A Mendelian predisposition to B cell lymphoma caused by IL-10R deficiency

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**Key points:**

1. Human inherited IL-10 receptor deficiency is associated with a very high risk of non EBV-related diffuse large B cell lymphoma.
2. IL-10 signaling may be involved in the immune control of germinal center B cell lymphoma.

**Abstract**

Monogenic interleukin (IL)-10 and IL-10 receptor (IL-10R) deficiencies cause very early-onset, severe inflammatory bowel disease. Here, we report that five patients with an IL-10R1 (n=1) or IL-10R2 (n=4) deficiency developed B cell non-Hodgkin’s lymphoma between the ages of 5 and 6 years (which were recurrent in one patient). These lymphomas had some of the characteristics of diffuse large B cell lymphomas and contained monoclonal, Epstein-Barr-virus-negative germinal center B cells. The tumors displayed a remarkably homogeneous signature, with original activation of the NF-κB pathway and a decrease in intratumor T cell infiltration. Hence, IL-10R deficiency is associated with a high risk of developing B cell lymphoma. Our results revealed an unexpected role of the IL-10R pathway in lymphomagenesis.

**Short Title:** IL10R deficiency predisposes to lymphomagenesis
Introduction

Inflammatory bowel disease (IBD) encompasses a heterogeneous group of diseases that are characterized by chronic intestinal inflammation with a complex etiology\textsuperscript{1,2,3}. Very-early-onset inflammatory bowel diseases (VEO-IBDs) are particularly severe, treatment-resistant conditions\textsuperscript{4,5}. Loss-of-function mutations in one or other of the IL-10 receptor's two chains (IL-10R1 and IL-10R2) or in IL-10 itself are detected in around 20\% of VEO-IBD patients\textsuperscript{6,7,8,9,10}. Studies of IL-10/IL-10R knock-out mice have shown that this cytokine is a key checkpoint for maintaining immune homeostasis towards intestinal microbiota\textsuperscript{11,12}. Chronic intestinal inflammation is a known risk factor for the development of malignancies\textsuperscript{13}. Infection with Epstein-Barr virus (EBV) and long-term administration of immunosuppressive medication are also associated with a slight increase in the risk of lymphoma in young children with colitis\textsuperscript{14} and in adults with IBD\textsuperscript{15}. A Mendelian predisposition to B-cell lymphoma\textsuperscript{16} has been observed in some primary immunodeficiencies related to DNA repair (such as ataxia-telangiectasia\textsuperscript{17}) and dominant-negative \textit{STAT3} mutations\textsuperscript{18,19} but not previously in monogenic form of IBD. Here, we report on the occurrence and recurrence of diffuse large B-cell lymphomas (DLBCLs) in five children with IL-10R1 (n=1) or IL-10R2 deficiencies (n=4). None of the DLBCLs was related to EBV infection. Our data strongly suggest the existence of a direct relationship between IL-10R deficiency and the development of B-cell lymphomas.
Patients and methods

Of 18 children being monitored for VEO-IBD at Necker Children's Hospital (Paris, France), seven were diagnosed with a defect in the IL-10 pathway (IL-10R1: n=1; IL-10R2: n=6). Similarly, IL-10 pathway defects were diagnosed in 18 children (IL-10R1: n=7; IL-10-R2: n=8; IL-10 n=3) out of 60 with VEO-IBD treated at Munich Children's Hospital (9 and personal data). Four of the 14 patients with IL-10R2 deficiency and 1 of the 8 patients with IL-10R1 deficiency developed lymphomas. The clinical characteristics of the five patients with VEO-IBD who subsequently developed lymphoma (P1 to P5) are summarized in supplementary Table 1. The first signs of colitis (bloody diarrhea) were observed between 2 and 12 weeks of age. The patients subsequently developed recurrent stomatitis and perineal inflammation. In P1, P2, P3 and P5, several episodes of peri-anal abscesses were observed. All of the patients except P5 had recurrent flare-ups of cutaneous folliculitis, which had also started during the first year of life. Immunophenotype, T cell function, immunoglobulin levels and antibody functions of these patients were normal. In view of the persistent colitis, four patients were treated with steroids and various immunosuppressive drugs (azathioprine: n=4; cyclosporine: n=2; mycophenolate mofetil and anti-tumor necrosis factor α monoclonal antibodies (infliximab)) at between 1.5 and 5 years of age. P5 did not receive any immunosuppressive drugs prior to lymphoma onset. In view of the poor observed long-term remission, colectomy was performed in P1, P3 and P4 at between 3 and 4 years of age (Fig. 1). Furthermore, P4 had brachysyndactyly of both hands. All participants or their parents/guardians gave their written, informed consent to participation, in accordance with the Declaration of Helsinki. The study was approved by CCPRB (comité consultatif pour la recherche biologique).

