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Review

Correlates of immune exacerbations in leprosy

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ABSTRACT

Leprosy is still a considerable health threat in pockets of several low and middle income countries worldwide where intense transmission is witnessed, and often results in irreversible disabilities and deformities due to delayed- or misdiagnosis. Early detection of leprosy represents a substantial hurdle in present-day leprosy health care. The dearth of timely diagnosis has, however, particularly severe consequences in the case of inflammatory episodes, designated leprosy reactions, which represent the major cause of leprosy-associated irreversible neuropathy.

There is currently no accurate, routine diagnostic test to reliably detect leprosy reactions, or to predict which patients will develop these immunological exacerbations. Identification of host biomarkers for leprosy reactions, particularly if correlating with early onset prior to development of clinical symptoms, will allow timely interventions that contribute to decreased morbidity. Development of a point-of-care (POC) test based on such correlates would be a definite game changer in leprosy health care.

In this review, proteomic-, transcriptomic and metabolomic research strategies aiming at identification of host biomarker-based correlates of leprosy reactions are discussed, next to external factors associated with occurrence of these episodes. The vast diversity in research strategies combined with the variability in patient- and control cohorts argues for harmonisation of biomarker discovery studies with geographically overarching study sites. This will improve identification of specific correlates associated with risk of these damaging inflammatory episodes in leprosy and subsequent application to rapid field tests.

1. Introduction

1.1. Leprosy

After tuberculosis, leprosy ranks second in the order of severe human mycobacterial diseases. This chronic, granulomatous disease is caused by *Mycobacterium leprae* (*M. leprae*), an obligatory intracellular organism which is preferentially found inside macrophages and Schwann cells (SC). The leprosy bacillus' tissue tropism leads to damage to peripheral nerves, skin and mucous membrane, which, in turn, causes sensorial impairment and wounding, often resulting in severe, life-long disabilities and deformities.

Despite decades of programs using multi drug therapy (MDT), leprosy still poses a threat in developing countries, afflicting individuals in their most productive stage of life, thereby imposing a significant social and financial burden on society in these economically underprivileged countries.

A characteristic epidemiological feature of leprosy is its virtually unwavering annual new case detection rates of roughly 200,000 for decades, which translates into more than 22 newly diagnosed patients per hour, implying persistent transmission [1–3]. Moreover, mathematical modelling has shown that we are likely missing millions of hidden leprosy cases [4], arguing for powerful interventions and improvements in current diagnostic approaches that would lead to improved detection rates.

Although leprosy is known to humans for many centuries [5,6], its pathology still represents a complex scientific challenge to clinicians as well as immunologists [7]. Nerve function impairment (NFI) is the key outcome of the pathological processes of infection with *M. leprae*, which can continue after completion of multidrug therapy (MDT) and lead to disability after leprosy patients are released from treatment. It has been established beyond doubt that host genetic factors (see 2.1) play a major role in controlling host resistance to *M. leprae* [8–10]. Moreover, the outcome of *M. leprae* infection is found to closely match the ability of the host to establish effective innate and adaptive immunity to the bacterium. Characteristic for leprosy is its unique disease spectrum, in susceptible individuals, reflecting the vast inter-individual variability in clinical manifestations which accurately parallel the hosts' abilities to mount effective immunity to the bacterium. On one hand of the spectrum this dichotomy of leprosy pathology, results in tuberculoid leprosy (TT) characterized by strong pro-inflammatory, T helper-1 (Th1) as well as Th17 immunity [11,12] leading to bacterial control but, also to

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collateral damage in the form of destruction of the body's own cells by the vigorous T cell response, mimicking autoimmunity. In lepromatous leprosy (LL) on the other part of the spectrum, *M. leprae* induces relatively later pathology characterized by bacterial multiplication inducing disseminated disease, with a lack of *M. leprae*-specific T cells but high *M. leprae*-specific antibody titres and increased levels of regulatory T cells [13–17]. Between the polar sites of the clinical spectrum instable borderline states occur representing the majority of the leprosy patients [14].

Due to the close parallel between the ability of the host to establish effective immunity to *M. leprae* and the inter-individual variability in clinical manifestations, leprosy is ideally suited as a model of human immunoregulatory disease using host immune markers as correlates of disease (state) [18,19].

