Clinical Immunology 203 (2019) 125–133

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

# Persisting enteropathy and disturbed adaptive mucosal immunity due to MHC class II deficiency

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ARTICLE INFO

*Keywords:*
MHC class II deficiency
Adaptive immunity
Mucosal immunity
Intestinal epithelial cells
Enteropathy
HLA-DR
Invariant chain
Hematopoietic stem cell transplantation

ABSTRACT

Intestinal epithelial cells (IECs) form a fundamental mucosal barrier and actively participate in tolerance and immunity against intestinal contents. Major histocompatibility complex class II (MHC II) and invariant chain (Ii) molecules are essential for adaptive immune response. MHC II deficiency often presents with gastrointestinal disorders.

Intestinal biopsy samples revealed an absence of HLA-DR, Ii, and local immunoglobulins in both hematopoietic immune cells and IECs accompanied by a lack of faecal sIgA. After successful hematopoietic stem cell transplantation (HSCT) absent HLA-DR and Ii expression persisted in IECs and faecal stool analysis indicated inflammation and high microbial activity.

We describe multifaceted disturbance of adaptive mucosal immunity in MHC II deficient patients suffering from enteropathy. HLA-DR and Ii expression on enterocytes is not restored by HSCT. This may account for increased susceptibility to enteric infections and intestinal inflammation leading to prolonged enteropathy reported in MHC II deficient patients.

## 1. Introduction

The intestinal immune system is challenged by an enormous load of antigens, derived from ingested nutrients, commensal bacteria or pathogens [1,2]. The gut-associated mucosal lymphatic tissue (GALT) discriminates between harmless environmental antigens and pathogen derived antigens [3]. The latter induce a protective local and systemic immune reaction whereas nutritional antigens and commensal bacteria are tolerated [3]. Dysfunction of the mucosal immune system leads to mucosal inflammation, tissue damage, and enteropathy.

MHC II (HLA-class II) molecules are essential for the adaptive immune response as they present peptides of internalized proteins on the cell surface, which are recognized by $CD4^+$ T cells and thereby promote pathogen specific cellular and humoral immune responses [4]. In humans, three different isoforms of the heterodimeric transmembrane glycoproteins (HLA-DR, HLA-DQ, and HLA-DP) are encoded by distinct α and β genes [5]. The expression of these isoforms is mainly controlled at the transcriptional level by an upstream MHC II regulatory *cis*-

*Abbreviations:* APC, Antigen-presenting cell; APECED, Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy; BLS, Bare lymphocyte syndrome; CD, Cluster of differentiation; DC, Dendritic cell; EPX, Eosinophil protein X; GALT, Gut associated lymphoid tissue; GvHD, Graft-versus-host-disease; HLA, Human leukocyte antigen; HSCT, Hematopoietic stem cell transplantation; IBD, Inflammatory bowel disease; IEC, Intestinal epithelial cell; IEL, Intra epithelial lymphocyte; Ig, Immunoglobulin; Ii, Invariant chain; IPEX, Immunodysregulation polyendocrinopathy enteropathy X-linked syndrome; LP, Lamina propria; MALT, Mucosa associated lymphoid tissue; MARSH, Grading system (I-IV) of small bowel lesions associated with celiac disease; MHC II, Major histocompatibility complex class II; PID, Primary immunodeficiency disease; PHA, Phytohaemagglutinin; SCID, Severe combined immunodeficiency; TCR, T-cell receptor; TLR, Toll-like receptor; VOD, Veno-occlusive disease

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https://doi.org/10.1016/j.clim.2019.04.012
Received 28 December 2018; Received in revised form 20 April 2019; Accepted 22 April 2019
Available online 24 April 2019
1521-6616/ 

module, known as the SXY module [6]. Thymic epithelial cells and professional antigen-presenting cells (APCs), namely dendritic cells, B cells and monocyte-macrophage lineage cells express MHC II dimers constitutively [5–7].

The invariant chain (Ii) and its exposed surface form CD74 mediate assembly and subcellular trafficking of the MHC II complex [4]. Similar to MHC II molecules, Ii is transcriptionally regulated by the SXY motif. The gene encoding Ii exhibits the SXY motif in its first intron, and Ii gene expression is similar to class II molecules [6,7]. The class II Ii peptide (CLIP) occupies the peptide-binding site of MHC II molecules and prevents premature peptide binding within the biosynthetic pathway [4,8]. Beyond facilitating ER exit, Ii directs the associated MHC II complex to the specialized endosomal MHC II compartment, where antigen-processing takes place [4]. Thus, a key role of Ii is the translocation of HLA-DR molecules to early endosomes for loading with peptides prior to export to the cell surface. The surface exposed form, CD74, is required for internalization of mostly empty MHC II molecules [8,9].

Absence of MHC II expression causes a rare form of an autosomal recessive primary immunodeficiency disease (PID; MIM 209920), also known as type II bare lymphocyte syndrome (BLS) [10,11]. In MHC II deficiency, causative mutations affecting the genes of the transcription factors in four distinct complementation groups (CG) A, B, C, and D have been identified [7,10,11]: The master regulator class II transactivator (CIITA) is mutated in CG A. The three subunits of regulatory factor X (RFX), i.e., regulatory factor X associated protein containing ankyrin repeats (RFXANK), the fifth member of the regulatory factor X family (RFX5), and regulatory factor X associated protein (RFXAP), are mutated in CG B, C, and D, respectively [7]. In cells constitutively lacking MHC II, their expression is induced by cytokines such as interferon gamma (IFN-γ) or in T cells upon activation by induction of CIITA expression [5,6,12].

Most MHC II deficient patients typically present with recurrent severe infections [11,13]. Infections of the gastrointestinal tract are most frequent and cause chronic diarrhoea, malabsorption, and failure to thrive [7,13,14]. The main immunological findings include a complete absence of MHC II expression on B cells, monocytes or activated T cells paralleled by decreased immunoglobulin serum levels [7,13]. As a consequence of absent MHC II-restricted antigen presentation, patients have inadequate antibody responses to immunization or microbial antigens [7].

MHC II deficiency is associated with a poor prognosis. Hematopoietic stem cell transplantation (HSCT) is the only curative treatment and may reduce mortality [15]. Unfortunately, HSCT in these patients has a less favourable outcome compared to other primary immune deficiencies with survival rates ranging from 40 to 80% [14,15]. However, it is reported that some patients with RFXANK mutations are stable without HSCT under supportive treatment based on immunoglobulin therapy and antibiotic prophylaxis [14]. Infections and graft versus host disease (GvHD) are the main causes of death after transplantation [15]. Interestingly, some patients are still susceptible to infections after successful HSCT [15].

We studied intestinal expression of HLA-DR, Ii and immunoglobulin (Ig) A, and faecal markers in seven patients with confirmed MHC II deficiency before and after HSCT suffering from enteropathy.