Details of the IL10RA and IL10RB gene mutations found in these five patients and the functional validation of these mutations (i.e. the absence of IL-10R expression or an in vitro response to IL-10) are given in Fig. 1 and Supplementary Data section. We further characterized these lymphomas by performing immunohistochemical studies, genome-wide array-based comparative genomic hybridization (aCGH) (n=4), cytogenetic tests, fluorescence in situ hybridization (FISH) analysis (n=4), and gene expression profiling (n=3) on tumor samples. Furthermore, whole exome sequencing (WES) of tumor DNA and counterpart genomic DNA was performed in three tumors from two patients. The methods are described in the Supplementary Data section. The study was conducted in accordance with the Declaration of Helsinki.
Results

Characteristics of lymphomas in five IL-10R-deficient patients

Patients 1 to 5 all developed high-grade B-cell non-Hodgkin’s lymphoma (NHL) at between 5.5 and 6.5 years of age (Fig. 1). Three further lymphomas occurred in P4 at 9.5, 12.1 and 16.5 years of age (referred to as P4-L2, P4-L3 and P4-L4, respectively) (Fig. 1). The B-cell lymphomas were variously located within abdominal lymph nodes (n=7), thoracic lymph nodes (n=3), spleen (n=5), liver (n=3) and bone (n=2) (Table 1). Lymphoproliferative mucosal lesions were not documented. All lymphomas were treated with chemotherapy, which was combined in all but one case (P1-L1) with administration of an anti-CD20 monoclonal antibody (Table 1). Patient 1 was in long-term remission when an ultimately fatal EBV-related lymphoproliferative disease occurred 4.5 years after chemotherapy. Patient 2 died during chemotherapy, due to meningoencephalitis of unknown etiology. Patient 3 underwent hematopoietic stem cell transplantation (HSCT) from a mismatched family donor 12 months after the completion of effective chemotherapy and is currently (twelve months post-HSCT) in remission. Two months after remission of his fourth lymphoma, P4 underwent HSCT from a geno-identical sibling and is currently (18 months post-HSCT) in remission. Patient 5 is presently in remission (3.3 years after the completion of chemotherapy) and HSCT is being considered. Data on IgH sequences, cytogenetic test results and aCGH data on tumor cells from P4 confirmed that the first three lymphomas (at least) were distinct, and had occurred sequentially.

Immunohistochemical characteristics of the B-cell lymphomas

Immunohistochemical studies were performed on lymph node, liver and spleen biopsies (Table 2 and Fig. 2). All the lymphomas had common histological features, with destruction of the normal architecture by diffuse proliferation of monomorphic, large, lymphoid cells (Fig. 2a and b). There were many mitotic figures and apoptotic bodies. Immunohistochemical staining revealed that the large, atypical cells were CD20-positive B-cells (Fig. 2c). Almost all the B-cells (80-90%) stained positive for the proliferation-associated nuclear antigen Ki67 (Fig. 2d). In situ hybridization assays based on an EBV-encoded RNA (EBER) probe were negative (Fig. 2e). All lymphomas were BCL6-positive (Fig. 2f) and four were CD10-positive, suggesting a germinal center origin (Table 2). In three BCL6-positive/CD10-negative tumors, MUM1/IRF4 was absent (n=1) or heterogeneous, positive in < 30% of cells (n=2). Hence, these B-cell lymphomas thus shared many of the features of diffuse large B-cell lymphomas (DLBCLs). CD3 staining revealed a predominantly perivascular distribution, with
very few lymphoma-infiltrating T-cells; these data contrasted with the detection of both perivascular and infiltrating T-cells in five control germinal-center-like B-cell (GCB)-DLBCLs (Fig. 2). Granzyme B staining was negative or scattered in all the tested IL-10R-deficient samples (DLBCLs: n=7) and was positive in five lymphoma-infiltrating T-cell control GCB-DLBCLs (Fig. 2).

Clonality
All tested specimens (6 out of 6) displayed monoclonal rearrangements of the IgH gene locus (Table 2 and data not shown). Somatic mutations in IgV regions were detected in two of three cases studied (Supplementary Table 2).

