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# Transcriptional regulator BOB.1: Molecular mechanisms and emerging role in chronic inflammation and autoimmunity

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## ABSTRACT

Lymphocytes constitute an essential and potent effector compartment of the immune system. Therefore, their development and functions must be strictly regulated to avoid inappropriate immune responses, such as autoimmune reactions. Several lines of evidence from genetics (e.g. association with multiple sclerosis and primary biliary cirrhosis), human expression studies (e.g. increased expression in target tissues and draining lymph nodes of patients with autoimmune diseases), animal models (e.g. loss of functional protein protects animals from the development of collagen-induced arthritis, experimental autoimmune encephalomyelitis, type 1 diabetes, bleomycin-induced fibrosis) strongly support a causal link between the aberrant expression of the lymphocyte-restricted transcriptional regulator BOB.1 and the development of autoimmune diseases. In this review, we summarize the current knowledge of unusual structural and functional plasticity of BOB.1, stringent regulation of its expression, and the pivotal role that BOB.1 plays in shaping B- and T-cell responses. We discuss recent developments highlighting the significant contribution of BOB.1 to the pathogenesis of autoimmune diseases and how to leverage our knowledge to target this regulator to treat autoimmune tissue inflammation.

## 1. Introduction

Lymphocyte-specific transcriptional regulation is a central feature of the generation, development, differentiation, maturation and function of immune effector and regulatory cells. Given that, control of gene expression in various cells of the immune system at a transcriptional level is achieved by the complex protein machinery comprising transcription factors, cofactors, chromatin regulators, and non-coding RNAs. Transcription factors bind to short recognition sites located in gene promoter or enhancer/silencer regions and recruit transcriptional cofactors, which in turn recruit and activate RNA polymerase II at core-promoters to enable transcription. Transcription cofactors generally do not bind DNA, but rather mediate protein-protein interactions between regulatory transcription factors and the basal RNA polymerase II transcription machinery. Some transcription factors and their associated cofactors are unique to certain immune cell lineages and their levels are determinative of whether their target genes are transcribed and to what

extent. This implies that these regulators of gene expression must be tightly controlled. Failure to achieve appropriate transcriptional regulation dramatically affects the development and function of immune cells and lies at the heart of the pathogenesis of many inflammatory and autoimmune disorders and cancers. Thus, a detailed characterization of transcriptional regulators of immune responses should advance our understanding of the molecular basis of these pathological conditions and may offer new therapeutic strategies. BOB.1/OCA-B/OBF.1 is a prototypic example of a lymphocyte-restricted transcriptional coactivator. Extensive efforts over the past years have been made to elucidate its role in the regulation of adaptive immune responses in both, physiological and pathological states. This review summarizes current knowledge and recent findings regarding the role of BOB.1 in the pathogenesis of autoimmune and chronic inflammatory diseases, as evidenced by genetic, animal model, and human *ex vivo* studies, as well as by the first evidence that targeting BOB.1 may offer new opportunities and potential benefits for patients with autoimmune disorders.

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## 2. The biology of BOB.1

### 2.1. BOB.1/OCA-B/OBF-1 protein

It has been nearly 30 years since Pierani and colleagues from the Robert G. Roeder's laboratory at Rockefeller University discovered in fractionated B cell nuclear extracts a component crucial for optimal octamer-dependent transcription from an immunoglobulin heavy chain (IgH) promoter [1]. Subsequent research revealed that this component works in concert with the ubiquitous DNA-bound activator OCT1 (or lymphoid-restricted OCT2) by forming a ternary complex on the conserved octamer sequence motif (5'-ATGCAAAT-3') [2,3] thereby contributing to the transcriptional activity of promoter and enhancer elements. Of interest, the interaction of this coactivator with either OCT1 or OCT2 activates transcription from promoter-proximal positions [3–6], whereas the activity from distal enhancer positions specifically requires the presence of OCT2 [7,8]. Shortly after, in 1995, *POU2AF1* (POU domain class 2-associating factor 1) gene encoding this novel factor was cloned based on biochemical and genetic screening by three research groups independently and variously termed BOB.1 (B cell Oct binding factor 1 [4], OCA-B (Octamer CoActivator from B cells) [5], or OBF-1 (Oct-Binding Factor 1) [3]. Besides, the murine homolog of human BOB.1 was isolated and characterized [6,9]. It has been shown that biochemically purified BOB.1/OCA-B/OBF-1 (hereafter referred to as BOB.1) is presented by two isoforms of 34 kDa and 35 kDa (translated from different translation initiation codons and designated as p34 and p35) that are expressed at similar levels and are equally able to stimulate octamer-dependent transcription in nuclear extracts [5]. However, further research revealed that p34 is much more potent than p35 on IgH promoters *in vitro* [10]. Moreover, in contrast to the p34 isoform, which is exclusively nuclear and functions as a transcriptional regulator, the p35 isoform may conceivably act as a signal transducer as it is post-translationally modified by myristoylation through the N-terminal glycine residue and can be found in both cytoplasmic/membrane and nuclear compartments [10]. Currently, there is limited knowledge on the exact role of cytoplasmic p35 isoform, though two studies suggested its involvement in the regulation of the B-cell receptor (BCR) signalling based on its direct interaction with the tyrosine kinase Syk [11] and Galectin-1 [12].

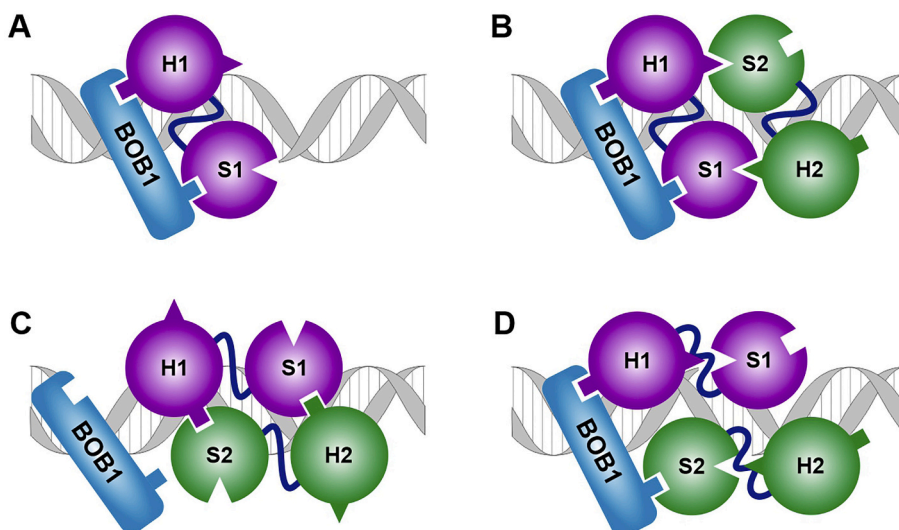
A detailed examination of BOB.1 protein structure and regulated gene transcription, thoroughly reviewed in [13–15] revealed a striking feature. This 256-residue polypeptide with unusually high proline content (16%) is intrinsically unstructured in solution and become partially structured upon recruitment into the ternary complex [16].

### 2.2. BOB.1 partnership with the POU-domain

BOB.1 interacts with the sequence-specific DNA-binding POU transcription factors (named after the founding family members PIT1, OCT1/2, and UNC86), the ubiquitously expressed OCT1 (POU2F1) and lymphoid-specific OCT2 (POU2F2).

