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Defects in mucosal immunity and nasopharyngeal dysbiosis in HSC transplanted SCID patients with IL2RG/JAK3 deficiency

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Abstract:

Both innate and adaptive lymphocytes have critical roles in mucosal defense that contain commensal microbial communities and protect against pathogen invasion. Here we characterize mucosal immunity in human severe combined immunodeficiency (SCID) patients receiving hematopoietic stem cell transplantation (HSCT) with or without myeloablation. We confirmed that pre-transplant conditioning impacted on innate (NK, ILC) and adaptive (B and T cells) lymphocyte reconstitution in these SCID patients and now demonstrate that this further extends to generation of Th2 and Tc2 cells. Using an integrated approach to assess nasopharyngeal immunity, we identify a local mucosal defect in type 2 cytokines, mucus production and a selective local IgA deficiency in HSCT-treated SCID patients with genetic defects in IL2RG/GC or JAK3. These patients have a reduction in IgA-coated nasopharyngeal bacteria and exhibit microbial dysbiosis with increased pathobiont carriage. Interestingly, IVIG replacement therapy can partially normalize nasopharyngeal Ig profiles and restore microbial communities in GC/JAK3 patients. Together, our results suggest a potential non-redundant role for type 2 immunity and/or of local IgA antibody production in the maintenance of nasopharyngeal microbial homeostasis and mucosal barrier function.

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Agreement to Share Publication-Related Data and Data Sharing Statement: Data Sharing Statement The raw 16S RNA sequence data for each patient were deposited in the NCBI Sequence Read Archive (SRA) under accession number PRJNA772582. All other datasets generated during the current study are available upon reasonable request via email to the corresponding author.

Clinical trial registration information (if any):

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- 35 type 2 responses and have mucosal IgA deficiency with dysbiosis

36 Abstract

37

Both innate and adaptive lymphocytes have critical roles in mucosal defense that 38 39 contain commensal microbial communities and protect against pathogen invasion. 40 characterize mucosal immunity in human Here we severe combined 41 immunodeficiency (SCID) patients receiving hematopoietic stem cell transplantation 42 (HSCT) with or without myeloablation. We confirmed that pre-transplant conditioning 43 impacted on innate (NK, ILC) and adaptive (B and T cells) lymphocyte reconstitution 44 in these SCID patients and now demonstrate that this further extends to generation of Th2 and Tc2 cells. Using an integrated approach to assess nasopharyngeal 45 46 immunity, we identify a local mucosal defect in type 2 cytokines, mucus production and a selective local IgA deficiency in HSCT-treated SCID patients with genetic 47 48 defects in IL2RG/GC or JAK3. These patients have a reduction in IgA-coated 49 nasopharyngeal bacteria and exhibit microbial dysbiosis with increased pathobiont 50 carriage. Interestinaly. IVIG replacement therapy can partially normalize nasopharyngeal Ig profiles and restore microbial communities in GC/JAK3 patients. 51 52 Together, our results suggest a potential non-redundant role for type 2 immunity 53 and/or of local IgA antibody production in the maintenance of nasopharyngeal 54 microbial homeostasis and mucosal barrier function.

55

56 Introduction

57 Hematopoietic stem cell transplantation (HSCT) for severe combined immune 58 deficiency (SCID) represents a life-saving therapy for this heterogeneous group of 59 hematopoietic disorders ^{1,2}. HSCT generates a variable degree of hematolymphoid reconstitution that depends on the pre-transplant conditioning regime (eq. 60 myeloablation) as well as the genetic defect being treated ^{2,3}. For example, T-B-61 62 natural killer cells (NK)+ SCID resulting from defects in the antigen receptor 63 recombination pathway harbor immature lymphoid precursors in the thymus and bone marrow. These patients may receive either myeloablation that can enhance 64 myeloid and lymphoid reconstitution following HSCT or reduced intensity conditioning 65 66 that may eliminate competitive but abnormal thymocyte precursor cells or NK cells. In contrast, patients with T-B+NK- SCID (caused by mutations in the common y chain 67 (yc) gene IL2RG or the Janus kinase JAK3) lack lymphoid precursors and are 68 generally not cytoreduced prior to HSCT³. As a result, lymphoid lineages engraft 69 rapidly but myeloid reconstitution is less robust. 70

Innate lymphoid cells (ILC) are tissue-resident lymphocytes, enriched at 71 mucosal barriers with roles in immune defense and tissue remodeling ^{4,5}. Diverse ILC 72 73 subsets (ILC1/2/3) produce a restricted range of cytokines that target hematopoietic 74 as well as non-hematopoietic (stromal, epithelial, endothelial...) cells. ⁶. Previous 75 studies have documented the developmental and functional parallels between ILCs 76 and T 'helper' (Th) cells ⁷. This homology suggests a potential functional redundancy 77 during immune responses but may also provide a means to synergistically promote 78 immune defense.

79 Recently it was shown that pre-transplant conditioning allows for better post-80 HSCT immune reconstitution with higher frequencies of donor NK and ILC subsets in 81 myeloablated T-B-NK+ SCID and cancer patients compared with non-conditioned T-B+NK- SCID recipients ³. The differential innate lymphoid cell engraftment in these 82 83 patients results from a combination of both absence of conditioning and inherent 84 genetic defects, providing a unique setting (ILC/NK+ versus ILC/NK- phenotypes) to 85 assess biological roles for ILC/NK cells in human immunity. Interestingly, both HSCT groups showed robust donor T cell engraftment with restoration of cellular immunity 86 87 and recovery of immune competence. In a long-term follow-up of these two HSCT-88 treated patient groups, no obvious differences in clinical course or disease 89 susceptibility were noted. As both HSCT groups showed similar clinical recoveries

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most aspects of normal human immunity ^{3,8} that more recently has b
 development and function of lymphoid tissues ⁹.