Tumor gene expression profiling
In an attempt to better characterize the lymphomas, gene expression profiles were determined for tumors P3-L1, P4-L2 and P5-L1 and compared with those in well-defined B-cell lymphoma entities and normal GCB cells from tonsils. Microarrays signatures from these various entities were downloaded from the open-access Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/), identifier: GSE 39503, and compared with our patients' samples in an unsupervised analysis. Remarkably, lymphomas P3-L1, P4-L2 and P5-L1 displayed similar profiles (R > 0.650) and clustered in the branch containing all the DLBCLs (despite the latter's known heterogeneity) and follicular lymphoma samples (R>0.125) (Fig 3a). This clustering fits with the initial diagnosis and the tumors' immunohistochemical phenotype. In view of the heterogeneity of DLBCLs, we sought to determine whether the patients' lymphomas were primarily ABC-like or GCB-like16,20. In fact, they all clustered in the GCB branch - confirming a major enrichment in the GCB signature (Fig. 3b) characterized by markers of germinal center differentiation (MME, BCL6, BCL7, LMO2, CD21 (CR2), CD22, CD27, CD86, XBP1, LRMP, SERPINA9, FAM3C, RGS13, LCK and IRS1; p<0.0001). Furthermore, the three lymphomas had additional functional pathways in common with GCB-DLBCLs, such as upregulation of the neurotrophin signaling pathway (p<0.0003), unsaturated fatty acid biosynthesis (p<0.0005), the MAPK-signaling pathway (p<0.003) and downregulation of the BCR signaling pathway (p< 0.02).
Even though GCB-DLBCLs were the closest malignant entities to lymphomas P3-L1, P4-L2 and P5-L1, a gene set enrichment analysis (GSEA) and a pathway analysis revealed that the latter also shared some features distinct from GCB-cell lymphomas (Supplementary Fig. 2
and Supplementary Table 3) and non-malignant, proliferative tonsil B cells (Supplementary table 4 and supplementary Fig. 3). When compared with GCB-DLBCLs, lymphomas P3-L1, P4-L2 and P5-L1 showed greater expression of the spliceosome pathway (p<2x10^{-12}) and ubiquitin-mediated proteolysis (p<2x10^{-11}). These characteristics fit with the observed active cell cycling and frozen GC phenotype. The activation of many pathways involved in cancer (such as NOTCH, mTOR, ERBB2 and NF-κB) (p<2x10^{-5}) is consistent with the high proliferation rate observed in these lymphomas. Strikingly, expression of molecules related to cytotoxicity and dendritic cell function was low and thus agreed with the immunohistological data on T cells. A comparison with the non-malignant, proliferating B-cells found in secondary lymphoid organs (Supplementary Fig. 3 and Supplementary Table 4) confirmed the lymphomas' monoclonality (relative to the polyclonal response observed in tonsils (p<4.10^{-6})) and, most importantly, revealed surprisingly low levels of AICDA, BACH2 and AFF3 - three molecules that are usually upregulated in GC B-cells. Lastly, it is noteworthy that the axon guidance pathway was also enriched (p <0.2 x 10^{-5}). Overall, this signature shows that the lymphomas had high proliferative and survival capacities. The low numbers of antitumor immune cells in the patients' samples (as assessed by immunohistochemical staining and relative to conventional DLBCLs) was confirmed by the results of a transcriptome analysis.

Amplification and expression of the cellular homolog of the reticuloendotheliosis viral oncogene (c-REL) and constitutive activation of NF-κB.

A cytogenetic analysis of the tumor cells revealed multiple chromosome rearrangements (Supplementary Table 5) and an aCGH analysis was performed on genomic DNA samples from four tumors (P3-L1, P4-L1, P4-L2 and P5-L1). The presence of high copy number variations in the aCGH analysis of tumor cells agreed in full or in part with the results from a standard karyotype. Of the many genomic variations detected, the only recurrent variation was a gain of the 2p16 chromosomal region (Supplementary Table 6 and Fig 4a). The minimum common amplified region defined by the breakpoint junctions of the P4-L1 sample spanned 0.25 Mb and included the \textit{PAPOLG}, \textit{FLJ6341}, \textit{REL} and \textit{PUS10} genes. P4-L2 notably exhibited full chromosome 2 trisomy and gain of the 2p16 chromosomal region (thus resulting in 2p16 tetraploidy). In P3-L1, gain of the 2p16 locus was confirmed by a FISH analysis with a probe that flanks the \textit{REL} locus (Fig 4b).
Given that REL locus amplification was found in all four tested cases, we assayed for expression of the cellular homolog cREL. The expression levels of cREL mRNA in P3-L1, P4-L2 and (above all) P5-L1 were similar to or greater than those found in control GCB-DLBCL samples (Fig. 5a). The expression and cellular localization of cREL were thus examined by immunohistochemistry in all specimens but P1-L1. Strikingly, all tested DLBCL specimens stained positive for cREL in the nucleus (Figs. 5b to d and data not shown). Only P4-L2 exhibited mixed, predominantly cytoplasmic cREL staining. A GSEA of P3-L1, P4-L2 and P5-L1 confirmed the upregulation of transcripts associated with REL functions (such as the cell cycle/proliferation, apoptosis/survival, adhesion/architecture and innate immune cell functions). The most activated NF-κB related pathways in the three lymphomas studied were Toll-like receptor signaling (p=9.7 x 10^{-22}) and apoptosis (p=5.09 x 10^{-17}) (Supplementary Table 7). The observed over-expression of directly cREL-responsive genes (such as CCL5, CXCL9, CCL19, TNFRSF17, TNFRSF9, and BCL2A1, BIRC3, etc.) suggested constitutive activation.