1.2. Leprosy reactions

While leprosy is no longer the scourge that it once was, and effective multidrug therapy (MDT) regimens are worldwide available for its cure, its clinical impact is aggravated by acute inflammatory episodes, referred to as reactions [20–23]. These immunological complications of exacerbated inflammation, representing the major cause of leprosy-related, irreversible nerve damage and anatomical deformities, can emerge spontaneously before, during and even years after treatment. Reactions can persevere as a major clinical concern and up to 50% of the leprosy patients experience a reaction at least once during their lifetime. Two distinct reactions are distinguished: reversal reactions (RR) or type 1 (T1R) and erythema nodosum leprosum (ENL) or type 2 (T2R) [24,25]. T1R represent local delayed hypersensitivity (DTH) reactions that occur in tuberculoid (TT/BT) and are characterised by $CD4^+$ T cell infiltrations of skin- and nerve lesions [26] leprosy and inflammation, leading to nerve-destructing granulomatous inflammation. T2R, better known as ENL, are associated with circulation- and tissue- deposition of immune complexes and occur in patients with dominant Th2 response histories (BL-LL) [7,25].

It is mainly the borderline states of the leprosy spectrum that are immunologically unstable and therefore prone to the occurrence of leprosy reactions. Prompt diagnosis and treatment improves recovery of these disease states significantly, thereby reducing risks for permanent disability and disfigurement [7,27]. However, if diagnosis and treatment is delayed beyond six months of symptoms initiation, neuropathy is likely to be permanent which represents a substantial problem in the light of the decreased leprosy expertise within integrated health services, as reactions are frequently misdiagnosed until they present as medical emergencies [24]. This situation renders the identification of correlates of protection and disease a priority in current leprosy research. This review will focus on correlates that can be determined in low or non-invasive samples in view of their application to field-friendly diagnostic tests.

2. Correlates of risk for (development of) leprosy reactions

The devastating consequences of leprosy reactions, if left untreated, along with the inverse relationship between the delay in diagnosis and the reversibility of clinical symptoms, unambiguously urge for novel tests based on correlates specific for these aggressive episodes. Implementation of such tests will not only allow their diagnosis but also facilitate personalized monitoring of (reactional) treatment efficacy and, in the best case scenario, prediction of occurrence of these immune exacerbations. This will enable timely drug treatment which will promote recovery and considerably reduce risks for permanent disability. Despite progress made in understanding immunopathology through numerous studies aimed at the identification of (epi) genetic and/ or environmental factors dictating the different outcomes of *M. leprae* infection, the exact triggers leading to reactions remain a challenging enigma in leprosy immunology.

2.1. Genetic risk correlates of leprosy reactions

The host genetic background has long been established as a risk factor for susceptibility to leprosy and numerous risk genes have been determined in various endemic populations including twin studies [28], hyper-endemic isolated populations [29,30], genome-wide association studies (GWASs) [9,30–33] and genome-wide linkage studies (GWLSs) [34]. For leprosy disease distribution of lepromatous- and tuberculoid leprosy is region-specific: e.g. in Bangladesh 43% of the new registered cases in 2015 were MB, whereas MB leprosy was more prevalent (67%) in Brazil among new cases in that same year [3]. Similarly, the incidence of leprosy reactions is highly variable across populations. Besides, the fact that most patients who undergo these tissue-destructive and painful episodes more than once, develop either T1R or ENL but rarely both types of reactions also indicates a genetic factor underlying development of reactions [35,36].

Some of these genes like HLA genes (HLA-DRB1 and HLA-DQA) [33,37], HLA-linked genes (*TAP*, *MICA*, and *MICB*), genes located in the HLA region (*TNFA*) as well as *PARK* [8] control susceptibility for leprosy per se [9], whereas other genes control clinical manifestation of leprosy such as the genetic tendency to develop reactions [38]. Since the family members Toll like receptor (TLR) 1, 2 and 6 form heterodimers that are involved in *M. leprae* pattern recognition [39] genetic variants of *TLR* genes are among the suspects. Indeed polymorphisms in *TLR2* [40] is associated with T1R whereas deficiency of *TLR1* was described to lead to reduced occurrence of this type of reaction [41]. During ENL, DNA sensing via TLR9 constitutes a key role as demonstrated not only by the higher expressed TLR9 levels in skin lesions and peripheral leukocytes (i.e. B-cells, monocytes, and plasmacytoid dendritic cells) but also by higher levels of TLR9 in the circulation of ENL patients compared to BL/LL controls without reactions [42]. Consistent with the role of TLR, a significant reduction in gene expression and protein levels of TLR2 and TLR4 has been noted during corticosteroid therapy in T1R patients [43].