- 93 Diverse ILC subsets are enriched at mucosal surfaces, and in conjunction with 94 adaptive T and B cell responses, promote barrier defense and tissue regeneration 95 after infection and inflammation and regulate microbial communities that have a symbiotic relationship with the host ^{10,11}. The reciprocal interactions and coordinated 96 regulation of ILCs versus T helper cells for immune defense remains unclear. A 97 98 better characterization of mucosal immunity in ILC/NK+ versus ILC/NK- HSCT 99 patients may shed light on the specific and/or redundant roles of innate lymphocytes 100 at barrier surfaces for protection from disease.
- Here we further analyzed a large cohort of HSCT-treated SCID patients to assess impact of hematopoietic reconstitution on mucosal barrier function. We document a selective deficiency in type 2 immunity, strong decreases in nasopharyngeal IgA and nasal microbial dysbiosis in IL2RG/JAK3-deficient patients receiving non-conditioned HSCT.

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106 Methods

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108 Patient and control cohorts

109 Healthy donors were recruited originally as part of the Milieu Intérieur cohort 110 (https://www.milieuinterieur.fr/en/; Supplementary Materials and Methods). HSCT-111 treated SCID patients were followed at Hôpital Necker-Enfants Malades (French 112 National Reference Center for Primary Immunodeficiencies). Pathogenic mutations 113 were identified in all cases (Supplementary Table 1). Written informed consent was 114 obtained from all patients and/or parents. Nasopharyngeal swabs were obtained concurrently with blood samples during routine visits (no evidence of ongoing 115 116 infection, autoimmunity or allergy; no antibiotic use) and were processed as 117 described ^{12,13}.

118

119 Cell isolation and FACS analysis

Human peripheral blood mononuclear cells (PBMC) were isolated using density
gradient centrifugation. For FACS analysis, cells were first stained with Flexible
Viability Dye eFluor 506 (eBioscience) following by surface antibodies staining on ice.
Fc receptors were blocked using IgG from human serum (MilliporeSigma). Samples
were acquired with an LSRFortessa (BD) and analyzed by FlowJ10.7.1 (TreeStar).
Bacterial species-specific antibody against microbiota were assessed as described
previous ¹⁵.

127

128 Analysis of nasopharyngeal proteins

Total IgA, IgM, IgG1, IgG2, IgG3 and IgG4 were determined using the Bio-Plex Pro Human Isotyping Assay Panel (Biorad, Hercule, CA, USA). Data were acquired on a Bio-Plex 200 System (Bio-Rad) and analyzed with Bio-Plex Manager v5 (Bio-Rad). IgA1 and IgA2 subclass were measured by Simoa (Quanterix). Total IgD were determined using a ELISA kit (MBS564048, Mybiosource, San Diego). Total IgE were determined using a ELISA kit (88-50610, Invitrogen, Massachusetts). Data were collected with the Multiskan Spectrum (Thermo Fisher Scientific).

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Cytokines were quantified by Simoa® Cytokine 3-Plex B, Discovery or Advantage
Kits (Quanterix) except IFN-γ and IL-17F that used Quanterix Homebrew assays.
Nasopharyngeal mucin levels were analyzed using a MUC5AC ELISA Kit (NBP2-

140 76703, Novus Biologicals, diluted 1/50). Eosinophil cationic protein (ECP) were
141 determined for swabs medium using an ELISA kit (MBS2602477, Mybiosource, San
142 Diego, USA, diluted 1/2). Total protein content of the supernatants by the Bradford
143 method ¹⁴.

144

145 **16S rRNA sequencing and analysis**

146 16S rRNA sequencing and sequence processing and statistical analysis was
147 described previous ^{12,13}. A total of 2.974.329 reads (90.131 reads on average per
148 sample) was obtained. Raw sequence data have been deposited in the NCBI
149 Sequence Read Archive (SRA) under accession number (PRJNA772582).

150

151 Bacterial quantification by quantitative RT-PCR assays

- Bacterial qPCR used universal 16S rRNA primers to measure total bacteria (16S_F:
 5'-ATTACCGCGGCTGCTGG-3' and 16S and 16S_R: 5'-ATTACCGCGGCTGCTGG3') and *Streptococcus pneumoniae* (*LytA* gene, F: 5'- ACGCAATCTAGCAGATGAAGC-
- 155 3' and R: 5'- TGTTTGGTTGGTTATTCGTGC -3').
- 156

157 Statistical analysis

Statistics were performed using GraphPad Prism (San Diego, USA). P values were determined by a Kruskal-Wallis test, followed by Dunn's post-test for multiple group comparisons with median reported; *P < 0.05; **P < 0.01; ***P < 0.001. Correlations between the different assays were calculated using Spearman test. Heatmaps were generated with Qlucore OMICS explore Version 3.5(26). Correlation matrices were built using the Spearman correlation and computed using R (v4.0.3).