To directly assess the status of NF-κB activation, Electrophoretic Mobility Shift Assay (EMSA) analysis was performed on protein extracts prepared from P3L1 and P5L1 patients. A strong constitutive NF-κB DNA binding activity composed of two major complexes was observed in both samples (supplem. Figure 2). The subunit composition of the NF-κB DNA-binding complexes was determined by supershift analysis. Antibody directed against RelA and p50 supershifted complex I almost completely. Instead complex II was effectively supershifted with anti-RelB and p50 antibodies (Figure X). Altogether, these results suggest that both the canonical (i.e. RelA) and non canonical (i.e. RelB) NF-κB activation pathways are constitutively activated in P3L1 and P5L1 patients.

To further characterize the key disease events leading to lymphomagenesis in our patients, we performed WES of P3-L1, P4-L1 and P4-L2 DNA and germline DNA in PBMCs from P3 and P4. We respectively detected 26, 22 and 9 somatic mutations in 24, 21 and 9 genes (Supplementary Table 8) in the three tumors. The numbers of somatic mutations found in these lymphomas are in agreement with data on DLBCL. Some of the somatic mutations previously associated with DLBCL were found in the patients' lymphomas, including a mutation in MYD88 (p.S219C) (P3-L1)\textsuperscript{24}. It is noteworthy that a somatic nonsense mutation in a master NF-κB regulator gene NFKBIA (p. Q68X) was identified in P4-L1, as previously
described in classical Hodgkin lymphoma. Remarkably, we did not find any somatic mutations in genes involved in histone and chromatin modifications - in contrast to events described in DLBCL. Overall, the atypical but homogeneous gene expression profiles, the amplification of 2p16 in all tumors tested, the non-conventional localization of c-REL, the constitutive activation of both canonical and non canonical NK-kB pathway, the presence of somatic mutations potentially conferring intrinsic activation of NF-kB suggested that the lymphomas in IL-10R-deficient patients represent a distinct/intermediate subtype of DLBCL.

Discussion
Here, we reported the occurrence of B cell lymphomas in five IL-10R-deficient patients. Strikingly, one patient (P4) developed at least three distinct lymphomas. This hitherto unrecognized association between IL-10/IL-10R-deficiency and lymphoma raises a number of questions. EBV-negative B-cell lymphomas were monoclonal. They contained highly proliferative, monomorphic, large B cells and showed several of the immunohistological and molecular characteristics of DLBCLs. A gene expression analysis confirmed the lymphomas' germinal center origin and revealed a strikingly homogeneous phenotype that was similar (but not identical) to that of GCB-DLBCL. A key finding was the amplification of the 2p16 chromosomal region in four tested tumors. This feature has been reported previously in between 17% and 26% of GCB-DLBCLs and in Hodgkin lymphoma. The minimal amplified region contained REL but not the closely located oncogene BCL11A. In fact, REL encodes c-REL, a member of the NF-κB transcription factor complex involved in driving cell survival and proliferation. It has been shown that mutations in REL lead to constitutive NF-κB activation in B-cell lymphomas, with the detection of NF-κB transcription factors (including c-REL) in the nucleus - as observed here for c-REL. This characteristic also distinguishes the patients' lymphomas from typical GCB-DLBCLs. The observed pattern of gene over-expression is consistent with aberrant triggering of the NF-κB pathway and is further suggested by the observation of somatic mutations that potentially lead to gain of function of the NF-κB pathway in two of the three lymphomas tested. In addition, EMSA combined with supershift analysis performed on two samples showed constitutive activation of both canonical and non canonical NF-κB pathways. This pattern in unusual for GCB-DLBCL.

