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IBD due to PID: Inflammatory bowel disease caused by primary immunodeficiencies -
Clinical presentations, review of literature, and proposal of a rational diagnostic algorithm

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Abstract

Inflammatory bowel diseases (IBD) including Crohn's disease (CD) and ulcerative colitis (UC) have a multifactorial pathogenesis with complex interactions between polygenetic predispositions and environmental factors. However, IBD can also be caused by monogenic diseases, such as primary immunodeficiencies (PID). Recently, an increasing number of these altogether rare diseases has been described to present often primarily, or solely as IBD. Early recognition of these conditions enables adaption of therapies and thus directly benefits the course of IBDs. Here, we discuss the different clinical presentations in IBD and characteristic features of patient's history, clinical findings, and diagnostic results indicative for a causative PID. Possible predictors are early onset of disease, necessity of parenteral nutrition, failure to respond to standard immunosuppressive therapy, parental consanguinity, increased susceptibility for infections, certain histopathologic findings, and blood tests that are atypical for classic IBD. We illustrate this with exemplary case studies of IBD due to NEMO deficiency, chronic granulomatous disease (CGD), common variable immunodeficiency (CVID), CTLA-4 and LRBA deficiency. Taking these factors into account, we propose a diagnostic pathway to enable early diagnosis of IBD due to PID.

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Key words:

Inflammatory Bowel Disease, Primary Immunodeficiency, Very Early Onset Inflammatory Bowel Disease, Immunological work-up, Diagnostic Algorithm, Case Series

Abbreviations:

Activated PI3KD syndrome (APDS)

B cell receptors (BCR)

Baculoviral IAP repeat-containing protein 4 (BIRC4)

Chronic granulomatous disease (CGD)

Cluster of differentiation 25 (CD25)

Common variable immunodeficiency (CVID)

Combined immunodeficiency (CID)

Crohn's disease (CD)

CT scan (computer tomography scan)

Cytomegalovirus (CMV)

Cytotoxic T-lymphocyte-associated protein 4 (CTLA4)

Dihydrorhodamine (DHR)

Epstein–Barr virus (EBV)

Fluorescence-activated cell sorting (FACS)

Forkhead box P3 (FOXP3)

Gain of function (GOF)

Gastrointestinal tract (GIT)

Gut associated lymphoid tissue (GALT)

Haematopoietic stem cell transplantation (HSCT)

Hemophagocytic lymphohistiocytosis (HLH)

Human leukocyte antigen (HLA)

Hyper-IgD syndrome (HIDS)

Hyper-IgM syndrome (HIGM)

I-kappa-B kinase (IKK)

Immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX)

Immunoglobulin (Ig)

Inducible T-cell costimulator (ICOS)

Inflammatory bowel diseases (IBD)

Interleukin-1 receptors (IL-1R)

Interleukin-10 (IL-10)

Interleukin-10 receptor (IL-10R)

Lipopolysaccharide-responsive and beige-like anchor protein (LRBA)

Mean platelet volume (MPV)

Mevalonic aciduria (MA)

Mevalonate kinase deficiency (MKD)

Mucocutaneous candidiasis (CMC)

Mucosal lymphoid tissue (MALT)

Muramyl dipeptide (MDP)

Natural killer cells (NK cells)

NF- κ B Essential Modulator (NEMO)

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B)

Nucleotide-binding oligomerization domain-containing protein 2 (NOD2)

Peripheral blood mononuclear cell (PBMC)

Primary immunodeficiencies (PID)

Signal transducer and activator of transcription (STAT)

Reactive oxygen species (ROS)

Receptor Activator of Nuclear Factor κ B (RANK)

Regulatory T cell (T_{reg})

Severe combined immunodeficiency (SCID)

T cell receptors (TCR)

Targeted next generation sequencing (tNGS)

Tetratricopeptide Repeat Domain 7A (TTC7A)

Toll-like receptors (TLR)

Total parenteral nutrition (TPN)

Tumor necrosis factor alpha (TNF α)

Ulcerative colitis (UC)

Very early onset inflammatory bowel disease (VEOIBD)

Whole exome sequencing (WES)

Wiskott-Aldrich syndrome (WAS)

X-linked inhibitor of apoptosis protein (XIAP)

Involved players in gut immunity

The approx. 30m² surface of the gastrointestinal tract (GIT) is constantly exposed to innumerable antigens and microorganisms (1). The gut associated lymphoid tissue (GALT) is the biggest lymphoid organ of the body. A constant equilibrium of physiological inflammation enables the intestine to digest and absorb nutrients and at the same time control potentially harmful bacteria (2). Thus, an immunological balance is of utmost importance for the nutritional supply and integrity of our organism. Different immunological barriers protect the integrity of the digestive tract. The first line of defence is the epithelial barrier with its intercellular junctions, mucus producing goblet cells and Paneth cells, which produce and secrete antimicrobial peptides into the gut lumen. Secondly, intraepithelial leukocytes participate in the complex cell-mediated local immunity. In addition, leukocytes resident in the lamina propria, including cells of the innate immune system, IgA producing plasma cells, and conventional as well as unconventional T cells orchestrate intestinal immunity (Fig 1)(3). Therefore, it is conceivable that dysregulation in this multifaceted network may result in susceptibility to infection or pathological inflammation.

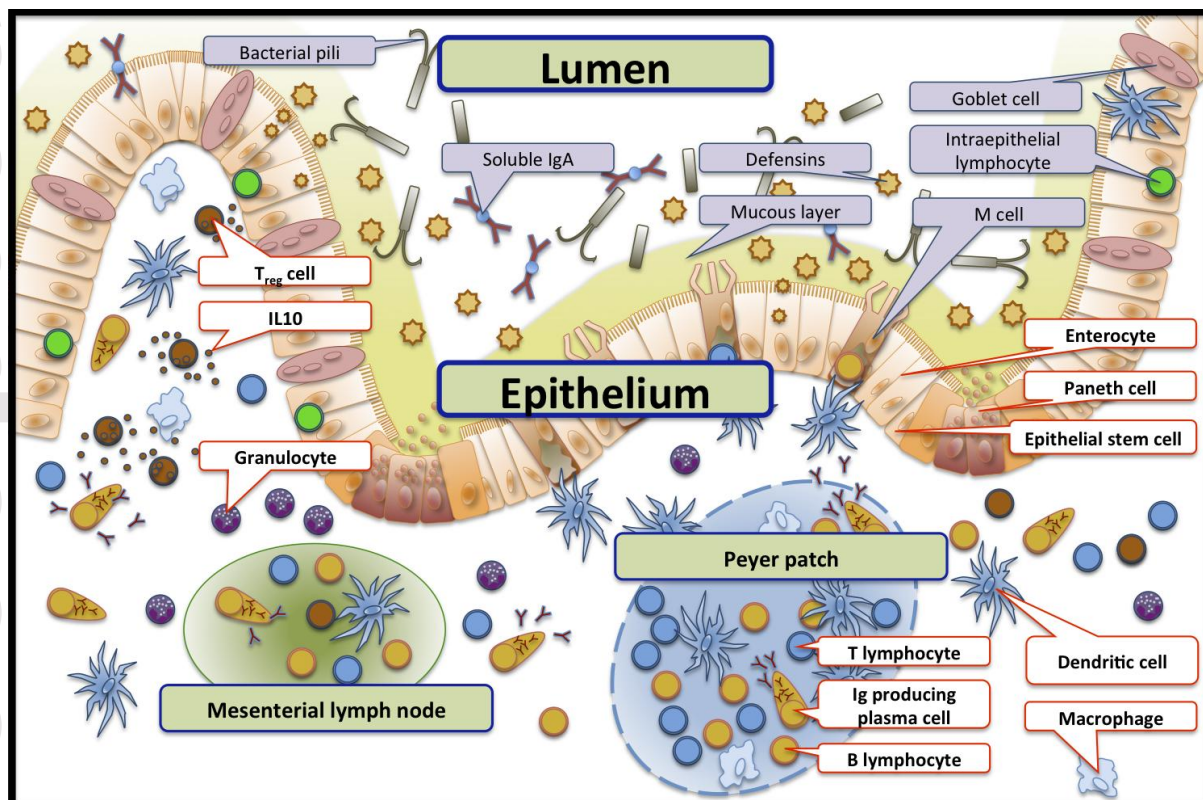


Figure 1: Simplified schematic gut immunity(4). *IgA* Immunoglobulin A, *IL-10* Interleukin 10, *T_{reg}* cell regulatory T cell

Classic IBD: Crohn's disease and ulcerative colitis

Inflammatory bowel disease is defined as a disorder with chronic idiopathic and recurrent inflammation of the GIT. Classic IBD typically presents during the third decade with recurrent or chronic diarrhoea and abdominal pain (5). In ulcerative colitis gut inflammation is limited to the colon and bloody diarrhoea is a common presentation (6). Endoscopic findings include a continuous ulcerative inflammation extending from anal to proximal parts of the colon. Histopathologically, inflammation is limited to mucosa and shows characteristic crypt abscesses (6). In contrast, Crohn's disease may segmentally affect any part of the GIT, typically the terminal ileum, resulting in abdominal pain and malnutrition as leading symptoms. The segmentally inflamed mucosa is often still intact and may show aphthous lesions, so non-bloody diarrhoea is common. However, Crohn's disease leads to a transmural inflammation that predisposes to a fistulating and stricturing course of disease and presents with characteristic granuloma formation (6). The granuloma formation is considered to be due to immunological dysregulation but the precise pathogenesis is incompletely understood (5). Extraintestinal manifestations of IBD like arthritis, erythema nodosum or primary sclerosing cholangitis illustrate the systemic immune dysregulation in IBD (7).