165 **Results**

166 This study analyzed a cohort of 21 SCID patients that have been successfully 167 treated with HSCT at Necker Hospital since 1977. SCID patients with X-linked as well 168 as autosomal etiologies were included, some patients required lg replacement 169 therapy (IgRT) by subcutaneous injection of IgG for specific clinical conditions (including recurrent respiratory tract infections ¹⁶⁻¹⁸; Supplemental Table 1). Several 170 patients have been previously reported ^{3,9}. The group 'GC/JAK3' were T-B+NK- SCID 171 172 (IL2RG, JAK3) patients that received non-myeloablative HSCT, and the group 'SCID other' were T-B-NK+ or T-B+NK+ SCID (RAG1/2, ARTEMIS, MHC Class II, IL7RA) 173 patients the majority of which received pre-transplant cytoreduction (Supplemental 174 175 Table 1). All HSCT-treated SCID patients showed successful donor hematopoietic 176 reconstitution and recovered T cell immunity. Once clinically stable, patients were 177 discharged and followed up periodically in our outpatient clinic. The follow up period 178 varied from 18 to 42 years.

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180 HSCT-treated GC/JAK3 patients have reduced circulating type 2 lymphocytes

181 While HSCT-treated SCID patients have stable T cell reconstitution with balanced CD4/CD8 ratios, naïve T cells and TRECs ^{1,3,19}, an in-depth analysis of 182 183 their differentiated T cell subsets has not been previously performed. ILC and NK cell 184 reconstitution in SCID patients receiving myeloablative conditioning for HSCT has only been reported in 2 RAG-deficient patients ³. As reciprocal interactions between 185 ILCs and T cells have been documented ²⁰⁻²³, previously reported lack of NK and 186 187 ILCs in the non-conditioned SCID recipients might be associated with perturbations 188 in their T cell compartment. We paid particular attention to differentiated T cell 189 subsets that can be identified by expression of specific chemokine receptors (see Methods; Supplemental Figure 1A, B for FACS gating) ^{24,25}. Non-conditioned 190 191 GC/JAK3 patients receiving IgRT post-HSCT (Supplemental Table 1) were analyzed 192 separately.

193 Circulating ILCs include CD56+ natural killer (NK) cells (CD56+++CD16- and 194 CD56+CD16+ cells) and CD127+ ILC2 (CRTH2+) and ILCP (ILC precursors, 195 CRTH2-CD117+ CD45RA+NKp44-) ²⁶. Using unsupervised clustering analysis, we 196 observed a significant reduction in frequencies of NK cells and ILC2 but not ILCP in 197 GC/JAK3 and GC/JAK3+IgRT patients compared with HC and SCID-other patients 198 (Figures 1A, B) confirming previous reports in HSCT-treated IL2RG/JAK3-deficient and RAG-deficient patients ³ and further extending this observation to other SCID

We next characterized naïve and differentiated CD4+ T cell subsets in this 201 202 SCID cohort. Unsupervised clustering analysis allowed us to identify T cell subsets 203 including naïve (CD45RA+), Treg (CD127loCD25+), Tfh (Treg-CD45RA-CXCR5+), 204 Th1 (Treg-CD45RA-CXCR5-CCR6-CXCR3+CCR4-), Th2 (Treg-CD45RA-CXCR5-205 CCR6-CXCR3-CCR4+CRTH2+/-) and Th17 (Treg-CD45RA-CXCR5-CCR6+). We 206 observed a significant reduction in clusters of CCR4+CRTH2+/- cells corresponding to the Th2 subset, as well as a reduction in naïve T cells (CD45RA+) in GC/JAK3 207 208 and GC/JAK3+IgRT patients compared to HC and SCID-other patients (Figures 1C, 209 D). Other Th subsets (Th1, Th17, Tfh) were normally present in all HSCT SCID 210 patients (Figure 1D, Supplemental Figure 2A, B).

A similar analysis was performed on CD8+ T cells. The unsupervised clustering analysis showed a significant decrease in the CCR4+CRTH2+ cluster corresponding to Tc2 subset in CD8+ T cells in GC/JAK3 and GC/JAK3+IgRT patients (Figure 1E, F) whereas other CD8+ T cell subsets were similar in SCID-other patients as compared to HC (Supplemental Figure 3).

216 The observations of a relative decrease in naïve CD4+ T cells with a 217 compensatory increase in activated CD8⁺ T cells following HSCT for SCID confirm earlier work ²⁸, while our detailed assessment of differentiated T cell subsets allowed 218 219 us to identify a selective deficiency in blood CD4⁺ Th2 cells and CD8+ Tc2 cells in 220 HSCT-treated GC/JAK3 patients. In all SCID etiologies, differentiation of other T 221 helper subsets (Th1, Th17, Treg) appeared largely intact. These results suggest that 222 non-conditioned HSCT-treated GC/JAK3 patients manifest a generalized and 223 selective deficiency in innate and adaptive lymphocytes involved in Type-2 immunity. 224

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etiologies.

# 225 HSCT-treated GC/JAK3 patients have reduced nasopharyngeal type 2 cytokines

We next assessed mucosal immune responses in HSCT-treated SCID patients. Nasopharyngeal swabs were obtained concurrently with blood samples and were processed as described <sup>12,13</sup> to yield nucleic acids as well as a soluble fraction that harbored cytokines, antibodies, anti-microbial peptides and various metabolites. As differentiated Th cells and ILCs maintain mucosal homeostasis <sup>4,5</sup>, we used digital ELISA (Simoa) to quantitate cytokines associated with Th1/ILC1, Th2/ILC2 and Th17/ILC3 responses in these nasopharyngeal samples.

