Characterization of IL-22BP expression during Crohn's disease shows lack of evidence for IL-22BP production by T cells

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Abstract

Background & aims: IL-22 binding protein (IL-22BP) is a soluble and specific inhibitor of IL-22, a cytokine with known protective actions on intestinal epithelial cells during inflammation. Although eosinophils and mononuclear phagocytes (MNP) were shown to be the producers of IL-22BP in the intestine *lamina propria* (LP), it has recently been proposed that $CD4^+$ T cells represent an additional source of IL-22BP in the gut during Crohn's disease (CD). In this study we sought to confirm these findings and to assess IL-22BP secretion levels.

Methods: We investigated IL-22BP cellular sources in CD gut samples using qPCR and ELISA on FACS-sorted LP and mesenteric lymph nodes (MLN) cell subsets. We also evaluated IL-22BP levels in *ex-vivo* culture of intestinal biopsies.

Results: MNP (HLA DR^+ $CD11c^+$ cells) and eosinophils isolated from LP CD tissues expressed *IL22RA2*, while the expression measured in the $CD4^+$ and $CD8^+$ T cells fractions was undetectable or 100-1000 times lower than in MNP. In MLN, *IL22RA2* expression in T cells was either undetectable of 1000-fold lower than in MNPs without differences between naïve and non-naive subsets. *IL22RA2* expression was undetectable after *ex vivo* activation of both $CD4^+$ and $CD8^+$ T cells from LP or MLNs and IL-22BP was not detected in their supernatant, either activated or not. LP eosinophils appeared capable of secreting IL-22BP but not DCs likely due to *in vitro* activation as confirmed with monocyte-derived DCs. We also identified higher production of IL-22BP from ileum as compared to colon biopsies and demonstrated that IL-22BP levels correlated with those of eotaxins but not with TNF and IL-6. Finally, high *ex vivo* production of IL-22BP in mucosa biopsies from CD patients was only observed in smokers.

Conclusion: We confirmed that eosinophils and MNPs are the major sources of IL-22BP in the gut during CD and found no clear evidence for IL-22BP expression by T cells. This work also provides new insights on factors regulating intestinal IL-22BP levels.

Introduction

Inflammatory bowel disease (IBD) is a group a chronic inflammatory conditions of the gastrointestinal tract, the main clinical entities of which are Crohn's disease (CD) and ulcerative colitis (UC)[1]. IBD progressively leads to bowel damage and disability that significantly alter the quality of life[2]. IBD prevalence has increased steadily worldwide[3]. IBD is thought to result from complex interactions between environmental[4], genetic[5], microbial[6] and immune factors responsible[7] for sustained inflammatory responses damaging the gut epithelium and leading to disease-associated intestinal remodeling[8]. Interleukin-(IL) 22, a member of the IL-10 family of cytokines, is strongly induced during IBD flares[9], as a result of increased production by populations of lymphocytes including $CD4^+$ T cells and group 3 innate lymphoid cells (ILC3)[9–11]. IL-22 receptor (IL-22R) expression is mostly limited to epithelial cells[12] and protective actions of IL-22 on the gut epithelium have been largely suggested in different rodent models of gut inflammation[13–17]. IL-22 contributes to increase epithelial barrier properties by inducing the secretion of antimicrobial peptides[15,18] and mucins[13] as well as intestinal epithelial cell (IEC) survival and proliferation to support epithelial regeneration and healing[19,20]. When unregulated, however, IL-22 actions on IEC can also promote tumor cell proliferation[21–24]. IL-22 binding protein (IL-22BP) is a soluble, specific and potent IL-22 inhibitor [25] which controls the level of IL-22-induced epithelial response *in vivo*[26–30]. We[31] and others[32] previously showed that IL-22BP expression is moderately increased during IBD but strongly down-regulated during infectious colitis. We also reported increased IL-22-dependent protection against DSS-induced colitis in IL-22BP-deficent rats[31]. Murine IL-22BP was shown by others to protect against T cell-dependent colitis[32] and to prevent lasting pro-proliferative actions of IL-22 that can support tumorigenesis[26]. *Il22ra2* expression is detected in subsets of dendritic cells (DCs)[26,32–35] and macrophages[35–38] in the gut *lamina propria* (LP) and gut-associated lymphoid structures in rodents. We confirmed the high expression of *IL22RA2* in human gut MNP and identified

74 eosinophils as another source of IL-22BP in the LP of healthy and IBD tissues[31], as later
75 confirmed by Pelczar et al[32]. Interestingly, the latter further revealed unsuspected expression of
76 IL-22BP by $CD4^+$ T cells in human and mouse gut tissues[32,39] and suggested T cells also
77 contributed to increased IL-22BP levels observed in the inflamed IBD intestine. This is of
78 particular importance in IBD given the major pathophysiological implications linked to the
79 increased production of IL-22BP observed in inflamed tissues[31,32]. In this study, we sought to
80 thoroughly revisit the cellular sources of IL-22BP in the LP and in mesenteric lymph nodes
81 (MLNs) of CD patients. While we verified the expression of IL-22BP in eosinophils and
82 mononuclear phagocytes (MNP), we could not obtain evidence for significant *IL22RA2*
83 expression in T cells. We also provided new insights on factors regulating intestinal IL-22BP
84 levels in healthy and CD tissues.