## 2. Methods

### 2.1. Patients

We investigated seven patients out of six families born between 1998 and 2013 who were referred to Ulm University Children's Hospital. Written informed consent was obtained from the parents in accordance with the Declaration of Helsinki. The diagnosis of MHC II deficiency was confirmed by sequencing *RFXANK* and *RFXAP* genes. In four patients, data were collected retrospectively after successful HSCT.

### 2.2. Immunologic analysis of peripheral blood

Blood lymphocyte subsets were evaluated by flow cytometry (10-colour Navios, Beckman Coulter, Krefeld, Germany) using antibodies from Beckman-Coulter, Krefeld, Germany. MHC II expression on peripheral blood $CD3^+$ T cells, $CD14^+$ monocytes and $CD19^+$ B cells was analysed (Navios v. 1.3).

### 2.3. Mutation analysis

Peripheral blood mononuclear cells (PBMCs) of the patients were obtained after written informed consent of the parents. Genomic deoxyribonucleic acid (DNA) was extracted using standard methods. All exons, including the exon-intron boundaries of the *RFXANK, CIITA, RFX5, and RFXAP* genes (NM_000246, NM_003721, NM_00449, NM_000538) were amplified by polymerase chain reaction (PCR) analysis using specific primers. The PCR products were directly sequenced in both directions using the ABI PRISM Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) and analysed on an ABI 3130xl Genetic Analyzer.

### 2.4. Histology and immunohistochemistry

Formalin fixed biopsy specimen were embedded in paraffin and sections of 3 µm were stained with hematoxylin-eosin (H&E), and for CD3 (F7.2.38, 1:100 dilution, DAKO, Glostrup, DK), CD38 (SPC32, 1:100 dilution, Menarini, Florence, IT), Ii (CD74) (LN2/Bu45, 1:100 dilution, Moldenhauer, Heidelberg, DE), lgA (polyclonal, 1:4.000 dilution, DAKO), and HLA-DR (1B5, 1,2.000, Moldenhauer). Briefly, deparaffinized tissue sections were incubated with the respective antibody at a dilution of 1:50 using standardized protocols after pre-treatment for 10 min of the slides in a steamer. A commercially available detection system set was used (DAKO REAL detection systems, DAKO). For negative controls the primary antibody was omitted.

### 2.5. Faecal analysis

Stool samples were collected and analysed. Faecal markers such as secretory IgA, lysozyme, lactoferrin, and calprotectin (KyperPlus, Institute for Mikroökologie, Herborn, Germany) were measured.

## 3. Results

### 3.1. Patients and clinical features

We investigated seven children out of six families with molecularly confirmed MHC II deficiency presenting with gastroenteropathy. One patient had a mutation in the *RFXAP* gene, and six in *RFXANK* (Table 1). Five patients were diagnosed in early childhood; however, in two patients the diagnosis was made late at the age of 7 and 15 years (Table 1).

Infections were the most prominent clinical feature in all patients affecting the gastrointestinal tract and the upper and lower respiratory tract (Table 1). Chronic diarrhoea was present in six out of seven patients leading to failure to thrive. Three patients had elevated liver enzymes and hepatomegaly. One of these patients presented with autoimmune hepatitis at age 3 years and developed hepatopathy and liver fibrosis without cholangitis (see Fig. 4 in [16]). Supportive treatment based on antibiotic prophylaxis and intravenous IgG substitution was provided. Five patients received HSCT from HLA-allele-matched unrelated voluntary donors and two from HLA-identical or compatible family members (Table 2). All surviving patients had sufficient immune reconstitution after HSCT in follow-up (Fig. 1).

The course after HSCT is depicted in Table 3. A summary of the patient's history is given in data in brief reference [16].

**Table 1**
patient molecular diagnosis, immune phenotype and characteristics before stem cell transplantation.

| Patient Sex/ origin | 1 w/Turkish | 2 w/Saudi Arabian | 3 m/Greek | 4 w/Saudi Arabian | 5 w/Saudi Arabian | 6 w/Saudi Arabian | 7 w/Saudi Arabian |
|---|---|---|---|---|---|---|---|
| MHC II defect | RFXAP<br>Exon 3<br>c.761C > T<br>p.Gln251*<br>homozygous | RFXANK<br>Exon 6<br>c.362A > T het<br>p.Asp121Val<br>Exon 7<br>c.477C > A het<br>p.Ser159Arg | RFXANK<br>Exon 6<br>c.451_452insC<br>p.His151ProfsX30<br>homozygous | RFXANK<br>Exon 7<br>c.477C > A<br>p.Ser159Arg<br>homozygous | RFXANK<br>IVS4<br>c.271 + 1delGins<br>TCAC homozygous | RFXANK<br>IVS4<br>c.271 + 1delGins<br>TCAC homozygous | RFXANK<br>IVS4<br>c.271 + 1delGins<br>TCAC<br>homozygous |
| Age at Dx | 15 years | 7 months | 4 years | 3 years | 3 months | 7 years | 18 months |
| Consanguinity | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Family history | one healthy brother | first child,<br>two siblings of the father died from PID | one healthy brother | two cousins died of MHCII deficiency | brother died at 8 months due to MCHII deficiency<br>Sister of #6 | brother died at 8 months due to MCHII deficiency<br>Sister of #5 | first child |
| Symptoms before HSCT | Failure to thrive,<br>Chronic diarrhoea (EPEC, Noro-, Adenovirus, Cryptosporidiosis, Campylobacter)<br>Candida-Keratitis<br>Delayed puberty<br>Osteopenia<br>Diabetes type I<br>Hypothyroidism | Failure to thrive<br>Chronic diarrhoea from birth (vaccination poliovirus and norovirus)<br>Recurrent pulmonary infections<br>Hepatopathy of unknown reason | Failure to thrive<br>Chronic diarrhoea<br>Recurrent otitis and upper airway infections,<br>urinary tract infection and pneumonia | Failure to thrive<br>Chronic diarrhoea<br>Recurrent otitis, cutaneous abscess and pneumonia,<br>Hepatitis with liver fibrosis | Failure to thrive<br>Chronic diarrhoea (Adenovirus, Salmonella)<br>Recurrent pulmonary infections<br>Hepatitis of unknown origin<br>Mild haemolytic anaemia | Recurrent pulmonary (4y/o) and urinary (1y/o) infections<br>One episode of diarrhoea<br>High CrP values | Failure to thrive<br>Recurrent pneumonia<br>Chronic diarrhoea (Norovirus, Echovirus 13)<br>Osteopenia |

Abbreviation: HSCT – hematopoietic stem cell transplantation; CrP – C-reactive protein; PID – primary immunodeficiency, PRES – posterior reversible encephalopathy syndrome;