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233 We found no significant differences in the levels of type-1 cytokines (IFN- $\gamma$ , TNF- $\alpha$ ) in normal controls compared to HSCT-treated GC/JAK3 or SCID-other 234 patients (Figure 2A). In contrast, levels of type-2 cytokines IL-5 and IL-13 were 235 236 clearly decreased in non-conditioned GC/JAK3 patients whereas IL-4 levels were 237 within healthy ranges (Figure 2B). While the deficiency in nasopharyngeal IL-13 238 persisted in GC/JAK3 patients receiving IVIG, IL-4 and IL-5 levels were somewhat 239 higher in these patients compared to non-conditioned GC/JAK3 patients that did not 240 receive IgRT (Figure 2B). Concerning inflammatory type-3 cytokines (IL-17A, IL-17F, IL-22), nasopharyngeal mucosal levels were not different from controls except in 241 242 GC/JAK3 patients being treated with IgRT (Figure 2C). Whether these increases in 243 nasopharyngeal cytokines result from IgRT treatment or are related to other 244 mechanisms that underlie the need to treat (eg. infections) with IgRT is unclear.

245 Type-2 cytokine secretion is regulated by stromal-derived factors and 246 promotes activation of hematopoietic (eosinophils, mast cells) as well as nonhematopoietic (goblet cells) targets.<sup>29,30</sup> We found that IL-33 (a major inducer of 247 248 recruitment, activation and IL-5 and IL-13 production by type-2 lymphocytes <sup>31-33</sup>) was not reduced in nasopharyngeal samples (Figure 2D), whereas eosinophil 249 cationic protein (ECP, marker for tissue eosinophilia <sup>34,35</sup>) was reduced in GC/JAK3 250 251 patients consistent with reduced IL-5 levels (Figure 2B, D). Nasopharyngeal IL-6 252 levels were not significantly elevated in any SCID patient although tended to be 253 higher in GC/JAK3+IgRT patients (Figure 2D). When all data was clustered, the 254 heatmap clearly distinguished the defective type-2 cytokine production (in particular, 255 IL-5 and IL-13) in the context of non-conditioned HSCT for GC/JAK3 and irrespective 256 of IgRT (Figure 2E).

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# 258 Systemic and mucosal Ig subtypes in HSCT-treated SCID patients

259 Previous studies have analyzed the impact of pre-HCST conditioning on systemic antibody levels following hematopoietic reconstitution for SCID<sup>18,36</sup>. In 260 261 particular, busulfan administration is correlated with higher donor chimerism, especially in the B cell compartment <sup>36</sup>. We found that non-conditioned GC/JAK3 262 263 patients demonstrated somewhat elevated total serum IgM, but all six patients 264 studied had normal total serum IgG levels (Figure 3A), confirming that GC/JAK3-265 deficient B cells can produce switched IgGs in the presence of normal T cells <sup>18</sup>. In 266 contrast, serum IgA was undetectable (selective IgA deficiency, SIgAD) in half of HSCT-treated GC/JAK3 patients, mirroring previous reports <sup>37,38</sup>. SCID-other patients harbored normal Ig subtype distributions and levels (Figure 3A). Interestingly, need for IgRT in GC/JAK3 patients did not clearly correlate with any selective serum Ig deficiency (Figure 3A) but was dictated by the clinical context (history of recurrent respiratory tract infections).

272 We next assessed mucosal lgs in HSCT-treated SCID patients by measuring 273 Ig isotypes and subclasses in paired nasopharyngeal samples. We found that total 274 nasopharyngeal IgM and IgG was elevated in GC/JAK3 patients compared to 275 controls but less so compared to SCID-other patients (Figure 3B) which appeared 276 related to higher levels of IgG3 (Supplemental Figure 4A). In contrast, all GC/JAK3 277 patients showed a strong reduction in total nasopharyngeal IgA that concomitantly 278 involved both IgA1 and IgA2 (Figure 3C, Supplemental Figure 4B) and a significant 279 reduction in total nasopharyngeal IgE (Figure 3D). Interestingly, total nasopharyngeal 280 IgD levels were significantly increased in GC/JAK3 patients. Lastly, GC/JAK3+IgRT 281 patients 'normalized' nasopharyngeal Ig distributions with increased IgA and IgE 282 (Figures 3C, D), although the mechanistic basis remains unclear. Together, our 283 results demonstrate distinct profiles of systemic and local mucosal IgA responses in 284 HSCT-treated SCID patients.

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# 286 IgG and IgD may provide mucosal protection in the context of IgA deficiency

287 We next analyzed the binding of different lg isotypes to nasopharyngeal microbiota using a flow cytometer-based assay <sup>12</sup>. We found a significant decrease in 288 289 the percentage of IgA-coated nasopharyngeal microbes as well as the density of IgA 290 coating (IgA MFI, not shown) in GC/JAK3 patients compared to SCID-other patients 291 and healthy controls (Figure 4A, B). Interestingly, GC/JAK3+IgRT patients showed a 292 partial but significant increase in IgA-coated nasopharyngeal microbes (Figure 4B) 293 consistent with the increase in nasopharyngeal IgA (Figure 3C). Whether the 294 increase in IgA activity following IgRT results from an indirect effect following stimulation of type 2 cytokine production <sup>39-41</sup> remains unclear. 295

296 Compensatory IgG responses to gut commensal bacterial communities may 297 operate in the absence of IgA-specific responses <sup>42</sup>. As other Ig isotypes and 298 subclasses were normally present (IgD) or elevated (IgM, IgG) in GC/JAK3 patients 299 (Figure 3B), we quantitated the fraction (%) and intensity (MFI) of nasopharyngeal 300 microbes that were coated with IgG, IgA or IgD using a recently reported multiplexing

301 technique <sup>12</sup>. The majority of nasopharyngeal microbes in healthy individuals are 302 coated with IgA in combination with IgD and to a lesser extent with IgG (Figure 4C) 303 <sup>12</sup>. SCID-other patients showed a similar pattern of nasopharyngeal microbe coating, 304 while nasopharyngeal microbes in GC/JAK3 patients were more abundantly coated 305 with IgG and IgD alone (Figure 4C). Finally, we observed an increase in IgA-/IgG-306 double coated nasopharyngeal microbes in GC/JAK3 patients receiving IgRT (Figure 307 4C), consistent with the increased nasopharyngeal IgA in these individuals. These results are consistent with the notion that IgG <sup>42</sup> and IgD <sup>43-45</sup> may provide a layer of 308 309 mucosal protection during IgA deficiency.