Materials and Methods

Human samples

For identification IL-22BP sources, specimens were obtained from surgical pieces from CD patients undergoing resection due to relapse (**Supp Table 1**). For *ex vivo* culture, colonic and ileum biopsies of mucosal areas were also obtained from 43 active CD and 6 active UC patients undergoing endoscopy (**Supp Table 2**). For RT-qPCR analysis, colonic and ileum biopsies of mucosal areas were also obtained from 24 active CD and 10 active UC patients undergoing endoscopy (**Supp Table 3)**. Control biopsies (n=16) from patients undergoing resection for CRC were also included. Normal colonic tissue samples were taken 10 cm downstream to the tumor. The tissue fragments were processed according to the French guidelines for research on human tissues. Informed patient consent was obtained according to the French bioethics law. Ethical approval was obtained from the local ethics committee (DC-2008-402).

Purification of human cells

Immune cells were isolated from fresh human intestine and MLN. First, in human intestine samples the mucosa was isolated. To remove epithelial cells, the tissue was cut in small pieces and further incubated for 15 min at 37°C under slow rotation (100 rpm) twice time with dissociation solution (HBSS without Ca^{2+} Mg^{2+} - HEPES 10mM – EDTA 5mM). The remaining tissue was washed in PBS, further minced with a scalpel and incubated for 40 min at 37°C in HBSS (with Ca^{2+} and Mg^{2+}) with Collagenase IV (0,5 mg/mL-Sigma ref C5138) and DNase I (0,1 mg/mL-Sigma) under 100 rpm rotation and vortexing every 20 min. Cells suspension was then filtered through 70µm pore size cell strainers (BD Biosciences) and processed for cell sorting. After removal adipose tissue, human MLN were cut in small pieces and incubated for 30 min at 37°C on a shaking incubator in Collagenase D solution (2mg/mL-

Sigma ref 1108882001) containing 0,1 mg/mL DNase I. Then, cell suspension was then filtered through 70µm pore size cell strainers (BD Biosciences) and processed for cell sorting.

Cells sorting from human LP and MLN

Human intestine LP and MLN cells were stained with antibodies specific for CD45 (clone 2D1/APCCy7), CD3 (clone UCHT1/FITC), CD4 (clone SK3/PECy7), CD19 (clone HIB19 /PE), CD11c (clone B-LY6/APC), HLA-DR (clone G46-6/V500) (BD Biosciences) and SIGLEC-8 (clone 7C9/PE) (BioLegend, San Diego, CA). The addition of the CD14 mAb (clone M5E2/PECy7) then allowed a precise separation between the DCs and macrophages. Cells isolated from MLN were also stained with the following CD45RA (clone HI100/APC) and CD62L (clone Dreg56/PE) mAbs to isolate naïve and non-naïve (memory/effector) T cells. Dead cells were excluded by gating on 4', 6-diamidino-2-phenylindole (DAPI)-negative cells. Cell sorting was performed on a BD FACS Aria Cell sorter (BD Biosciences).

Real-time quantitative PCR

After sorting, cells were suspended in TRIzol reagent (Thermo Fisher Scientific) and frozen at 80°C. Total RNA was prepared using an RNeasy mini kit (Qiagen,Valencia, CA) according to manufacturer's instructions. Reverse transcription was performed using Murine Moloney Leukemia Virus Reverse Transcriptase (Thermo Fisher Scientific) following manufacturer's instructions. Gene expressions were assessed with the TaqMan Fast Advanced Master Mix reagent (Applied Biosystems, Foster City, Calif). Primers and probes were purchased from Applied Biosystems (see **Supp Table 4** for complete list). Real-time PCR was performed using the StepOne Plus System (Applied Biosystems). Then, relative expression was normalized to hypoxanthine-guanine phosphoribosyltransferase and calculated using $2^{-\Delta\Delta Ct}$. Results were expressed in arbitrary unit (a.u.).

Monocyte-derived dendritic cells

Dendritic cells were generated from monocytes isolated from healthy volunteers. Monocytes were isolated either by elutriation of PBMCs (Clinical Development and Transfer Platform, Nantes, France) or by magnetic labelling (untouched cells, Human monocyte Isolation kit II). To obtain monocytes-derived dendritic cells (moDC), $2.5x10^6$ monocytes were incubated in a 6-well plate in 5 mL of complete medium (RPMI 1640 medium containing 10% Fetal Calf serum (FCS), 1% L-glutamin, 1% antibiotics, 1mM Sodium Pyruvate, 1mM HEPES, 1% non-essential amino acids) supplemented with recombinant human IL-4 (200U/mL) and recombinant human GM-CSF (100U/mL) for 6 days at 37°C with 5% CO_2. When indicated, cells were treated with retinoic acid (RA) (100nM, Sigma Aldrich), LPS (1 µg/mL, Sigma Aldrich) and TNFα (50 ng/mL, Miltenyi). After 6 days, moDC and supernatants were collected and frozen at −80°C until use.

Ex- vivo culture of gut biopsies

Biopsies from healthy and CD patients were cultured *ex vivo* during 6 hours at 37°C – 5% Co2 under slow rotations. Biopsy were placed (1 biopsy per well) in 4-well Petri dishes in 500µL serum-free medium (Media 1640, Gibco) supplemented with BSA (0.01%), 200µg/mL Penicillin/Streptomycin (Gibco ref 15140-122) and 0.25µg/mL Fungizone (Gibco ref 15290-026). After 6 hours, supernatants were collected and frozen -80°C until use.

ELISA assay for 22BP

Measurement of IL-22BP in the supernatant of stimulated biopsy was performed with the Human IL-22BP ELISA DuoSet kit (R&D System, DY-1087-05) according to manufacturer's instruction. Briefly, a 96-well microplate was coated with a rabbit monoclonal anti-IL-22BP [4µg/mL] and incubated at room temperature (RT) overnight. The next day, after blocking 100-

µL standard dilutions and pure samples were added to each well and incubated 2 hours at RT. Then, 100µL/well of goat anti-IL-22BP [70ng/mL] were added and 2 hours incubation at RT was performed. Finally, ELISA was revealed, and plates were read at 450 nm with TECAN Spark instrument. According to the supplier's instructions (R&D Systems), the detection limit was 94 pg/mL.

