**SUPPLEMENTARY FIGURES**



**Supplementary Figure 1**: **Basal levels of cytokine secretion by colonic biopsies from IBD patients.** Levels of cytokine secretion by colonic biopsies from IBD patients in active phase (Crohn’s Disease, CD; Ulcerative Colitis, UC) and healthy controls (HC) in basal condition (Live Bacteria). No statistical significance was found.



**Supplementary Figure 2**: L**evels of cytokine secretion by colonic biopsies from IBD patients, in the presence of antibiotics.** Levels of cytokine secretion by colonic biopsies from IBD patients in active phase (Crohn’s Disease, CD; Ulcerative Colitis, UC) and from healthy controls (HC) in the presence of antibiotic mixture (+PS). Statistical significances are denoted using alphabetical letters ( a=p < 0.05; b=p < 0.01; c=p< 0.001).

**SUPPLEMENTARY TABLES**

**Supplementary Table 1. Clinical characteristics of patients suffering from Inflammatory Bowel Disease**

|  |  |  |
| --- | --- | --- |
| **Clinical characteristics** |  | **Inflammatory markers** |
| PATIENT | SEX/AGE | DIAGNOSIS | AGE AT DIAGNOSIS | DISEASE ACTIVITY | THERAPIES | PUCAI/PCDAI | DISEASE LOCATION | CRP | ESR | FECAL CALPROTECTIN |
| **01** | M/17 | UC | 12 | ACTIVE | AZA. 5-ASA | 0 | LEFT-SIDED UC |  | 0.06 | 9 | - |
| **02** | M/7 | UC | 6 | ACTIVE | - | 20 | PANCOLITIS |  | 0.82 | 47 | 1522 |
| **03** | M/17 | UC | 17 | ACTIVE | CIPX | 45 | LEFT-SIDED UC |  | 0.4 | 10 | - |
| **04** | F/14 | UC | 12 | ACTIVE | 5-ASA. CS | 30 | LEFT-SIDED UC |  | 0.3 | 44 | - |
| **05** | M/17 | UC | 13 | ACTIVE | Sulfasalzine | 25 | PANCOLITIS |  | 2.05 | 67 | 2060 |
| **06** | F/15 | UC | 14 | ACTIVE | CS. 5-ASA. AZA | 40 | LEFT-SIDED UC |  | 0.04 | 33 | 2120 |
| **07** | F/11 | UC | 2 | ACTIVE | CS. 5-ASA. AZA | 40 | PANCOLITIS |  | 0.59 | 108 | 1648 |
| **08** | F/14 | UC | 9 | ACTIVE | AZA | 5 | PANCOLITIS |  | 0.26 | 94 | 33 |
| **09** | M/17 | UC | 7 | ACTIVE | AZA | 5 | PANCOLITIS |  | 0.03 | 4 | - |
| **10** | F/12 | UC | 7 | ACTIVE | AZA 5-ASA | 20 | ULCERATIVE PROCTITIS |  | 0.02 | 12 | 1746 |
| **11** | M/12 | UC | 10 | ACTIVE | 5-ASA | 20 | ULCERATIVE PROCTITIS |  | 0.03 | 6 | 21 |
| **12** | M/18 | UC | 17 | ACTIVE | 5-ASA | 65 | LEFT-SIDED UC |  | 0.16 | 17 | - |
| **13** | M/18 | UC | 18 | ACTIVE | 5-ASA. MNZ | 15 | PANCOLITIS |  | 0.18 | 3 | - |
| **Clinical characteristics** |  | **Inflammatory markers** |
