnosis		-	37.3 $\pm$ 13.1	28.7 $\pm$ 13.7
Disease duration		-	6.8 $\pm$ 4.5	7.6 $\pm$ 7.2
Extent	E1	-	3 (21.4%)	7 (7.9%)
	E2		6 (42.9%)	31 (35.2%)
	E3		5 (35.7%)	50 (56.8%)
Clinical Mayo score		-	0.73 $\pm$ 0.90	5.34 $\pm$ 2.01
Rectal bleeding score		-	0 $\pm$ 0	1.81 $\pm$ 0.77
CRP		-	1.86 $\pm$ 2.2	21 $\pm$ 38.7
Concomitant treatments	None	43 (100%)	5 (35.7%)	18 (20.5%)
	5-ASA	-	5 (35.7%)	45 (51.1%)
	Budesonide or prednisone < 20mg	-	2 (14.3%)	9 (10.2%)
	Prednisone $\geq$ 20mg	-	0 (0%)	13 (14.8%)
	Thiopurines	-	1 (7.1%)	11 (12.5%)
	Methotrexate	-	0 (0%)	3 (3.4%)
	TNFi	-	1 (7.1%)	2 (2.3%)
Blood		32 (74.4%)	11 (78.6%)	64 (72.7%)
Biopsies		18 (41.9%)	4 (28.6%)	30 (34.1%)

Supplementary Table 1 - Clinical characteristics of the patients of the primary cohort

5-ASA: 5-amino salicylic acid; CRP: C-reactive protein; E: Extent according to the Montreal classification; UC: Ulcerative Colitis;

TNFi: Tumor Necrosis Factor α inhibitor

Validation Cytof cohort		Quiescent UC (n=31)	Active UC (n=42)
Sex	female, n (%)	16 (51.6%)	22 (52.4%)
Age	years, average $\pm$ SD	44.1 $\pm$ 17.0	40.5 $\pm$ 15.6
Age at UC diagnosis		29.8 $\pm$ 15.2	29.0 $\pm$ 12.3
Disease duration		14.3 $\pm$ 14.1	11.5 $\pm$ 11.6
Extent	E1	2 (6.5%)	6 (14.3%)
	E2	12 (38.7%)	16 (38.1%)
	E3	17 (54.8%)	20 (47.6%)
Concomitant treatments	None	3 (9.7%)	4 (9.5%)
	5-ASA	20 (64.5%)	27 (64.3%)
	Budesonide or prednisone < 20mg	0 (0%)	7 (16.7%)
	Prednisone $\geq$ 20mg	0 (0%)	2 (4.8%)
	Thiopurines	8 (25.8%)	16 (38.1%)
	Methotrexate	0 (0%)	2 (4.8%)
	TNFi	8 (25.8%)	10 (23.8%)
	Ustekinumab	1 (3.2%)	0 (0%)
	Vedolizumab	1 (3.2%)	6 (14.3%)
Endoscopic Mayo score at site of biopsies	0	31 (100%)	0 (0%)
	1	0	21 (50.0%)
	2	0	12 (28.6%)
	3	0	9 (21.4%)

Supplementary Table 2 - Clinical characteristics of the 73 patients with Ulcerative Colitis of the VCo1 with follow-up data

Study Id	Sex	Age (years)	Duration of UC (years)	Concomittant treatment	Disease extent	Site of biopsies	Endoscopy mayo score at the site of biopsies
UC_164	M	30	0.1	5-ASA	E3	Left colon	3
UC_200	F	67	42.2	5-ASA	E3	Left colon	2
UC_205	F	60	13.6	5-ASA	E2	Left colon	3
UC_281	M	77	8.3	0	E2	Left colon	2
HC_96	F	29	-	-	-	Left colon	-
HC_99	M	51	-	-	-	Left colon	-
HC_157	F	30	-	-	-	Left colon	-
HC_158	F	23	-	-	-	Left colon	-
HC_324	M	31	-	-	-	Left colon	-

Supplementary Table 3 - Clinical characteristics of the 4 patients with Ulcerative Colitis and 5 healthy controls who were characterized by single-cell RNA sequencing

Study Id	Sex	Age (years)	Duration of UC (years)	Concomitant treatment	Disease extent	Site of biopsies	Endoscopic mayo score at the site of biopsies
UC_108	M	23	0.8	0	E3	Left colon	3
UC_164	M	30	0.1	5-ASA	E3	Left colon	3
UC_201	M	37	11.3	5-ASA	E3	Left colon	3
UC_206	F	33	5.3	0	E2	Left colon	2
HC_92	M	49	-	-	-	Left colon	-
HC_96	F	29	-	-	-	Left colon	-
HC_177	M	33	-	-	-	Left colon	-
HC_178	F	36	-	-	-	Left colon	-

Supplementary Table 4 - Clinical characteristics of the 4 patients with Ulcerative Colitis and 4 healthy controls who were characterized by IgH gene sequencing