### 3.2. Gastrointestinal histology and faecal analysis

Intestinal biopsies of the patients show inflamed colonic and duodenal tissue before and after HSCT (Fig. 2, Fig. 3 and Fig. 4, and see Figs. 3 and 5 in [16]). Histologically, no typically associated signs of intestinal acute or chronic GvHD were found, such as high frequency of apoptotic enterocytes, “exploding crypts”, apoptotic abscesses, degenerating crypts, collagen deposition in the lamina propria or regenerative changes of the lamina propria. Duodenal histology showed disturbed duodenal villous morphology, and crypt hyperplasia with enhanced intraepithelial lymphocytes (IEL) resembling celiac disease (Fig. 2). Faecal analysis revealed an increase in faecal water fraction reflecting diarrhoea with liquid or mushy stools of the patients (see Fig. 8 in [16]). There was only mild protein leakage of the gastrointestinal tract as normal or mildly elevated excretion of plasma alpha-1-antitrypsin in the faeces (see Fig. 8 in [16]). Other faecal markers such as calprotectin, lactoferrin, and lysozyme (released from neutrophils), and eosinophil protein X (EPX) (from degranulated eosinophils), were elevated in some patients indicating inflammation (Fig. 5 and see Fig. 8 in [16]). Cytotoxic EPX is strongly released from activated eosinophils and indicate inflammation and tissue damage (patients 1, 2, 5, and 7). In contrast to only mildly elevated faecal calprotectin and lactoferrin levels, high levels of lysozyme were detected in the faeces of patients 1, 2, 5, and 7, indicating increased antimicrobial activity (Fig. 5). This was accompanied by an increase of β-defensin 2, a common antimicrobial peptide usually induced by various types of bacteria in patients 2, 5, 6, and 7 (see Fig. 8 in [16]). Patient 5, and 6 suffered from chronic infectious diarrhoea due to adenovirus

**Table 2**
Immune phenotype at presentation, HSCT donor, and last chimerism.

| Patient | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Immune phenotype before HSCT | | | | | | | |
| $CD3^+$ (/µl) [39] | 222 [1000 − 2200] | 234 [1400–3700] | 522 [1400–3700] | 133 [1400–3700] | 682 [1400–3700] | 844 [1200–2600] | 637 [1400–3700] |
| $CD3^+/CD4^+$ (/µl) [39] | 189 [530–1300] | 183 [700–2200] | 163 [700–2200] | 54 [700–2200] | 318 [700–2200] | 168 [650–1500] | 403 [700–2200] |
| $CD4^+/CD45RA^+/RO^-$ (%) [39] | 4 [33–66] | 13 [53–86] | 31 [53–86] | 21 [53–86] | 40 [53–86] | 15 [46–77] | 40 [53–86] |
| $CD3^+/CD8^+$ (/µl) [39] | 15 [330–920] | 34 [490–1300] | 272 [490–1300] | 62 [490–1300] | 333 [490–1300] | 654 [370–1100] | 195 [490–1300] |
| $CD19^+$ (/µl) [39] | 54 [110–570] | 308 [390–1400] | 304 [390–1400] | 580 [390–1400] | 758 [390–1400] | 211 [270–860] | 455 [390–1400] |
| $CD3^-CD56^+CD16^+$ (/µl)* | 18 [90–700] | 34 [100–600] | 228 [100–600] | 78 [100–600] | 60 [100–600] | 10 [80–600] | 208 [100–600] |
| HLA-DR + /CD3 + (%)* | 0 [0–3.7] | 0 [0–4.3] | 0 [0–4.3] | 0 [0–4.3] | 0 [0–4.3] | 1.5 [0–4.1] | 0 [0–4.3] |
| HLA-DR + /CD14 + (%)* | 0 [100] | N/A [100] | 0 [100] | 0 [100] | 1 [100] | 0 [100] | 0 [100] |
| HLA-DR + /CD19 + (%)* | 0 [100] | N/A [100] | 0 [100] | 0 [100] | 0 [100] | 8 [100] | 0 [100] |
| IgA (g/l) [40]° | < 0.1 [0.61–3.48] | < 0.05 [0.27–1.95] | < 0.05 [0.21–3.21] | < 0.05 [0.27–1.95] | < 0.05 [0.20–1.00] | < 0.05 [0.34–3.05] | < 0.05 [0.20–1.00] |
| IgM (g/l) [40]° | < 0.05 [0.23–2.59] | 0.26 [0.24–2.10] | 0.33 [0.40–2.30] | < 0.05 [0.24–2.10] | < 0.05 [0.19–1.46] | 0.18 [0.31–2.08] | < 0.05 [0.19–1.46] |
| Age at HSCT (years) | 16 $^1/_{12}$ | 3 | 5 $^5/_{12}$ | 4 | 2 $^2/_{12}$ | 7½ | 2 $^1/_{12}$ |
| Donor | HLA-compatible unrelated (10/10) | HLA-compatible unrelated (10/10) | HLA-compatible unrelated (10/10) | HLA-compatible unrelated (10/10) | HLA-compatible unrelated (9/10) | HLA-identical sibling | HLA-compatible family (9/10) |
| Chimerism on last F/U | N/A | full donor | full donor | N/A | 80% donor | full donor | full donor |

HSCT – hematopoietic stem cell transplantation; F/U - follow up; N/A – not available; age appropriate reference values are in brackets []; * own reference values; ° reference values are adapted to IFCC/BCR/CAP reference preparation CRM 470 (RPPHS 91/0619) by applying a conversion factor of 0.83.