| PATIENT | SEX/AGE | DIAGNOSIS | AGE AT DIAGNOSIS | DISEASE ACTIVITY | THERAPIES | PUCAI/PCDAI | DISEASE LOCATION |  | CRP | ESR | FECAL CALPROTECTIN |
| **14** | F/18 | UC | 17 | ACTIVE | - | 30 | LEFT-SIDED UC |  | 0.12 | 27 | 50 |
| **15** | M/13 | UC | 11 | ACTIVE | - | 30 | PANCOLITIS |  | 0.19 | 31 | 1600 |
| **16** | F/10 | UC | 9 | REMISSION | 5-ASA | 5 | LEFT-SIDED UC |  | 0.01 | 4 | 602 |
| **17** | F/14 | UC | 12 | REMISSION | CS. 5-ASA | 0 | LEFT-SIDED UC |  | 0.01 | 8 | 126 |
| **18** | F/18 | UC | 13 | REMISSION | AZA. 5-ASA | 0 | PANCOLITIS |  | 0.06 | 13 | - |
| **19** | M/17 | UC | 11 | REMISSION | 5-ASA | 0 | PANCOLITIS |  | 0.11 | 10 | 124 |
| **20** | M/10 | UC | 6 | REMISSION | AZA. 5-ASA | 0 | PANCOLITIS |  | 0.01 | 11 | 35 |
| **21** | M/20 | UC | 17 | REMISSION | 5-ASA. IFX | 0 | LEFT-SIDED UC |  | 0.06 | 6 | - |
| **22** | F/24 | UC | 13 | REMISSION | CS. 5-ASA. AZA | 0 | PANCOLITIS |  | 0.02 | 6 | - |
| **23** | M/13 | CD | 13 | ACTIVE | - | 15 | PANENTERIC CD |  | 3.1 | 92 | 1915 |
| **24** | F/11 | CD | 9 | ACTIVE | ADA | 15 | PANENTERIC CD |  | 0.08 | 11 | 1080 |
| **25** | F/10 | CD | 9 | ACTIVE | IFX | 2.5 | PANENTERIC CD |  | 0.53 | 35 | - |
| **26** | F/13 | CD | 9 | ACTIVE | ADA | 10 | PANENTERIC CD |  | 0.71 | 66 | - |
| **Clinical characteristics** |  | **Inflammatory markers** |
| PATIENT | SEX/AGE | DIAGNOSIS | AGE AT DIAGNOSIS | DISEASE ACTIVITY | THERAPIES | PUCAI/PCDAI | DISEASE LOCATION |  | CRP | ESR | FECAL CALPROTECTIN |
| **27** | M/11 | CD | 9 | ACTIVE | 5-ASA | 12.5 | ILEOCOLONIC CD |  | 0.4 | 27 | 475 |
| **28** | M/10 | CD | 10 | ACTIVE | CIPX | 27.5 | PANENTERIC CD |  | 5.01 | 33 | 8688 |
| **29** | M/15 | CD | 14 | ACTIVE | - | 0 | PANENTERIC CD |  | 0.04 | 5 | 0 |
| **30** | M/10 | CD | 10 | ACTIVE | MNZ | 35 | ILEOCOLONIC CD |  | 2.88 | 51 | 2772 |
| **31** | M/15 | CD | 12 | ACTIVE | IFX | 17.5 | GASTRIC AND ILEOCOLONIC CD |  | 2.64 | 38 | 1295 |
| **32** | M/16 | CD | 12 | ACTIVE | CS. SSZ | 45 | GASTRIC AND ILEOCOLONIC CD |  | 4.9 | 75 | - |
| **33** | F/11 | CD | 9 | ACTIVE | - | 32.5 | PANENTERIC CD |  | 2.96 | 120 | 1372 |
| **34** | F/16 | CD | 11 | ACTIVE | MTX. SSZ | 40 | ILEOCOLONIC CD |  | 0.2 | 46 | 55483 |
| **35** | M/11 | CD | 5 | ACTIVE | IFX | 25 | ILEOCOLONIC CD |  | 1.0 | 37 | 1050 |