In addition to TLR genes, other genes that are associated with innate immunity and were identified by GWAS [9] are nucleotide-binding oligomerisation domain containing 2 (NOD-2), vitamin D receptor (VDR), natural resistance-associated macrophage protein 1 (NRAMP-1), C4B and IL6. These genes were reported to be associated with one or more reactional outcomes in leprosy as well [25,38,44,45]. Some of these associations such as between TNFSF8 and T1R, may, however, vary with age [46].

Finally, as reactions can be considered as 'immunity out of control', genes associated with regulation also play a substantial role in the aetiology of reactions as evident from the association of IL-10 genetic variants with development of reactions [47,48].

Besides susceptibility genes (**2.1**) other factors definitely play a role as well as shown by assessment of correlates of reactions in a study including leprosy patients from Nepal, Brazil and the Philippines. This study showed that males, adults and lepromatous leprosy patients have a higher risk of developing reactions than females, children and tuberculoid patients, respectively [22]. Despite the predominance of men among reactional patients, women were overrepresented in the category of patients suffering from T1R while ENL occurs equally among the two sexes [35]. Furthermore, age and duration of leprosy disease are risk factors for both reactions.

2.2. Proteomic (serum) correlates of leprosy reactions

Although susceptibility genes can definitely shed more light on the mechanism of pathology of reactions, correlates of disease that can be measured in a point-of-care fashion using low or non-invasive samples, would offer a more practical tool for application in leprosy health care in field settings. Additionally, such correlates would more accurately reflect the dynamics of an individual's health status, thereby offering options for monitoring of treatment, specifically detection of

occurrence of reactions during MDT [49].

Leprosy reactions are dynamic and unpredictable immunological complications mostly occurring during MDT. The bactericidal effect of this antibiotic treatment on *M. leprae* leads to a huge reservoir of bacterial breakdown products (antigenic determinants) that are recognized by the innate immune response receptors leading to activation of pro-inflammatory cytokines and attraction of $CD4^+$ T cells to the site of infection. Upon recognition of *M. leprae*-derived antigenic determinants these skin- and nerve infiltrating T cells [26] can become activated and able to kill *M. leprae* infected Schwann cells leading to nerve damage [50].

Although the clinical presentations of T1R and ENL are distinct, it is suggested based on the similar pro-inflammatory cytokine profiles presented during both reactions, that they probably share certain aspects in their disease mechanisms [18] including enhanced Th1 responses to *M. leprae* and macrophage activation as reflected by augmented levels in blood, skin or nerve of soluble IL-2 receptors, IFN-γ, TNF-α, IL-1β and IL-6 [21,25,26,51–57]. For ENL, high serum TNF-α levels correlated with disease and decreased significantly during thalidomide treatment [58]. For T1R, IP-10 could function as a correlate of risk as it is substantially increased during this reaction. Moreover, longitudinal studies showed that increased IP-10 levels correlate with T1R onset and decreased upon successful treatment [49,59,60]. Contrasting data on the specificity for the type of reaction were reported, however: one study suggested specific association of IL-7 and PDGF-BB with ENL in Brazil [61], while these cytokines were reported to be similarly increased in T1R in Indian patients [62]. For IL-17 the studies reported are not conclusive with respect to its specificity for one type of reaction, as either IL-17 F was found to increase upon development of T1R [63,60,64] or IL-17 A for ENL onset [11]. In view of the alleged reciprocity between regulatory T cells (Tregs) and Th17 cells [65], disappearance of Tregs in favour of Th17 cells is a plausible underlying mechanism for reactions. Besides classical αβ T cells, γδ T cells are established as main source of IL-17 in many diseases [66]. Since frequency of γδ T cells was shown to be 5-8-fold higher in the peripheral blood and normal skin of reactional patients [19], production of IL-17 by these unconventional T cells may offer a useful correlate for reactions that merits further investigation.