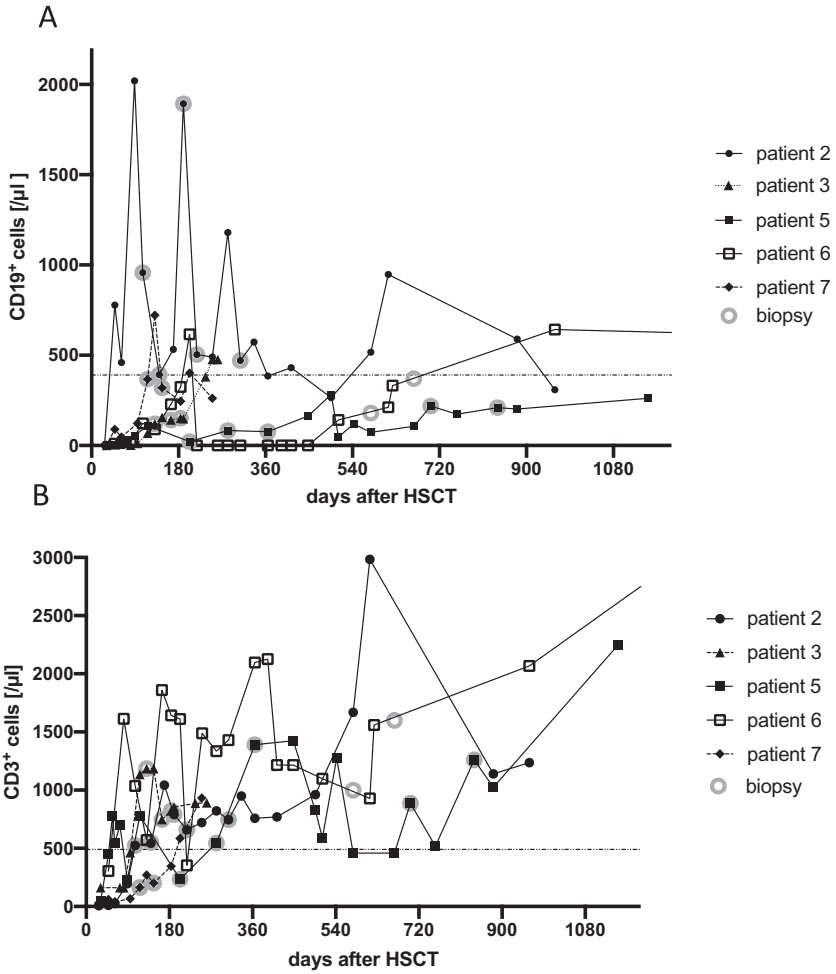


**Fig. 1.** Immune reconstitution after HSCT in MHC II deficient patients.
Time course of immune reconstitution of CD $19^+$ (A) and CD $3^+$ cells (B) after HSCT of patient 2, 3, 5, 6, and 7 measured by flow cytometry. Patient 6 received an anti-CD20 antibody (rituximab) depleting CD20 expressing B cells 217 days after HSCT explaining the loss of $CD19^+$ B cells. Grey circles indicate time points of endoscopies and biopsy sampling.

(Table 3).

### 3.3. HLA-DR expression

Peripheral blood flow cytometric analyses showed a reduction of all lymphocyte subpopulations and naïve T cells ($CD45RA^+/RO^-$) and an absence of HLA-DR expression on T cells ($CD3^+$), B cells ($CD19^+$), and monocytes ($CD14^+$) before HSCT (Table 2 and see Fig. 1 in [16]). An inverse $CD4^+$ to $CD8^+$ ratio typical for MHC II deficiency was found in 5 of 7 patients.

Immunohistochemistry of the inflamed gut revealed a complete absence of HLA-DR expression in intestinal epithelium cells (IECs) of MHC II deficient patients, whereas some HLA-DR positive lymphocytes were interspersed in the lamina propria and lymph follicles of patients 1 and 7 prior HSCT (Fig. 2 and Fig. 3). Interestingly, residual HLA-DR expression could be detected on stimulated peripheral B cells in patient 1 (see Fig. 2 in [16]). Still, following successful HSCT, HLA-DR expression was undetectable in IECs (Fig. 2 and Fig. 3). In addition, patient 4 did not express HLA-DR on liver cells (see Fig. 4 in [16]).

### 3.4. Invariant chain expression

Interestingly, the expression of the invariant chain (Ii), was also absent in IECs of MHC II deficient patients before HSCT (Fig. 4). Compared to other patients after HSCT only few $Ii^+$ mononuclear cells were found in the lamina propria of the duodenum and colon and no $Ii^+$ IECs were detectable in these patients (Fig. 4 and data not shown).

### 3.5. Immunoglobulin expression and intestinal B-cell compartment

IgG, IgA, and IgM blood levels were very low or undetectable prior HSCT in all patients and plasma cells expressing IgA, IgM, or IgG were absent in the lamina propria (Table 2, see Fig. 5 in [16]). In addition, we found only few or no $CD20^+$ B lymphocytes, and $CD38^{high}$ plasma cells in the lamina propria and organized lymphoid tissues before HSCT (see Fig. 6 in [16]). Several months after HSCT we detected few $CD20^+$ and $CD38^+$ cells in the LP and focally enhanced in lymph follicles (see Fig. 6 in [16]).

Tissue content of IgA, IgM, IgG, and IgD is typically elevated in inflamed duodenal tissue, e.g., celiac disease (see Fig. 5 in [16]). However, the production of intestinal IgA was not restored promptly and sufficiently in most of the MHC II deficient patients after HSCT with inflamed tissue (Fig. 3, Fig. 5). In patient 3 almost no intestinal IgA was detectable five and six months after HSCT despite emergence of low serum IgA levels (0.76 and 0.51 g/l) and the presence of HLA-DR expressing mononuclear cells in the lamina propria (LP) (Fig. 2, see Fig. 5 in [16]). Similar, in patient 1 there was no intestinal IgA expression present after HSCT, despite detectable HLA-DR expressing mononuclear cells in the lamina propria (LP) (Fig. 3).

In contrast, patient 2 displayed high levels of all immunoglobulin

**Table 3**
Course after HSCT.

| Patient | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Age at FU (years) | $15\,^{8}/_{12}$ | $5\,^{3}/_{12}$ | $6\,^{2}/_{12}$ | 4 | $5\,^{5}/_{12}$ | $9\,^{4}/_{12}$ | $2\,^{10}/_{12}$ |
| Status after HSCT | Dead<br>+5 months HSCT, MOF | Alive<br>F/U 33 months | Alive<br>F/U 10 months | Dead<br>+3 weeks HSCT, intracranial haemorrhage | Alive<br>F/U 56 months | Alive<br>F/U 49 months | Alive<br>F/U 8 months |
| GVHD | aGvHD °III<br>(°2 skin, °2 liver, °3 gut) | aGvHD °I<br>(°2 skin)<br>cGvHD<br>(liver, lung, alopecia) | aGvHD °II<br>(°3 skin) | | aGvHD °III<br>(°3 skin, °2 gut) | aGvHD °II<br>(°2 skin, °1 gut) | aGvHD °I<br>(°1 skin) |
| Other complications | Several viral infections | VOD<br>Hashimoto thyroiditis | Viral infections<br>Encephalopathy of unknown origin | Capillary leakage | Recurrent infections<br>PRES | Viral infections (CMV, Influenza A, H1N1, Polyoma BK-virus)<br>Neuritis optica | |
| Gastrointestinal characteristics after HSCT | Severe bloody diarrhoea Sepsis<br>Campylobacter jejuni | Chronic diarrhoea with malabsorption<br>Gastroesophageal Reflux | Severe diarrhoea, haemorrhagic gastritis (Astro-, Adenovirus) | Bloody diarrhoea | Chronic diarrhoea<br>Adenovirus<br>MR-Salmonella species | Chronic diarrhoea<br>Adenovirus | Enteral nutrition via gastrostomy |

HSCT – Hematopoietic stem cell transplantation; MOF - multi-organ failure; MR – multi-resistant; F/U – follow-up; aGvHD – acute Graft-versus-Host-Disease; cGvHD – chronic GvHD; VOD – veno occlusive disease; PRES – posterior reversible encephalopathy syndrome; CMV – cytomegaly virus.

subclasses in the lamina propria eight months after HSCT accompanied by normal serum IgM levels, and even elevated serum IgA levels (Fig. 3, see Fig. 5 in [16]). Faecal soluble IgA was investigated in five patients confirming that patient 2 at eight months after HSCT secreted IgA into the faeces, unlike patients 1, 5, and 6 (Fig. 5).