As a transcriptional co-activator, BOB.1 itself does not bind DNA but is rather recruited into transcriptional regulation *via* interaction with DNA-bound POU-domain transcription factors OCT1 and OCT2. The POU-domain is a unique bipartite structure allowing DNA recognition with remarkable flexibility (reviewed in [17,18]). This flexibility is largely inferred from the presence of two autonomous DNA-binding structures, the POU-specific (POUs) and POU-homeodomains (POUH), connected by a flexible linker (Fig. 1). Historically, BOB1 was first shown to interact with OCT1 or OCT2 bound to the octamer site (5'-ATGCAAAT-3') as a monomer *via* simultaneous contacts with POUH, POUH and adenine at position five of the octamer thus acting as a molecular clamp that holds together three molecules [19–21] (Fig. 1A).

Next level of BOB.1 functional diversity is provided by its selective interactions with POU-domain dimers (Fig. 1B,C). Alternative arrangements of the POUH and POUH sub-domain on DNA expose different surfaces for interaction with proteins partners, eventually resulting in different functional readouts. A striking illustration of this principle has been provided about 20 years ago during the studies of dimeric arrangements of the POU-domain proteins on PORE- (Palindromic Oct-Recognition Element, 5'-ATTTGAAATGCAAAT-3') and MORE-class DNA elements (More of PORE, 5'-ATGCATATGCAT-3') [22–24]. It was found that PORE-type arrangement was highly permissive for this interaction (Fig. 1B), whereas MORE-type arrangement of OCT1 POU (POU1) dimer was refractory to BOB.1 interaction (Fig. 1C). Computer modelling and X-ray studies provided the structural rationale of this selectivity by showing that MORE-mediated dimerization and BOB.1 binding are mutually exclusive as it turned out that BOB.1 interaction interfaces both in the POU-domain and DNA major groove were engaged in MORE-type POU1 dimerization [23]. MOREs have been found to mediate transcription of the number of genes, including the IgH genes. Ironically, the *BCL1* VH promoter was the first regulatory DNA element reported to mediate interaction and synergism between OCT1 (OCT2) and BOB.1 [2]. This apparent discrepancy arose from a deletion in the DNA constructs of a subpart of MORE, resulted in the ablation of MORE-mediated dimerization of OCT1 (OCT2) with retention of BOB.1-permissive octamer site [2,5]. Although this deletion was crucial for BOB.1 discovery, it also led to the conclusion that this protein was involved in the regulation of IgH gene transcription *via* the octamer



**Fig. 1.** Modes of BOB.1 interaction with the POU-domains. (A) BOB.1 is recruited *via* interaction with DNA-bound POU-domain transcription factor monomer as described in [20]. Selective BOB.1 recruitment by POU-domain factor dimers formed on the POREs (B), but the MOREs which are refractory to BOB.1 since BOB.1-interaction surface (square shapes) is used for dimerization. (D) A PORE-like configuration with altered linker connectivity, suggested by Lins et al. [26]; the POU-specific sub-domains (S1 and S2) are moved away from POU-homeodomains (H1 and H2), as it occurs on the PORE<sup>+1</sup> [26], to emphasize alleviated requirements of PORE-specific protein-protein interaction interface (triangle shapes) for the ternary complex formation. See text for further details.

motif within the VH promoters, subsequently contravened by largely unaffected IgH expression in BOB.1 knockout mice [9,25].

The ability of BOB.1 to form a ternary complex with PORE-type OCT1 (OCT2) dimer is due to the fact that this dimer is present as two-fold repeated, however, unexpectedly, this complex includes only one molecule of BOB.1. Computer modelling showed altered geometric parameters of OCT1 monomer bound to the non-octamer half-site of the PORE, which is incompatible with BOB.1 interaction [24] (Fig. 1B).

Perhaps the most intriguing and least understood molecular feature of BOB.1 resides in its ability to empower OCT-factors to bind to unfavourable non-consensus DNA motifs in murine and human promoters. Lins and colleagues have shown that BOB.1 (1) stabilizes its complex with OCT1 dimer on the PORE to a much greater extent than with OCT1 monomer on the octamer site, (2) compensates for the loss of the PORE-type dimerization interface that is caused by amino acid mutation or by nucleotide insertion, (3) alters sequence requirements of DNA recognition [26]. As an extreme example of the last statement, PORE-derived sequences that do not bind OCT1 either as a monomer or as a dimer, in the presence of BOB.1 become an efficient platform for ternary complex assembly, which includes two OCT1 and one BOB.1 molecule. These data prompted the idea that the PORE-type OCT1 or OCT2 dimers, rather than the monomers, are the primary targets of the cofactor BOB.1. Furthermore, altered connectivity of POU and POUh sub-domains, when these sub-domains belonging to one molecule bind parallel to the DNA strand and clamped together by BOB.1, has been proposed [26] (Fig. 1D).

These listed literature data show that BOB.1 functioning *via* POU dimers in certain configuration provides a unique level of the structural and functional plasticity of transcriptional regulation. Clearly, there are still many open questions with regard to molecular mechanisms of BOB.1 action, as well as to *bona fide* BOB.1/OCT1(OCT2) genomic targets. These questions await further answers, urged by the looming impact of BOB.1 on autoimmune diseases, which is in the focus of the current review.

### 2.3. Role in B cells

BOB.1 plays an essential role in B-cell ontogeny. It is expressed throughout all stages of the developing B lymphocyte axis, albeit at different levels. The highest BOB.1 expression is found in germinal centre (GC) B cells in both mouse [27] and man [28,29]. Accordingly, the key signals that regulate the formation of GCs, namely CD40-CD40L cross-talk in conjunctions with IL-4 or IL-21 cytokines, boost BOB.1 expression in B cells *in vitro* and *in vivo* [27,28,30].

Probably the most striking feature of BOB.1 emerged from *in vivo* studies. In 1996, the laboratories of Roeder, Matthias and Schaffner simultaneously established its critical role for the development of T cell-dependent (TD) immune responses. They demonstrated that targeted BOB.1 knockouts in mice led to a complete lack of GCs in spleen and lymph nodes, as well as to a massive defect in the production of isotype-switched immunoglobulins [9,25,31], ultimately linking the function of BOB.1 to GC maturation and plasma cell differentiation. However, the interpretation of the BOB.1 knockout phenotype is complicated by the subsequent findings demonstrated that BOB.1 is required for B-cell development at multiple stages. First of all, BOB.1 plays a significant role at early stages of B cell development in bone marrow as the number of transitional B220<sup>+</sup> IgM<sup>high</sup> B cells and mature recirculating IgD<sup>high</sup> B cells are dramatically reduced in the bone marrow of mice lacking BOB.1 [32,33]. Furthermore, an increased number of apoptotic cells was detected in both the immature and mature B cell compartments [33], which was partially explained by the strong reduction of the expression of the anti-apoptotic protein Bcl2, a direct target of BOB.1 [34]. Besides, the marginal zone B cell compartment, crucial for mounting innate and adaptive responses, is affected in the absence of BOB.1 as both, the numbers and the function of marginal zone (MZ) B cells are dramatically reduced in BOB.1<sup>-/-</sup> animals [34,35]. Second, recent data