PID manifesting with IBD

25% of IBD present in childhood or adolescence and only 1% before the age of 6, then referred to as very early onset IBD (VEOIBD). In some PIDs gastrointestinal inflammation due to immune dysregulation is the initial or leading symptom and may manifest before other characteristics of PID (8). In other cases, IBD is the result of an increased susceptibility for infections. The majority of severe PID manifests during early childhood (8,9). However, a relevant number of PIDs (with or without isolated IBD manifestation) manifests as late-onset variants illustrated by various educative cases of XIAP deficiency (Fig. 2) (10–12).

It is difficult to obtain systematic data on PID causing IBD, as both VEOIBD and PID are rare diseases and thus systemic or prospective studies are challenging to perform. In addition, a selection and referral bias skews estimation of the true prevalence of PID causing IBD. A recent investigation of 147 paediatric IBD patients revealed 4 monogenic PID as causative (13), but due to the bias the true incidence remains elusive. However, an increasing number of reports suggest different 'red flags' of when to consider PID being causative for IBD.

Initially proposed by Uhlig et al. (and summarized in Table 1) some predictors seem indicative for PID (8). Among those are a very early onset of IBD, especially in infancy, an very severe course of disease, often indicated by the need of multiple immunosuppressive agents, the need for total parenteral nutrition as well as a suggestive family history or consanguinity (Table 1).

The gut symptoms in polygenic IBD versus monogenic IBD due to PID are indistinguishable. Also, histological findings do generally not allow differentiation of both entities. To reach a diagnosis of an underlying PID, it is therefore pivotal to be aware of possible predictors of PID and initiate further immunological investigations. Establishment of a monogenic diagnosis results in a better understanding of the pathogenesis and severity of the disease. Moreover, making a diagnosis of an underlying PID enables estimation of the prognosis and natural course of disease. Most importantly, establishing diagnosis of a PID can have a fundamental impact on the therapeutic regimen.

Medical history and physical findings

Possible predictors of a PID are an early onset of disease, a particularly severe course of disease, failure to standard immunosuppressive therapy and the manifestation of (extra)intestinal complications (8). Furthermore, parental consanguinity, a positive family history, and additional syndromic, immunological or infectious findings may give hints to an underlying PID. Especially consanguinity should be evaluated carefully, as it increases the likelihood of autosomal-recessive disorders. Table 1 summarises the most relevant aspects of the medical history when dealing with possible PID.

Important findings raising suspicion towards PID:

- 1.) Family history for IBD or Immunodeficiency
- 2.) Consanguinity
- 3.) Food allergies
- 4.) Celiac disease excluded
- 5.) Refractory course under conventional therapy
- 6.) Necessity of TPN
- 7.) Recurrent or unusual infections/fever
- 8.) Associated autoimmunity
- 9.) Perianal abscesses or fistulas
- 10.) Skin lesions, nail dystrophy, hair abnormalities [präziser]

Table 1: Important aspects of medical history when dealing with possible PID.

Gastroenterology

Naturally, given the presentation the anamnesis in IBD focuses on the gastrointestinal symptoms at first. As CU and CD present very heterogeneously (see section “classic IBD”) only a limited amount of clues can be drawn regarding a potentially underlying PID. For example, XIAP deficiency, defects in the Interleukin-10 pathway or chronic granulomatous disease (CGD), typically cause severe perianal inflammation, often accompanied by fistula (10,14,15). In TTC7A deficiency pathognomonic multiple intestinal atresia are found (16).

Infectiology

PID with impaired production of immunoglobulins or combined immunodeficiencies (impaired T and B cell function) often manifest with a pathologic susceptibility to bronchopulmonary infections, severe courses of infections, and infections with unusual or opportunistic pathogens (17). Chronic mucocutaneous candidiasis (CMC) should always trigger thorough investigations for an underlying

immunodeficiency, especially CID and IPEX(like) diseases e.g. gain of function mutations in *STAT1* or other defects in the IL-17 pathway (18–21). Also, patients with chronic norovirus or rotavirus infections (the latter esp. after vaccination) should be carefully evaluated for PID. Severe or chronic postnatal CMV infections may also be indicative for PID (22).

Hypogammaglobulinaemia, regardless of its molecular background, predisposes to intestinal infections with *Giardia lamblia*, *Salmonella spp.*, and *Shigella spp.* (17). A lack of immunoglobulin M may lead to chronic infections with *Campylobacter spp.*.

Occurrence of invasive mycosis, multiple abscesses, especially affecting the liver, lymph nodes and skin, and granuloma formation are indicators for chronic granulomatous disease (CGD). Common pathogens in CGD are *Staphylococcus spp.*, *Mycobacterium spp.* and *Aspergillus spp.* (23,24). In CGD IBD is not a cause of increased susceptibility to infections, but rather a result of immune dysregulation (25). Immune dysregulation is seen in XIAP deficiency, which is often associated with recurrent fever, splenomegaly and prolonged, severe or incompletely controlled EBV infections culminating in the development of hemophagocytic lymphohistiocytosis (10,26,27).

In future, the interpretation of local gut microbiota (microbiome) will possibly help identify an underlying immunodeficiency in patients with IBD and contribute to a better understanding of the pathogenesis of IBD in general. Recently, a cohort of CVID patients was shown to have reduced bacterial diversity and increased levels of plasmatic LPS and pro-inflammatory soluble CD25. Interestingly, these findings were most pronounced in the subgroup of CVID patients with immune dysregulation including IBD (28,29). Currently, routine evaluation of a patient's microbiome for diagnostic purposes is not recommended.

Dermatology:

Some genetic defects not only affect the immune system but also the epithelial development and function leading to conspicuous skin manifestations. Hair, nail or skin dystrophy as well as leucoplakia are typical findings in patients with dyskeratosis congenital or Hermansky-Pudlak syndrome (30,31). In NEMO deficiency signs of ectodermal dysplasia (xeroderma, thin hair, dental

abnormalities) are common. Female carriers of NEMO mutations may show incontinentia pigmenti (32). Patients with IPEX syndrome present besides IBD often with severe, early onset eczema and in addition food allergy, elevated IgE and numerous autoimmune (AI) phenomena (AI-cytopenias, -endocrinopathias, -liver, and -kidney disease) (33).

Severe eczema and an increased tendency to petechial bleeding (due to microcytic thrombopenia) are described in Wiskott-Aldrich syndrome (34). Also ADAM17 deficiency causes extended epithelial defects with eczema and hair anomalies (35). Pyogenic and pustular skin manifestations are common symptoms of an immunodeficiency and an increased susceptibility to bacterial, viral and/or fungal infections (see section “*Infectiology*”). However, skin manifestations are also observed in CU and CD exemplified by aphthous stomatitis, gingivitis, cheilitis granulomatosa, erythema nodosum, and pyoderma gangrenosum (36,37). Also, it should be kept in mind that skin manifestations are sometimes side effects of immunosuppressive drugs used in IBD therapy. For example, psoriasiform skin inflammation is seen in therapies blocking TNF α (38).

Autoimmunity and lymphoproliferation:

Further common anamnestic and clinical features of several PID are endocrinologic or autoimmune phenomena (e.g. autoimmune thyroiditis, diabetes mellitus type 1, adrenal insufficiency), classically found in IPEX syndrome (33) and the expanding field of IPEX-like diseases exemplified by deficiency of CTLA4, LRBA, CD25 or STAT5b as well as dominant gain-of-function (GOF) mutations of STAT3 or STAT1. Further autoimmune manifestations as haemolytic anaemia, arthritis and vasculitis also appear in patients with Wiskott-Aldrich syndrome (39). If lymphoproliferation with splenomegaly or lymphadenopathy is observed CVID, CID, and XIAP should be considered early as a differential diagnosis. Furthermore, autoimmune lymphoproliferative syndromes such as deficiency of LRBA or CTLA4 as well as gain of function mutations in *STAT3* should be excluded.