The occurrence of one or more B-cell lymphomas in five patients with IL-10R deficiency cannot be viewed as a chance event, given that (i) the incidence of NHL in childhood is
1x10^{-5} per year and (ii) DLBCLs account for only 15% of childhood NHLs\textsuperscript{39}. Furthermore, the characteristics shared by all the patients' lymphomas argue strongly against random occurrence. This increased risk of lymphomas might have conceivably been related to the immunosuppressive therapy that four out of the five patients were receiving as treatment for IBD. In adult IBD, a five-fold increase in the risk of lymphoproliferative disorders has been observed in thiopurine-exposed patients\textsuperscript{15,40}. The CESAME study of almost 20,000 IBD patients reported 23 lymphoproliferative disorders (12 of which were EBV-related)\textsuperscript{15}. However, none of the 53 children with idiopathic VEO-IBD in our series (who were also exposed to thiopurine-based immunosuppression for a comparable period) developed lymphomas\textsuperscript{8}. Likewise, patients with monogenic IBDs (such as chronic granulomatous disease or XIAP deficiency) do not develop lymphoma more frequently that would be expected\textsuperscript{41,42}. Thus, in the context of IL-10R deficiency, azathioprine therapy is probably at most a weak risk factor for lymphomagenesis.

Our data therefore point to a direct relationship between the occurrence of lymphoma and a deficiency in the IL-10 pathway. Taking in account all reported patients with IL-10 (n=5), IL-10R1 (n=11) or IL-10R2 (n=19) deficiencies\textsuperscript{6,8,7,9,10,43}, the frequency of developing lymphoma is estimated to be 36% (5 out of 14) at the age of 7 years (in the absence of a previous HSCT). It is noteworthy that lymphomagenesis has not been reported in mice with impaired IL-10-mediated pathways, despite the onset of IBD\textsuperscript{11,44} - suggesting either the existence of marked differences between mice and humans or insufficient follow-up of the mice in an adequate environment. A role for the IL-10 pathway in human lymphomagenesis has been suggested by several studies showing a significant association between genetic variants in IL-10 and IL-10RB on one hand and NHL (and particularly DLBCL) on the other\textsuperscript{45,46}. Since disruption of the IL-10/IL-10R axis in both humans and mice leads to severe inflammatory enterocolitis\textsuperscript{12}, one can hypothesize that chronic inflammation may create a favorable milieu for B-cell lymphomagenesis - perhaps through protracted B-cell activation\textsuperscript{47}. However, few of the patients' nodal lymphomas were in the gut, no mucosa-associated lymphomas were detected and an activated B cell lymphoma phenotype was not observed. Furthermore, VEO-IBD was not found to be associated with lymphoma when the IL-10/IL-10R pathway was unaffected. We thus conclude that either (i) gut inflammation is not a main driver of lymphomagenesis or (ii) a distinct pattern of inflammation (related to IL-10/IL-10R deficiency) is involved in lymphomagenesis. Future research will have to evaluate these possibilities.

Hence, two mutually non-exclusive models may account for the occurrence of B cell lymphomas in the absence of a fully competent IL-10 signaling pathway. Given that IL-10
controls the proliferation of non-activated B-cells, defective signaling may lead to an increase in DNA replication and, in turn, acquisition of rearrangement events and somatic mutations. An alternative mechanism might involve a local T-cell immunodeficiency caused by impairment of the IL-10/IL-10R pathway. Surprisingly, in view of IL-10’s immunosuppressive role, recent studies of murine models have found that this cytokine promotes CD8 T-cell infiltration into tumors, local interferon γ secretion, cytotoxicity, antigen presentation and thus tumor suppression. Furthermore, IL-10 can directly induce cytotoxicity in intratumor CD8 T cells. Overall, these experimental data tent to confer a role of IL-10 in immunosurveillance. This hypothesis is attractive but remains to be tested. The scarcity of intratumor, infiltrative, granzyme-B-positive T-cells in lymphomas from IL-10R-deficient patients (relative to control DLBCLs) shown in immunohistochemical analyses and confirmed at transcriptomic level is consistent with the presence of a deficiency in an IL-10-triggered local antitumor immunity pathway. It is noteworthy that patients with heterozygous STAT3 mutations (resulting in partial loss of function) are also prone to the development of B-cell lymphomas (albeit to a lesser extent). The observation that IL-10 signaling is STAT3-dependent supports the hypothesis in which the IL-10R/STAT3 pathway is involved in controlling lymphomas. An additional role of the IL-10R2 signaling pathway could be suggested by the fact that the IL-10R2 chain is common to the IL-22, IL-26 and interferon λ (IL-28A, IL-28B and IL-29) receptors. However, the occurrence of B cell lymphoma with the very same characteristics in a patient with IL-10R1 deficiency makes this hypothesis very unlikely.