As an antagonist of inflammation, it is not surprising that considerable levels of IL-10 produced by e.g. Tregs and type 2 macrophages [67] is found in lepromatous leprosy [17,68]. Similarly, reduction of relative levels of IL-10 compared to pro-inflammatory cytokines can play a role in the conversion of *M. leprae* specific T cell unresponsiveness in LL/BL patients to excessive inflammation during reactions [17]. In the sporadic animal models that leprosy research can build on, a borderline tuberculoid-like murine model, suppression of IL-10 significantly augmented $CD4/44^+$ and $CD8/44^+$ longitudinal infiltrative responses specific to *M. leprae* antigens and permitted $CD4^+$ T-cells to penetrate and fragment nerve tissue [69]. Other evidence supporting breakdown of (IL-10 induced) tolerance as a general mechanism for reactions was observed in the form of reduced IL-10 levels produced by *M. leprae* antigen stimulated PBMC at onset of T1R in longitudinal studies monitoring MDT in patients from endemic areas on 3 continents [64]. Partly conflicting data exist, however, on the link between Tregs and reactions: one study reported an association between reduced Treg levels in circulation and in situ during ENL but not during T1R in Brazilian leprosy patients [70], whereas a detailed, longitudinal study on a patient originating from Africa with delayed diagnosis and treatment showed reduction of expression of Treg-associated genes (*FOXP3*, *LAG3*) as well as *IL10* during onset of T1R.

Besides cytokines, acute phase proteins belonging to the pentraxin family such as CRP as well as the stress hormone cortisol can be detected in elevated levels in T1R patients [62]. Another pentraxin family member, PTX3, also known as TNF-inducible gene 14 protein, is specifically increased in ENL patients [71]. This protein binds with high affinity to the complement component C1q, possibly explaining why C1q levels in the circulation are inversely correlated with ENL (see also **2.3**).

Other molecules belonging to the complement system were also found to contribute to a risk signature as systemic levels of complement factors such as terminal complement complex (TCC) and iC3b were valuable for stratification of patients from Bangladesh and Ethiopia into those with and without T1R [72].

2.3. *M. leprae*-specific antibodies as correlates of leprosy reactions

Humoral immunity in the form of antibodies (Ab) against *M. leprae* antigens, particularly phenolic glycolipid I (PGL-I), has been intensely investigated and typically used as a proxy for infection with the leprosy bacillus [73]. Since reactions are fuelled by the presence of *M. leprae* antigens and bacterial load corresponds to anti-*M. leprae* Ab, initial Ab levels at diagnosis of leprosy could represent correlates of risk for reactions. This is in line with the finding that (borderline) lepromatous patients are more prone to reactions and the bacterial load (BI) being a risk factor for multiple reactions in one patient [74]. In a Brazilian study including 452 reaction-free leprosy patients at diagnosis, anti-PGL-I Ab and anti-LID (Leprosy IDRI antigen)-1 levels at intake showed some prognostic value for ENL in patients with a positive BI at diagnosis, but low sensitivity and specificity for T1R prediction. In 80 Colombian leprosy patients, Ab against LID-1 were associated with reactions in general and with neuritis [75]. Although these findings contrasted with an earlier study in Nepal [76], the negative association of increased humoral immunity and T1R onset was in agreement with a study monitoring anti-PGL-I Ab levels during MDT treatment in Nepal, Ethiopia, Bangladesh and Brazil showing no predictive value for T1R either [60,64]. Thus, although anti-*M. leprae* Ab can be used to monitor effective MDT and high levels (indicating high BI) at initiation of MDT is a plausible risk factor for ENL, they do not have any prognostic or diagnostic value for onset of reactions. Furthermore, it is of note that quantification of Ab levels in POC tests, allowing usage of a stringent threshold, may be applied as a prognostic tool for ENL [73]. However, application of ELISAs or qualitative rapid test that are prone to false-positives should be avoided as predictive tools.