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# 1 HSCT-treated GC/JAK3 patients have nasopharyngeal microbiota dysbiosis

312 Secretory IgA (sIgA) produced locally at mucosal sites plays an essential role in host defense <sup>12</sup> and shapes commensal microbiota composition and activity in 313 each individual <sup>46</sup>. To characterize local nasopharyngeal microbial communities, we 314 315 performed 16S ribosomal RNA (rRNA) gene sequencing from nasopharyngeal 316 samples, calculated bacterial beta diversity and subjected sequenced OTU to 317 Principal Coordinates Analysis (PCoA). We found that nasopharyngeal samples 318 derived from HSCT-treated GC/JAK3 patients without IVIG replacement therapy 319 clustered distinctively from the healthy controls and SCID-other patient samples 320 (Figure 5A) and had elevated beta diversity based on Bray-Curtis, Euclidian and 321 Jaccard distance matrices (Figure 5B). Accordingly, Shannon and Simpson diversity 322 indices were reduced in these GC/JAK3 patients (Figure 5C). We further applied a 323 non-metric multidimensional scaling (NMDS) using Bray-Curtis distances and found a 324 similar distinctive clustering of GC/JAK3 patients not treated with IVIG 325 (Supplementary Figure 5A). In contrast, SCID-other patients with more complete 326 hematopoietic reconstitution showed nasopharyngeal microbial communities that 327 were more similar to healthy controls (Figures 5A-C, Supplementary Figure 5B). 328 Total bacterial load was also increased in GC/JAK3 patients not treated with IgRT 329 compared to healthy controls and other SCID patients (Supplementary Figure 5C).

Nasopharyngeal microbial communities in these different SCID patients were further characterized by annotation of the 16S rRNA datasets. It has been reported that the nasopharyngeal microbiota of healthy individuals is enriched in commensal bacteria including *Corynebacterium* and *Dolosigranulum* genera <sup>47,48</sup>. We found a general reduction in *Dolosigranulum* and *Comamonas* genera in HSCT SCID

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335 patients compared to healthy controls and a selective reduction in Corynebacterium, 336 Cutibacterium and Staphylococcus genera in GC/JAK3 patients compared to other 337 SCID patients and healthy controls (Figure 5D, E). These 'cornerstone' bacterial 338 communities (particularly Corynebacterium and Dolosigranulum genera) can reduce 339 carriage of several pathobionts (Streptococcus pneumoniae, Haemophilus influenzae 340 and Moraxella catarrhalis) that may be present in normal healthy individuals.<sup>49,50</sup> 341 Relative abundance of Streptococcus, Haemophilus and Moraxella were not 342 significantly increased in HSCT SCID-other patients compared to healthy controls, 343 although a higher abundance of Streptococcus and Haemophilus genera were 344 detected in GC/JAK3 patients that were not treated with IgRT (Figure 5D, F). This 345 was in part due to the increased abundance of S. pneumoniae (Figure 5G; 346 Supplementary Figure 5D). Finally, a significant negative correlation of S. 347 pneumoniae abundance with Dolosigranulum genus abundance could be detected in 348 the nasopharynx (Figure 5H).

349 IgRT is an established treatment to combat infections in HSCT-treated SCID 350 patients for which *S. pneumoniae* is a leading cause of disease.<sup>51</sup> Nasopharyngeal 351 samples from GC/JAK3 patients receiving IgRT clustered closer to SCID-other 352 patients and healthy controls (Figure 5A). Moreover, IgRT in GC/JAK3 patients 353 'normalized' microbiota diversity (beta Shannon and Simpson diversity indices) with 354 higher representation of Corynebacterium and reduced levels of Streptococcus 355 (Figure 5B-D). Moreover, we could confirm a decrease in S. pneumoniae in 356 GC/JAK3+IgRT patients (Figure 5G). Intriguingly, IgA level under IgRT correlated 357 with microbial  $\alpha$ -diversity indicating the intricate interplay between immune selection 358 and maintenance of complex commensal communities (Supplementary Figure 5E). 359 Taken together, these data suggest that HSCT-treated GC/JAK3 patients without 360 IgRT can present persistent nasopharyngeal microbial dysbiosis with expansion of bacteria associated with increased morbidity and mortality risk <sup>51</sup>. As IgRT appears to 361 362 ameliorate the nasopharyngeal dysbiosis, one may consider potential use of IgRT in 363 all GC/JAK3 patients to pre-empt or correct this abnormality.

364

Role for type 2 immunity and nasal IgA in maintaining commensal microbiota
diversity and protection against 'pathobiont' carriage

367 Secretory IgA is implicated in human nasopharyngeal microbial homeostasis <sup>52</sup> 368 and may protect mucosal surfaces from pathogen invasion through agglutination <sup>53,54</sup>.