demonstrated that BOB.1 together with OCT2 and MEF2B is required to drive expression of BCL6, the master regulator of the GC B cell state, by recruiting and concentrating transcription factors and cofactors, including chromatin/epigenetic factors that facilitate enhancer-promoter interactions [36]. As BOB.1 is expressed preferentially in GC B cells, the complete loss of these sites of cognate B and T cell interaction and expansion upon BOB.1 deletion is responsible for the lack of high titres of switched antibody isotypes and disturbed TD memory responses in BOB.1-deficient animals. Additionally, *in vitro* data revealed that the presence of functional BOB.1 is critical for the final stages of TD B cell differentiation as the loss of BOB.1 under these conditions blocks the genetic program of plasma cell differentiation [37]. Third, BOB.1 is essential for optimal BCR-derived signals as mature splenic B-cell proliferative responses to BCR stimulation are reduced in BOB.1 knock-out animals [25]. Later, Siegel et al. proposed that BOB.1 has a dual function in B cell signalling, playing, on one hand, a role in early events in intracellular signalling by non-transcriptional regulation of SYK stability by the cytoplasmic form of BOB.1 and functioning, on the other hand, as a transcription coactivator of downstream target genes at later stages of B cell activation [11]. Finally, the complexity of the role of BOB.1 in TD GC formation is further highlighted by findings that it is not only crucial for the efficient transcription of immunoglobulin heavy-chain and V(D)J recombination of a subset of immunoglobulin kappa genes [38,39], but also is important for regulation of many B-cell essential genes that belong to multiple categories, including, cell survival and proliferation, metabolism, ion channel, cell adhesion, cytokines, and chemokines [34,40–45]. All these B-cell-intrinsic defects in the absence of BOB.1 might be accountable for the absence of GC formation and impaired production of switched Ig *in vivo*. Last but not least, although studies of adoptive transfers of BOB.1-sufficient and deficient B and T cells to Rag1<sup>-/-</sup> mice demonstrated B-cell autonomous defect [27,29], one cannot formally exclude additive T-cell specific effects of BOB.1 loss on the absence of GCs and block of memory and plasma cell differentiation in the BOB.1 knockout mice. Indeed, its pivotal role in the differentiation of T follicular helper (Tfh) and in the formation of memory T cells, important players in mounting a humoral response in GCs, has been demonstrated [46].

A couple of excellent reviews covering the role of BOB.1 in B-cell development and function in great detail have been published [45,47], therefore it will be not discussed here further.

### 2.4. Role in T cells

BOB.1 has long been considered a B cell-specific factor, however, emerging evidence that BOB.1 plays an indispensable role in the T-cell compartment led to a re-evaluation of the established paradigm. BOB.1 is not expressed during T cell development or in naïve T cells, yet its expression can be induced in T cells upon TCR engagement or by the treatment with PMA/ionomycin [41,48,49]. It has been demonstrated that posttranslational modifications, such as phosphorylation at Ser184, are required for the inducible activation of OBF.1 [49]. Later it was noticed that BOB.1<sup>-/-</sup> mice are not able to control experimental leishmaniasis, and detailed re-examination of T cell compartment revealed a reduction of both CD4-positive and CD8-positive subpopulations in lymph nodes and spleen in the absence of BOB.1 [50]. Further analysis demonstrated, that in contrast to unpolarised CD4-positive T cells, BOB.1 is expressed in Th1 and Th2 cells and that cytokine secretion of both subtypes of T helper cells was affected by the BOB.1 deficiency: the secretion of Th1 cytokines was reduced, whereas the secretion of Th2 cytokines was increased [50]. Mechanistically, BOB.1 shapes the balance of Th1- *versus* Th2-mediated immunity through cooperative binding with OCT1 to the IFN $\gamma$  and IL2 promoters. In the absence of BOB.1, this balance is shifted towards Th2 as another target of BOB.1, the transcriptional factor PU.1, is repressed, resulting in increased GATA3 activity and consequently, increased Th2 cytokine release and susceptibility to *Leishmania major* infection [50]. The consequent study

confirmed this concept by demonstrating that selective blockade of microRNA (miR)-126, a negative regulator of BOB.1, leads to an augmented expression of BOB.1, which activates PU.1 and thereby alters Th2 cell function *via* negative regulation of GATA3 expression [51]. BOB.1 is also required for the differentiation of Th17 cells [52], presumably *via* blockade of IL-2 production, a known endogenous repressor of Th17 polarization [53]. Accordingly, subsequent gene and protein analyses confirmed the expression of BOB.1 in Th1, Th2, and Th17 cells in human tonsils or peripheral blood [54]. However, within the T-cell compartment, the highest BOB.1 level was found in Tfh cells in human [55] and mouse [54,56], suggesting its role in the development and/or function of this cellular subset. It was suggested that BOB.1, in cooperation with OCT1(OCT2), can directly bind Bcl6 and BTLA promoters to regulate their expression and, correspondingly, Tfh cell development [56]. Indeed, a strong reduction of Tfh cells frequency after immunization with TD antigen was observed in secondary lymphoid organs of mice lacking BOB.1 in the T cell compartment [56]. However, other studies contradicted these data, reporting either similar [57] or even increased [54] Tfh frequencies in the absence of BOB.1 in T cells. The latter study showed that (a) early processes accompanying Tfh cell differentiation, including Bcl6 expression, occur normally in BOB.1-deficient animals and, (b) the overexpression of BOB.1 in primary human naïve CD4-positive T cells does not result in the expression of Tfh signature genes such as *BCL-6*, *PD-1*, *IL-21*, and *CXCR5* [54], arguing against the notion that BOB.1 is critical for differentiation of Tfh cells. The authors proposed that the function of BOB.1 is to restrict the TCR-mediated expansion of Tfh [54]. Future research is needed to identify the exact role of BOB.1 in Tfh cells, yet a recent study has further stressed the importance of this transcriptional regulator in T-cell biology by demonstrating that BOB.1 together with OCT1 is selectively required for the *in vivo* generation of CD4-positive memory T cells [46] (discussed later in this review). Collectively, these findings indicate a pivotal role of BOB.1 in the biology of CD4-positive cells. As to CD8-positive cells, scarce data suggest that an elevated expression of BOB.1 is a hallmark of hypo-functional tumour-infiltrating CD8-positive T cells (WO/2017/075451), yet the contribution of BOB.1 to exhaustion of these cells remains to be investigated.

### 2.5. Role in other cell types

The expression of BOB.1 is considered to be lymphocyte restricted, however, Zhou et al. detected its expression in normal human airway epithelium [58]. Using a combination of RNA-seq analysis, immunohistochemistry, and analysis of differentiating single basal cell clones the authors confirmed that BOB.1 is increased upon differentiation of airway basal stem/progenitor cells, where it controls expression of its multiple known and novel target genes, related to a host defence [59]. Accordingly, another recent meta-analysis study of publicly available gene expression profiles in synovial tissue and single-cell RNA-seq data from isolated fibroblasts-like synoviocytes identified BOB.1 as a putative disease-related master regulator in RA fibroblasts [60]. However, experimental validation is required to confirm these *in silico* data.