Multiplex assay

Cytokines (IL-6 and TNFα) and chemokines (MIP-1β, CXCL1, CCL11 (eotaxin-1), CCL24 (eotaxin-2) and CCL26 (eotaxin-3)) were measured in 6 hours culture supernatants of biopsies from normal (n=13) and CD mucosa (n=29 colons) with a multiplex assay from Biotechne (Rennes, France) according to the supplier's instructions. Analysis was carried out using a Luminex MAGPIX® instrument.

Statistical analysis

Statistical analysis was performed with GraphPad Prism Software (GraphPad Software, San Diego, CA). Means comparisons of unpaired samples were performed using the Mann–Whitney U-test or the Kruskal–Wallis test with Dunn's post-test. The Wilcoxon signed-rank test was used for paired samples. P-values <0.05 were considered statistically significant.

Results

High levels of IL-22BP expression is limited to MNP and eosinophils in human

We previously showed that human MNP (DCs and macrophages) and eosinophils purified from uninflamed colons of controls expressed significant levels of *IL22RA2* mRNA, whereas almost no expression was detected in epithelial cells, lymphocytes and neutrophils[31]. To revisit the cellular distribution of IL-22BP mRNA expression in CD patients, we FACS-sorted populations of HLA-DR$^+$ CD11c$^+$ MNP, eosinophils, CD4$^+$ and CD8$^+$ T cells and B cells from uninflamed and inflamed LP tissues (n= 5 CD patients with only 2 of them having matched areas, **supp Table 1**) (**Supp Figure 1**) and analyzed them by RT-qPCR for *IL22RA2* expression (**Figure** 1A). HLA-DR$^+$ CD11c$^+$ MNP presented the highest levels of *IL22RA2* levels followed by eosinophils, in which average expression was about 3-fold lower, though with more variability, as 2 out of the 5 samples analyzed had low-to-no IL-22BP mRNA detected. Compared to MNP and eosinophils, IL-22BP expression in CD4$^+$ T cells was 100-1000-fold lower and two samples had undetectable levels (**Figure** 1A). In addition, IL-22BP expression in CD4$^+$ T cells was similar to that detected in CD8$^+$ T cells and B cells (**Figure** 1A).

Pelczar et al. reported *Il22ra2* expression in mouse MLN CD4$^+$ T cells which was especially high in memory cells[32]. We FACS-sorted naïve and non-naïve populations of CD4$^+$ and CD8$^+$ T cells, MNP and B cells from MLN collected on CD surgical resections (n=5). In order to dissect standard naïve vs effector/memory T cells populations in lymph node, we stained for CD45RA and CD62L. The common presence of both CR45RA and CD62L is routinely and used here to identify naïve T cells[40] (**Supp Figure 2**). The absence of both markers is characteristic of effector memory T cells while the singular expression of CDR45RA and CD62L is associated to T_{EMRA} cells and central memory T cells, respectively[40]. Cells not positively stained for CD45RA and CD62L were sorted together and will later be called non-naïve T cells. As shown in **Figure 1**A, significant expression of *IL22RA2* was consistently

detected in MNP whereas very low levels were observed in lymphocytes, which did not differ between naïve and non-naïve subsets of $CD4^+$ and $CD8^+$ T cells. Again, similar expression existed between T and B lymphocytes (**Figure** 1A). To assess whether IL-22BP expression could be induced in T cells upon TCR-mediated activation, we FACS-sorted T cells from CD intestine and MLN and stimulated them with PMA plus Ionomycine (PMA+Iono). T cell activation was verified by the induction of *IL2RA* (encoding for CD25) expression (**Figure 1**B). However, upon stimulation, neither $CD4^+$ and $CD8^+$ T cells upregulated *IL22RA2* expression in intestine, nor naïve and non-naïve fractions T cells in MLN (**Figure 1**C).

It is possible that *IL22RA2* mRNA expression is transitorily induced upon TCR-dependent stimulation and was not captured in our single time point analysis. We thus quantified the levels of secreted IL-22BP protein in cell supernatants by an ELISA assay that we first validated using monocyte-derived DCs (moDC), which express *IL22RA2*[33,41]. MoDC were differentiated with or without retinoic acid (RA) (moDC-RA), which we[33] and others[41,42] identified as a major inducer of IL-22BP expression in moDC, a finding we confirmed here at the protein level in **Figure 2**A. As previously demonstrated[33,41], LPS or TNF-induced maturation of moDCs dramatically decreased *IL22RA2* mRNA expression, independently of RA preconditioning (**Figure 2**A). Secreted IL-22BP was only detected in supernatants of moDC-RA and higher levels were observed in unstimulated vs. LPS-stimulated cells (**Figure 2**B). Secreted IL-22BP reflected IL22RA2 expression except for moDC-RA and LPS-stimulated cells, as confirmed by the significant correlation existing between IL-22BP levels quantified by RT-PCR and ELISA (**Figure 2**C).