Still, other 'non-specific' barriers, including a dense mucus layer, restrict commensal 369 and pathobiont colonization at mucosal sites <sup>55</sup>. While total protein levels in 370 nasopharyngeal samples from SCID patients and healthy controls were not 371 372 significantly different (Supplemental Figure 3B), we found that GC/JAK3 patients 373 (treated or not with IgRT) had markedly reduced MUC5AC levels compared to SCID-374 Other and healthy controls (Figure 6A). MUC5AC levels were positively correlated 375 with  $\alpha$ -diversity, *Dolosigranulum* and *Corynebacterium* genera abundance and 376 strongly negatively correlated with S. pneumoniae abundance (Figures 6B, C). These 377 results suggest that nasopharyngeal mucus plays a role in homeostasis of local 378 commensal microorganisms and is selectively reduced following HSCT for GC/JAK3.

379 We next looked for possible correlations in the cytokine dataset that could 380 associate with reduced MUC5AC levels in these SCID patients. The reduction in 381 nasopharyngeal IgA and type 2 cytokine levels in GC/JAK3 patients (Figures 2, 3) 382 paralleled the observed reductions in MUC5AC (Figure 6D, E), suggesting a possible 383 link. Type 2 cytokines are known to regulate barrier immunity by promoting mucus production <sup>29,30</sup> but other factors stimulate epithelial cell renewal and differentiation, 384 including IL-22 <sup>56</sup>. IL-22 levels were correlated with MUC5AC levels (Supplemental 385 386 Figures 6C; see Supplemental Figures 6D for all 2-parameter correlations). Together, 387 these results identify critical soluble factors that coordinate local mucosal immune 388 defense in the nasopharynx via mucus production. Finally, a supervised analysis of 389 nasopharyngeal factors that were significantly different in GC/JAK3 patients reiterate 390 the key parameters that integrate the unique mucosal immune profile of these 391 individuals (Figure 6F), including reduced type 2 cytokines, reduced MUC5AC levels, 392 reduced IgA and microbial dysbiosis.

#### 394 **Discussion**

395 In this report, we assess local mucosal immunity in the human nasopharynx in 396 a well-characterized cohort of SCID patients treated with curative HSCT. Previous 397 studies on systemic immune reconstitution after BMT have highlighted differences in homeostasis of peripheral pools of innate and adaptive lymphocytes <sup>1,3,18,19</sup>. Pre-398 399 transplant conditioning regimes may allow for differential engraftment of donor 400 hematopoietic precursor cells and downstream myeloid and innate lymphocyte pools, the latter including natural killer (NK) and innate lymphoid cells (ILCs)<sup>3</sup>. The impact 401 402 of differences in 'innate reconstitution' on overall systemic immune responses 403 appeared limited given the similar clinical profiles of these HSCT-treated SCID 404 patients<sup>3</sup>. These studies raised questions concerning the specific versus redundant 405 functions of innate lymphocytes in human immunity.

406 As ILCs abundantly populate mucosal sites, we explored their potential 407 immune roles by comparing HSCT-treated SCID patients that show variable innate lymphocyte reconstitution<sup>3</sup>. We observed clear differences in mucosal immune 408 409 parameters in a subset of SCID patients with genetic defects in GC or JAK3 which 410 were not conditioned prior to HSCT. We documented a generalized reduction in mucosal type 2 immunity and IgA production and an inability to maintain 'healthy' 411 412 commensal bacterial communities consistent with a defect in nasopharyngeal 413 mucosal barrier function. In contrast, other SCID etiologies (with or without 414 myeloablative protocols prior to HSCT) showed normal type 2 cytokines and IgA 415 production without nasal dysbiosis. Thus the underlying SCID etiology is apparently a 416 major factor for the observed defects in mucosal immunity.

417 Local IgA production is a hallmark of mucosal immunity and is largely driven 418 by specific immune responses to resident micro-organisms <sup>57</sup>. Cytokines promote IgG and IgE production <sup>58</sup>. In contrast, the key soluble factors that regulate switch to 419 IgA are a matter of debate <sup>57,59</sup>. Previous studies have documented absence of 420 serum IqA in about half of HSCT-treated GC/JAK3 patients <sup>18,36-38</sup>. Here we show 421 422 that mucosal IgA is absent in all HSCT-GC/JAK3 patients examined, including those 423 with normal circulating IgA. Interestingly, nasopharyngeal type 2 cytokines were most 424 strongly perturbed in these patients, suggesting a causative link to mucosal IgA 425 production. Type 2 cytokines can promote the survival and differentiation of tissue resident memory B cells and IgA secreting plasma cells <sup>60-62,63,64</sup> thereby increasing 426 IgA production <sup>30,65-68</sup>. Along these lines, a recent report described a major role for 427

ILC2-derived IL-5 in promoting local mucosal IgA production in mice <sup>69</sup>. Whether locally generated IL-5 (by ILC2 or Th2 cells) regulates mucosal IgA production in

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humans remains unclear.

431 An alternative explanation for the observed IgA deficiencies may result from 432 defective Ig switch in residual GC/JAK3-deficient host B cells, the latter not being 433 fully replaced by donor HSCT after non-ablative conditioning <sup>36</sup>. The inability of GC/JAK3-deficient B cells to respond to any  $\gamma_c$ -dependent cytokine might also reduce 434 435 IgA switch mechanisms and result in selective IgA deficiency. Still, nasopharyngeal 436 IgG subclasses were not decreased in HSCT-treated GC/JAK3 patients, and serum 437 IgG levels were normal consistent with the ability of GC/JAK3-deficient host B cells to switch Ig isotypes <sup>18</sup>. However, these patients have an intrinsic B cell deficiency 438 (defective response to IL-2, IL-9 and in part to IL-4) that can affect antibody 439 production.  $\gamma_c$ -dependent signals in epithelial cells <sup>70</sup> may also play a role. These 440 observations suggest a contribution of impaired  $\gamma_c$ -dependent signaling pathways to 441 442 the defective mucosal IgA production in these patients.