Although $CD8^+$ IEL and $CD8^+$ lamina propria T cells were found frequently, $CD4^+$ T cells and FOXP3 expressing regulatory T cells were reduced in the LP of all patients (see Fig. 3 in [16] and data not shown).

## 4. Discussion

We demonstrate that MHC II deficiency is accompanied by a multifaceted disturbance of intestinal mucosal immunity affecting the crosstalk between IECs and adaptive immune cells: by the absence of HLA-DR expression and Ii expression, accompanied by a paucity of immunoglobulins, especially secretory IgA. Most importantly, despite full donor engraftment, adequate immune reconstitution and detectable HLA-DR expressing mononuclear cells in the lamina propria after HSCT, insufficient restoration of the intestinal mucosal immunity may explain the intestinal pathology.

Inducible MHC II expression in IECs has been described for several decades although the functional consequences are not fully understood [17,18].

Here, we describe the lack of HLA-DR and Ii expression in highly inflamed tissue of three patients before stem cell transplantation in all sections of the gastrointestinal tract. Absence of HLA-DR expression on immune and epithelial cells in the rectal mucosa in patients with MHC II deficiency has been reported [14].

We detected a persisting lack of HLA-DR expression after HSCT on IECs of our patients suffering from enteropathy. This resembles the phenotype of $pIV^{-/-}$ K14 CIITA Tg mice that are deficient in non-hematopoietic MHC II expression, but harbour normal thymic and peripheral CD4+ T-cell populations [19]. These mice develop overt colitis during chronic *helicobacter hepaticus* infection upon anti-IL10R antibody treatment with increased innate effector cell infiltration and elevated pro-inflammatory cytokine expression [19]. Induction of MHC II on IECs correlated with protection against colitis in heterozygous mice demonstrating an anti-inflammatory, MHC II-dependent crosstalk of IECs with adaptive immune cells [19]. In addition, $Rag2^{-/-}$ mice exclusively expressing MHC II either on DCs or IECs develop colitis. Mice with MHC II expressing DCs develop severe colitis whereas mice with MHC II expressing IECs develop only mild inflammation in the presence of T cells and microorganisms [20]. Comparably, we observed inflammation and prolonged enteropathy in MHC II deficient patients still lacking MHC II expression on IECs after HSCT. Furthermore, we detected residual expression of MHC II molecules on mononuclear cells in the lamina propria within the inflamed tissue in two MHC II deficient patients investigated before HSCT and described inducible HLA-DR expression on B cells of one patient. Residual HLA-DR expression on epidermal Langerhans cells and a subset of peripheral blood monocyte-derived dendritic cells has been reported in twin brothers with MHC II deficiency demonstrating cell type specific regulation and residual "leaky" MHC II molecule expression [21]. Indeed, the twins' monocyte-derived dendritic cells were capable of presenting antigen and induce proliferation in CD4+ T cells in a MHC II-dependent fashion explaining their relatively benign clinical symptomatology [21].

Moreover, we describe that absence of HLA-DR expression is accompanied by a lack of invariant chain (CD74) in IECs of patients with MHC II deficiency due to *RFXANK* and *RFXAP* mutations. We assume that these genetic defects may additionally affect Ii transcription as the interferon-gamma responsive region of the Ii gene intron 1 and also the upstream enhancer of Ii have S, X/X2 and Y boxes highly similar to those in the MHC II gene promotors [22]. There is further evidence that Ii is essential for MHC II-dependent adaptive immune response as Ii knockout mice ($Ii^{-/-}$) have a defect in MHC II-restricted activation of CD4+ T cells similar to MCH II knockout mice ($H2^{dlAB1\text{-}Ea}$) [23].