We then specifically isolated and cultured overnight HLA DR^+, $CD11c^+$, $CD14^-$ cells, corresponding to DCs, eosinophils and T cells from three CD LP tissues (**supp Figure 3**). When applying this ELISA assay to the quantification of IL-22BP in culture supernatants, only one eosinophil supernatant returned detectable levels (**Table 1**). We did not detect DC-derived IL-

22BP likely because of rapid *ex vivo* associated maturation induced by tissue processing, cell sorting and cell culture, a phenomenon well-described for tissue DC[33,43]. Concordant with RT-qPCR data in **Figure 1C**, IL-22BP was not detected in supernatants of both resting and stimulated LP T cells, whereas the production of IFNγ was strongly induced upon stimulation (**Table 2**). Similarly, in MLN, IL-22BP was not detected in supernatants of both resting and stimulated T cells, naïve and non-naïve fractions (data not shown). While the sensitivity of the ELISA assay remains low, these data, together with the mRNA expression ones, do not support human gut T cells as significant sources for IL-22BP.

IL-22BP levels correlate with eosinophil attracting eotaxins in human gut tissues.

We previously reported variable levels of *IL22RA2* mRNA expression in IBD biopsies accross patients[31] and we sought here to assess protein levels by measuring IL-22BP in the supernatant of biopsies from non-IBD intestinal tissues (healthy) and CD lesions (both uninflamed and inflamed biopsies) after 6h culture (**Figure 3**A) (**Supp Table 3&4**). High amounts of IL-22BP (ranging from 94 to 812 pg/ml in normal tissues and up to 8795 pg/mL in CD lesions) were detected in some samples again with wide inter-sample variability. Compared to colonic biopsies, levels of IL-22BP were significantly higher in ileum biopsies from both healthy and IBD tissues (**Figures 3**A), ~~as confirmed by RT-qPCR~~ (**Figure 3**B). IL-22BP levels were not significantly different between non-IBD and CD tissues in both ileum and colon, as well as between CD and UC biopsies (**Figures 3**A). Interestingly, we observed small but significant correlations between levels of IL-22BP and eotaxins (**Figure 3**C), chemokines involved in eosinophil recruitment in the gut LP[44], but not between IL-22BP and proinflammatory cytokines (TNF, IL-6) / chemokines (MIP-1β and CXCL1) (**Figure 3**D). Because eotaxin levels reflect eosinophil abundance in LP mucosa[45], these results suggest that higher production of IL-22BP observed in some biopsies could reflect increased infiltration of eosinophils. We finally

252 investigated whether clinical parameters could contribute to IL-22BP heterogeneity observed
253 in CD tissues. We observe no correlation between secreted IL-22BP levels and disease activity
254 at the time of collection, nor at 3 years post-endoscopy with disease progression or response to
255 anti-TNF. Unexpectedly, we found that all high IL-22BP producers, in both colon and ileum
256 CD tissues, were active smokers (**Figure 3**E).

Discussion

Data presented in this study confirm the production of IL-22BP by MNP and eosinophils in CD gut tissues, but do not support a production by T cells in LP nor in MLN as recently reported by Huber's group[32,39]. The reason for the discrepancies regarding expression of *IL22RA2* in human remain to be elucidated. *IL22RA2* expression was either undetected or detected at extremely low levels in T cells than MNP from CD mucosa, similar to what we observed previously in normal colonic mucosa[31]. *IL22RA2* expression levels in T cells were in fact equivalent to those in B cells which are not currently identify as a cellular source of IL-22BP. In Pelczar's study, results for *IL22RA2* expression in IBD mucosa were reported on two separate graphs, making thus the comparison between $CD4^+$ T cells and MNP impossible[32]. They also assessed *Il22ra2* expression in $CD4^+$ T cell subsets from mice LN and reported increased expression in memory as compared to naïve fractions. IL-22BP expression by murine T cells, however, was not confirmed by several other groups[34,36,46,47] or by the analysis of the ImmGen database (www.immgen.org)[48]. In our study, we could not confirm these data in human MLN in which *IL22RA2* expression levels in both naïve and non-naïve $CD4^+$, as well as $CD8^+$ T cells, were either undetectable or 1000 times less than DCs. Although we could not exclude that a rare subset of LP T cells expresses *IL22RA2* during IBD, mining of our recently published single cell analysis data of CD inflammatory lesions[49] as well as other public data revealed a lack of *IL22RA2* mRNA expression by T cells.

Several assays have been used to measure IL-22BP protein production. We previously used tissue immunofluorescence to demonstrate that LP eosinophils were by far the most abundant IL-22BP expressing cells in normal and CD mucosa with few DCs expressing IL-22BP and none of IL-22BP-expressing cells expressing CD3[31]. Huber group used intracellular flow cytometry to demonstrate IL-22BP expression by DCs, eosinophils and $CD4^+$ T cells from LP[32,39], the levels of which were reported to be enhanced during IBD only in $CD4^+$ T cells[32].

Although not mentioned by authors, DCs actually also appear to upregulate intracellular IL-22BP in their data. Strikingly, virtually all DCs appear to express IL-22BP in their report which is contradictory with the previously described restricted expression of *IL22RA2* by a specific subset of DCs (DC2)[31,50]. Here, we used an ELISA assay for IL-22BP but found no evidence for production by T cells from LP or MLN in line with RT-qPCR results yet the sensitivity of this immunoassays was rather low.

IL-22BP levels in culture supernatants from normal and CD biopsies are consistent with the fact that eosinophils are the major source of IL-22BP in the gut. Indeed, higher levels of IL-22BP in the ileum compared to the colon is consistent with the known intestinal distribution of eosinophils[44,51–53] and IL-22BP levels ae weakly but significantly correlated with eotaxins ones [44]. We found no correlation between TNF and IL-22BP as shown by Pelczar et al at the mRNA level[32], suggesting that IL-22BP levels are not modulated by the state of inflammation.