Endoscopy and histopathology

In contrast to adult IBD that differentiates UC and CD, paediatric IBD often presents as unspecific intestinal inflammation encompassing endoscopic and histopathologic features of both CD and UC. This is often referred to as IBD unclassified (IBDu). When the patient gets older and disease evolves, IBDu can usually be classified. This condition complicates the recognition of an underlying PID. Also,

IBD in PID are endoscopically and histopathologically poorly characterised. The few existing case reports show a very heterogeneous manifestation of intestinal inflammation which in most cases does not enable a PID diagnosis by itself. Few characteristics may prompt further PID investigations, but macro- and microscopic findings are rarely specific: A villous atrophy, typically associated with celiac disease, is also commonly seen as unspecific finding in IBD. Furthermore, it can be caused by autoimmune enteropathy. Examples are IPEX(-like) syndromes or CVID (40,41). To differentiate PID associated enteropathy from celiac disease a negative HLA-DQ2/DQ8 can be indicative. In CVID associated enteropathy a typical lack of mucosal plasma cells is described (Case 3) (42). Additionally, CVID often presents with a pronounced nodular lymphoid hyperplasia in the small intestine (43). Missing mucosal lymphoid tissue (MALT) or impaired migration of lymphocytes to the site of inflammation may hint to combined immunodeficiencies (44,45). Opportunistic bacterial, viral or fungal infections can develop in classic IBD due to immune suppressive therapies but also due to inborn (19,46,47). Chronic granulomatous disease presents typically with discontinuous ulcerative colitis, but characteristic granuloma formation is present only in half of the cases (15). Most XIAP deficient patients with IBD show a discontinuous, often ulcerative and fistulating inflammation. The inflammation affects especially perianal regions and the colon but can also be found in other parts of the GIT. There it may present as aphthae or villous atrophy and can thus be misclassified as CD. Multiple crypt abscesses seen in XIAP deficiency may also question the diagnosis of CD (10,11). However, crypt abscesses are characteristic (but non-specific) for UC and may also be seen in CD and other –also infectious- bowel inflammations.

Besides CGD and XIAP, other PID with pronounced perianal and fistulating IBD are defects in the IL-10 receptor pathway. Clinically, IL-10 or IL-10R deficiency present almost invariably as neonatal or infantile IBD (48).

In summary, endoscopic and histomorphologic features of PID associated IBD are insufficient to either confirm or exclude an underlying PID. However, taking into account the patient's history and immunologic findings, endoscopy and histopathology play a central role in characterising the suspected PID and determine the extension and severity of the IBD.

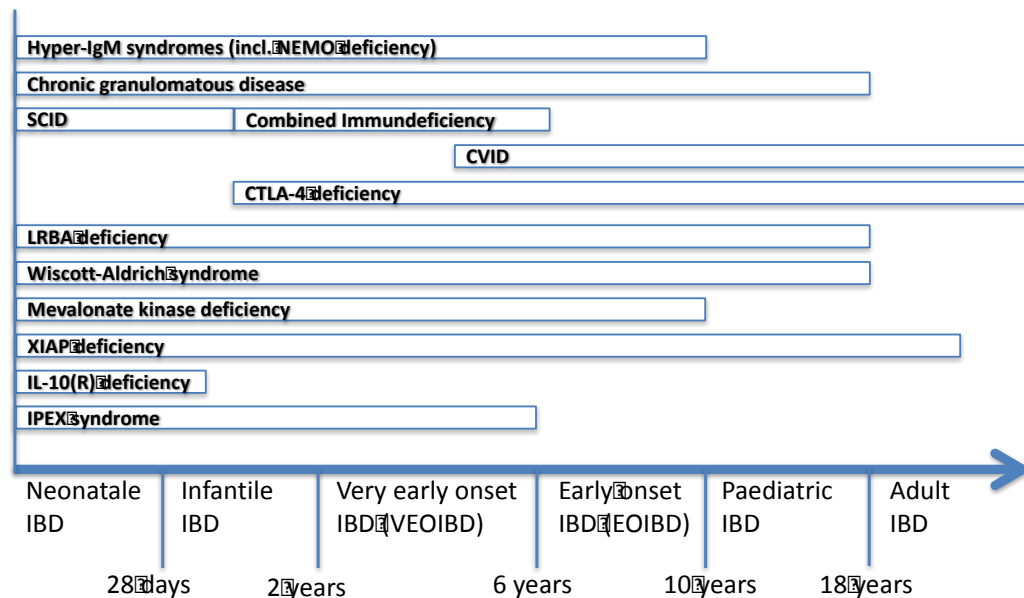


Figure 2: Early onset IBD is a predictor of PID. Adapted from Klemann and Tegtmeyer et al.(4). NEMO NF-kappa-B essential modulator, SCID severe combined immunodeficiency, CVID common variable immunodeficiency, CTLA-4 cytotoxic T-lymphocyte-associated Protein 4, LRBA LPS Responsive Beige-Like Anchor Protein, XIAP X-linked inhibitor of apoptosis protein, IL-10(R) Interleukin-10 (Receptor), IPEX immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome.

Possible predictors	Comment
Early onset of disease	PID with IBD phenotype mainly manifest during infancy (8).
Consanguinity	Parental consanguinity increases the risk for inherited autosomal-recessive diseases (49).
Severe disease	Severe disease, necessity of parenteral nutrition, complications and therapy refractory course are commonly seen in PID (8).
Untypical histopathologic findings	Untypical macroscopic and microscopic findings, e.g. non-celiac villous atrophy or lacking plasma cells may lead to an underlying PID (42,50).
Extraintestinal manifestations	Hair/nail/skin dystrophy(31), granuloma(25), autoimmunity(33), tumours(51), etc.
Recurrent or unusual infections	Many PID show an increased susceptibility for severe, prolonged, opportunistic or recurrent infections and fever(17,43,52).
Abnormal blood tests	Neutro-/lymphopenia(19,53), reduced mean platelet volume (MPV)(39), abnormal immunoglobulin levels(43).

Table 2: Summary – When to consider PID. Adapted from Klemann and Tegtmeyer et al.(4).

Overview of representative immunological disorders that may cause IBD

At least 50 monogenic defects with IBD-like phenotype are known (54). In order to better overview this very heterogeneous group, Uhlig et al. and Kammermeier et al. proposed 5 groups of IBD-related immunodeficiencies based on their different implications in systemic immunity(8,55). As a detailed review of every known monogenic cause of IBD is beyond our scope, we outline some representative PIDs from each group.

Defects of the epithelial barrier:

A key characteristic of all forms of IBD is the chronic mucosal inflammation. Any PID that compromises the integrity of the epithelial barrier may lead to an increased translocation of pathogens across the epithelium triggering an inflammatory response locally.

NEMO-Deficiency

In a large series of X-linked NF-kappa-B essential modulator (NEMO) deficient boys, IBD was the most frequent inflammatory complication affecting 13 of 61 patients (21 %). IBD was frequently associated with intractable diarrhoea and/or failure to thrive (56). Age of onset was commonly infancy or early childhood and was often characterised by severe pyogenic or mycobacterial infections (32). 90% of NEMO-deficient patients showed signs of ectodermal dysplasia, such as thin, sparse hair and eruption of conical teeth (Case 1). Female carriers may present with incontinentia pigmenti or Behçet-like immune dysregulation, the latter partly due to skewed X-inactivation (32,57,58).

At a molecular level, NEMO enables the I-kappa-B kinase (IKK) complex to activate NF-kappa-B-mediated cellular responses (32,59). The NF-kappa-B pathway accounts for multiple pro-inflammatory genes encoding e.g. toll-like receptors (TLR), T and B cell receptors (BCR, TCR), interleukin-1 receptors (IL-1R), and tumour necrosis factor receptors (TNFR) (32,59). At the same time NF-kappa-B promotes the expression of cell adhesion molecules, defensins and anti-apoptotic proteins (Bcl-2, IAPs) (60,61). Thus, the NF-kappa-B pathways play a crucial role for maintaining homeostasis between intestinal microflora and host immunity of the intestine (62). In NEMO-deficient patients, therefore, defects of the epithelial integrity favour the translocation of commensal bacteria (57). Apart from the IBD phenotype, NF-kappa-B accounts for developmental pathways (ectodysplasin, RANK, etc.).

There is no immunological assay reliably testing for NEMO deficiency. The immunological workup may reveal hypogammaglobulinaemia and impaired production of specific antibodies in some patients (56). Thus, with a clinical presentation suggestive for NEMO deficiency, genetic analysis of the NEMO encoding *IKBKG* gene is indicated and necessary for diagnosis. A haematopoietic stem cell transplantation (HSCT) reconstitutes the defect in immune cells, but does not affect the epithelial component and thus bacterial translocation (63). Therefore, after successful HSCT the persisting bacterial translocation is met by a re-established immune system. Thus, the IBD may be worsened after HSCT (57). TNF α antagonists have been described to successfully treat IBD in NEMO deficiency (and female carriers with Behçet-like immune dysregulation, too) (57).

Case 1: NEMO deficiency

In a German family 3 females suffered from several autoimmune phenomena including uveitis, erythema nodosum, polyarthritis, hepatitis and recurrent oral ulcers. As two of them were HLA-B51 positive, they were diagnosed with familial Behçet's disease. Two females displayed abnormal skin pigmentation (incontinentia pigmenti).

The first son of one of them suffered from neonatal-onset IBD. Apart from enteropathy, the boy suffered from recurrent septic episodes which were believed to be due to a strong immunosuppressive treatment with steroids and cyclosporine. Following a blunt abdominal trauma, he died at 4 years of age with septic peritonitis.