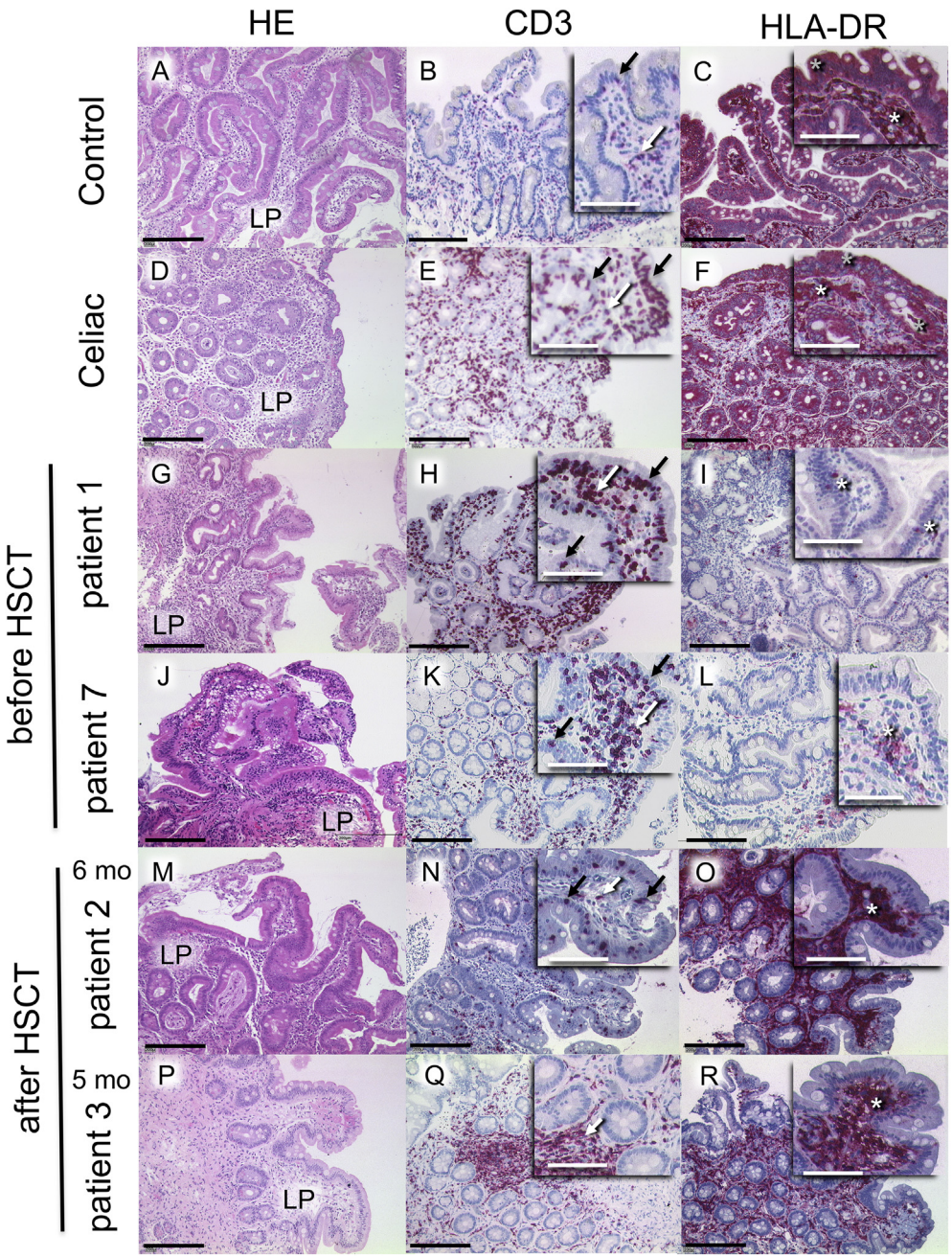


**Fig. 2.** Lack of duodenal HLA-DR Expression before and after HSCT in MHC II deficient patients:
A patient with functional abdominal pain served as a control demonstrating normal villus and crypt morphology in the HE staining (A), $CD3^+$ intraepithelial lymphocytes (IEL) (black arrow) found scattered in the epithelium (B) and regularly expressed HLA-DR in intestinal epithelial cells (IEC) (grey asterix) and lamina propria stromal cells (white asterix) (C). Inflamed duodenal tissue from a celiac disease patient before gluten-free diet with complete villous atrophy and crypt hyperplasia (D), dramatically increased IELs (E) (black arrows) and an induced expression of HLA-DR in IECs (grey and white asterix) (F). Although patient 1 displayed inflamed tissue before HSCT similar to the celiac disease patient with villous blunting and crypt hyperplasia in (G), CD3+ T cell infiltrates and pronounced IELs (H) (black arrows) there are only scattered HLA-DR positive cells in the lamina propria (white asterix) and intraepithelial but no HLA-DR staining of IEC (I). Patient 7 suffered from chronic viral enteritis and showed inflamed duodenal tissue (J), some CD3+ T cells and IELs (K), few HLA-DR positive cells in the lamina propria but no HLA-DR expressing enterocytes (L) before HSCT.
Patient 2 still suffered from intractable diarrhoea and malabsorption six months after successful HSCT and needed parenteral nutrition. He displayed disturbed villus morphology, an increased villous to crypt ratio (M), and several CD3+ lymphocytes in the lamina propria and intraepithelial (black arrows) (N). HLA-DR expression is detectable intensively in cells of the lamina propria (white asterix) and rarely intraepithelial, but not in IEC (O). Similarly, patient 3 dependent on parenteral nutrition due to malabsorption five months after HSCT shows inflamed tissue with villous blunting and crypt hyperplasia (P), a mildly increased inflammation demonstrated by infiltrated CD3 in the lamina propria (white arrow) (Q). After successful HSCT there is still a lack of HLA-DR expression on IEC although HLA-DR expressing is reconstituted in the LP (white asterix) shown in (R). Black bars indicating 200 µm and white bars 100 µm.

We also describe disturbed immunoglobulin expression and IgA secretion in MHC II deficient patients before and after HSCT.

Induction of $IgA^+$ B cells in the gut requires conventional T cell-dependent B-T-cell interactions via the MHC II complex to peptides and the T cell receptor (TCR) or alternatively T cell-independent stimulation through Toll like receptors (TLR) and co-receptors such as CD40 [1]. $CD20^+$ B cells and $CD38^{high}$ plasma cells were dramatically reduced or absent before HSCT in MHC II deficient patients and slowly repopulate the LP after HSCT, accounting for a long lasting reduced sIgA secretion in the gut of some patients. This may affect antimicrobial activity, antigen sampling and the quality of immune response [24].

Both MHC II deficiency and IgA deficiency may disturb the microbial colonization in the gut as IgA maintains a certain diversity of the microbiome [1] and MHC II polymorphism contribute to defining the host microbiome [25–28]. In addition, both conditions are associated with an increased susceptibility to autoimmune diseases e.g. IBD or celiac disease [29,30]. Thus, in MHC II deficiency defective IgA response and secretion may increase the susceptibility to enteric infections and predispose for developing an inflammatory milieu in the gut. Accordingly, we observed elevated levels of faecal inflammatory markers, e.g., calprotectin and lactoferrin as well as an up-regulation of factors of the innate mucosal immunity such as lysozyme and beta defensin 2.

Viral infections of the pulmonary and gastrointestinal tract are common in MHC II deficiency. They impede and limit the success rate of HSCT as they may persist and trigger inflammation and GvHD. Thus, an incomplete restoration of MHC II and Ii expression on epithelial cells may account for a relatively poor outcome of HSCT in contrast to other