Surprisingly, we did not detect higher secreted IL-22BP in IBD biopsies as compared to normal tissue, but rather a large interindividual variability. Intriguingly, all CD biopsies with high levels of IL-22BP were harvested from active smokers patients both in ileal and colonic mucosa. Although the same observation was made in our small cohort of UC patients, these findings remain to be confirmed in larger number of samples. Although the exact mechanism remains unknown, it provides an additional argument for a damaging effect of smoking during CD.

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References

1. Kaplan, G. G. The global burden of IBD: from 2015 to 2025. *Nat Rev Gastroenterol Hepatol* **12**, 720–727 (2015).
2. Baumgart, D. C. & Sandborn, W. J. Inflammatory bowel disease: clinical aspects and established and evolving therapies. *Lancet* **369**, 1641–1657 (2007).
3. Ananthakrishnan, A. N. Epidemiology and risk factors for IBD. *Nat Rev Gastroenterol Hepatol* **12**, 205–217 (2015).
4. Ananthakrishnan, A. N. *et al.* Environmental triggers in IBD: a review of progress and evidence. *Nat Rev Gastroenterol Hepatol* **15**, 39–49 (2018).
5. Jostins, L. *et al.* Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* **491**, 119–124 (2012).
6. Turpin, W., Goethel, A., Bedrani, L. & Croitoru Mdcm, K. Determinants of IBD Heritability: Genes, Bugs, and More. *Inflamm. Bowel Dis.* **24**, 1133–1148 (2018).
7. Friedrich, M., Pohin, M. & Powrie, F. Cytokine Networks in the Pathophysiology of Inflammatory Bowel Disease. *Immunity* **50**, 992–1006 (2019).
8. de Souza, H. S. P. & Fiocchi, C. Immunopathogenesis of IBD: current state of the art. *Nat Rev Gastroenterol Hepatol* **13**, 13–27 (2016).
9. Andoh, A. *et al.* Interleukin-22, a member of the IL-10 subfamily, induces inflammatory responses in colonic subepithelial myofibroblasts. *Gastroenterology* **129**, 969–984 (2005).
10. Brand, S. *et al.* IL-22 is increased in active Crohn's disease and promotes proinflammatory gene expression and intestinal epithelial cell migration. *Am. J. Physiol. Gastrointest. Liver Physiol.* **290**, G827-838 (2006).
11. Geremia, A. *et al.* IL-23-responsive innate lymphoid cells are increased in inflammatory bowel disease. *J. Exp. Med.* **208**, 1127–1133 (2011).

12. Sabat, R., Ouyang, W. & Wolk, K. Therapeutic opportunities of the IL-22-IL-22R1 system. *Nat Rev Drug Discov* **13**, 21–38 (2014).

13. Sugimoto, K. *et al.* IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. *J. Clin. Invest.* **118**, 534–544 (2008).

14. Zenewicz, L. A. *et al.* Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. *Immunity* **29**, 947–957 (2008).

15. Zheng, Y. *et al.* Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nat. Med.* **14**, 282–289 (2008).

16. Monteleone, G. *et al.* Phase I clinical trial of Smad7 knockdown using antisense oligonucleotide in patients with active Crohn's disease. *Mol. Ther.* **20**, 870–876 (2012).

17. Couturier-Maillard, A. *et al.* Interleukin-22-deficiency and microbiota contribute to the exacerbation of Toxoplasma gondii-induced intestinal inflammation. *Mucosal Immunol* **11**, 1181–1190 (2018).

18. Coorens, M. *et al.* Innate lymphoid cell type 3-derived interleukin-22 boosts lipocalin-2 production in intestinal epithelial cells via synergy between STAT3 and NF-κB. *J. Biol. Chem.* **294**, 6027–6041 (2019).

19. Lindemans, C. A. *et al.* Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration. *Nature* **528**, 560–564 (2015).

20. Gronke, K. & Diefenbach, A. Regenerative biology: Innate immunity repairs gut lining. *Nature* **528**, 488–489 (2015).

21. Hernandez, P., Gronke, K. & Diefenbach, A. A catch-22: Interleukin-22 and cancer. *Eur. J. Immunol.* **48**, 15–31 (2018).

22. Jiang, R. *et al.* IL-22 is related to development of human colon cancer by activation of STAT3. *BMC Cancer* **13**, 59 (2013).

354 23. Kirchberger, S. *et al.* Innate lymphoid cells sustain colon cancer through production of
355 interleukin-22 in a mouse model. *J. Exp. Med.* **210**, 917–931 (2013).
356 24. Kryczek, I. *et al.* IL-22(+)CD4(+) T cells promote colorectal cancer stemness via STAT3
357 transcription factor activation and induction of the methyltransferase DOT1L. *Immunity* **40**,
358 772–784 (2014).
359 25. Dumoutier, L., Lejeune, D., Colau, D. & Renauld, J. C. Cloning and characterization of IL-
360 22 binding protein, a natural antagonist of IL-10-related T cell-derived inducible factor/IL-
361 22. *J. Immunol.* **166**, 7090–7095 (2001).
362 26. Huber, S. *et al.* IL-22BP is regulated by the inflammasome and modulates tumorigenesis in
363 the intestine. *Nature* **491**, 259–263 (2012).
364 27. Broquet, A. *et al.* Interleukin-22 level is negatively correlated with neutrophil recruitment
365 in the lungs in a Pseudomonas aeruginosa pneumonia model. *Sci Rep* **7**, 11010 (2017).
366 28. Martin, J. C. *et al.* Limited Presence of IL-22 Binding Protein, a Natural IL-22 Inhibitor,
367 Strengthens Psoriatic Skin Inflammation. *J. Immunol.* **198**, 3671–3678 (2017).
368 29. Kleinschmidt, D. *et al.* A Protective Function of IL-22BP in Ischemia Reperfusion and
369 Acetaminophen-Induced Liver Injury. *J. Immunol.* **199**, 4078–4090 (2017).
370 30. Perriard, G. *et al.* Interleukin-22 is increased in multiple sclerosis patients and targets
371 astrocytes. *J Neuroinflammation* **12**, 119 (2015).
372 31. Martin, J. C. *et al.* IL-22BP is produced by eosinophils in human gut and blocks IL-22
373 protective actions during colitis. *Mucosal Immunol* **9**, 539–549 (2016).
374 32. Pelczar, P. *et al.* A pathogenic role for T cell-derived IL-22BP in inflammatory bowel
375 disease. *Science* **354**, 358–362 (2016).
376 33. Martin, J. C. J. *et al.* Interleukin-22 binding protein (IL-22BP) is constitutively expressed
377 by a subset of conventional dendritic cells and is strongly induced by retinoic acid. *Mucosal*
378 *Immunol* **7**, 101–113 (2014).