Th2 differentiation is considered as a 'default' pathway which can be subverted to alternative Th fates by environmental signals <sup>71,72</sup>. Still, Th2 differentiation requires reinforcing signals through STAT6 (IL-4, IL-13) to upregulate GATA3 expression and seal Th2 cell fate <sup>73</sup>. The defect in generation of Th2 cells in HSCT-treated GC/JAK3 patients may result from the absence of these STAT6dependent signals, perhaps delivered by innate lymphocytes (NKT cells, ILC2). In addition, ILC2 can prime tissue Th2 responses via DC recruitment <sup>20-23</sup>.

450 The mucus layer that lines mucosal surfaces provides a physical barrier to 451 commensal micro-organisms as well as pathogens and segregates 'niches' harboring complex microbial biofilms <sup>74</sup>. Secreted mucins (MUC5AC) are produced by goblet 452 453 cells in the nasopharyngeal mucosa and tracheobronchial surface epithelium of the 454 lower respiratory tract that acts as a "scaffold" to present and organize secreted proteins such as sIgA, antimicrobial peptides (AMPs) and cytokines <sup>74</sup>. Type 2 455 cytokines (including IL-13) activate goblet cells to produce mucus <sup>29,30</sup>. Whether the 456 457 loss of MUC5AC in GC/JAK3 patients secondary to reduced IL-13 predisposes these 458 individuals to microbial dysbiosis will require further study.

459 Finally, nasopharyngeal IgA deficiency in HSCT-treated GC/JAK3 patients is 460 associated with local microbial dysbiosis that may have been present prior to HSCT. It is well established that mucosal IgA plays a major role in regulating bacterial communities in the gut <sup>46,75</sup> and the reduction in nasopharyngeal IgA observed in GC/JAK3 patients is correlated with loss of microbial diversity and frequently accompanied by increased pathobiont carriage. While other Ig subclasses are present (and even elevated) in the nasopharynx in these patients and are able to coat bacteria, IgA remains a non-redundant immune factor required for microbial mucosal mucosal

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#### 468 **Acknowledgments**

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## 478 **Contributions**

P.G. conducted experiments, analyzed the data and prepared the manuscript. J.M.D., T.E., V.B., C.P., A.L., A.C., B.C. performed experiments. C.L. processed samples. D.D. provided logistical support and access to samples from normal individuals via the Milieu Intérieur Consortium. B.N. and A.F. were responsible for patient care, collected samples, designed experiments and prepared the manuscript. J.P.D. designed experiments, analyzed the data, obtained funding, supervised research and prepared the manuscript.

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#### 487 **Competing interests**

488 The authors declare no competing interests.

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## 490 **Data Sharing Statement**

491 The raw 16S RNA sequence data were deposited in the NCBI Sequence Read
492 Archive (SRA) under accession number PRJNA772582. All other datasets generated

493 during the current study are available upon reasonable request via email to the494 corresponding author.

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708 Figure Legends

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710 FACS analysis of peripheral blood mononuclear cells (PBMCs) Figure 1. 711 reveal a defect in differentiation of type-2 lymphocytes in HSCT-treated 712 GC/JAK3 patients. A, Unsupervised Uniform Manifold Approximation and 713 Projection (UMAP)<sup>27</sup> of single live CD45+Lin-CD3-CD4-CD7+ cells was applied for 714 CD56, CD16, CD94, NKp46, CD94, CD127, CD161, CD25, CD117 and CRTH2 715 fluorescence parameters. **B**, Supervised analysis of circulating innate lymphoid cells 716 (ILCs) including two CD56+ subsets of natural killer (NK) cells and two CD127+ 717 subsets denoted as ILC2 (CRTh2+) and ILCP (ILC precursors, CRTh2-718 CD117+CD45RA+NKp44-) (manually gating strategy on Supplemental Figure 1A). C, 719 UMAP analysis on CD4+ T cells including CXCR3, CCR4, CRTH2, CCR6, CXCR5, 720 CD25, CD127 and CD45RA fluorescence parameters (Supplemental Figure 2A). D, 721 Supervised analysis of circulating T cell populations. The different subsets were 722 identified as follow: Naïve (CD45RA+), Th1 (Treg-CD45RA-CXCR5-CCR6-723 CXCR3+CCR4-), Th17 (Treg-CD45RA-CXCR5-CCR6+), Th2 (Treg-CD45RA-724 CXCR5-CCR6-CXCR3-CCR4+CRTH2+/-) and Treg (CD127loCD25+) (manually 725 gating strategy on Supplemental Figure 1B). E, UMAP analysis on CD8+ Tc2 subset 726 including CCR4 and CRTH2 fluorescence parameters (Supplemental Figure 3). F, 727 Supervised analysis of circulating CD8+ Tc2 subset defined as CD45RA-CD25-728 CD94-CD56-CXCR5-CCR6-CXCR3-CCR4+CRTH2+/-. In (**B**), (**D**) and (**F**), box plots 729 with median ± minimum to maximum. P values were determined with the Kruskal 730 Wallis test followed by with Dunn's post test for multiple group comparisons; \*P < 731 0.05, \*\*P < 0.005, \*\*\*P < 0.001.