His younger brother suffered from severe neonatal staphylococcal scrotal cellulitis and necrotising fasciitis. In the following weeks he also experienced an interstitial pneumonia and two episodes of *Enterobacter cloacae* meningitis. Dental abnormalities and pustulous skin lesions were noted. The boy was referred to an immunological centre and diagnosed with NEMO deficiency based on family history and clinical presentation of mother and child. Following allogeneic HSCT, the patient developed inflammatory bowel disease. Endoscopy showed an ulcerative colitis and later pseudopolyposis. Histopathologically, weak neutrophilic infiltrates in duodenum and abundant neutrophils at the site of the colon were



Dysfunctional neutrophil granulocytes:

Neutrophils are part of the innate immune system and play a major role in eliminating bacteria and fungi (64). Reduced absolute numbers rarely ever result in neutropenic colitis (53). By far the most common cause of IBD due to neutrophil dysregulation is chronic granulomatous disease.

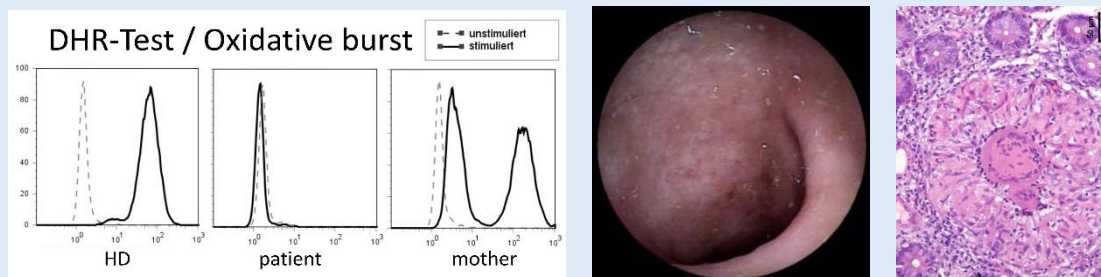
Chronic granulomatous disease (CGD)

Among PID, CGD is relatively frequent with a prevalence estimated to be 1/250.000 (65). Causative are mutations in the NADPH oxidase (phox) complex rendering neutrophils unable to produce reactive oxygen species (ROS) and thus unable to kill phagocytosed bacteria or fungi. There are 5 subunits of phox (*CYBB*, *CYBA*, *NCF1*, *NCF2*, *NCF4*). Mutations in the x-linked gp91-phox subunit encoded by *CYBB* are the most common cause of CGD, as the other subunits are inherited autosomal-recessively (25). The exact mechanisms of granuloma formation in CGD are not fully understood, but thought to be an attempt by monocytes prevent infectious agents from spreading (25). About 40 % of patients with CGD develop non-infectious IBD mimicking CD (65,66). A cohort of 87 patients with CGD and GI symptoms revealed a histopathologically heterogeneous picture with granuloma formation, eosinophilia, pigmented macrophages, acute neutrophilic or chronic lymphoplasmocytic inflammation and ulcers (67). Even though lesions could be found in any part of the GIT, colon was the most affected part of the GIT. No infectious trigger was identified in these lesions (67). Therefore, IBD in CGD represents a autoinflammatory syndrome caused by impaired regulatory functions of the NADPH oxidase: The lack of ROS impairs autophagy and leads to an increase of IL-1 production (68). Impaired neutrophilic function seems to trigger and uphold a dysregulation of the local immune response which, however, does not appear to result in local infections with commensal microflora. This is consistent with the observation that levels of anti-microbial antibodies do not correlate with the presence of GIT symptoms (69). CGD is diagnosed by the Dihydrorhodamine (DHR)-Test based on changes of a dye by neutrophilic oxidative burst reaction. This test is a sensitive, specific and low-priced screening method and should be performed in all (VEO)IBD, even in absence of other symptoms typical for CGD, such as abscesses or invasive fungal infections. The clinical course of CGD correlates with the degree of (residual-) activity of the phox which depends on the mutation. It tends to be milder a condition in autosomal recessive variants (65). Therapeutically, prophylactic treatment with antibiotics and antifungals is indicated. Facing the often life-threatening infectious complications in CGD, HSCT is advisable in most cases. HSCT leads to a complete reconstitution of neutrophil function and may lead to resolution of all CGD symptoms including IBD (25). In line with the observed increase of IL-1 production, clinical

improvement of CGD-colitis was achieved after IL-1 blockade (70). Due to the significant increase of infectious complications the indication for anti TNF- α directed therapies should be used with caution.

Case 2: Chronic granulomatous disease (CGD)

At 15 months of age the first son of non-consanguineous German parents developed lymphadenitis which healed without complications under antibiotic treatment. In the diagnostic work-up a microcytic anemia became apparent and was treated with oral iron substitution. At 24 month of age the boy presented with loose stools 6-8x/day and was treated as a presumed paradoxical diarrhea. At 30 months of age the child showed failure to thrive and a gastroenterological work-up was initiated. Calprotectin was markedly elevated without evidence of infectious agents. Endoscopy showed macroscopic signs of ulcerative colitis, while histology was compatible with medium active Crohn's disease. The immunological workup that was performed due to the early onset of IBD showed a compromised oxidative burst. The mother was subsequently assessed and had a typical pattern of a conductor with only one allele affected (see below image). The diagnosis of X-linked CGD was confirmed genetically showing a mutation in Exon 7 in the gene *CYBB*. Subsequently, the boy successfully underwent allogenic HSCT from a 10/10 donor. Currently, 200 days post-HSCT, the boy is alive and well. Gastroenterological symptoms resolved completely.



Defects in T and B cell function:

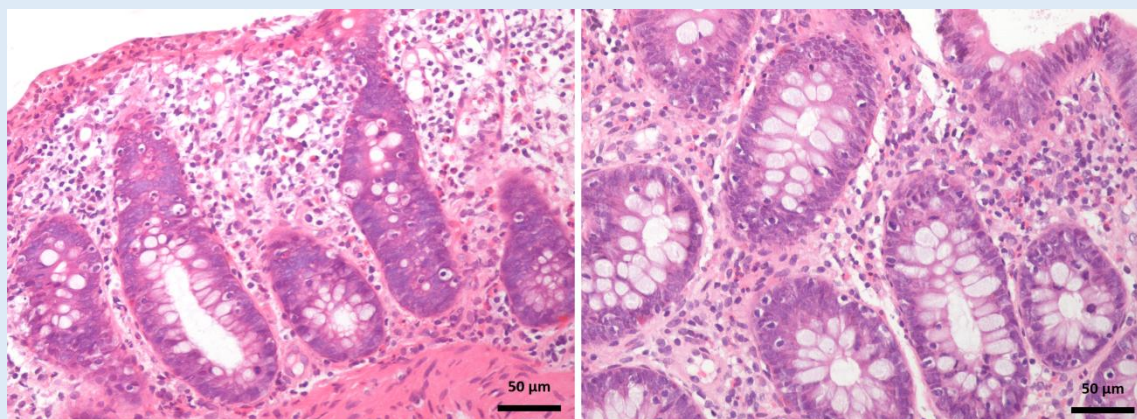
Lymphocytes are the backbone of adaptive immunity. Dendritic cells process and present pathogens to B and T cells. Helper T cells activate and recruit B cells that mature within the follicles of the Peyer's patches to class-switched immunoglobulin secreting plasma cells or long-lived memory cells. Others migrate to nearby lymph follicles and initiate a systemic immunological reaction. Cytotoxic T cells are able to kill virus-infected cells. T regulatory T cells have multiple regulative functions and are discussed separately further below.

Chronic variable immunodeficiency (CVID)

CVID is the most frequently diagnosed primary immunodeficiency requiring therapy. Its prevalence is estimated at 1 in 25,000 to 50,000 (18). CVID is a heterogeneous group of conditions with antibody deficiency. CVID is diagnosed if levels of at least 2 immunoglobulin isotypes (IgG and IgA, IgM, or both) are below the normal range, together with an reduced specific antibody response to vaccination or recent infections (71). This heterogeneous group of diseases presents often with normal total B cell counts but disturbed maturation and differentiation to plasma cells and switched

Case 3: Chronic variable immunodeficiency (CVID)

At 6 years of age the daughter of consanguineous Lebanese parents developed a type 1 diabetes mellitus. Also, she suffered from recurrent episodes of diarrhea of unclear origin. From age 8 to 10 she suffered from pneumonia four times. Immunological investigations showed IgG was normal, but IgM, IgA and IgG2 & IgG4 subclasses were reduced. Despite vaccination no titers for tetanus, diphtheria, haemophilus influenzae B, measles or pneumococcus could be found. Thus, the diagnosis of Common Variable Immunodeficiency (CVID) was established. After initiation of IgG substitution, the susceptibility for infections was initially controlled. At 14 years of age the patient suffered of interstitial pneumonia. At age 19 to 21 the patient suffered from chronic recurrent Campylobacter-associated diarrhea. At 25 years of age the patient developed severe distal colitis with >20 bloody stools per day. Infectiology work-up was negative. Colonoscopy showed severely inflamed mucosa with aphtous lesions. Histology showed an autoimmune colitis with multiple apoptosis and cryptitis. Typically, plasma cells were absent. Rectal budesonide and systemic steroids failed to control the symptoms and the patient is currently undergoing further immunological testing.



memory B cells (72). In some patients, T cell function is mildly affected. An increasing number of different monogenic defects have been described to cause CVID. Clinically, patients with CVID suffer from recurrent bacterial infections, particularly of the respiratory tract (72). 20%-60% of patients with CVID show GIT manifestations, mostly chronic diarrhoea, weight loss and malabsorption (18). Clinically and endoscopically CVID may mimic other pathologies e.g. celiac disease and IBD (73,74). However, hypogammaglobulinaemia and histopathologic biopsies with few or absent plasma cells and low intestinal IgA and IgM are suspicious of a CVID enteropathy (75).