379 34. Laaksonen, H. *et al.* The multiple sclerosis risk gene IL22RA2 contributes to a more severe
380 murine autoimmune neuroinflammation. *Genes Immun.* **15**, 457–465 (2014).
381 35. Jinnohara, T. *et al.* IL-22BP dictates characteristics of Peyer's patch follicle-associated
382 epithelium for antigen uptake. *J. Exp. Med.* **214**, 1607–1618 (2017).
383 36. Savage, A. K., Liang, H.-E. & Locksley, R. M. The Development of Steady-State
384 Activation Hubs between Adult LTi ILC3s and Primed Macrophages in Small Intestine. *J.*
385 *Immunol.* **199**, 1912–1922 (2017).
386 37. Leonardi, I. *et al.* CX3CR1+ mononuclear phagocytes control immunity to intestinal fungi.
387 *Science* **359**, 232–236 (2018).
388 38. Baratin, M. *et al.* T Cell Zone Resident Macrophages Silently Dispose of Apoptotic Cells
389 in the Lymph Node. *Immunity* **47**, 349-362.e5 (2017).
390 39. Kempski, J. *et al.* IL22BP Mediates the Antitumor Effects of Lymphotoxin Against
391 Colorectal Tumors in Mice and Humans. *Gastroenterology* **159**, 1417-1430.e3 (2020).
392 40. Geißler, K. *et al.* Functional characterization of T-cells from palatine tonsils in patients with
393 chronic tonsillitis. *PLoS One* **12**, e0183214 (2017).
394 41. Lim, C., Hong, M. & Savan, R. Human IL-22 binding protein isoforms act as a rheostat for
395 IL-22 signaling. *Sci. Signal.* **9**, ra95–ra95 (2016).
396 42. Gómez-Fernández, P. *et al.* Long Interleukin-22 Binding Protein Isoform-1 Is an
397 Intracellular Activator of the Unfolded Protein Response. *Front Immunol* **9**, 2934 (2018).
398 43. Schlecht, G., Mouriès, J., Poitrasson-Rivière, M., Leclerc, C. & Dadaglio, G. Purification
399 of splenic dendritic cells induces maturation and capacity to stimulate Th1 response in vivo.
400 *International Immunology* **18**, 445–452 (2006).
401 44. Loktionov, A. Eosinophils in the gastrointestinal tract and their role in the pathogenesis of
402 major colorectal disorders. *World J Gastroenterol* **25**, 3503–3526 (2019).

403 45. Adar, T. *et al.* The Importance of Intestinal Eotaxin-1 in Inflammatory Bowel Disease: New
404 Insights and Possible Therapeutic Implications. *Dig Dis Sci* **61**, 1915–1924 (2016).
405 46. Fukaya, T. *et al.* Pivotal Role of IL-22 Binding Protein in the Epithelial Autoregulation of
406 Interleukin-22 Signaling in the Control of Skin Inflammation. *Front Immunol* **9**, 1418
407 (2018).
408 47. Guendel, F. *et al.* Group 3 Innate Lymphoid Cells Program a Distinct Subset of IL-22BP-
409 Producing Dendritic Cells Demarcating Solitary Intestinal Lymphoid Tissues. *Immunity* **53**,
410 1015-1032.e8 (2020).
411 48. Shay, T. & Kang, J. Immunological Genome Project and systems immunology. *Trends*
412 *Immunol* **34**, 602–609 (2013).
413 49. Martin, J. C. *et al.* Single-Cell Analysis of Crohn's Disease Lesions Identifies a Pathogenic
414 Cellular Module Associated with Resistance to Anti-TNF Therapy. *Cell* **178**, 1493-
415 1508.e20 (2019).
416 50. Watchmaker, P. B. *et al.* Comparative transcriptional and functional profiling defines
417 conserved programs of intestinal DC differentiation in humans and mice. *Nat. Immunol.* **15**,
418 98–108 (2014).
419 51. Lee, E. H., Yang, H. R. & Lee, H. S. Quantitative Analysis of Distribution of the
420 Gastrointestinal Tract Eosinophils in Childhood Functional Abdominal Pain Disorders. *J*
421 *Neurogastroenterol Motil* **24**, 614–627 (2018).
422 52. Matsushita, T. *et al.* The number and distribution of eosinophils in the adult human
423 gastrointestinal tract: a study and comparison of racial and environmental factors. *Am J*
424 *Surg Pathol* **39**, 521–527 (2015).
425 53. DeBrosse, C. W., Case, J. W., Putnam, P. E., Collins, M. H. & Rothenberg, M. E. Quantity
426 and distribution of eosinophils in the gastrointestinal tract of children. *Pediatr Dev Pathol*
427 **9**, 210–218 (2006).
428

Legends to figures:

Figure 1: IL-22BP is expressed by human MNP and eosinophils but not T cells.