| **36** | F/10 | CD | 9 | ACTIVE | - | 25 | GASTRIC AND ILEOCOLONIC CD |  | 2.07 | 56 | 2079 |
| **37** | F/11 | CD | 11 | ACTIVE | CS | 30 | COLONIC CD |  | 5.76 | 88 | 2083 |
| **38** | F/11 | CD | 11 | ACTIVE | IFX. SSZ | 0 | COLONIC CD |  | 0.03 | 15 | 402 |
| **39** | F/10 | CD | 10 | ACTIVE | - | 20 | COLONIC CD |  | 3.18 | 77 | 1477 |
|  |  |  |  |  |  |  |  |  |  |  |  |
| **Clinical characteristics** |  | **Inflammatory markers** |
| PATIENT | SEX/AGE | DIAGNOSIS | AGE AT DIAGNOSIS | DISEASE ACTIVITY | THERAPIES | PUCAI/PCDAI | DISEASE LOCATION |  | CRP | ESR | FECAL CALPROTECTIN |
| **40** | F/10 | CD | 9 | ACTIVE | Thd. SSZ | 30 | GASTRIC AND ILEOCOLONIC CD |  | 1.6 | 25 | 2175 |
| **41** | M/6 | CD | 6 | ACTIVE | - | 20 | COLITIS IN CGD |  | 3.12 | 88 | 28311 |
| **42** | M/7 | CD | 6 | ACTIVE | 5-ASA | 17.5 | COLITIS IN CGD |  | 0.42 | 35 | 3305 |
| **43** | M/7 | CD | 6 | ACTIVE | MNZ | 52.5 | JEJUNALILEOCOLONIC CD |  | 1.04 | 98 | 648 |
| **44** | M/12 | CD | 10 | ACTIVE | MNZ | 17.5 | PANENTERIC CD |  | 0.19 | 41 | 807 |
| **45** | M/16 | CD | 16 | ACTIVE | MNZ, CIPX | 17.5 | PANENTERIC CD |  | 2.19 | 34 | 1087 |
| **46** | M/18 | CD | 15 | REMISSION | IFX | 10 | PANENTERIC CD |  | 0.35 | 18 | 151 |
| **47** | M/11 | CD | 6 | REMISSION | AZA | 0 | ILEOCOLONIC CD |  | 0.03 | 8 | - |
| **48** | M/7 | CD | 1 | REMISSION | Thd | 0 | NASAL, COLONIC CD |  | 0.05 | 13 | - |
| **49** | F/16 | APHTHOSIS, AUTOIMMUNE GASTRITIS | - | NORMAL COL | - | - | - |  | 0.05 | 40 | 25 |
| **50** | M/15 | PREVIOUS INTESTINAL POLYP | - | NORMAL COL | - | - | - |  | 0.14 | 5 | - |
| **Clinical characteristics** |  | **Inflammatory markers** |
| PATIENT | SEX/AGE | DIAGNOSIS | AGE AT DIAGNOSIS | DISEASE ACTIVITY | THERAPIES | PUCAI/PCDAI | DISEASE LOCATION |  | CRP | ESR | FECAL CALPROTECTIN |
| **51** | M/10 | JUVENILE INTESTINAL POLYPOSIS | - | INTESTINAL POLYPS | - | - | COLON |  | - | - | - |
| **52** | M/11 | FUNCTIONAL ABDOMINAL PAIN | - | NORMAL COL | - | - | - |  | 0.24 | 39 | - |
| **53** | M/3 | JUVENILE INTESTINAL POLYP | - | INTESTINAL POLYPS | - | - | COLON |  | 0.03 | 21 | - |
| **54** | F/11 | VIRAL INFECTION | - | NORMAL COL | - | - | - |  | 4.86 | 110 | 22 |
| **55** | F/4 | FUNCTIONAL ABDOMINAL PAIN | - | NORMAL COL | - | - | - |  | 0.03 | 19 | 6.5 |
| **56** | F/10 | FUNCTIONAL ABDOMINAL PAIN | - | NORMAL COL | - | - | - |  | 0.09 | 8 | - |