### 2.6. Regulation of BOB.1 expression

A gene targeting in mice revealed that aberrant expression of BOB.1 has serious functional consequences on the immune system. Thus, the absence of BOB.1 leads to a partial block of B-cell differentiation at multiple stages, lack of germinal centre formation, and severely reduced immune response to T cell-dependent antigens, as reviewed elsewhere [45,47]. Besides, overexpression of BOB.1 in B cells leads to impairment of early-stage differentiation of B cells in the bone marrow, and cells can only escape this block by post-transcriptional downregulation of the BOB.1 expression [33,61]. These findings suggest that aberrant expression of BOB.1 in B cells has major functional consequences and, therefore, has to be strictly controlled. As discussed above, BOB.1

protein level is strongly increased in GC B cells and can also be induced to high levels by stimulation that mimics T-cell help or responses to bacterial or viral infections (TLR4-, TLR7-, or TLR9-triggering) [28,62,63]. Later, it has been demonstrated that canonical and non-canonical NF- $\kappa$ B, as well as, to some extent, NFAT signalling pathways are involved in the activation of both constitutive and inducible BOB.1 expression [64,65]. Also, TNF, a pro-inflammatory cytokine elevated in many chronic and autoimmune disorders can upregulate BOB.1. Consistently, analyses of BOB.1 proximal and B-cell specific distal promoter regions [26] revealed the presence of TATA box, a binding site for the cAMP regulatory element-binding protein/activating transcription factor (CREB/ATF), NFAT-binding site, and three composite and consecutive NFAT/NF- $\kappa$ B sites [64,65] with different degrees of importance for constitutive and inducible BOB.1 expression. Furthermore, BOB.1 is induced in response to classical unfolded protein response (UPR) activation through spliced XBP-1 bound to conserved unfolded protein response element present in the promoter of BOB.1 [66]. Also, microRNA miR-126 was shown to regulate expression of BOB.1, however, the mechanism of this regulation has to be elucidated as BOB.1 does not contain putative binding elements for miR-126 in the 3'- or 5'-untranslated regions [51].

The expression level of BOB.1 is not only regulated at the level of transcription but also at the level of protein stability. It has been found that, in contrast to the protein level, which is increased in GC-B cells, the mRNA levels of BOB.1 are not different in GC- versus non-GC-B cells [67]. This phenomenon was explained by two research teams published back-to-back. They showed that BOB.1 interacts with the SIAH proteins 1 and 2 and that this interaction leads to ubiquitin-proteasome-dependent degradation of BOB.1 [67,68]. However, the subsequent study argued the specific role of SIAH and the importance of ubiquitination in the degradation of BOB.1 in B cells [69]. Instead, the authors proposed that charged residues in the BOB.1C-terminus regulate its stability by showing that changes, which make the C-terminus more acidic, including tyrosine phosphorylation-mimetic mutations, stabilize otherwise unstable murine BOB.1 protein [69].

## 3. BOB.1-OCT1 axis in autoimmune and chronic inflammatory diseases autoimmune diseases

### 3.1. Genetic association

OCT1(2) and BOB.1 are highly conserved in humans and the evidence accumulates that genetic variants either within genes encoding these factors or near their binding sites within the genome are associated with a variety of human autoimmune diseases (Table 1, Fig. 2). A comprehensive study by Maurano and colleagues performed to systematically localize common disease-associated variations in regulatory DNA revealed that a high proportion of SNPs repeatedly perturb recognition sequences of common transcription factors. Strikingly, polymorphisms within OCT1 binding sites confer a predisposition to celiac disease (CD), human systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), type 1 diabetes (T1D), and primary biliary cirrhosis (PBC) [70] (Fig. 2). A role of OCT1 in autoimmune diseases is further supported by the genetic study that links HLA-C cell surface levels to a polymorphism in consensus OCT1 binding site located in the HLA-C promoter region [71]. This is particularly intriguing as high levels of HLA-C are associated with increased risk of developing Crohn's disease (CRD) [72]. Similarly, -857C/T SNP in the OCT1 binding site, which is located in the promoter of TNF and appears to alter constitutive TNF expression, is associated with susceptibility to inflammatory bowel disease [73]. The authors have proposed that OCT1 physically interacts with the proinflammatory nuclear factor kappa-B (NF- $\kappa$ B) at an adjacent binding site inhibiting its transactivating effects and affecting the susceptibility to IBD [73].

Also, a study based on the combination of high-density genotyping and epigenomic data found that CD-associated SNPs are enriched in

**Table 1**  
The evidence of involvement of BOB.1 in autoimmune and chronic inflammatory diseases.

Disease	Genetics	Ex vivo	In vivo
Rheumatoid arthritis	OCT1 binding site polymorphisms are associated with predisposition to RA (70).	Expression of BOB.1 is decreased in PB of RA patients compared with HCs (97, 98). Expression of BOB.1 is 3-fold lower in citrulline-specific B cells isolated from RA PB than in influenza antigen hemagglutinin-specific B cells sorted from HC PB (99). BOB.1 levels are increased in RA as compared to SpA synovium (29). BOB.1 expression is increased in draining lymph nodes of RA patients in comparison to those of HCs (30). Expression of BOB.1 is elevated in synovial membranes obtained from patients with RA compared with that of patients with joint trauma (108).	BOB.1-deficient mice failed to form GCs, produce anti-CII antibodies and are resistant to CIA (29). This protection was shown to be B-cell intrinsic (29).
Lupus	OCT1 binding site polymorphisms are associated with predisposition to SLE (70).		Loss of BOB.1 prevented GC formation, production of autoantibodies and reversed severe "SLE"-like phenotype in mice lacking the zinc finger transcription factor Aiolos (82). The lack of BOB.1 protected MRL- <i>lpr</i> mice from the development of hypergammaglobulinemia, immune complex-mediated glomerulonephritis, and premature mortality (83). BOB.1 T cell-deficient NOD mice are fully protected from the development of spontaneous T1D (89). Targeting BOB.1 with rationally-designed membrane-penetrating peptide inhibitors normalized glucose levels, reduced T-cell infiltration and proinflammatory cytokine expression and blocked T1D in NOD mice (89).
Type 1 diabetes	OCT1 binding site polymorphisms are associated with predisposition to T1D (70).	BOB.1 expression is strongly elevated in islet-reactive CD4+ T cells -infiltrating T1D pancreas ( <a href="http://www.immgen.org/databrowser/index.html">http://www.immgen.org/databrowser/index.html</a> )	
Primary biliary cirrhosis	OCT1 binding site polymorphisms are associated with predisposition to PBC (70). <i>POU2AF1</i> is the significant susceptibility loci for PBC in the Japanese population (78).		
Multiple sclerosis	<i>POU2AF1</i> was identified by GWAS as a candidate gene for MS susceptibility in Belgian population (75). Meta-analysis study identified <i>POU2AF1</i> as a susceptibility gene for MS in Europeans (76).		Deletion of BOB.1 protected mice from the development of EAE (91). A decrease in EAE score in BOB.1 knockout animals challenged with MOG <sub>35-55</sub> peptide was paralleled by a reduction in infiltrating cells, lesser demyelination in spinal cords and a decline in the proportion of IL-17A-producing CD4-positive T cells.
Celiac disease	CD-associated SNPs are enriched in OCT2 binding sites (74). Fine mapping of common immune-mediated diseases-associated variants previously identified by GWAS using dense resequencing data from the 1000 Genomes Project found <i>POU2AF1</i> as a new loci for CD (77).	Gluten-specific CD4+ PD-1+ ICOS+ CXCR5- cells present in PB and intestines of CD patients with celiac disease express high levels of BOB.1 (109).	
Sjogren's syndrome		BOB.1 mRNA levels are increased in salivary glands of SS patients as compared to sicca controls (29).	
Disease	Genetics	Ex vivo	In vivo
Inflammatory bowel disease	OCT1 binding site polymorphism in the HLA-C promoter is associated with HLA-C cell surface levels (18), which is an increased risk for developing CRD (72). -857C/T polymorphic SNP in the OCT1 binding site in the promoter of TNF appears to alter constitutive TNF expression and associated with susceptibility to IBD (73).	BOB.1 mRNA is elevated in mesenteric adipose tissue located near the affected intestinal area in CRD patients (112).	
Chronic inflammatory diseases of lungs and kidney	A meta-analysis of GWAS data found nominally significant association of rs2282637 SNP annotated to <i>POU2AF1</i> with a COPD (79). An intergenic region of <i>POU2AF1</i> polymorphism is associated with serum urea and kidney function (80). The 3' UTR region of <i>POU2AF1</i> polymorphism is associated with increased risk of kidney stones (81).	The downregulation of <i>POU2AF1</i> can predict the development of BOS, the main manifestation of chronic lung allograft dysfunction at least 6 months before the onset (100). The decrease in BOB.1 expression levels in PB was observed in patients with cystic fibrosis, IPF and pulmonary hypertension (100,102,103). BOB.1 levels are specifically increased in B cells infiltrating the lungs of patients with IPF (96). BOB.1 is a key regulator of profibrotic transcriptional program in IPF lung (96). BOB.1 expression is increased in emphysema (111).	Mice lacking BOB.1 revealed suppressed CI expression, reduced hydroxyproline content and morphological changes in the lungs in responses to bleomycin and were protected from bleomycin-induced lung injury as compared to wild-type littermates (96).