(**A**) *IL22RA2* expression was analysed by RT-qPCR on FACS-sorted MNP, eosinophils and T cells from LP and MLN CD tissues (n=5). For gut results, red circles correspond to inflamed mucosa and the black one to uninflamed mucosa. Concerning MLN graph, naïve lymphocytes refer to $CD45RA^+$ $CD62L^+$ cells while non-naïve to all the others. Each point represents the ratio of *IL22RA2* to HPRT (hypoxanthine guanine phosphoribosyl transferase) expression, as determined by the 2^{-ddct} method of relative quantification. (**B**)&(**C**) Fresh isolated T cells from LP and MLN CD tissues have been stimulated with media culture or PMA (50ng/m) + Ionomycine (250ng/mL). After 20 hours, *IL22RA2* and *IL2Rα* gene expression were evaluated by RT-qPCR in resting and stimulated T cells. Each symbol represents one sample. Means comparisons of unpaired samples were performed using the Kruskal–Wallis test. P-value < 0,05 were considered statistically significant. CD: Crohn's disease; DCs: dendritic cells, Mθ: macrophages; MLN: mesenteric lymph node, MNP: mononuclear phagocytes.

Figure 2: IL-22BP level assessed using ELISA in monocytes-derived DCs.

Human monocytes from peripheral blood of healthy donors were differentiated into DCs (moDCs) in complete medium with GM-CSF and IL-4 for 6 days. When indicated, ligands were added of culture. **(A)** On day 6, *IL22RA2* expression was analysed by quantitative RT–qPCR. Bars represent mean±s.e.m. ratio of *IL22RA2* gene to *HPRT* expression as determined by the 2^{-ddCt} method of relative quantification from n=11 independent experiments. **(B)** At day 6, supernatants were collected and frozen -80°C until measurement of IL-22BP level with ELISA. **(C)** Correlation between *IL22RA2* expression and IL-22BP protein levels. Means comparisons of paired samples were performed using the Wilcoxon test. Correlation were calculated using the Spearman rank correlation test. P-value < 0,05 were considered statistically significant. ***

454 p<0,001; ** p<0,01; * p<0,05; moDCs: monocyte-derived dendritic cell; RA: retinoic acid;
455 LPS: Lipopolysaccharide; TNFα: Tumor necrosis factor alpha.
456
457 **Figure 3: In human gut tissue, IL-22BP levels are stronger in the ileum and correlate with**
458 **those of eotaxins.**
459 (**A**) ELISA assay of IL-22BP in 6 hours culture supernatants of biopsies from normal mucosa
460 (He) (n=4 ileum and n=12 colons), CD mucosa (n=11 ileum and n=32 colons) and UC mucosa
461 (n=6 colons) biopsies. (**B**) *IL22RA2* gene expression evaluated by RT-qPCR in fresh normal
462 gut tissue (He) (n=7 ileum and n=15 colons), CD mucosa (n=14 ileum and n=10 colons) and
463 UC mucosa (n= 10 colons). Correlation between secreted IL-22BP and eotaxins (**C**) or IL-6,
464 TNFα, CXL1 and MIP-1β (**D**) measured in 6 hours culture supernatants of biopsies from normal
465 (n=13) and CD tissues (n=29). **(E)** ELISA assay of IL-22BP in 6 hours culture supernatant of
466 biopsies from CD gut mucosa (n=11 ileum and n=32 colons) according to smoking status. Each
467 symbol represents one sample. Means comparisons of unpaired samples were performed using
468 the Mann Whitney test. Correlations were calculated using the Spearman rank correlation test.
469 P-value < 0,05 were considered statistically significant. *** p<0,001; * p<0,05 n.s: non-
470 significant. He: normal tissue; CD- Crohn's disease; UC: ulcerative colitis.

Table 1 : IL-22BP protein assay in culture supernatant of fresh sorted cells from CD LP

Data are representative of three independent experiments on three different donors. IL-22BP dosages are expressed in pg/mL and values below the detection limit, according to the supplier's instructions, are reported as <94pg/mL. n.d: not done.

CD-LP	DCs	Eosinophils	T cells
Inflamed Ileum#1	n.d	321	<94
Inflamed Ileum#2	<94	<94	<94
Inflamed Ileum#3	<94	<94	<94

476 **Table 2: IL-22NP and IFNγ protein assays in culture supernatant of fresh sorted T cells**
477 **from CD LP.**