M: Male; F: Female; CD: Crohn’s disease; UC: Ulcerative colitis; AZA: azathioprine; ADA: Adalimumab; Thd: Thalidomide; 5-ASA: mesalamine; IFX: Infliximab;

CS: corticosteroids; CIPX: ciprofloxacin; SSZ: sulfasalazine; MZN: metronidazole; COL: Colonoscopy.

Age (year); CRP (mg/dl), ESR (mm/h); Fecal Calprotectin (mg/Kg)

**Supplementary Table 2. Clinical characteristics of patients suffering from Inflammatory Bowel Disease**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| PATIENT | SEX/AGE | DIAGNOSIS | DISEASE ACTIVITY | BMI | BMI PERCENTILE |
| **01** | M/17 | UC | ACTIVE | 21 | 36 |
| **02** | M/7 | UC | ACTIVE | 15.4 | 50 |
| **03** | M/17 | UC | ACTIVE | 24 | 80 |
| **04** | F/14 | UC | ACTIVE | 17.5 | 23 |
| **05** | M/17 | UC | ACTIVE | 23 | 70 |
| **06** | F/15 | UC | ACTIVE | 23.8 | 80 |
| **07** | F/11 | UC | ACTIVE | 14 | 5 |
| **08** | F/14 | UC | ACTIVE | 16.5 | 10 |
| **09** | M/17 | UC | ACTIVE | 19.9 | 40 |
| **10** | F/12 | UC | ACTIVE | 16.6 | 25 |
| **11** | M/12 | UC | ACTIVE | 15.6 | 12 |
| **12** | M/18 | UC | ACTIVE | 22.8 | 60 |
| **13** | M/18 | UC | ACTIVE | 24.4 | 75 |
| **14** | F/18 | UC | ACTIVE | 23.5 | 75 |
| **15** | M/13 | UC | ACTIVE | 16.6 | 15 |
| **16** | F/10 | UC | REMISSION | 12 | 1 |
| **17** | F/14 | UC | REMISSION | 20.3 | 62 |
| **18** | F/18 | UC | REMISSION | 24 | 75 |
| **19** | M/17 | UC | REMISSION | 25.8 | 92 |
| **20** | M/10 | UC | REMISSION | 17.5 | 65 |
| **21** | M/20 | UC | REMISSION | 24 | 60 |
| **22** | F/24 | UC | REMISSION | 21.5 | 50 |
|  |  |  |  |  |  |
| PATIENT | SEX/AGE | DIAGNOSIS | DISEASE ACTIVITY | BMI | BMI PERCENTILE |
| **23** | M/13 | CD | ACTIVE | 16 | 10 |
| **24** | F/11 | CD | ACTIVE | 16 | 28 |
| **25** | F/10 | CD | ACTIVE | 20.6 | 90 |
| **26** | F/13 | CD | ACTIVE | 23.8 | 91 |
| **27** | M/11 | CD | ACTIVE | 15.7 | 23 |
| **28** | M/10 | CD | ACTIVE | 17.6 | 66 |
| **29** | M/15 | CD | ACTIVE | 19 | 35 |
| **30** | M/10 | CD | ACTIVE | 16 | 40 |
| **31** | M/15 | CD | ACTIVE | 20 | 50 |
| **32** | M/16 | CD | ACTIVE | 19 | 25 |
| **33** | F/11 | CD | ACTIVE | 15 | 10 |
| **34** | F/16 | CD | ACTIVE | 20.7 | 50 |
| **35** | M/11 | CD | ACTIVE | 17.8 | 60 |
| **36** | F/10 | CD | ACTIVE | 15.5 | 30 |
| **37** | F/11 | CD | ACTIVE | 15.8 | 22 |
| **38** | F/11 | CD | ACTIVE | 16 | 25 |
| **39** | F/10 | CD | ACTIVE | 17.1 | 50 |
| **40** | F/10 | CD | ACTIVE | 14.9 | 22 |
| **41** | M/6 | CD | ACTIVE | 14.5 | 20 |
| **42** | M/7 | CD | ACTIVE | 14.1 | 10 |
| **43** | M/7 | CD | ACTIVE | 13.1 | 2 |
| **44** | M/12 | CD | ACTIVE | 24.8 | 96 |
| **45** | M/16 | CD | ACTIVE | 20.2 | 50 |
| **46** | M/18 | CD | REMISSION | 20.5 | 45 |
| **47** | M/11 | CD | REMISSION | 16.9 | 45 |
| **48** | M/7 | CD | REMISSION | 14.2 | 15 |
| PATIENT | SEX/AGE | DIAGNOSIS | DISEASE ACTIVITY | BMI | BMI PERCENTILE |
| **49** | F/16 | APHTHOSIS, AUTOIMMUNE GASTRITIS | NORMAL COL | 21.5 | 60 |
| **50** | M/15 | PREVIOUS INTESTINAL POLYP | NORMAL COL | 16.2 | 30 |
| **51** | M/10 | JUVENILE INTESTINAL POLYPOSIS | INTESTINAL POLYPS | 16.4 | 25 |
| **52** | M/11 | FUNCTIONAL ABDOMINAL PAIN | NORMAL COL | 16.4 | 33 |
| **53** | M/3 | JUVENILE INTESTINAL POLYP | INTESTINAL POLYPS | 16 | 50 |
| **54** | F/11 | VIRAL INFECTION | NORMAL COL | 16 | 25 |
| **55** | F/4 | FUNCTIONAL ABDOMINAL PAIN | NORMAL COL | 14.2 | 15 |
| **56** | F/10 | FUNCTIONAL ABDOMINAL PAIN | NORMAL COL | 18 | 45 |

M: Male; F: Female; CD: Crohn’s disease; UC: Ulcerative colitis; COL: Colonoscopy.