RA, rheumatoid arthritis; PB, peripheral blood, HCs, healthy controls; SpA, spondyloarthritis; CIA, collagen-induced arthritis; SLE, *systemic lupus erythematosus*; T1D, type 1 diabetes; NOD, non-obese diabetic; MS, multiple sclerosis; CD, celiac disease; SS, Sjogren's syndrome; GCs, germinal centres; CII, collagen type II; CI, collagen type I; PBC, primary biliary cirrhosis; EAE, experimental autoimmune encephalomyelitis; CRD, Crohn's disease; IBD, inflammatory bowel disease; BOS, bronchiolitis obliterans syndrome; IPF, idiopathic pulmonary fibrosis.

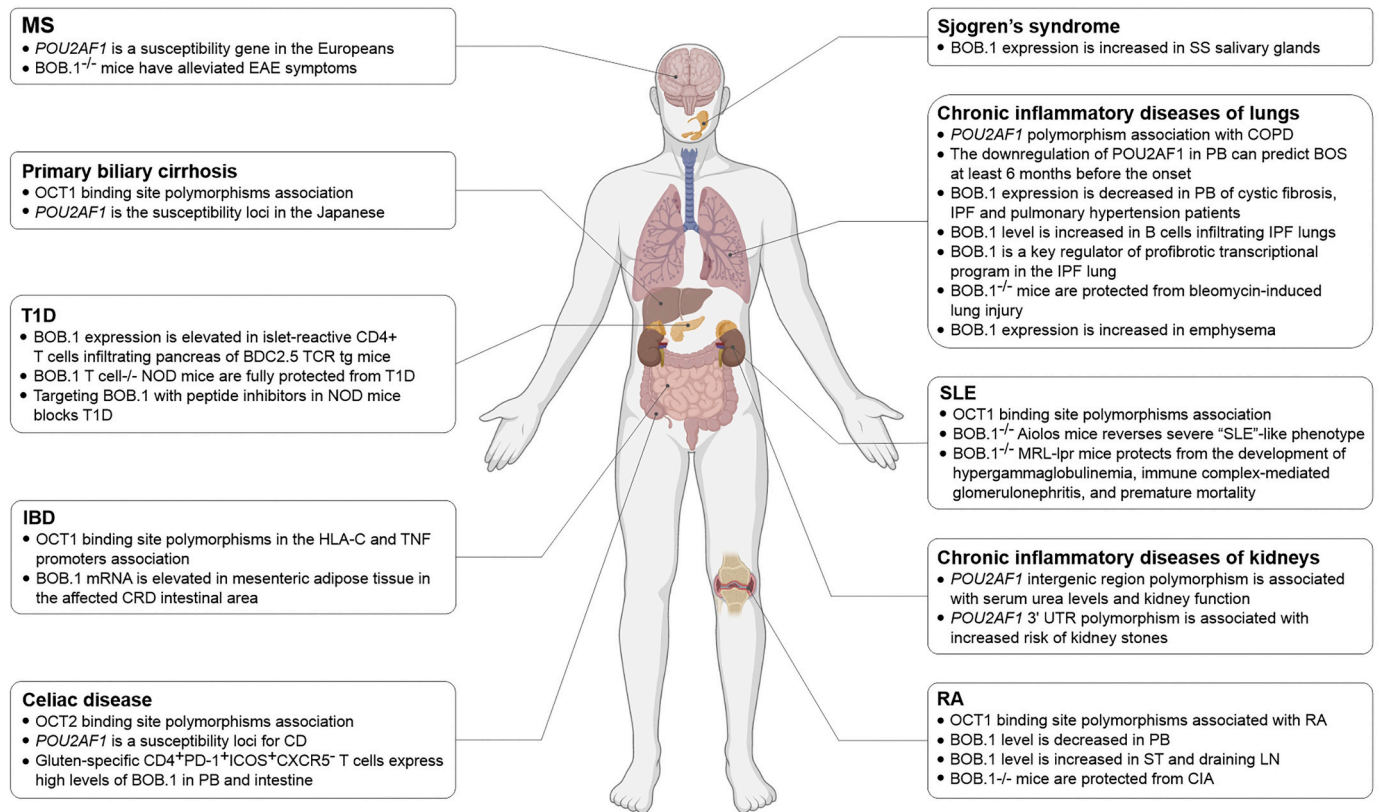


Fig. 2. Genetics, *ex vivo* and *in vivo* evidence of involvement of BOB.1 in autoimmune and chronic inflammatory diseases.

MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis; T1D, type 1 diabetes; NOD, non-obese diabetic; CD, celiac disease; PB, peripheral blood; CRD, Crohn's disease; SS, Sjogren's syndrome; BOS, bronchiolitis obliterans syndrome; IPF, idiopathic pulmonary fibrosis; SLE, systemic lupus erythematosus; IBD, inflammatory bowel disease; RA, rheumatoid arthritis; CIA, collagen-induced arthritis.

OCT2 binding sites [74]. Moreover, other studies implicate polymorphisms in or near *POU2AF1* locus itself into autoimmune and immune-mediated diseases. Thus, the whole-genome screen for association with Multiple sclerosis (MS) in a Belgian population identified potential susceptibility loci including *POU2AF1* [75]. Subsequently, the meta-analysis study of all available data from the Genetic Analysis of MS in Europeans (GAMES) project followed by the individual genotyping of the most promising markers confirmed previous data and implicated *POU2AF1* as an MS susceptibility gene in Europeans [76]. Several years later, fine mapping of common immune-mediated disease-associated variants by GWAS of dense resequencing data from the 1000 Genomes Project identified 13 new loci for CD including *POU2AF1* [77]. Another study based on genome-wide association analysis of 512 cases and 500 controls identified *POU2AF1* as significant susceptibility loci for PBS in the Japanese population [78], further supporting the notion that genetic variants in BOB.1 contribute to human autoimmune disorders. Somewhat surprising that no association with PBC was observed for *POU2AF1* in a Han Chinese Population [58] despite the close relationship of these two East Asian groups. Besides, accumulating data indicate that polymorphisms at loci encoding transcriptional regulator BOB.1 may confer susceptibility to chronic inflammatory and metabolic diseases of lungs and kidney. In particular, the meta-analysis of genome-wide association study data from several cohorts found a nominally significant association of rs2282637 SNP in *POU2AF1* with a chronic obstructive pulmonary disease [79]. Additionally, two novel associations, one in an

intergenic region of *POU2AF1* with serum urea and kidney function [80], and the other in the 3' UTR region of *POU2AF1* with increased risk of kidney stones [81] have been reported recently.