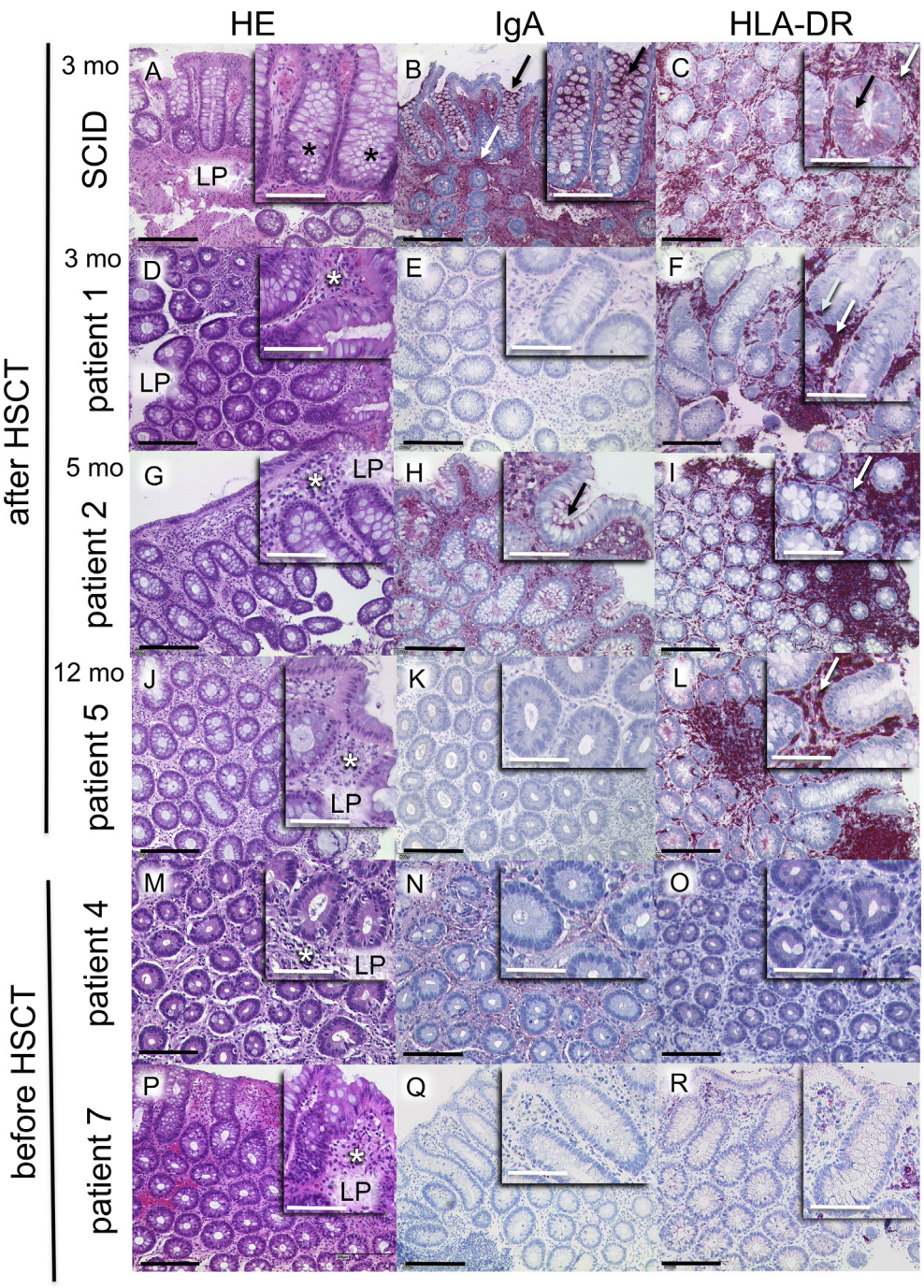


**Fig. 3.** Lack of colonic IgA and HLA-DR expression in MHC II deficient patients before and after HSCT:
Colonic biopsies of a severe combined immunodeficiency (SCID) ($T^-$, $B^+$, $NK^+$) patient three months after stem cell transplantation (HSCT) suffering from a mild graft versus host disease (GvHD) of the gut (A-C) and MHC II deficient patients before (4 and 7) and after (1, 2 and 5) HSCT are stained with HE, IgA, and HLA-DR.
There is only mild inflammation indicated by some lymphocytes in the lamina propria (LP) and only sporadic apoptotic bodies (black asterix) in the crypts of the control patient (A). In contrast, lymphocyte infiltration is more pronounced in the inflamed colonic mucosa of the MHC II patients before (M, P) and after (D, G, J) HSCT (white asterix).
IgA is abundantly expressed by plasma cells of the colonic mucosa (white arrow) and absorbed by intestinal epithelial cells through receptor mediated endocytosis on the basolateral surface and apically released as receptor IgA complex into the intestinal lumen (black arrow) of the SCID patient three months after HSCT (B). Before HSCT neither IgA is expressed by plasma cells nor secreted into the lumen by intestinal epithelial cells of patient 4 (N) and 7 (Q). IgA is detected five months after HSCT in the lamina propria and apically on intestinal epithelial cells of Patient 2 (H), whereas IgA is still absent in the colonic mucosa three after HSCT of patient 1 (E) and 12 months of patient 5 (K).
HLA-DR is expressed by cells of the lamina propria (white arrow) and by colonic enterocytes (black arrow) of a SCID patient with mild GvHD of the gut after HSCT (C). In contrast, HLA-DR expression lacks on colonic enterocytes in MHC II deficient patients before (O, R) and after HSCT (F, I, L), while it is expressed on intraepithelial lymphocytes (IEL) (blue arrow) and lamina propria cells (white arrow) after HSCT (F, I, L). Black bars indicating 200 μm, white bars 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

primary T cell immunodeficiencies [14].

In analogy, HSCT of X-linked recessive ectodermal dysplasia with immunodeficiency caused by hypomorphic mutations of the *IKBKG* gene encoding the nuclear factor κB essential modulator (NEMO) protein sufficiently reconstitute the defect in immune cells [31–33]. However, the defect of epithelial integrity in NEMO deficiency could not be restored in intestinal epithelial cells and accounts for the variable outcome following HSCT [31,32]. In addition, HSCT corrects the immune defect of *tetratricopeptide repeat domain 7 A (TTC7A)* deficiency but does not cure the intestinal epithelial defects and inflammatory phenotype [34,35].

Notably, the recognition of severe enteropathy due to MHC II deficiency is challenging as it may phenotypically and histologically resemble other primary immunodeficiencies (e.g. NEMO deficiency, CTLA 4 deficiency, TTC7A deficiency, IPEX), celiac disease, inflammatory bowel disease or autoimmune enteropathy [34,36–38]. Here, we report a case of severe enteropathy and polyendocrinopathy due to MHC II deficiency. Thus, MHC II deficiency should be considered as a differential diagnosis in disorders associated with severe inflammation of the gastrointestinal tract, e.g., autoimmune enteropathy, IPEX-like syndrome or certain cases of IBD.

## 5. Conclusions

Expression of molecules directly involved in antigen processing and presentation pathways, i.e., HLA-DR and invariant chain (Ii) are disturbed in hematopoietic cells and intestinal epithelial cells in patients with MHC II deficiency. This suggests a shared transcriptional regulation of HLA-DR and Ii. However, MHC II and Ii expression are not restored in IEC after successful HSCT. This may affect immune response