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733 Figure 2. HSCT-treated GC/JAK3 patients have a generalized defect in nasopharyngeal type 2 immunity. A, Nasopharyngeal levels of type 1 cytokines 734 735 (IFN-y and TNF- $\alpha$ ). **B**, Nasopharyngeal levels of type 2 cytokines (IL-4, IL-5 and IL-736 13). C, Nasopharyngeal levels of type 3 cytokines (IL-17A, IL-17F and IL-22). D, 737 Nasopharyngeal levels of IL-33, IL-6 and Eosinophil cationic protein (ECP)/RNase 3. 738 **E**, Heatmap representation of the mean of the concentration of all nasopharyngeal 739 cytokines in healthy and HSCT-treated SCID patients. In (A), (B), (C), and (D), box 740 plots with median ± minimum to maximum. P values were determined with the

741 Kruskal Wallis test followed by with Dunn's post test for multiple group comparisons; 742 \*P < 0.05, \*\*P < 0.005, \*\*\*P < 0.001.

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744 Figure 3. HSCT-treated GC/JAK3 patients without lqs replacement therapy 745 have decrease in nasopharyngeal secretory IgA (SIgA). A, Serum Igs 746 concentration in healthy and HSCT-treated SCID patients. **B**, Nasopharyngeal 747 concentrations of total IgM, IgD and IgG in healthy and HSCT-treated SCID patients. **C**, Nasopharyngeal concentrations of total IgA and IgA1 and IgA2 in healthy and 748 749 HSCT-treated SCID patients. D, Nasopharyngeal concentrations of total IgE in healthy and HSCT-treated SCID patients. In (A), (B), (C), and (D), box plots with 750 751 median ± minimum to maximum. P values were determined with the Kruskal Wallis 752 test followed by with Dunn's post test for multiple group comparisons; \*P < 0.05, \*\*P753 < 0.005, \*\*\*P < 0.001.

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755 Figure 4. HSCT-treated GC/JAK3 patients without IVIG replacement therapy 756 have decreased IgA-coating of nasopharyngeal bacteria. A, Representative dot 757 plots of IgA, IgG and IgD binding to nasopharyngeal microbiota in healthy and HSCT-758 treated SCID patients. **B**, % of IgA/IgG/IgD coated nasopharynx microbes in healthy 759 and HSCT-treated SCID patients. C, Double IgA/IgG or IgA/IgD coated nasopharynx 760 microbes in healthy and HSCT-treated SCID patients. In (A), (B), (C), box plots with 761 median ± minimum to maximum. P values were determined with the Kruskal Wallis 762 test followed by with Dunn's post test for multiple group comparisons; \*P < 0.05, \*\*P < 0.005, \*\*\*P < 0.001. 763

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765 HSCT-treated GC/JAK3 patients without IVIG replacement therapy Figure 5. have nasopharyngeal microbiota dysbiosis. A, Unsupervised principal coordinate 766 767 analysis (PCoA) of 16S RNA sequencing Operational Taxonomic Units (OTU) of the 768 healthy controls and HSCT-treated SCID patients along the first two principal 769 coordinate (PC) axes, based on Bray-Curtis distances. The respective PERMANOVA 770 test showing that nasopharynx microbiota from SCIDX/JAK3 patients is significantly 771 different from healthy controls. **B**, The beta ( $\beta$ ) diversity, calculated using the Bray-772 Curtis, Jaccard and Euclidean distances, among subjects by group. **C**, The alpha ( $\alpha$ ) 773 diversity, calculated using the Shannon index and Simpson index in healthy and 774 HSCT-treated SCID patients. Mean and standard error of mean (SEM) values are

775 indicated. **D**, Bar plot showing the mean of the microbiota Genus abundance (%) in 776 healthy and HSCT-treated SCID patients. (E) and (F) Individual Genus abundance 777 (%) plots in healthy and HSCT-treated SCID patients. **G**, Individual Streptococcus 778 pneumoniae (%) plot in healthy and HSCT-treated SCID patients. **H**, show individual 779 correlation plot between Streptococcus Genus abundance (%) and Dolosigranalum 780 Genus abundance (%). In (B), (E), (F) and (G) box plots with median ± minimum to 781 maximum. P values were determined with the Kruskal Wallis test followed by with 782 Dunn's post test for multiple group comparisons; \*P < 0.05, \*\*P < 0.005, \*\*\*P < 783 0.001. In (**H**),  $\sigma$  represents Spearman coefficient and p the p value.

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Defective type 2 immunity underlies nasopharyngeal dysbiosis in 785 Figure 6. 786 **HSCT-treated GC/JAK3 patients. A**, Nasopharyngeal MUC5AC concentration in 787 healthy and HSCT-treated SCID patients. **B**, Individual correlation plot between 788 nasopharyngeal MUC5AC concentration and  $\alpha$ -diversity. **C**, Individual correlation plot 789 between nasopharyngeal MUC5AC concentration and *Dolosigranalum* genus 790 abundance (%) or Streptococcus pneumoniae abundance (%). D, Individual 791 correlation plot between nasopharyngeal concentration MUC5AC and 792 nasopharyngeal IL-4, IL-5, and IL-13 concentration. E, Individual correlation plot 793 between nasopharyngeal MUC5AC concentration and nasopharyngeal secretory IgA 794 (SIgA). F, Heatmap representation of statistically different (P<0.05) nasopharyngeal 795 features between GC/JAK3 patients and the other patients and the 3D PcoA 796 representation. In (A) box plots with median  $\pm$  minimum to maximum. P values were 797 determined with the Kruskal Wallis test followed by with Dunn's post test for multiple 798 group comparisons; \*P < 0.05, \*\*P < 0.005. In (**B**), (**C**), (**D**) and (**E**)  $\sigma$  represents 799 Spearman coefficient and p the p value.









Figure 5





3 (10 %)