The pathogenesis of chronic intestinal inflammation in CVID remains to be elucidated. A disturbed T cell homeostasis may be involved in the development of intestinal inflammation (75). This notion is supported by the observation that GIT symptoms are more frequent in CVID patients with T cell defects, for example ICOS deficiency. ICOS is expressed on activated T cells and orchestrates the formation of germinal centres and differentiation of B cell to antibody-producing plasma cells (76). Furthermore, low mucosal IgA levels may lead to luminal bacterial overgrowth (40). According to numerous reports about the impact of alterations of the microbiota for the pathogenesis of IBD, intestinal inflammation in CVID may also be triggered by infectious agent (5). Whether or not this hypothesis can be further supported remains to be determined. Immunoglobulin replacement therapy, at least in our experience, reduces enteric infections in CVID, but does not appear to ameliorate the course of IBD in CVID, while others have reported a positive impact in IBD in this patient group (74,77).

Severe combined immunodeficiency (SCID)

SCID is the most severe form of PID and is characterised by the absence of functional T cells and frequently of B cells, too. This complete failure of the adaptive immunity renders the affected infants prone to severe invasive infections. Affected patients may present with severe diarrhoea and malabsorption early in life (18). Intestinal biopsies show a hypocellular lamina propria, without plasma cells or lymphocytes (45). Early HSCT is always indicated for infants die within the first 1-2 years of life without an adequately functional immune system (78).

Combined immunodeficiency (CID)

The clinical presentation of the combined immunodeficiencies (CID) ranges between SCID and CVID. In contrast to CVID, T cell compartment is more markedly affected in CID, but the condition is less severe thus allowing survival past infancy. T cell numbers, especially naïve T cells, are reduced and their function impaired. The clinical spectrum of CID is more variable. Any paediatric patient presenting with “CVID” should thus be evaluated for CID. A rapidly increasing number of monogenetic defects resulting in ‘bona fide’ CID has been described recently (79). In addition, heterogeneous hypomorphic mutations in SCID genes may present as CID, but would better be termed “atypical SCID” or “leaky-SCID”. In these SCID variants residual numbers and functions of B and T cells result in a milder immunodeficiency than in SCID (19). Chronic diarrhoea is found in 29% and IBD in 6% of cases which may be considered as atypical SCID (19,80). T-cell dysfunction can act

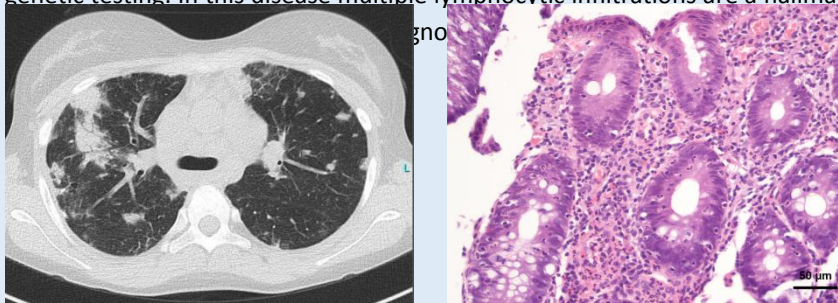
as a double-edged sword: It renders the patient susceptible to infections by an immune response too weak to eliminate pathogens, while causing autoimmunity by an immune response, strong enough to harm autologous tissue, for example the gut. A condition that often requires immunosuppressant treatment. Isolated IBD due to CID is rare; most times IBD is accompanied by recurrent or severe infections and symptoms due to immune dysregulation. Hypogammaglobulinaemia or low lymphocyte counts should prompt early investigation of (naïve) T, B, NK cells. CID patients should be carefully evaluated for curative HSCT (19).

CTLA4 deficiency

Cytotoxic T lymphocytic antigen-4 (CTLA4) is constitutively expressed on regulatory T cells and is a dominant suppressor of pro-inflammatory effector T cell function. CTLA4-deficiency results in impairment of regulatory T cells, hyperactivation of effector T cells, and lymphocyte infiltration of organs including the intestines (81). CTLA4 deficiency usually manifests in children and adults with diarrhoea, enteropathy, granulomatous-lymphocytic interstitial lung disease, recurrent respiratory infections, severe autoimmunity, and lymphoproliferation (82). Hypogammaglobulinaemia, impaired differentiation to class-switched memory B cells, and a progressive decrease in absolute B cell numbers is commonly observed (82). Treatment includes antibiotic prophylaxis and immunoglobulin substitution. In patients with lymphocytic organ infiltrations a combined therapy of immunosuppressive medication (e.g. prednisone, rapamycin, and rituximab) has reported to be effective. Abatacept, a CTLA4 fusion protein mimicking the body's own CTLA4 protein has shown promising results. Successful HSCT cures the disease (83).

Case 4: CTLA4 deficiency

A previously healthy 12-year-old girl of Georgian descent presented with EBV associated lymphadenopathy and autoimmune hemolytic anemia. Diagnostic work-up showed lung involvement and diagnosis of a sarcoid-like immune dysregulation was established. In the following years the girl suffered of numerous pneumonias and further autoimmune phenomena. Decreased IgG and IgA lead to the diagnosis of CVID. Lung function decreased and CT scans showed deteriorating interstitial lung disease. Lung biopsy showed massive lymphocytic infiltrates, establishing the diagnosis of an interstitial pneumonia. Additionally, the spleen was severely enlarged. Multiple immunosuppressive regimens including steroid-pulses, MMF, rituximab, cyclosporine, and sirolimus failed to control the disease. Despite continuing immunosuppressive therapy she developed refractory bloody diarrhea. Endoscopy showed duodenal and colonic inflammation. Histology showed autoimmune enteropathy with duodenal villous atrophy and intraepithelial lymphocytosis. Plasma cells were present. In the colon a marked florid inflammation with crypt abscesses predominated, without significant intraepithelial lymphocytes. Autoantibody titers were negative. Ultimately, the diagnosis of a CTLA4 deficiency could be established by genetic testing. In this disease multiple lymphocytic infiltrations are a hallmark, explaining all symptoms of



LRBA deficiency

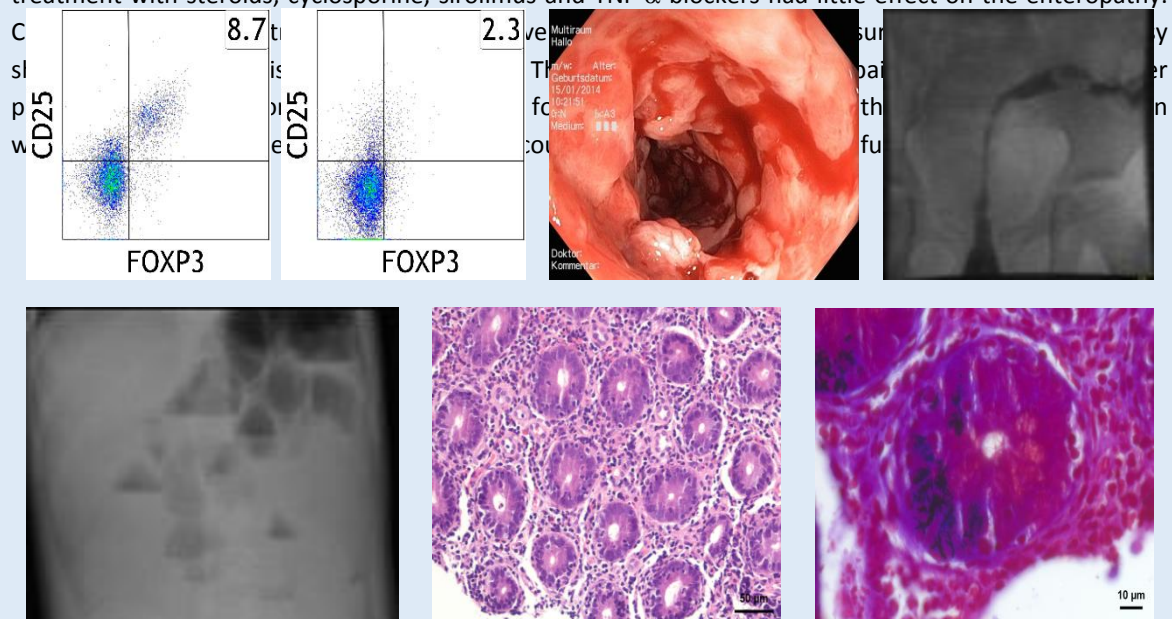
LRBA is a recently detected widely expressed protein that has been implicated in regulating endosomal trafficking of ligand-activated receptors (84–86). Specifically, LRBA was shown to co-localize with anti-inflammatory CTLA4 in endosomal vesicles and regulate CTLA4 turnover in regulatory and activated conventional T cells. Thus, LRBA deficiency results in reduced levels of CTLA4 and consecutively impaired control of inflammation (87,88). A cohort of 22 LRBA deficient patients mainly presented in childhood with immune dysregulation/autoimmunity (95%), organomegaly (86%) and recurrent infections (71%) (89). Enteropathy was the most common immune dysregulation (65%). Typical findings in the immunological workup are hypogammaglobinaemia, a defective specific antibody response, low B cell counts, predominantly of class switched plasma and memory cells, and decreased regulatory T cell numbers while overall T cell counts are normal (89). Treatment includes immunoglobulin substitution and various immunosuppressive regimens (steroids, cyclosporine, mycophenolate mofetil, sirolimus, rituximab, or abatacept) (87,89,90). Again, successful HSCT is curative.