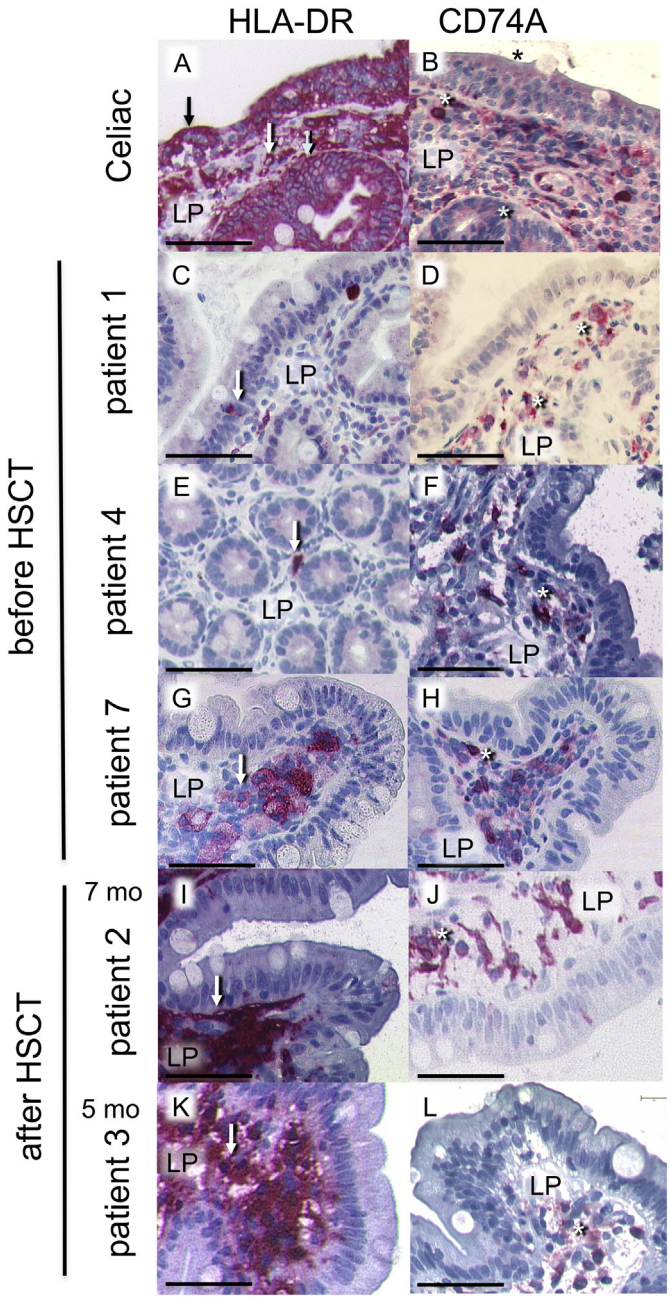


**Fig. 4.** Lack of duodenal CD74 expression before and after HSCT in MHC II deficient patients:
A celiac patient serves as a positive control. High-power magnification showing HLA-DR (A) and Ii (CD74) (B) not only on mononuclear cells in the lamina propria (white arrow and asterix) but also apically in intestinal epithelial cells (IEC) (black arrow and asterix) (A, B). Granular intracellular HLA-DR staining localizes apical (black arrow) and basolateral (aquamarine arrow) (A). Immunohistochemistry of the invariant chain reveals staining of the apical (black asterix) and the peri-nuclear ER region (aquamarine asterix). In contrast, MHC II deficient patients (1, 4, 7) before and after (2, 3) HSCT do not express HLA-DR and the invariant chain in their IEC (C-L). Compared to the SCID patient after HSCT only few $CD74^+$ B and DC cells were found in the duodenum (white asterix) of patient 1 (D), 4 (F) and 7 (H) before HSCT, whereas patient 2 seven and patient 3 five months after HSCT display HLA-DR (white arrow) (I, K) and CD74 (white asterix) (J, L) on mononuclear cells in the lamina propria, but not in the IECs. Black bars indicating 50 µm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

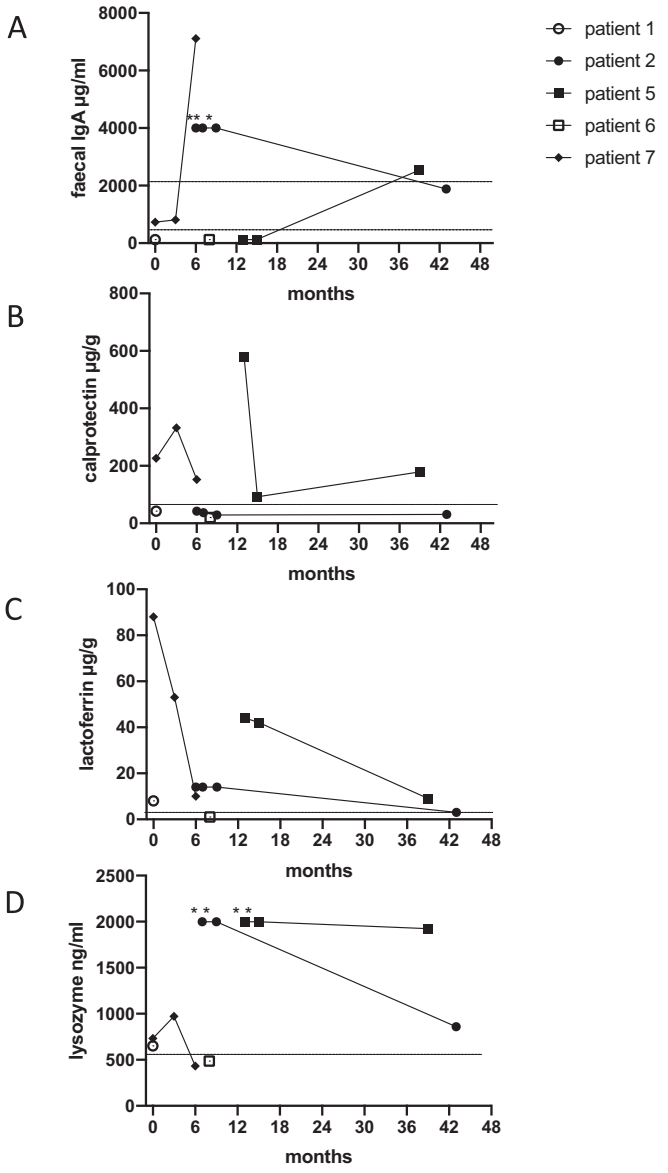


**Fig. 5.** Faecal IgA and faecal inflammatory markers before and after HSCT.
Faecal IgA (A) is low before HSCT and reaches normal values after 5 months after HSCT in patient 2 and 7 (*maximum value > 4000). However, IgA was still dramatically reduced in patient 6 and 5. Faecal Calprotectin (B) and Lactoferrin (C) indicate inflammation. Patient 5 and 7 displayed elevated Calprotectin and Lactoferrin values. Patient 1, 2, 5 and 7 also expressed elevated Lysozyme (D) (*maximum value > 2000) indicating anti-microbial and inflammatory activity. X axis displays months after HSCT and dotted line indicating cut-off values.

towards gastrointestinal infections and account for intestinal inflammation and prolonged enteropathy reported in MHC II deficient patients after HSCT. Both disturbed IgA secretion and HLA-DR expression in the gut may affect the gut microbial composition and increase susceptibility to autoimmune diseases in MHC II deficiency. Our findings support a crucial role of MHC II and Ii expression for the crosstalk between epithelial cells and adaptive immune cells in the gut.

## Authorship contribution

All authors have made substantial contributions to the manuscript, revised it critically with important intellectual content and approved the final version. CP is responsible for the conception and design,

acquisition and together with TFB and PM, interpreting of data and drafting the manuscript; EJ did flow cytometry analysis and lymphocyte proliferation assays of the patients; MR and KS performed the molecular genetic analysis; TFB and PM the immunohistochemistry workup; AS-C, TR, CS, MH, MS and AS were involved in patient workup and reports.

## Disclosure of conflicts of interest

This work has not been published before. There is no conflict of interest to be declared regarding the manuscript.

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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