In conclusion, our results indicate that IL-10R deficiency predisposes to the development of a sub-type of diffuse large B-cell lymphomas with germinal center origin characterized by original constitutive activation of the NF-κB pathway and a defective local T cell immune response. Further work will be needed to fully elucidate the IL-10R pathway's protective effect. Meanwhile, our data support early HSCT in patients with an impaired IL-10 pathway, this procedure is able to cure IBD and may well prevent the occurrence or recurrence of lymphoma.
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Conflict of interest: None

Author contributions: B.N. designed the research, collected and analyzed data, participated in drafting of the paper and clinical care; E.M. performed and interpreted microarray studies and participated in drafting of the manuscript, J.B., D.C. and N.B. performed pathology and immunohistochemical studies and participated in drafting of the manuscript, S.K., J.M-P. and I.R-W. performed cytogenetic and micro-array studies and participated in drafting of the manuscript; F.R-L. and C.P. participated in genetic testing, functional analysis and critical review of the manuscript, V.A. and F.D. performed clonality and somatic mutations in IgV regions studies and participated in critical review of the manuscript, C.B. and P. N. performed whole exome sequencing; F.S., S.B., O.G., J-L.C. O.H., participated in clinical care and critical review of the manuscript; D.K., D.M., C.K. performed genetic and functional studies, participated in clinical care of patients and critical review of the manuscript, K.B. performed analysis of the NF-κB activation status, V.B. designed NF-κB activation study, participated in data analyses and drafting of the manuscript. B.N. designed the research and participated in data analyses and drafting of the manuscript, F.R. participated in data analyses, drafting of the paper and clinical care; A.F. designed the research and participated in data analyses, drafting of the manuscript and clinical care.
References


Table 1: Characteristics of lymphomas, treatment and outcome in 5 IL10R deficient patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Lymphoma</th>
<th>Age at Onset (years)</th>
<th>IS at Lymphoma Onset</th>
<th>Organ Involvement</th>
<th>Treatment</th>
<th>Outcome (post HSCT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>P1-L1</td>
<td>5.8</td>
<td>0</td>
<td>liver, spleen, retroperitoneal and intra-abdominal LN, bone</td>
<td>COP, COPADMx2, CYMx2</td>
<td>remission of lymphoma, EBV related LPD at 10.3 years, died from progression</td>
</tr>
<tr>
<td>P2</td>
<td>P2-L1</td>
<td>5.3</td>
<td>azathioprin</td>
<td>liver, bone</td>
<td>COP, R-COPADM</td>
<td>died during treatment</td>
</tr>
<tr>
<td>P3</td>
<td>P3-L1</td>
<td>6.5</td>
<td>azathioprin</td>
<td>liver, intra-abdominal LN</td>
<td>COP, R-COPADMx2, R-CYMx2, HSCT</td>
<td>remission, alive (+12 months)</td>
</tr>
<tr>
<td>P4</td>
<td>P4-L1</td>
<td>5.5</td>
<td>0</td>
<td>spleen, pelvis and inguinal LN</td>
<td>COP, COPADMx2, CYMx2</td>
<td>remission, alive (+18 months)</td>
</tr>
<tr>
<td></td>
<td>P4-L2</td>
<td>9.7</td>
<td></td>
<td>spleen, mediastinal and intra-abdominal LN</td>
<td>COP, R-COPADMx2, R-CYMx2</td>
<td>remission, alive (+18 months)</td>
</tr>
<tr>
<td></td>
<td>P4-L3</td>
<td>12.1</td>
<td></td>
<td>spleen, mediastinal and intra-abdominal LN</td>
<td>COP, R-COPADMx2, R-CYMx2</td>
<td>remission, alive (+18 months)</td>
</tr>
<tr>
<td>P4</td>
<td>P4-L4</td>
<td>16.5</td>
<td>6MP and MTX</td>
<td>spleen, mediastinal and intra-abdominal LN</td>
<td>R-ICEx4, HSCT</td>
<td>remission, alive (+18 months)</td>
</tr>
<tr>
<td>P5</td>
<td>P5-L1</td>
<td>6.5</td>
<td>0</td>
<td>abdominal LN</td>
<td>up-front R prephase* - 4x(2A4-2BA)</td>
<td>remission, alive</td>
</tr>
</tbody>
</table>

**P** denotes Patient; **L**, Lymphoma; **IS**: immunosuppressive therapy; **6MP**: 6 Mercapto-Purin; **MTX**: methotrexate; **LN**: lymph Node; **BM**: bone marrow; **COP**: Cyclophosphamide, Vincristine, Prednisone; **CHOP**: Cyclophosphamide, Adriamycine, Vincristine, Prednisone; **COPADM**: Cyclophosphamide, Vincristine, Prednisone, Doxorubicine, Methotrexate; **R-**: rituximab; **CYM**: Cytarabine, Methotrexate; **ICE**: Ifosfamide, Carboplatine, Etoposide; *prephase: dexamethasone, cyclophosphamide, 2A4: dexamethasone, vincristine, cytarabine, etoposide, methotrexate, Iphosphamide, 2B4: dexamethasone, vincristine, doxorubicine, methotrexate, cyclophosphamide. HSCT: hematopoetic stem cell transplantation; LPD: lymphoproliferation.
Table 2: Immunopathological features and IgH clonality of lymphomas in IL-10R deficient patients