Case 5: LRBA deficiency

The first daughter of non-consanguineous Kazakh parents developed at 15 month of age chronic secretory diarrhea. No infectious agent could be identified. Endoscopy revealed duodenal villous atrophy. Autoantibodies were negative. Until the 4th year of life diarrhea persisted with little response to the treatment with steroids and cyclosporine. Additionally, the girl suffered from severe eczema. Immunological investigations showed low IgG, absent IgA, and revealed normal T cells counts, while B and NK cells were low. As the T cell compartment appeared normal, combined immunodeficiency was unlikely. Due to the severity of the clinical presentation and the refractory course to all immunosuppressant regiments, the child was selected for HSCT. She died during preparation of stem cell therapy due to a catheter sepsis at 7 years of age.

Her sister developed persistent enteritis with noro-, adeno-, and rotavirus at 4 years of age. In the following years variable autoimmune cytopenias and hepatosplenomegaly suggested an immunological disease. At 5 years of age the girl developed chronic diarrhea. Endoscopy showed follicular hyperplasia in the duodenum.

At 10 years of age the enteropathy worsened with increasing amounts of bloody diarrhea. Endoscopy revealed a severe ulcerative colitis. Histopathologically, a predominantly lymphocytic, partly granulocytic inflammation was noted. An autoimmune aetiology was suggested. Stromal cells were absent. In immunological workup, total IgG, specific IgG-antibodies and B cell counts were decreased. B cell differentiation showed absence of class switched memory B cells and plasma cells. FOXP3 expression was markedly decreased. Genetic diagnosis of LRBA deficiency could be established. Immunosuppressive treatment with steroids, cyclosporine, sirolimus and TNF- α blockers had little effect on the enteropathy.



Hyper- or autoinflammatory disorders:

Classic IBD is a state of intestinal hyperinflammation triggered by a multifactorial aetiology, including multiple genes (91). In contrast to classic IBD some monogenic disorders specifically affect the physiological tolerance of the adaptive system and cause autoinflammatory reactions against intestinal mucosa. This autoinflammation is typically mediated by T cells and hypersecretion of pro-inflammatory cytokines.

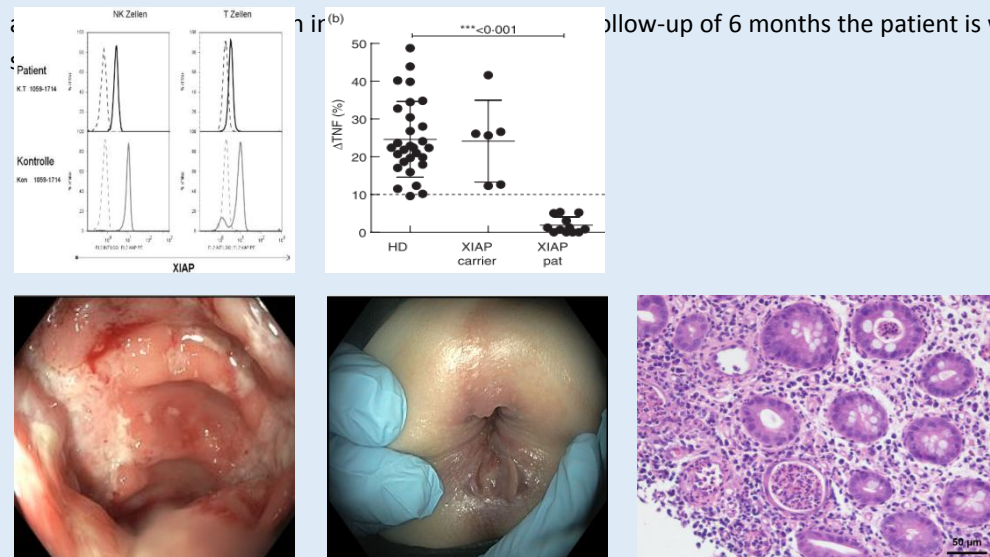
X-linked inhibitor of apoptosis protein (XIAP) deficiency

XIAP is a protein encoded by the *birc4* gene, which is ubiquitously expressed. Numerous functions have been identified illustrating a central role for the enhancement of an effective immune response and control of inflammation. As inhibitor of caspase mediated apoptosis in activated T cells, XIAP enables a sufficient expansion of cytotoxic T cells (92). This defective anti-viral defence predisposes for prolonged viral infections, e.g. EBV. Additionally, in myeloid cells XIAP has been shown to enhance NF- κ B dependent immune responses triggered by pathogen binding to NOD2 (93). Also, XIAP inhibits the inflammasome (94). Impaired regulation of the inflammasome causes hyper- and autoinflammatory conditions that can manifest as HLH and IBD, but also uveitis, arthritis, erythema nodosum or nephritis (10,26,95,96). 20% of XIAP-deficient patients develop a CD-like phenotype that often may evolve as late-onset IBD and even be their first and sole manifestation of XIAP associated symptoms (97). The course of disease is particularly complicated by extensive fistulas. Of note, the frequency of XIAP deficiency may be underestimated as recent studies show an prevalence of *birc4* mutations in 1,5-4 % in male paediatric cohorts (11–13). Currently, there is no clear therapeutic concept for this rare disease. To our experience, some patients respond well to conventional regimens such as TNF-antagonists, while in others HSCT (and thus restoring XIAP expression in leukocytes) proved successful to control the disease, including IBD (98).

Case 6: XIAP deficiency

At the age of 9 years a boy of non-consanguineous German parents suffered from a prolonged EBV infection. Because of this atypical manifestation of disease immunological workup was performed. Via FACS analysis a defective XIAP expression on monocytes was seen. Also, via 18-MDP stimulation assay, a loss of XIAP mediated NOD2 signaling could be demonstrated. The diagnosis of XIAP deficiency was confirmed genetically. At 14 years of age, months after a first episode of hemophagocytic lymphohistiocytosis, the boy developed IBD with chronic diarrhea, proctitis, enoral aphthae, massively increased calprotectin and a weight loss of 14 kg body weight within 2 months. Endoscopy revealed multiple oesophageal ulcers and a severe ulcerative pancolitis. Histopathologically, crypt abscesses and increased stromal lymphocytes were present. Without the immunological diagnosis the gastroenterological findings could not have been discriminated from classic IBD. An anti-TNF- α directed therapy (infliximab) was started. Already after the first dose diarrhea and aphthae completely resolved

After follow-up of 6 months the patient is well and the IBD



Mevalonate kinase deficiency

Mevalonate kinase deficiency (MKD) was formerly referred to as hyper-IgD syndrome (HIDS) and its more severe variant of mevalonic aciduria (MA). MKD is inherited in an autosomal recessive fashion and causes an increased caspase-1 activity leading to hyper-secretion of pro-inflammatory IL-1 β mainly by monocytes. IL-1 β orchestrates the acute phase response (99,100). MKD manifests in most patients with recurrent febrile attacks with lymphadenopathy, diarrhoea, skin lesions, hepatosplenomegaly and abdominal pain. Half of the patients develop an inflammatory bowel disease, autoimmunity and/or suffer from recurrent infections (99). Symptoms occur in 50% before the age of 6 months and 92% before the age of 6 years (99). In almost all patients increased urinary mevalonic acid concentrations and serologic IgD levels are found. Half of the patients also present

elevated levels of IgA (99). Conventional treatment options are non-steroidal anti-inflammatory drugs and corticosteroids. In 30% of patients anti-IL-1 antibodies (e.g. anakinra and canakinumab) are a successful treatment option (99). Successful HSCT has been reported (101,102).

Defects of regulatory T cells:

Regulatory T cells are a subpopulation of T cells that modulate the immune response. Their immunosuppressive functions maintain tolerance to self-antigens and prevent autoimmune or hyperinflammatory diseases. Mainly, they suppress or downregulate activation and proliferation of effector T cells by secreting IL-10 in a FOXP3 dependent manner or expression of CTLA4 (103).