As apparent from the sometimes conflicting data in various studies, leprosy reactions are multifactorial complicated events. This makes it highly unlikely that only one serum marker will suffice to identify onset or predict development of reactions before these have already caused loss of sensation or nerve motor function [77,78]. Instead, biomarker profiles consisting of multiple factors including cellular and humoral markers but also gender and age bear more promise as specific correlates for reactions. In fact, this has been demonstrated for tuberculosis as a combination of seven host serum markers could be used to diagnose active TB in a HIV-co-infected cohort in a highly TB endemic area (South Africa) with a sensitivity of 93,8% and specificity of 73,3% [79]. Also, for distinguishing *M. leprae* infected from non-infected test groups, patients from household contacts and endemic controls, or MB from PB patients [80] biomarker signatures improved diagnostic potential compared to single markers. In this respect, ratios of pro-inflammatory cytokines (e.g. IFN-γ, IP-10 or IL-17) versus IL-10 rather than the absolute cytokine levels may provide early indicators of impending clinical reactions and evaluating treatment [60,64,81].

2.4. Transcriptomic correlates of leprosy reactions

Since host transcriptomic biomarkers reflect actively ongoing biological processes they have been widely used to profile the host response in many diseases, including tuberculosis [82–85]. Multicomponent host biomarker signatures could be derived that were able to predict development of disease in retro- and prospective cohorts [86,87]. For leprosy disease per se the use of transcriptomics for classification was acknowledged more than a decade ago [15]. Similarly, transcriptomic analysis of skin tissue, blood or PBMC of leprosy patients

has identified several differentially expressed genes characteristic for leprosy reactions: in line with proteomics studies, pro-inflammatory genes were upregulated in independent studies that either assess RNA expression in blood or in *M. leprae* stimulated PBMC [64,88,89]. For T1R a unique 44 gene signature including genes associated with arachidonic acid metabolism was identified using PBMC stimulated with *M. leprae* antigen [88]. T1R lesions showed augmented interferon (IFN) α pathway transcripts suggesting its involvement in pathogenesis [90]. Further transcriptomic analysis at the side of disease (skin lesions) using integrative bioinformatics identified neutrophil and endothelial cell gene networks specific for ENL as part of the vasculitis that results in tissue injury, while T1R transcriptomic signatures clustered with tuberculoid leprosy [16].

The potential of *C1qC* gene expression has been shown for tuberculosis based on increased expression in patients with active tuberculosis compared to healthy controls and individuals with latent infection [91]. In a small study (11 EN L; 11 T1R), gene expression in PBMC showed increased expression of C1q for both T1R and ENL which was confirmed by immunohistochemical staining of skin lesions [89]. Additionally, in a study including 60 untreated Ethiopian leprosy patients (30 EN L; 30 L L) C1q was recently postulated as a correlate for active ENL based on increased RNA expression in both skin lesions and peripheral blood although circulating serum C1q was decreased compared to LL patients without reactions [92].

Despite inclusions of reference household genes, comparison of transcriptomic data can be cumbersome not in the least since patient classification, severity of disease as well as methods of analysis may vary in different studies. Monitoring of leprosy patients by longitudinal RNA expression profiles during their monthly visit while receiving MDT, avoids comparison with other individuals and allows personalized treatment. Using this strategy, monitoring whole blood transcriptomics of leprosy patients before, at onset and after treatment of T1R revealed that IFN inducible transcripts (*OAS1/2, GBP1/5*, *IFI44, IFI44 L, IFIT5, IFIH1*), *VEGF* and genes associated with cytotoxic T-cell responses (*GNLY, GZMA/B, PRF1*) were upregulated during T1R, whereas expression of *IL4* and *IL13* was hardly affected and T cell regulation-associated genes (*FOXP3, IL10*) were downregulated [64]. In summary, gene expression analysis studies support the notion that transcriptomic biomarkers reflect the collapse of regulation, in favour of inflammation as the underlying aetiology of reactional tissue damage as correlates of both ENL and T1R, though further studies to define signatures applicable to field use are still warranted with emphasis on longitudinal transcriptomic analyses and comparison of patients with different ethnic backgrounds to allow prediction and timely intervention of these immune exacerbations.