Age (year); BMI: Body Mass Index; BMI Percentile: underweight (<5); healthy (5-85);

overweight (85-95); obese (>95).

**Supplementary Table 3. Cytokine values in the absence of antibiotics, with or without the addition of MDP**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|   |   | CD |    | UC  |    | HC  |
| **-MDP** | **+MDP** | **-MDP** | **+MDP** | **-MDP** | **+MDP** |
| G-CSF |  | 2192 (3745±3282) | 2580 (6738±10109) |  | 4536 (7301±7694) | 7525 (9804±8562) |  | 436 (645.8±572.4) | 385.5 (364.2±220.4) |
| GM-CSF |  | 748.1 (883.4±712.1) | 775 (1217±1247) |  | 1247 (1549±867.3) | 1717 (1715±759.7) |  | 504.4 (495.8±215) | 393.2 (505.9±206.1) |
| IFNγ |  | 402.7 (510.5±434.3) | 377.6 (526.8±427.4) |  | 414.6 (620.6±525.3) | 1121 (909.7±479.1) |  | 115.4 (144.6±139.2) | 72.89 (122.8±107.5) |
| IL1β |  | 408.8 (1275±1535) | 215.8 (843.6±1238) |  | 502.8 (1627±2835) | 498.3 (1741±2217) |  | 97.27 (377.9±536.2) | 126.8 (235.5±335.5) |
| IL2 |  | 23.69 (33.92±32.62) | 22.82 (35.17±37.17) |  | 36.64 (65.21±66.51) | 86.3 (85.65±60.97) |  | 6.405 (6.74±4.601) | 1.875 (2.508±2.258) |
| IL4 |  | 7.08 (10.25±12.55) | 7.76 (10.34±9.249) |  | 11.41 (14.07±11.09) | 15.42 (18.61±13.08) |  | 1.93 (3.267±3.687) | 1.02 (2.917±2.898) |
| IL6 |  | 5512 (8428±8573) | 5774 (11007±14697 |  | 10788 (20660±28559) | 17345 (28516±31326) |  | 2015 (1775±1576) | 793.2 (1096±848.6) |
| IL8 |  | 34163 (35988±26282) | 19170 (44152±48640) |  | 21678 (37553±33836) | 73990 (81093±63573) |  | 3408 (8853±14711) | 5080 (6931±6421) |
| IL10 |  | 32.44 (62.44±75) | 46.69 (69.82±73.54) |  | 65.98 (68.16±48.2) | 57.28 (63.06±29.97) |  | 10.57 (12.81±8.041) | 12.36 (14.09±6.162) |
| IL12 |  | 92.17 (155.8±140.5) | 94.54 (141.3±143.8) |  | 78.12 (161.9±169.6) | 97.51 (221.1±223.7) |  | 11.98 (21.91±24.42) | 20.35 (23.26±23.46) |
| IL17 |  | 128 (134.3±82.9) | 104.8 (131.3±96.54) |  | 118.2 (175.4±159.7) | 234.1 (204.8±157) |  | 30.21 (59.22±60.62) | 47 (53.88±53.89) |
| MCP1 |  | 268.9 (645.9±1333) | 268.1 (308.8±195.3) |  | 370.7 (435.6±323) | 408.5 (572.4±498.1) |  | 412.4 (370.9±238.5) | 273.7 (439.1±479.9) |
| MIP1β |  | 1121 (2564±3640) | 2864 (2519±2220) |  | 797.1 (3139±3655) | 2081 (3531±3241) |  | 59.58 (131.4±194.5) | 80.83 (155.1±165.6) |
| TNFα |  | 90.83 (135.7±163.6) | 72.83 (91.8±82.98) |  | 158 (184±161.7) | 170.9 (182.2±109.5) |  | 15.03 (21.79±21.48) | 27.27 (21.66±14.89) |