IPEX syndrome

In immune dysregulation, polyendocrinopathy, enteropathy X-linked (IPEX) syndrome a mutation of FOXP3 factor leads to a loss of functional regulatory T cells and their IL-10 mediated anti-inflammatory role (104). The consequences are autoinflammation, typically autoimmune enteropathy, alopecia, skin eczema and polyendocrinopathy (e.g. diabetes, adrenal insufficiency) (33). Symptoms usually manifest during infancy with the triad of intractable watery and sometimes bloody diarrhoea, type-1 diabetes, eczema and food allergy. Common laboratory findings are elevated IgE titers and eosinophilia. Due to the enteropathy low plasma protein levels including IgG, IgA and IgM are seen. Differential blood counts may initially show a moderate lymphocytosis with normal subsets (105). Depending on the type of FOXP3-gene mutation regulatory T cell counts may be absent or severely reduced. In rare cases a normal expression can be seen (106). IPEX syndrome often has a fatal course regardless of the type of mutation (33). The enteropathy causing anti-AIE-75 and anti-villin autoantibodies that have been found in a number of patients with IPEX appear to be highly specific for IPEX enteropathy (107,108). Yet, only few laboratories have established assays to detect them. Gut biopsies show villous atrophy with mucosal lymphocytic and eosinophil infiltration, which are not IPEX-specific and may mimic graft-versus-host or celiac disease (109). As FOXP3 is encoded on the X chromosome generally only male infants are affected. The only curative therapeutic option is an allogeneic HSCT, which in the last couple of years has become increasingly successful. Survival rates remarkably increase when patients undergo HSCT before the age of 5 years (110). In few patients immunosuppressive treatment with steroids and calcineurin inhibitors could control the disease (41).

IL-10 deficiency and IL-10 receptor deficiency

IL-10 is an anti-inflammatory protein that binds to the IL-10 receptor (IL-10R) and restrains activated cells of the innate and adaptive immunity, thus preventing hyperinflammation and autoimmunity (111). A IL-10/IL-10R deficiency causes a severe form of neonatal onset IBD with pronounced perianal disease and fistulas (14,112). Two cohorts of 9 and 13 patients with infant IBD revealed 4 and 2 cases of IL-10R deficiency (14,113). A third cohort of 66 patients with VEOIBD revealed 13 cases of IL-10R and 3 cases of IL-10 deficiency (112). Histopathology often shows ulcers of the intestinal mucosa with inflammatory infiltrates of the epithelium as well as abscesses and granulomas (14,114). Extraintestinal symptoms include folliculitis, recurrent infections, arthritis and B-cell lymphoma (14,51,112,114). Typically, routine immunological work-up appears normal in both conditions. IL-10R deficiency can be easily tested by functional assays. Upon stimulation with IL-10 PBMC of patients show phosphorylation of STAT3 (115). Even more rare than IL-10R deficiency is lack of IL-10 itself. Since *in vitro* assays testing IL-10 synthesis are not established for routine diagnostics, sequencing of the IL-10 gene is recommended (115). IBD in IL-10(R) deficient patients manifests very early in life and is unresponsive to conventional anti-inflammatory treatment including anti-TNF-antibodies. Thus, early surgical interventions such as colectomy, resection of fistulas and stoma formation as well as need for parenteral nutrition are common (112,114). HSCT is the treatment of choice leading to sustained remission of the disease (114). Therefore, in all forms of neonatal onset IBD early functional and genetic testing of the IL-10 pathway is recommended. If the diagnosis is confirmed, HSCT within the first year of life is indicated.

Which diagnostics and when?

For paediatric patients with a diagnosed IBD we suggest a rational, step by step diagnostic approach to determine the potential risk of an underlying PID. The rationale for each investigation is outlined below and summarised in *Figure 3*. Routine gastroenterological diagnostics include patient's history and careful examination with a focus on the findings discussed above. Obviously, endoscopic findings, inflammatory markers, potential microbiological affiliations or diagnostics for celiac disease should be reviewed for abnormalities. Next, basic immunological investigations should be performed.

Basic immunology: Differential blood count and immunoglobulin titers

A differential blood count and immunoglobulin levels are easily available in clinical routine and should always be done in patients with IBD. Especially neutrophil and lymphocyte counts and levels of the different immunoglobulin isotypes IgG, IgA, IgM, and IgE may be altered in several PID. In the differential blood count myeloid and lymphoid defects may stand out with neutro- or lymphopenia. A reduced mean platelet volume or thrombocytopenia together with eczema is suggestive for Wiskott-Aldrich syndrome. Anaemia is sometimes not only due to chronic inflammation but also manifests as haemolytic anaemia in PID with autoimmunity (e.g. IPEX(-like) syndromes such as CTLA4 or LRBA deficiency). Antibody titres broadly reflect the function of the B cells compartment. It has to be kept in mind that low IgG titres may also be secondary to protein loss via an inflamed gut mucosa. As the different antibody isotypes represent different stages of B cell development, pathologic levels may give hints to different defects in B cell differentiation. For example, in Hyper-IgM syndromes (HIGM) or CVID low IgG levels are found (71,116). Of note, in HIGM the GIT problems are mostly due to infections (*Cryptosporidium parvum*, *Giardia lamblia*, *Salmonella*, *Entamoeba histolytica*), while in CVID the cause of IBD commonly is immune dysregulation. The hypogammaglobulinaemia in both conditions is due to defects of B cell differentiation and class switch into IgA and IgG producing plasma cells and long-lived memory cells. A lack of IgG mediated immune responses predisposes to respiratory infections and bronchiectasis (72). Deficiency of IgA that is produced by mucosal plasma cells and secreted into the intestinal lumen may weaken the local mucosal immune response. An isolated IgA deficiency is quite common with a prevalence of 1:400-800 in the general population and 2-4% in patients with celiac disease (117). The relatively high prevalence of IgA deficiency in celiac disease complicates the diagnosis of celiac disease, since most disease-related antibodies are of IgA nature (117). In most cases, however, isolated IgA deficiency is of no clinical significance. We do not recommend to measure mucosal IgA levels which is of no additional value. There is no known condition with a mucosal IgA deficiency and normal systemic levels (118). In patients with IPEX-syndrome increased levels of IgE and eosinophilia are common findings (41). Patients with mevalonate kinase deficiency present in early infancy with flares of fever and elevated IgD and IgA titers, which are present in 100% and 50% of cases, respectively (99). If hypogammaglobulinaemia is noted, specific IgG antibody titres against vaccine antigens (e.g. tetanus & measles) as well as IgM alloagglutinins should be assessed. This may detect an impaired antigen-specific immunoglobulin production requiring a detailed immunological work-up.

Advanced immunology: Specific antibody titers, oxidative burst, lymphocyte subsets, specific protein staining

In VEOIBD or when history, physical or basic immunologic findings are suggestive for an underlying PID, diagnostics should include a more detailed immunologic workup (Figure 3). We suggest a collaboration with a paediatric immunologist with specialised laboratories. In the future, genetic screening tools may replace our present rational and stepwise diagnostic approach.

Characterisation of lymphocyte subsets aids to diagnose defects of the adaptive immune system. Decreased numbers of T cells, especially naïve T cells, with or without B cell lymphopenia are found in combined immunodeficiencies (CID), but also in a number of other defects which may also have a CID component e.g. CTLA4 (119). In CIVD and CID impaired B cell maturation can be found (72,119). Absent or severely reduced regulatory T cells are a hallmark characteristic of IPEX-syndrome, but may also occur in CTLA4 and LRBA-deficiency (case 5) (89,106). FACS analysis of FOXP3 is a feasible tool to measure the protein expression, albeit some rare mutations resulting in sustained but dysfunctional protein expression may be missed.

In patients with chronic granulomatous disease (CGD) the defective neutrophilic oxidative burst can be tested functionally by stimulating macrophages and assessing release of oxygen radicals. Of all IBD associated PID CGD appears to be most common condition. In XIAP deficiency aberrant protein expression can be tested by FACS analysis and impaired protein function by stimulating monocytes with muramy dipeptide (MDP) that signals via NOD2 and XIAP (L18-MDP assay) (120). FOXP3 (IPEX syndrome) and XIAP are X-linked conditions and thus should be tested in male patients. Defects of the IL-10(R) pathway present early in infancy with severe perianal IBD (48). Thus, IL-10 tests are appropriate in infant IBD. Functionally, leukocytes from IL-10R deficient patients fail to phosphorylate STAT3 upon stimulation with IL-10 (115). In contrast, IL-10 deficiency is diagnosed genetically, because currently functional assays are not available (115).

Genetic analysis: Single gene analysis, targeted next generation sequencing (tNGS), whole exome sequencing (WES).

Currently, immunologic tests as outlined above should be the basis of any immunological diagnostic workup. In contrast to genetic analysis, functional tests are relatively easy accessible and inexpensive. If a specific immunological disorder is suspected, sequencing of the candidate gene is

performed to prove the diagnosis and determine the type of mutation. Recently, major efforts were made to establish panel diagnostics that tests up to 100 PID associated genes via tNGS. This targeted approach differs from WES, which decodes the whole genome and may also reveal new unknown genetic disorders. However, WES produces extensive amounts of data that requires a substantial bioinformatics for correct interpretation. In healthy individuals WES reveals up to 40-100 mutations in approximately 5-10% of the genome that is currently characterised. The relevance of these findings remains frequently uncertain. Functional test that in many cases have to be established are necessary to prove or exclude the relevance of the detected mutations (121–123). Also, in WES poor coverage of certain genes like *IKBKG* in NEMO deficiency and *BIRC4* in XIAP deficiency can lead to false negative results (55).