### 3.2. Insights from experimental animal models of autoimmunity and chronic inflammation

The exact role of BOB.1 in the pathogenesis of autoimmune and chronic inflammatory diseases is not precisely understood. Up-to-date, multiple animal models of human autoimmune and inflammatory diseases have been generated to investigate the function of BOB.1. However, most of these studies have been focused on conventional BOB.1-knockout mice that exhibit multiple defects in B cell lineage since ablation of BOB.1 compromises B cell development [33]. Moreover, it is hard to discriminate between the contribution of B and T-cell intrinsic effects in these mice, because, although BOB.1 is not expressed along the developing T lymphocyte axis or in naive T cells, its expression is induced upon T cell activation [48,49,65] and plays a crucial role in the memory development [46] and the Tfh cell differentiation [56]. These factors hamper direct interpretation of the BOB.1 role in autoimmune tissue inflammation, and further studies evaluating conditional and inducible models of selective BOB.1 knockout or overexpression in B or T cells are needed to advance our knowledge. Yet, the data from experimental animal models so far provide substantial evidence that BOB.1 plays a key role in the pathogenesis of the autoimmune disease

(Table 1, Fig. 2). Studies in 2003 and 2005 showed that BOB.1 is essential for the development of an autoimmune disorder that resembles human SLE in two different animal models: (1) in mice lacking the zinc finger transcription factor Aiolos [82] and (2) in MRL-*lpr* mouse strain [83]. In the first model, loss of functional BOB.1 protein prevented GC formation and production of autoantibodies including anti-dsDNA antibodies and reversed severe phenotype characterized by immune complex-mediated glomerulonephritis and tissue injury, eventually leading to chronic renal failure [82]. Since the combined loss of Aiolos and BOB.1 proteins blocks the transition from small pre-BII cells to immature B cells and significantly impairs immunoglobulin light chain DNA rearrangement, resulting in a severe reduction of the immature B cell pool in the bone marrow of double-knockout mice [84], the absence of autoantibodies in Aiolos/BOB.1 double-deficient mice was explained by block or deletion of autoreactive B cells at the stage of the immature B cells in this model [82]. A subsequent examination of the spontaneous murine lupus model MRL-*lpr* by the same group demonstrated that lack of BOB.1 completely abolished production of multiple pathological autoantibodies, including lupus-specific anti-dsDNA and anti-Sm antibodies, and consequently protected the animals from the development of hypergammaglobulinemia, immune complex-mediated glomerulonephritis, and premature mortality [83]. However, in contrast to Aiolos/BOB.1 double-deficient mice, loss of BOB.1 on the MRL background did not impair the differentiation of immature and mature B cells. Therefore, the failure to produce antibodies against nuclear antigens in MRL-*lpr* mouse lacking BOB.1 was explained by the absence of GC formation and a dramatic reduction in IgG-secreting cells rather than by defects in B-cell development [83]. Similar to the lupus models, BOB.1 is a critical factor influencing resistance to collagen-induced arthritis (CIA), an experimental model for RA, as BOB.1 deficiency completely abrogated GC formation, production of pathogenic anti-collagen type II antibodies and arthritis development [29]. As it has been shown that loss of BOB.1 during T-cell dependent immune responses prevent GC formation, as well as resulted in the significant reduction of Tfh cell numbers [57], the observed resistance to CIA could reflect either a B-cell-intrinsic failure to stimulate Tfh formation or T-cell-intrinsic defects. Importantly, an adoptive transfer of B and T cells from wild-type (WT) or BOB.1<sup>-/-</sup> animals to RAG null mice lacking endogenous B and T cell compartments revealed that a marked protection of BOB.1-deficient mice from the development of CIA was dependent on B cells as the presence of functional BOB.1 in B cells regardless of its expression in T cells was crucial for the GC formation, production of autoantibodies and development of clinical disease [29]. Similarly, B cell-inherent inability to generate a robust T cell-dependent immune response, including defective GC formation and reduced Tfh cell development in the absence of functional BOB.1 was demonstrated during influenza infection [57]. This study demonstrated that IL-6 induction in B cells, which is necessary for IL-21-dependent differentiation of activated CD4-positive T cells into Tfh cells and GC formation during an antiviral immune response, is dependent on the presence of BOB.1 and its transcriptional partner OCT2 in B cells [57]. In contrast, BOB.1-deficient T cells were not impaired in their ability to differentiate into Tfh cells and to provide sufficient help for GC B cell development in response to viral infection *in vivo* [57] and during autoimmune response [29]. However, the function of BOB.1 is more complex and is not limited to the regulation of GC formation as deletion of BOB.1 can result in antibody-mediated autoimmune disease in the absence of high-affinity IgG, as revealed evaluation of *sanroque* mice [85]. Since in *sanroque* background constitutive signalling through co-stimulatory receptor ICOS caused by expression of the modified version of Roquin1 protein results in spontaneous T-cell activation, GC formation, production of high levels of autoreactive antibodies of all isotypes leading to hypergammaglobulinemia and immune complex-mediated glomerulonephritis [86], it was reasoned that loss of BOB.1 would prevent GC formation and therefore the over-reactive GC responses. Indeed, the previous study demonstrated that reduction of GCs due to the loss of just one allele of BCL6, a master

regulator of GC formation, substantially reduced disease prevalence in *sanroque* mice [87]. However, unexpectedly and in sharp contrast to described above lupus models and to *sanroque/Bcl6*<sup>+/-</sup> mice, the deletion of BOB.1 from *sanroque* phenotype did not prevent but even exacerbated autoimmune glomerulonephritis [85]. The detailed evaluation revealed that this autoimmune disease was GC-independent and mediated by autoreactive IgM [85]. Since BCR repertoire is skewed in BOB.1-deficient B cells [38,88], it was proposed that the autoreactive IgM was derived from inappropriately selected and therefore autoreactive *sanroque/BOB.1*<sup>-/-</sup> B cells, in response to either exuberant activity of *sanroque* CD4-positive T cells or B cell-intrinsic defects resulting from the combination of BOB.1 and *sanroque* mutations. Although many questions still have to be answered to elucidate the complex role of BOB.1, cumulatively, these studies indicate the crucial impact of BOB.1 in autoimmune antibody responses and development of B-cell-driven autoimmune disorders.

In 2015 Shakya and colleagues demonstrated that in CD4-positive T cells, BOB.1 together with OCT1 regulates a set of multiple immunomodulatory target genes, including IL-2, IFN $\gamma$ , IL-17A, and IL-21 [46]. The authors proposed that, mechanistically, BOB.1 removes inhibitory chromatin modifications by recruiting the histone lysine demethylase Jmjd1a/Kdm3a to prevent stable repression of target genes and to maintain thereby transcriptionally poised states associated with immunological memory. Accordingly, in the absence of BOB.1, hardly any memory cells were generated and those which were formed responded poorly to the antigen re-encounter [46]. As a persistent antigen exposure is a typical feature of autoimmune responses, and because memory T cells can promote autoimmune disease, the recognition that BOB.1 is indispensable for the formation of T cell memory and response to re-challenge, steered re-evaluation of its role in T-cell-mediated autoimmunity. Since BOB.1 levels are strongly elevated in CD4-positive T cells infiltrating pancreas of T1D mice (<http://www.immgen.org/databrowser/index.html>), it was hypothesised that T cell-specific BOB.1 deletion would protect mice from T1D.