Values (pg/ml) are expressed as Median (Mean±SD). CD: Crohn’s disease; UC: Ulcerative Colitis; HC: Healthy Controls;

MDP: Muramyl dipeptide

**Supplementary Table 4. Cytokine values in the presence of antibiotics, with or without the addition of MDP**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|   |    | CD |    | UC |    | HC |
| **-MDP** | **+MDP** | **-MDP** | **+MDP** | **-MDP** | **+MDP** |
| G-CSF |  | 3377 (6330±7510) | 2407 (3133±2371) |  | 5954 (10270±12725) | 8187 (9128±8209) |  | 675.3 (642.9±354.8) | 1224 (1199±1080 |
| GM-CSF |  | 927.1 (921.3±489.6) | 853.1 (1186±1804) |  | 1515 (1788±853.7) | 1457 (1615±933.3) |  | 436.7 (519±202.1) | 526.1 (550.5±258.6) |
| IFNγ |  | 660.3 (599.3±399.4) | 365.6 (434.6±294) |  | 977.2 (835.3±521.5) | 452.8 (645.9±509.6) |  | 123 (108.9±56.97) | 161.3 (162.1±85) |
| IL1β |  | 199.2 (373.2±567.8) | 363.2 (759.3±947.7) |  | 308.5 (1475±2023) | 478.1 (1606±2436) |  | 89.52 (136±154.5) | 53.66 (87.21±112) |
| IL2 |  | 41.21 (40.66±27.39) | 21.05 (27.43±27.43) |  | 39.33 (75.02±74.49) | 59.79 (76.39±66.97) |  | 1.18 (4.608±6.054) | 9.56 (10.25±4.785) |
| IL4 |  | 10.02 (11.25±9.659) | 7.64 (9.761±10.29) |  | 12.51 (14.41±11.08) | 11.1 (13.55±10.45) |  | 1.13 (3.491±4.66) | 1.13 (3.818±6.83) |
| IL6 |  | 5550 (9737±11609) | 5576 (9543±11874) |  | 13072 (24974±26737) | 14588 (22221±22516) |  | 1704 (2144±1789) | 2610 (2934±3254) |
| IL8 |  | 42294 (36328±27994) | 46697 (57161±67755) |  | 98115 (95571±48577) | 23748 (63080±95386) |  | 8075 (8773±6897) | 7501 (19984±28397) |
| IL10 |  | 54.94 (68.84±45.13) | 28.17 (85.95±209.8) |  | 62.33 (61.6±48.43) | 58.75 (75.9±60.62) |  | 14.55 (17.88±18.22) | 25.55 (27.85±18.57) |
| IL12 |  | 102.4 (129.1±111.6) | 80.86 (133.3±123) |  | 112.5 (179.3±175.2) | 119.2 (146.5±123.5) |  | 14.94 (26.19±25.67 | 18.92 (28.33±29.12) |
| IL17 |  | 111.7 (138.2±86.12) | 103.7 (140.6±108.2) |  | 152.9 (174.5±146.2) | 134.6 (177.8±129.1) |  | 46.3 (66.64±54.21) | 44.29 (67.46±72.89) |
| MCP1 |  | 314.8 (394.6±265.4) | 254 (848.3±2575) |  | 337.3 (360±264.3) | 235.1 (678.1±1112) |  | 560.2 (519±333.5) | 608.9 (1228±1730) |
| MIP1β |  | 1844 (1965±1521) | 2723 (3683±3561) |  | 1502 (3167±3530) | 3570 (4325±3739) |  | 195.5 (218.4±175.7) | 273.2 (408.3±429.1) |
| TNFα |  | 110.1 (92.81±57.47) | 64.84 (98.78±110.3) |  | 173.7 (183.3±135.9) | 165.9 (188.6±161.3) |  | 19.89 (27.02±26.79) | 16.73 (25.36±31.78) |

Values (pg/ml) are expressed as Median (Mean±SD). CD: Crohn’s disease; UC: Ulcerative Colitis; HC: Healthy Controls;

MDP: Muramyl dipeptide