Marker	Fluorochrome	Company	Isotype	Clone
CD19	PE.Cy7	Biolegend	Mouse IgG1, k	SJ25C1
CD38	APC	Biolegend	Mouse IgG1, k	HB-7
CD10	APC.Cy7	Biolegend	Mouse IgG1, k	HI10a
CD27	PerCP.Cy5.5	Biolegend	Mouse IgG1, k	O323
IgD	PB	Biolegend	Mouse IgG2a, k	IA6-2
IgM	A700	Biolegend	Mouse IgG1, k	MHM-88
IgA	FITC	SouthernBiotech	Goat IgG	Polyclonal
Beta7-integrin	PE	Biolegend	Rat IgG2a, k	FIB504
Beta7-integrin	FITC	Biolegend	Rat IgG2a, k	FIB504
CD3	PB	Biolegend	Mouse IgG1, k	UCHT1
CD4	A700	Biolegend	Mouse IgG1, k	RPA-T4
CD8	APC.CY7	Biolegend	Mouse IgG1, k	SK1
CD45RA	PE.Cy7	Biolegend	Mouse IgG2b, k	HI100
TIGIT	BV605	Biolegend	Mouse IgG2a, k	A15153G
ICOS	APC.Cy7	Biolegend	Armenian Hamster IgG	C398.4A
CXCL13	APC	Invitrogen	Mouse IgG1	53610
PD1	PE	Biolegend	Mouse IgG1, k	EH12.2H7
PD1	APC	Biolegend	Mouse IgG1, k	EH12.2H7
CXCR3	PE	Biolegend	Mouse IgG1, k	G025H7
CCR9	PE	Biolegend	Mouse IgG2a, k	L053E8
CXCR4	PE	Biolegend	Mouse IgG2a, k	12G5

Supplementary Table 5 - List of flow cytometry antibodies

Isotope	Target	Clone	Vendor
In113	CD57	HCD57	Biolegend
In115	CD45	HI130	Biolegend
Pr141	Va7.2	3C10	Biolegend
Nd142	CD19	HIB19	Biolegend
Nd143	CD45RA	HI100	Biolegend
Nd144	CD62L	DREG-56	Biolegend
Nd145	CD4	RPA-T4	Biolegend
Nd146	CD8	RPA-T8	Biolegend
Sm147	CD49d	9F10	Biolegend
Nd148	CD16	3G8	Biolegend
Sm149	CD127	A019D5	Biolegend
Nd150	CD1c	L161	Biolegend
Eu151	CD123	6H6	Biolegend
Sm152	CD66b	G10F5	Biolegend
Eu153	PD-1	EH12.2H7	Biolegend
Sm154	CXCR4	12G8	Biolegend
Gd155	CD27	O323	Biolegend
Gd156	PD-L1	29E.2A3	Biolegend
Gd158	BTLA	MIH26	Biolegend
Tb159	TLR2	TL2.1	Biolegend
Gd160	CD14	M5E2	Biolegend
Dy161	CD56	B159	BD Biosciences
Dy162	CD64	10,1	Biolegend
Dy163	CCR4	205410	R&D Systems
Dy164	CCR9	L053E8	Biolegend
Ho165	CCR6	G034E3	Biolegend
Er166	CD25	M-A251	Biolegend
Er167	NKG2D	1D11	Biolegend
Er168	CD3	UCHT1	Biolegend
Tm169	Beta7	FIB504	Biolegend
Er170	CD38	HB-7	Biolegend
Yb171	CD161	HP-3G10	Biolegend
Yb172	CD11b	M1/70	Biolegend
Yb173	CXCR3	G025H7	Biolegend
Yb174	HLADR	L243	Biolegend
Lu175	CD29	TS2/16	Biolegend
Yb176	CD26	BA5b	Biolegend

Supplementary Table 6 - List of mass cytometry (CyTOF) antibodies

IgH first PCR	
5' L-VH 1	ACAGGTGCCCCACTCCCAGGTGCAG
5' L-VH 3	AAGGTGTCCAGTGTGARGTGCAG
5' L-VH 4/6	CCCAGATGGGTCCTGTCCCAGGTGCAG
5' L-VH 5	CAAGGAGTCTGTTCCGAGGTGCAG
3' Cu CH outer	GGAAGGAAGTCCTGTGCGAGGC
3' Cy CH1	GGAAGGTGTGCACGCCGCTGGTC
3' Ca CH1	TGGGAAGTTTCTGGCGGTCACG
IgH second PCR	
5' AgeI VH1	CTGCAACCGGTGTACATTCCCAGGTGCAGCTGGTGCAG
5' AgeI VH1/5	CTGCAACCGGTGTACATTCCGAGGTGCAGCTGGTGCAG
5' AgeI VH3	CTGCAACCGGTGTACATTCTGAGGTGCAGCTGGTGGAG
5' AgeI VH3–23	CTGCAACCGGTGTACATTCTGAGGTGCAGCTGTTGGAG
5' AgeI VH4	CTGCAACCGGTGTACATTCCCAGGTGCAGCTGCAGGAG
5' AgeI VH 4–34	CTGCAACCGGTGTACATTCCCAGGTGCAGCTACAGCAGTG
3' Cu CH1	CAG GAA ACA GCT ATG ACGGGAATTCTCACAGGAGACGA
3' IgG (internal)	CAG GAA ACA GCT ATG ACGTTCGGGGAAGTAGTCCTTGAC
3' Ca CH1–2	CAG GAA ACA GCT ATG ACGTCCGCTTTCGCTCCAGGTCACACT

Supplementary Table 7 - List of primers used for the IgH amplification and sequencing