<table>
<thead>
<tr>
<th>P</th>
<th>L biopsy</th>
<th>classification</th>
<th>CD20</th>
<th>BCL2</th>
<th>CD10</th>
<th>BCL6</th>
<th>CD30</th>
<th>KI67</th>
<th>MUM-1</th>
<th>EBER</th>
<th>c-REL</th>
<th>IgH</th>
<th>MYC</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>L1 liver</td>
<td>DLBCL</td>
<td>+++</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>P2</td>
<td>L1 liver</td>
<td>DLBCL</td>
<td>+++</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>C++/N++ monoclonal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>L1 LN</td>
<td>DLBCL</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>C++/N+ monoclonal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P4</td>
<td>L1 spleen</td>
<td>DLBCL</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+/-</td>
<td>C++/N++ monoclonal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L2</td>
<td>LN</td>
<td>DLBCL</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>C++/N+/- monoclonal</td>
<td></td>
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</tr>
<tr>
<td>L3</td>
<td>LN</td>
<td>DLBCL</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+++</td>
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<td>C++/N+ monoclonal</td>
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<tr>
<td>L4</td>
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<td>+++</td>
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<td>+</td>
<td>+++</td>
<td>+</td>
<td>C++/N+ ND</td>
<td></td>
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</tr>
<tr>
<td>P5</td>
<td>L1 LN</td>
<td>DLBCL</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>+/-</td>
<td>C++/N+ monoclonal</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P denotes patients; L lymphoma; DLBCL: diffuse large B-cell lymphoma; LN lymph node; C: cytoplasm; N: nuclear; ND not done; - negative staining, +/- heterogeneous staining, +++: strongly positive staining; Classification of lymphomas according to WHO: World Health Organisation; MYC: rearrangement of MYC locus detected by FISH.
Figure legends

Figure 1: Genetic characteristics, time course of lymphoma development and treatment in patients with IL-10R deficiency

(a) Pedigrees of the five patients with IL-10R1- and IL-10R2-deficient patients. Consanguinity (double horizontal bars), affected individuals (black boxes and circles), carriers (half-filled boxes and circles) and subjects not available for participation in the study (asterisks) are indicated. Patient 1 carried a homozygous missense mutation in exon 2 (p.Y59C) of the IL-10RB gene. This mutation was absent from genome databases (including HGMD, Ensembl and 1000 Genomes). Patient 2 was a heterozygous composite, with a frameshift mutation in exon 7 (F269fsX275) and a missense mutation in exon 5 (p.W204C) of IL-10RB. Patient 3 carried an homozygous nonsense mutation in exon 3 of IL-10RB (p. E141X, as previously reported by Begue et al). Patient 4 displayed homozygous deletion (g.11930-17413 del) in IL-10RB. Patient 5 (born to consanguineous parents) carried a homozygous c.368-10C>G mutation in intron 3 of IL-10RA.

(b) Positions of the IL-10RA and IL-10RB mutations within the gene sequence.

(c): time course of lymphoma development and treatment: P: patient, L: diffuse large B-cell-like lymphoma; LPD: EBV-positive Hodgkin-like lymphoproliferative disease; yrs: years, with occurrence shown by arrows (↓); HSCT: hematopoietic stem cell transplantation; Immunosuppressive treatment with azathioprine is represented as a grey rectangle and administration of an anti-tumor necrosis factor antibody is shown in blue. Maintenance therapy with 6-mercaptopurine and methotrexate was given in P4 after remission of L3 and is represented by a yellow rectangle. P1 (IL-10R2) died due to the progression of an EBV-related lymphoproliferative disease (despite chemotherapy); P2 (IL-10R2) died of meningoencephalitis (of undetermined etiology) that occurred during chemotherapy; P3 (IL-10R2) was in remission from lymphoma when he was transplanted from a mismatched family donor and is now alive and well 12 months after HSCT; P4 (IL-10R2) was in remission from L4 when he was transplanted from a geno-identical sibling. He is alive and well 18 months after HSCT. P5 (IL-10R1) is in remission from lymphoma and is alive and well 3.4 years after completion of chemotherapy (HSCT is pending).