To address this, BOB.1 conditional allele was developed and backcrossed onto a diabetes-prone NOD background [89]. This elegant study demonstrated that while prediabetic BOB.1 T cell-deficient NOD mice have a normal T cell number and similar TCR specificities in their lymph nodes, they are fully protected from spontaneous T1D [89]. This protection was associated with a reduction of T cells and macrophage infiltration in the pancreas and a decrease of the expression of proinflammatory cytokines and chemokines, including IFN $\gamma$  and CCL1, previously identified as direct targets of BOB.1 [46]. Interestingly, prediabetic NOD mice lacking BOB.1 in T cells harboured more Tregs, however, they were still protected from T1D after Tregs depletion, albeit to a lesser extent, suggesting that Tregs only partly contributed to the T1D protection. Strikingly, loss of BOB.1 significantly modified the landscape of islet effector/memory T cells, in particular CD8-positive T cells, towards anti-diabetogenic clonotypes [89]. CD4-positive clones associated with diabetes were present, however, exhibited anergic phenotype. These results are concordant with previous results of gene expression profiling showing that genes associated with anergy, such as *Ctla4*, are overexpressed in CD4-positive cells lacking functional BOB.1 [46]. Remarkably, protection from T1D conferred by the loss of BOB.1 varied among different TCR transgenic and monoclonal antigen models and was highest in polyclonal ones [89]. Notably, BOB.1 ablation also attenuates the development of age-induced glucose intolerance, fat accumulation and insulin resistance in the diet-induced murine model of type 2 diabetes [90]. The authors suggested that reduction of B-cell maturation together with an impediment of B2 cell activity and Ig production, particularly IgG2c, induced by loss of BOB.1 underlay tolerance to glucose and sensitivity to insulin in diet-induced obesity model. This study was performed using conventional knockout mice and therefore, contribution of T cell-specific BOB.1 loss to the development of age-induced fat accumulation and insulin resistance has to be further assessed in tissue-specific and inducible models.



Another contribution to the concept that BOB.1 is a potent autoimmune regulator was made by Japanese researchers, who have demonstrated its pivotal role in Th17-mediated autoimmune response as loss of BOB.1 protected mice from the development of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis [91]. A decrease of EAE score in BOB.1 knockout animals challenged with MOG<sub>35–55</sub> peptide was paralleled by a reduction in infiltrating cells, lesser demyelination in spinal cords and a decline in the proportion of IL-17A-producing CD4-positive T cells. The authors have proposed that the formation of a ternary DNA-OCT1-BOB.1 complex and interaction of BOB.1 with the ligand-binding domain of the retinoic acid receptor-related orphan receptor (ROR) gamma t (RORγt) is necessary for sufficient for the disease induction IL-17A expression by T cells [91]. This is consistent with the previous study, which identified BOB.1 and OCT1 as positive regulators of Th17 differentiation by a combination of transcriptional profiling, computational algorithms and a gene knockdown screen *in silico* and *in vitro* [52]. Accordingly, another study supported the notion that OCT1 can control the threshold of IL-17 induction in activated Th17 effectors in cooperation with BOB.1 [92]. Cumulatively, these studies provide evidence that BOB.1 may be involved in Th17 cell-mediated disease by modulating IL-17A induction *in vivo*, yet the drawback of the work of Ikegami and colleagues is that the study is based on conventional BOB.1 knockout, which precludes unbiased conclusions. Although B cells have shown to be dispensable for disease progression in a model of acute EAE, induced by MOG peptide [93,94], in contrast to EAE induced by MOG protein, where the antigen-presentation function of B cells is critical for the disease induction [95], further investigation utilizing T cell-specific BOB.1-deficient mice that retain intact B cell compartment is required to elucidate the exact impact of T-cell specific BOB.1 loss on Th17 cell-mediated autoimmune disease.

Beyond its role in the prototypic autoimmune diseases, BOB.1 contributes to bleomycin-induced lung injury, an experimental model for lung fibrosis [96]. This study demonstrated that in response to this damage, mice lacking BOB.1 exhibited suppressed type I collagen expression, reduced hydroxyproline content and morphological changes in the lungs and were protected from fibrotic development as compared to WT littermates [96].

Cumulatively, these studies demonstrate that BOB.1 is emerging as an essential factor in the pathogenesis of many autoimmune and chronic inflammatory diseases.

### 3.3. Human expression studies

The most abundant information emphasizing the importance of BOB.1 in the development of autoimmune responses came from murine models, however, whether these findings apply to human autoimmune diseases remains to be determined. Yet, corroborating evidence continues to accumulate that BOB.1 expression is altered in the peripheral blood and target tissue of patients with autoimmune and chronic inflammatory diseases.

#### 3.3.1. Peripheral blood

We and others have reported that expression of BOB.1 is significantly decreased in peripheral blood of patients with RA compared with healthy controls (HCs) [97,98] (Table 1, Fig. 2). In addition, BOB.1 mRNA levels in the blood were found to inversely correlate with the onset of clinical symptoms and RA disease progression [98]. Furthermore, a recent study utilizing a comprehensive gene expression profiling of antigen-specific B cells revealed that expression of BOB.1 is 3-fold lower in citrulline-specific autoreactive B cells isolated from the peripheral blood of RA patients than in influenza antigen hemagglutinin-specific B cells sorted from the blood of healthy individuals [99]. However, the results of this study may be biased as citrulline-specific B cells were obtained from RA patients, who received anti-TNF therapy and other disease-modifying anti-rheumatic drugs. Another evidence of

the association of a reduced *POU2AF1* mRNA expression levels in the blood with pathological immune-related mechanisms in the tissue has been reported [100]. In that study, transcription profiling of whole blood of lung transplant recipients was applied to identify early biomarkers of bronchiolitis obliterans syndrome (BOS), the main manifestation of chronic lung allograft dysfunction (CLAD). Downregulation of *POU2AF1* along with two other genes, *TCL1A* and *BLK*, predicts BOS at least 6 months before the onset of its manifestation [100]. A decrease in the frequency of CD24<sup>hi</sup>CD38<sup>hi</sup> transitional B cells harbouring regulatory properties was described in the same patients before BOS appearance [101], however, whereas BOB.1 decreased expression is associated to the diminution of such regulatory B cells remain to be investigated. A decrease in BOB.1 expression level in the blood has also been reported in patients with cystic fibrosis, idiopathic pulmonary fibrosis (IPF), pulmonary hypertension [100,102,103], end-stage chronic respiratory diseases that share common pathogenic mechanisms with BOS [104]. These transcriptomic studies the rationale for investigating the role of BOB.1 in pathological processes leading to respiratory dysfunction.