2.5. Metabolomics biomarkers of leprosy reactions

Metabolites (small molecules and natively occurring peptides) provide a biochemical thumbprint of the events occurring within cells and the body as a whole. Metabolites are universal and not dependent on the genetic makeup, animal species or body fluid/ tissue and thus metabolomics is used to understand the biology of an organism and the way it responds to environmental stimuli. Disease and inflammatory states correlate with changes in the biochemistry of host and pathogen that can be monitored or measured via metabolomics approaches [93]. Importantly, through the rapidly evolving field of metabolomics it is now widely accepted that there is considerable crosstalk between metabolic pathways on one hand and both innate and adaptive immune mechanism on the other hand [93]. Thus, metabolomics provides insights into how the human host or host's tissues reacts to environmental stimuli. Numerous host metabolic pathways associated with various viral and bacterial infections have been identified, including e.g. tryptophan metabolism associated with immune suppression, polyamine metabolism related to macrophage phenotype, fatty acid metabolism, lipid and lipid mediator metabolism [94–100]. The signatures generated by disease associated metabolic profiles may be exploited as correlates of disease or protection or efficacy of treatment.

Using sera of leprosy patients from both polar sites of the spectrum (bacterial load (BI) < 1 or BI > 4) high concentrations of polyunsaturated fatty acids and phospholipids were detected in lepromatous patients with high numbers of bacteria [101]. More extended serum metabolomics studies on reactional patients showed that 40 metabolic pathways were perturbed in patients with T1R, with 71 dysregulated metabolites mapping to pathways for lipid mediators of inflammation [100]. This coincided with an increase in the abundance of the proinflammatory leukotriene B4, prostaglandin D2 and lipoxin A4 but a decrease in proresolving resolvin D1 and prostaglandin E2. The shifts in levels from proresolving lipid mediators to proinflammatory clearly links metabolic and cellular immune responses that jointly cause Th1/Th17-mediated pathology of T1R (see **2.2**).

As an alternative to blood or serum, urine represents an easily accessible and non-invasive body fluid. Exploratory metabolomic analysis on a prospective cohort consisting of Nepalese leprosy patients with and without reactions as well as healthy controls from the same area showed that cross-sectional, urinary metabolic signatures at the time of diagnosis of T1R, distinctly differed from those before reactions [102]. This indicates that urinary and serum metabolic profiles that contribute to T1R pathology can be promising correlates for states of acute inflammation in leprosy.

3. Triggers of hyperimmune states of leprosy

3.1. Effect of interventions on occurrence of leprosy reactions

Reactions can be diagnosed concurrently with leprosy but mostly these aggressive episodes occur during MDT. It is hypothesized that the amount of antigen that becomes available to the immune system by killing of the bacterium during antibiotic treatment gives rise to overactivation of the immune system in an attempt to clear these bacterial antigens which leads to an inflammatory state, especially in those with high bacillary load at initiation of MDT. Besides MDT other interventions may also cause development of T1R as described for two arthritis patients from the southern part of the United States who received anti-TNF therapy (infliximab) for arthritis. Although without clinical symptoms of leprosy they subsequently developed borderline lepromatous (BL) leprosy and after discontinuation of infliximab both presented with T1R [103]. A conceivable explanation for these immunopathologic sequellae is the decrease of Th1 immunity in first instance by infliximab allowing growth of previously latently present *M. leprae* bacteria causing leprosy. Removal of anti-TNF caused excessive inflammation as a result of the body's attempt to remove an at that stage considerable amount of bacteria.

Notwithstanding its beneficial immunoprophylactic outcomes regarding leprosy and childhood tuberculosis, BCG vaccination can also lead to immune exacerbations as exemplified by a BCG vaccination trial in Bangladesh: within 3 months after receiving BCG an unexpectedly high proportion among apparently healthy contacts of leprosy patients developed borderline tuberculoid (BT) leprosy, 43% of whom even presented with signs of T1R [104]. Although exact immune correlates were not determined for those who developed T1R, further investigation of individuals with skin complications after BCG vaccination in the same population showed elevated IFN-γ levels along with decreased levels of sCD40 L and GRO (CXCL1) in response to *M. leprae,* indicating reduced T cell regulation which can be causatively related to uncontrolled Th1-cell immunity damaging the skin [105].