Figure 2: the lymphomas' histochemical characteristics

a.b.: hematoxylin-eosin staining (HES; original magnification: x400 of P3-L1 (intra-abdominal lymph node biopsy) and P4-L2 (inguinal lymph node biopsy), showing diffuse, massive infiltration by monomorphic large lymphoid cells.
c. to f.: immunohistochemical studies on P3-L1 biopsy showing CD20 staining (c), Ki67 staining (d), in situ hybridization for EBV-encoded RNA (e) and BCL6 staining (f).

g. to l.: CD3, CD8 and granzyme B immunohistochemical studies: in control GCB-DLBCL biopsy, CD3 (g) and CD8 (h) T cells were detected at perivascular and intratumor sites. Granzyme B (i) was detected in many T cells. In the IL10R-deficient DLBCL (P4-L3) biopsy, CD3/CD8 T cells were found in the perivascular region (j,k), whereas no granzyme B was detected within the tumor (l) (x200 magnification).

m.: Counts of granzyme-B-positive cells in 5 control GCB-DLBCLs and 7 lymphomas from IL-10R deficient patients are shown. Counts per optic field (x400 magnification). Horizontal bars represent mean values.

Figure 3: Gene expression profiles

a.: Unsupervised, hierarchical clustering of IL-10R lymphoma samples and other lymphoma samples. The dendrogram is based on expression of the most variable genes. Genes whose median expression was above background and whose standard deviation across all samples was > 2 in at least one lymphoma entity were used for unsupervised hierarchical clustering (n=3364). CB denotes centroblasts, CC: centrocytes, Ton: tonsils, BL: Burkitt’s lymphoma, PMBL: primary mediastinal B-cell lymphoma, cHL: classic Hodgkin's lymphoma, DLBCL: diffuse large B-cell lymphoma, FL: follicular lymphoma, MCL: mantle cell lymphoma, NLPHL: nodular predominance lymphocytic Hodgkin's lymphoma, THRBL: T-cell/histiocyte-rich large B-cell lymphoma. Samples from P3-L1, P4-L2 and P5L1 are shown in white and are highlighted by arrows.

b.: Hierarchical clustering of IL-10R lymphoma samples and ABC- (light pink) and GCB- (dark pink) DLBCL samples. Samples from P3-L1, P4-L2 and P5L1 are shown in white and are highlighted by arrows. A list of 227 genes used to discriminate between ABC- and GCB-DLBCL was extracted via a non-parametric test implemented in the LIMMA software package (p<0.05). Gene lists were submitted to the Cluster program for calculation of the Pearson correlation coefficient (as a similarity metric) and centroid linkage clustering. The results were visualized with TreeView software.

Figure 4: Duplication of 2p16 in four lymphomas

a.: Schematic representation of chromosome 2, with the various duplicated regions spanning the 2p16 chromosomal band in samples P3-L1, P4-L1, P4-L2 and P5L1. The expanded region is indicated by a large blue column.
b.: involvement of the *REL* locus in IL-10R deficiency-associated lymphomas, as detected by FISH. The FISH analysis (using an RP11-1118K19 probe for the *REL* locus (red signals) and a chromosome 2 centromere probe (green signals)) revealed duplication of *REL* in metaphase-spread and interphase nuclei from P3-L1.

**Figure 5: Expression of cREL mRNA and protein**

a.: cREL transcript levels in tumor cells from P3-L1, P4-L2 and P5L1 in comparison with ABC-DLBCL and GCB-DLBCL controls. The horizontal bar represents the mean value.

b. to e.: immunohistochemical staining of cREL on tumor biopsies (b, d: original magnification, x400; c and e: detail from b and d, respectively), b and c: a P4-L4 sample showing nuclear expression of cREL in tumor cells and the absence of staining in endothelial cells; d and e: a P4-L1 sample showing cREL expression in both the nucleus and cytoplasm of tumor cells.
1c

P1: Colectomy, L1, LPD → Death (LPD progression)

P2: L1 → Death (meningoencephalitis during chemotherapy)

P3: Colectomy, HSCT, L1 → Alive and well

P4: Colectomy, L1, L2, L3, L4, HSCT → Alive and well

P5: L1 → Alive and well
Figure 2

The figure shows a scatter plot comparing Granzyme B+ cells in controls and IL10R deficiency DLBCL. The y-axis represents the number of Granzyme B+ cells, ranging from 0 to 70. The x-axis indicates different groups: controls and IL10R deficiency DLBCL, with subgroups labeled GCB-DLBCL (n=5) and IL10R deficiency DLBCL (n=7).

A horizontal line with data points above it represents the controls group, while a vertical line with points below it represents the IL10R deficiency DLBCL group. The p-value for the comparison is p < 0.0001.
Figure 5

(a) Scatter plot showing relative transcript levels (linearized Affymetrix values) for P5-L1, P4-L2, P3-L1. The x-axis represents DLBCL, with subcategories ABC and GCB.

(b) Image showing a specific section or region of interest.

(c) Close-up image of another area.

(d) Another image with detailed views.

(e) An additional close-up view.
A Mendelian predisposition to B cell lymphoma caused by IL-10R deficiency

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