#### 3.3.2. Target tissue

Recent evidence indicates that, in contrast to peripheral blood, levels of BOB.1 are aberrantly increased in the target tissue of patients with autoimmune and chronic inflammatory diseases (Table 1, Fig. 2). Gene expression analysis study comparing synovial biopsies from RA and spondyloarthritis (SpA) identified BOB.1 as one of the top hits in the genes overexpressed in RA synovium, where its expression levels correlated strongly with the presence of ectopic GCs [29]. Interestingly, elevated expression of BOB.1 has been also detected in lymph nodes that drain inflamed joints of RA patients [30]. Analysis at single-cell resolution has to determine whether the increased transcript levels of BOB.1 reflect a shift in the cellular composition of target tissues or it is a result of changes in gene expression underlying activation of specific molecular and cellular pathways. However, a similar degree of synovial infiltration with T, B, and plasma cells, as well as the presence of equal amounts of lymphocyte aggregates and ectopic lymphoid neogenesis in RA and SpA synovitis [105–107], and comparable numbers of GC reactions between RA and healthy lymph nodes [30] advocate for the second scenario. Significant overexpression of BOB.1 in RA synovitis was also revealed by an integrative meta-analysis of multiple gene expression profiles in synovial membrane obtained from patients with RA compared with that of patients with joint trauma [108]. Although the relationship between lymphocytes in the blood *versus* those in target tissues is unknown, these data may indicate that during the onset/development of the disease the population of BOB.1-expressing lymphocytes relocate from the bloodstream to target tissue, where they may drive autoimmune tissue inflammation. This notion is supported by recent data demonstrating that autoreactive T cells express high levels of BOB.1. The comprehensive study combining HLA-DQ–gluten tetramers with mass cytometry and RNA sequencing analysis identified a population of gluten-specific CD4-positive T cells with distinct and relatively rare phenotype expressing aberrantly high levels of BOB.1 in the blood and intestines of CD patients [109]. Strikingly, cells with similar phenotype are also elevated in systemic sclerosis, SLE [109] and RA [110]. These studies suggest that this subset is not only present in multiple autoimmune disorders, but also that these cells might be the key disease-driving T cells in these pathological conditions.

Furthermore, several seminal studies provide evidence that high levels of BOB.1 are detected in B cells infiltrating the target tissue. Thus, while the data on BOB.1 expression in lung transplants with relation to BOS development is still lacking, a recent study reported that BOB.1 level is specifically increased in B cells infiltrating lungs of patients with idiopathic pulmonary fibrosis (IPF) [96], a chronic scarring lung disease where mechanisms leading to the fibrotic processes exhibit striking similarities to those in the engrafted lung [104]. Importantly, the comprehensive dynamic regulatory model of human IPF based on mRNA and microRNA expression profiles of affected microenvironments of IPF

lungs combined with immunohistochemistry, single-cell RNA sequencing and animal model found that BOB.1 is a key regulator of a profibrotic transcriptional program [96]. Increased BOB.1 expression was also shown to be a signature of emphysema, where B cells play a key mediator role in the immune response against foreign or self-antigens [111]. Additionally, elevated BOB.1 transcription is also observed in mesenteric adipose tissue located near the affected intestinal area in patients with CRD [112] and in salivary glands of patients with primary Sjogren's syndrome [29]. The exact role of lymphocytes expressing high levels of BOB.1 remains to be investigated. Given that BOB.1 plays an indispensable role in the establishment of immunological memory in both, B and T-cell compartments, future work has to elucidate the role of BOB.1 in the autoimmune tissue inflammation and transplant graft rejection where constant exposure to (self)antigens may reinforce pathogenic memory responses.

### 3.4. Human *in vitro* models

Experimental *in vitro* models indicate that overexpression of BOB.1 in B cells in the conditions that mimic TD immune responses (CD40L-L + IL-21) resulted in the suppression of plasma cell differentiation and promoted the generation of cells characterized by increased expression of costimulatory molecules and an increased BCR signal strength mirrored by an enhanced costimulatory capacity, phenotypically and functionally resembling memory B cells [30]. Although *in vivo* validation is required, these results indicate that BOB.1 plays important role in determining cell-fate decision during TD immune responses and suggest that increased expression of BOB.1 in inflamed tissues may lead to pathologic accumulation of activated memory B cells.

### 3.5. Targeting BOB.1 in disease

During the last decade, important findings emerged from studies of animal models of autoimmunity, which positioned BOB.1 as a promising therapeutic target for human autoimmune diseases. Recent advances in the field of small-molecule inhibitors of protein-protein interactions and availability of the co-crystal structure of BOB.1-N-terminus/OCT1 DNA binding domain/octamer binding DNA complex allowed designing drugs that exploited vulnerable unique interface not present in other proteins and, enabled the generation of a membrane-permeable competitive peptide inhibitor of the interaction between BOB.1 and a downstream effector, Jmjd1a/Kdm3a [89]. As discussed above, BOB.1 interacts with Jmjd1a/Kdm3a, a histone lysine demethylase that removes inhibitory histone H3 lysine 9 methyl marks to establish permissive chromatin environments and poised gene expression state of previously activated targets to be robustly re-activated upon antigen-re-encounter [46]. The authors postulated that this property becomes damaging due to the persistent response to self-antigen(s), such as in T1D, and tested the effect of this inhibitor, termed JumOCA (OCA-B is an alias name for BOB.1), on T1D onset and severity [89]. Indeed, strikingly, peptide administration into NOD mice significantly reduced islet-infiltrating T cell numbers and proinflammatory IFN $\gamma$  and IL-17A cytokine production, reversed elevated blood glucose levels and protected animals from newly-arisen T1D [89] (Table 1, Fig. 2). Although future research is needed to investigate how interference with BOB.1 would affect B cell axis, these data provide first proof-of-concept evidence that targeting BOB.1 is a promising therapeutic approach to treat emerging T1D effects and potentially other T-cell dependent autoimmune diseases, where BOB.1 plays a prominent role.

## 4. Conclusions and perspectives

Since the discovery of the OCT1-BOB.1 axis three decades ago, immunologists, molecular and structural biologists around the globe have extensively worked to understand its role in mice and man, yet there are still novel aspects of its biology being uncovered in healthy and diseased

conditions. In this review, we summarised cumulative evidence from genetics, *in vitro* models, human expression studies, and animal models that all link BOB.1-OCT1(OCT2) axis to the pathogenesis of autoimmune and chronic inflammatory disorders. This strong albeit circumstantial evidence has recently been confirmed by the proof that targeting BOB.1 suppresses autoimmune conditions. These promising results certainly open new avenues, however, many fundamental and clinical questions remain to be answered. Key aspects to be considered during the pre-clinical validation of the interference with the ternary OCT1-BOB.1-DNA complex include (1) detailed characterization of BOB.1 target genes in B, T, and other cellular sources, (2) a better understanding whether and how interference with BOB.1 may affect functions of OCT1 (OCT2) transcription factors, (3) identification of other, potentially targetable, components of this pathway, (4) the definition of the therapeutic window for maintenance of the delicate balance between the proper response to pathogens and formation of pathogenic memory directed towards self-antigens, allowing impediment of autoimmunity while keeping normal immune homeostasis intact. Strikingly, elevated expression of BOB.1 is a hallmark of dysfunctional CD8-positive T cells incapable of efficient combating tumour cells. Given that BOB.1 is a promising target for pharmaceutical inhibition in autoimmune conditions, targeting this factor in the context of the tumour environment may rescue T cell dysfunction, promoting/enhancing anti-tumour immunity without inducing autoimmune side effects [113].

### Author contributions

NY and AT drafted the manuscript, drew illustrations, and discussed the content with the other authors. RD participated in the drafting and review of the manuscript. SB and DB critically revised the content of the manuscript. All authors approved the publication of the manuscript. D. B. is an employee of Union Chimique Belge. The other authors have no financial conflicts of interest.

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