Today, WES and tNGS offer a research-oriented and currently not clinically validated approach in cases of suspected underlying immunodeficiencies. They are offered by specialised research laboratories and not refunded by the health insurances. Therefore, anamnestic, clinical and immunologic details are of paramount importance for the diagnostic workup. In future, genetic screening tests are likely to become more affordable and part of clinical routine. But even more sophisticated genetic analysis can only be interpreted in context with clinical and immunological findings.

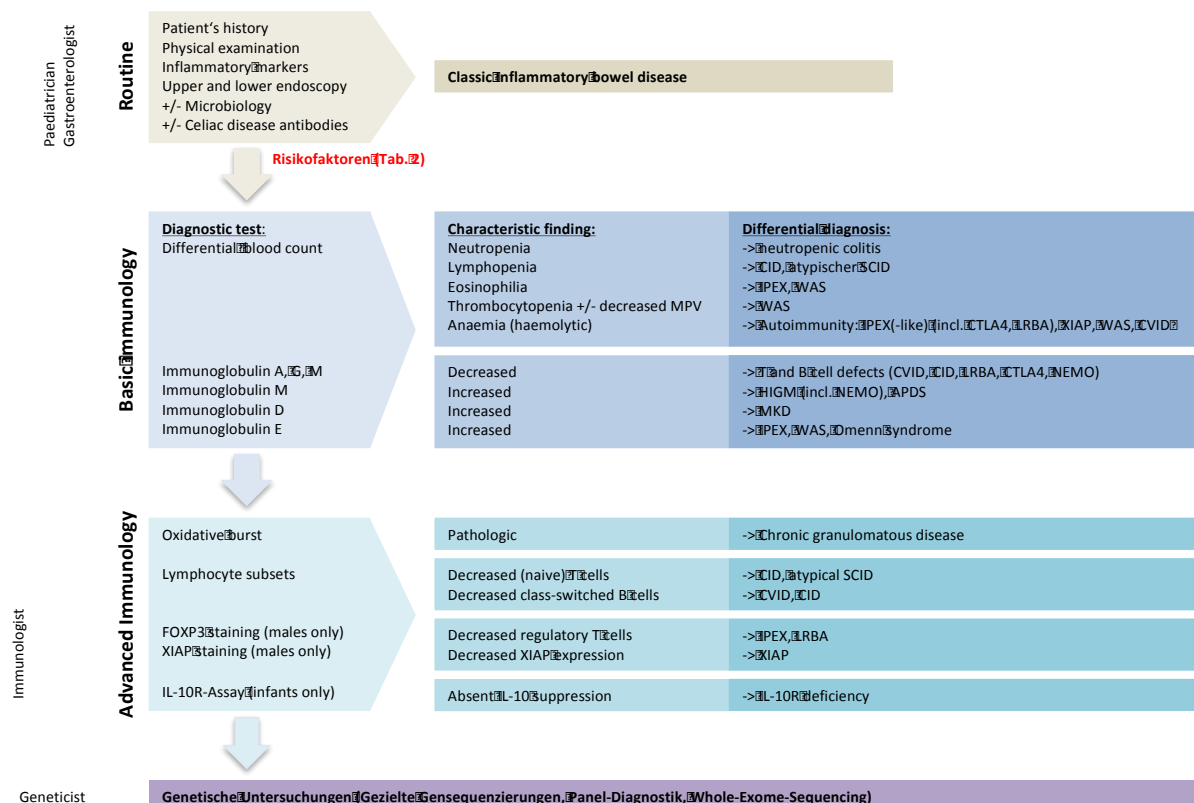


Figure 3: Diagnostic algorithm for suspected immunodeficiency in IBD. Adapted from Klemann and Tegtmeyer et al. (4). *MPV* mean platelet volume; *(S)CID* (severe) combined immunodeficiency; *WAS* Wiskott-Aldrich syndrome; *IPEX* immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome; *CVID* common variable immunodeficiency; *XIAP* X-linked inhibitor of apoptosis protein deficiency; *LRBA* LPS responsive beige-like anchor protein deficiency; *APDS* activated PI3KD syndrome; *CTLA4* cytotoxic T-lymphocyte-associated protein 4 deficiency; *NEMO* NF-kappa-B essential modulator deficiency; *HIGM* Hyper IgM syndrome; *MKD* mevalonate kinase deficiency; *IL-10R* interleukin 10 receptor deficiency. *FOXP3* Forkhead Box Protein P3.

Diagnostic and therapeutic consequences

The identification of an underlying PID often has significant implications for the further course of the IBD.

Early recognition of associated clinical symptoms and complications:

Depending on the immunodeficiency several gastrointestinal and extra intestinal symptoms and complications may occur. A better awareness would enable early targeted diagnostics. This is of utmost importance since many complex PID can primarily manifest as IBD. For example, patients with XIAP deficiency have a high risk to develop other inflammatory disorders and often potentially life-threatening haemophagocytic lymphohistiocytosis (10). Multiple PID have a remarkable susceptibility for severe infectious complications (19,65).

Prophylactic therapy:

In some PID a direct consequence of the diagnosis of a PID may be initiation of prophylactic therapies. In PID with an increased risk of infection a prophylactic antibiotic (and sometimes antifungal) therapy may be indicated. In case of antibody deficiency immunoglobulin replacement therapy is indicated (72). In our patients with CVID associated IBD substitution of immunoglobulin therapy did not improve gastrointestinal symptoms up to now but significant improvement of gastrointestinal symptoms was described by others and thus may play a role (74).

Protection from psychologic and iatrogenic damage:

An early diagnosis of an IBD causing immunodeficiency saves the patient from a prolonged search for the cause of an often insufficiently controlled IBD. The patient's uncertainty concerning the genesis of his physical complaints is a substantial psychological burden and possibly predisposes for iatrogenic damage. For instance, unnecessary computer tomography is accompanied with radiation exposure, surgical procedures may cause short bowel syndrome, and immunosuppressive therapy of patients with CGD, e.g. with TNF α blockers, substantially increase the risk of fatal infections.

Therapeutic consequences:

The diagnosis of an underlying PID has a substantial impact on therapeutic decisions in IBD. In some PID specific treatment options are available and have proven their positive effect on IBD. For example, IBD in CGD has been significantly improved after targeted therapy with IL-1 β blockers (70). For many other immunodeficiencies targeted therapies are currently developed or in clinical testing. Currently, clinical trials for the CTLA4 fusion protein Abatacept in CTLA4 and LRBA deficiency and specific inhibitors of PI3kinase in APDS are ongoing (87,124,125). Some PID, e.g. XIAP deficiency, IL-10(R) deficiency or CGD, can be cured with haematopoietic stem cell transplantation (HSCT) (25,114,126). Positive effects on IBD via HSCT have also been described in CTLA4 and LRBA deficiency (83,127). In contrast, HSCT in NEMO deficiency reconstitutes the immune system but may worsen the IBD because of the associated mucosal/epithelial defects that remain after HSCT (57). Generally, if indicated, early HSCT before infectious complications or irreversible organ damage occur is associated with a better prognosis (128). For every patient the indication for HSCT should always be carefully evaluated and is based on many factors including the patient's clinical condition and the donor's characteristics.

Prognostic considerations:

The knowledge of an underlying PID allows careful prognostic considerations of expected complications and severity of the IBD and associated IBD. If there is a close genotype-phenotype correlation, like in Wiskott-Aldrich syndrome, depending on the mutation different therapeutic options apply (34,39). While IBD is often perceived as significant restriction of life-quality, associated immunologic complications can be life-threatening. For instance, HLH in XIAP deficiency, severe infections in NEMO deficiency, CID and CGD, or endocrine and autoimmune complications in IPEX(-

like) syndromes. Early diagnosis and treatment of these severe complications are of considerable relevance for the further course of disease. Also, further therapeutic decisions should be made in the context of the expected course of disease.

Cost efficacy and resource management:

Every child with a diagnosed PID should be introduced to a paediatric immunologic centre. These centres bring together immunological, gastroenterological and general paediatric expertise and facilitate an interdisciplinary approach to PID associated IBD. Some centres for immunodeficiencies and their associated research laboratories provide further immunological tests to screen for PID. Another aspect of centre-based patient care is the possibility of participation in clinical trials that may allow implementation of innovative therapeutic options.

Conclusion

PID are a rare but important cause of inflammatory bowel disease. IBD may be first or sole symptom of an underlying PID. Possible predictors are an early onset of disease, failure to respond to standard immunosuppressive therapy, parental consanguinity, an increased susceptibility to infections, and concomitant symptoms, histopathologic findings and blood results that are atypical for classic IBD. With the present overview of IBD associated PID and illustrative cases we propose an algorithm for diagnosis of PID in IBD. If an IBD causing PID is suspected, we suggest early initiation of stepwise immunological diagnostics that allow the assessment of key immunological functions and related disorders. In the future, genetic analysis, especially targeted next generation sequencing methods, will become more important in clinical routine. However, genetic analysis is currently not recommended for all IBD patients because of its lack of diagnostic standards, uncovered costs, and the time-consuming workup. An early diagnosis of an underlying immunodeficiency implicates substantial diagnostic, therapeutic and prognostic consequences for the patient with IBD due to PID.

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