BCG vaccination induces histone modifications and epigenetic reprogramming of human monocytes at the promoter sites of genes encoding for inflammatory cytokines such as TNF-α and IL-6, resulting in a more active innate immune response upon restimulation, a process called trained immunity with a key role for IL-1β [106,107]. BCG-induced trained immunity is not specific in the sense that it can also lead

to enhanced reduction of unrelated pathogens not related to BCG as described for yellow fever virus vaccine strain [107]. Thus, it is plausible that the live BCG vaccine causes inflammatory 'unmasking' of a previously untreated *M. leprae* infection by amplifying existing innate immunity. Currently, a phase IV chemoprophylactic clinical trial in MB patients' contacts is currently ongoing in Brazil to assess whether administrating single dose rifampicine (SDR) prior to BCG vaccination is can prevent onset of leprosy (reactions) in cases that develop PB/T1R within 3 months after receiving BCG as a post exposure prophylactic strategy [108].

Another example of BCG causing too vigorous immunity that leads to reaction-associated pathology was described for *M. leprae* infected nine-banded armadillos that precipitated motor nerve conduction abnormalities more rapidly and severely after post *M. leprae* exposure BCG vaccination than infected armadillos without BCG vaccination [109]. This finding stresses that, in order to prevent development of T1R, leprosy vaccines will need to meet stringent requirements in endemic populations where the majority of individuals is exposed to *M. leprae.*

3.2. Effect of coinfections on leprosy reactions

For HIV infected individuals or AIDS patients on highly active antiretroviral therapy (HAART), the immune reconstitution inflammatory syndrome (IRIS) was proposed as the causative mechanism for the development of both T1R and ENL within 6 months after initiation of HAART [110,111]. Similarly to reactions occurring after BCG vaccination in contacts of leprosy patents (see **3.1**), this phenomenon in these patients is considered an inflammatory revealing of a previously undetected *M. leprae* infection. Secondly, a less commonly occurring phenomenon in HIV-infected individuals is a paradoxical clinical deterioration in pre-existing leprosy during which HAART-associated T1R develops [112–120].

Chronic infections with soil-transmitted helminths (STH) are known to induce systemic immune dysregulation towards a Th2 bias affecting e.g. the efficacy of BCG vaccination [121]. Since over 94% of the annual new leprosy cases originate from areas co-endemic for STH [122], simultaneous (latent) infection with *M. leprae* as well as STH is probably more common than anticipated. Indeed higher relative risks for leprosy have been detected in Brazil in areas with schistosomiasis [123]. Furthermore, in India, the country with the most leprosy patients, lymphatic filariasis is endemic as well [124]. A phenotypic consequence of STH co-infections is that they occur more frequently in lepromatous than tuberculoid leprosy patients, both in Nepal as well as in Indonesia [125,126]. This is presumably due to suppressed Th1 and high Th2 facilitating *M. leprae* growth and progression to the multibacillary site of the spectrum. Moreover, in a Nepalese cohort STH co-infection was recently found to be inversely associated with reactions in a co-endemic setting [125]. This epidemiological finding implicates that the development of leprosy reactions is associated with absence or disturbance of a chronic STH co-infection. Thus, during helminths prophylaxis campaigns in areas where both infections are endemic it is vital to monitor for leprosy reactions. In this respect, the availability of diagnostic tests based on correlates for leprosy reactions will positively impact leprosy surveillance.

3.3. Flares in non-mycobacterial diseases

Besides comparable paradoxal reactions observed for patients with buruli ulcer [127] or tuberculosis [128], immunological complications in this ancient, poverty-associated disease have significant overlap with destructive flares in contemporary chronic diseases in high income countries such as rheumatoid arthritis (RA) and inflammatory bowel disease (IBD) including Crohn's disease. It has been recognized by several studies that these diseases share genetic susceptibility genes with leprosy [28,129–133]. A recent IBD GWAS showed that single nucleotide polymorphisms (SNPs) encoding susceptibility for IBD