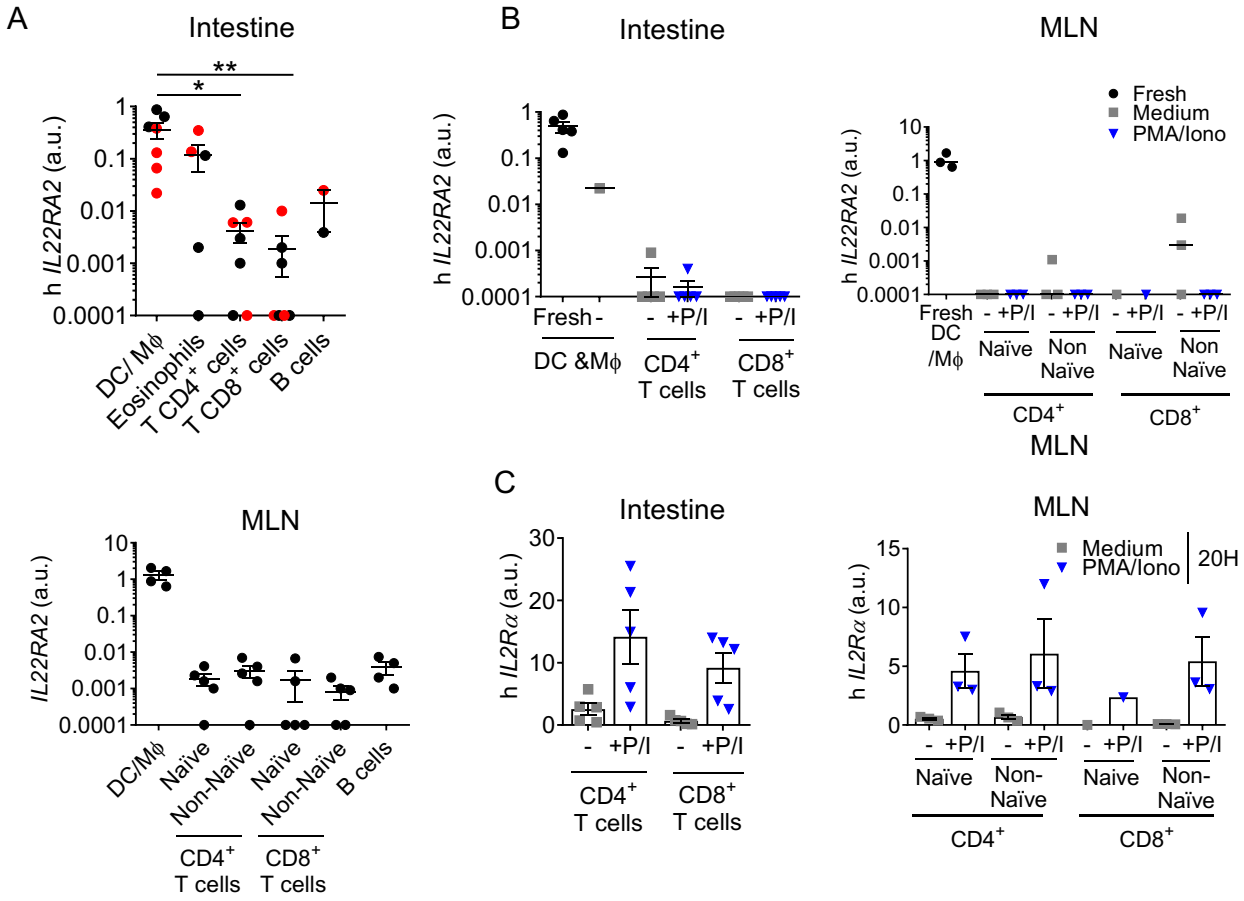
478 Data are representative of five independent experiments on three different donors. IL-22BP and
479 IFNγ dosages are expressed in pg/mL and values below the detection limit, according to the
480 supplier's instructions, are reported as < 94 pg/mL and < 17 pg/mL respectively. n.d: not done.

CD LP	CD4+ T cells			
	medium		PMA+Ionomycine	
	IFNγ (pg/mL)	IL-22BP (pg/mL)	IFNγ (pg/mL)	IL-22BP (pg/mL)
Uninflamed colon-CD#1	<17	<94	8946	<94
Inflamed colon-CD#1	<17	<94	2060	<94
Uninflamed ileum-CD#2	<17	<94	36275	<94
Inflamed ileum-CD#2	<17	<94	6138	<94
Uninflamed colon-CD#3	<17	<94	23440	<94

481

Figures

FIGURE 1



485 **FIGURE 2**

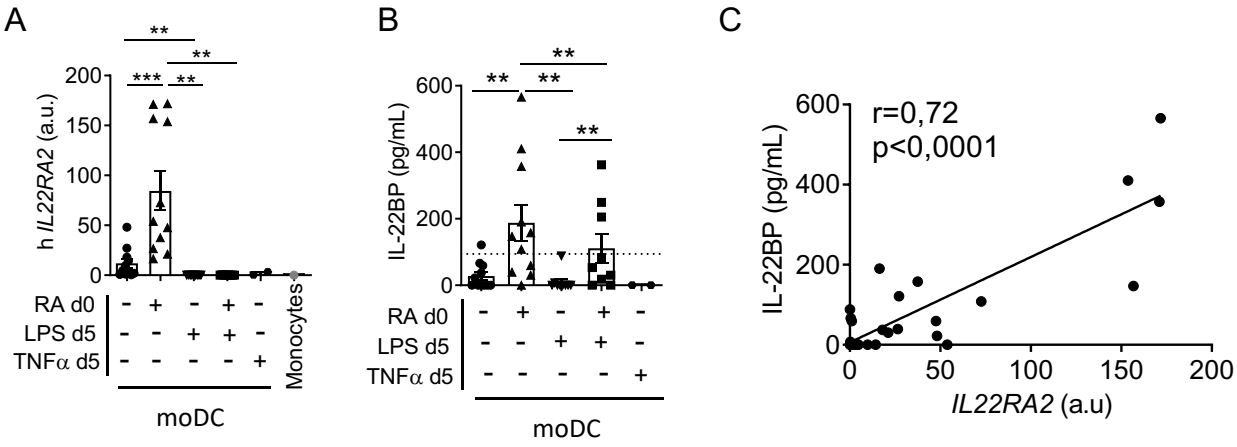


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FIGURE 3