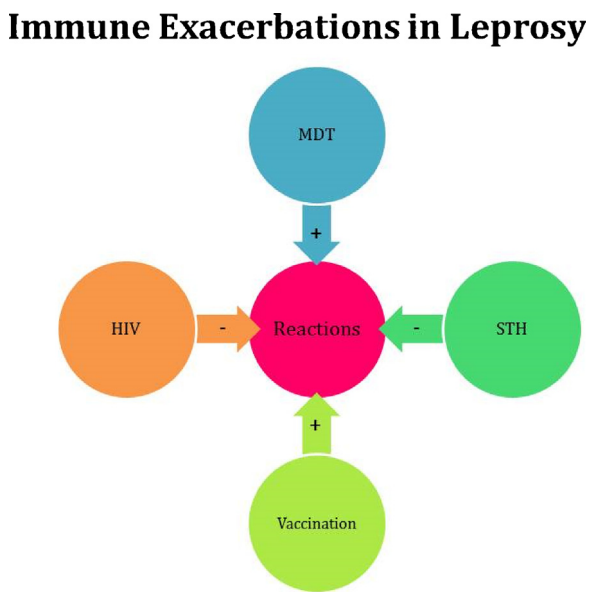

Fig. 1. Schematic overview indicating the effect of treatment, vaccines and co-infections on the development of leprosy reactions in *M. leprae* infected individuals.

shared a common risk-allele with T1R indicating a substantial overlap in the genetic control of clinically diverse inflammatory disorders [32]. Thus, identification and diagnostic application of correlates of leprosy reactions, will also be advantageous to help monitoring disease progression including acute episodes of exacerbated inflammation in other diseases as was demonstrated recently by the application of a serum IP-10-based POC test developed for monitoring T1R, for detection of disease severity in RA [49,132].

4. Conclusions

Despite the fact that certain mechanisms of leprosy reactions have become less enigmatic than they used to be (Fig. 1), these acute nerve-destructive immune exacerbations due to *M. leprae* infection still include unresolved issues.

The lack of specific correlates of leprosy reactions impedes diagnosis of these flares prior to onset of clinical symptoms, such that they often cause irreversible pathology. The ability to detect and treat these episodes timely will significantly improve the patients' quality of life. Since leprosy can present across the Th1-Th2 immunological spectrum, the balance of pro- and anti-inflammatory immune responses determines the outcome of infection.

To date research supports excessive innate and adaptive inflammatory immunity combined with perturbed T cell regulation, vasculoneogenesis and T cell cytotoxicity as the aetiological mechanism of pathology observed during leprosy reactions. However, proteomics, transcriptomics and metabolomics studies have produced conflicting data with respect to host biomarkers that can specifically discriminate T1R from TT/BT or T1R from ENL. The lack of generic clinical classification methods along with variable numbers of and heterogeneity in patients or different male/ female ratios in these studies often hamper combination of data to identify general biomarker signatures for either type of reaction. Furthermore, due to low disease incidence and long incubation times, numbers of leprosy patients are frequently limited hampering sufficient power of most studies.

Moreover, studies that aim to identify correlates specific for reactions should include appropriate control groups such that ENL patients' profiles are compared with LL/BL patients without reactions, and T1R patients with BT patients without reactions. Comparison of reactional patients with other controls groups such as healthy individuals can

negatively influence specificity of such markers [25,134,135].

For translation of identified correlates in diagnostic tools for reactions, techniques including serum markers are most far advanced. In this respect, it is of note that inter-individual normal levels for serum proteins may vary considerably for some biomarkers [25,49,60,132]. Thus, algorithms (e.g. ratios or number of a minimal number of markers above cut-off for positivity) based on serum biomarker signatures instead of single markers are expected to be more reliable as correlates of risk for reactions and should preferably be applied instead of measuring merely absolute levels [19,60,102]. However, for detection of onset of reactions during MDT in high risk patients or for treatment efficacy of reactions, intra-individual longitudinal comparison of absolute numbers of biomarkers could still be applicable as a monitoring tool.

Identification of predictive correlates for application of practical diagnostic tools thus urge for longitudinal studies overarching ethnic backgrounds that are based on standardized laboratory assays monitoring leprosy patients during MDT. Correlates allowing immunodiagnosis of exacerbated episodes in leprosy and other chronic inflammatory diseases will greatly improve patients' quality of life.

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Conflicts of interest

The author declares to have no conflict of interest.

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