HLA region contains a set of genes that play crucial roles in the immune system. In addition to the central function of antigen-presentation, which is conducted by HLA class I and II genes, function of the other HLA-linked genes may also contribute to the immune regulation. *IKBL*, alternatively named as *NFKBIL1*, mapped within the HLA class III region is a newly emerged gene, of which sequence variations are associated with the susceptibility or resistance to autoimmune and/or inflammatory diseases. We recently have revealed that the *IKBL*-coded protein, IkBL, is involved in the regulation of alternative splicing in human immune-related genes and a viral gene, which unravel an unexpected function of the HLA-linked gene and provided a novel understanding of HLA in the regulation of immunity and infection. In this review, we summarize the latest trends in the study of *IKBL*.

**Key Words:** NFKBIL1, CLK1, alternative splicing, susceptibility, autoimmune disease, influenza virus
provided with the function in antigen presentation to CD4⁺ or CD8⁺ T cells, respectively. On the other hand, HLA class III region contains a number of genes not involved in the antigen-presentation. It has been well known that these non-antigen-presentation genes are also important in the immune regulation. For examples, genes in the HLA class III region encode components of complement system, Bf, C2, and C4, which are involved in the clearance of pathogens. In addition, lymphotoxins including TNF-α are encoded by the genes in the HLA class III region and play roles as central mediators in the inflammatory response as well as in the programmed cell death.

**A potential role of IkBL in the immune regulation**

Inhibitor of κB-like (IKBL), also named as NF-κB inhibitor-like 1 (NFKBIL1), is mapped within the HLA class III region about 25 kb telomeric to TNFA. A considerable number of studies reported the association between genetic variations of IKBL with the susceptibility or resistance to autoimmune and/or inflammatory diseases, suggesting that IKBL might mediate underlying mechanisms in the immune regulation.

As far as we know, the genetic variations of IKBL, which are reported to link with immune-related diseases, include five different single nucleotide polymorphisms (SNPs); −421 8T/9T (rs3219186), −324 C/G (rs3219185), −262 A/G (rs3219184), −62 A/T (rs2071592) and +738 T/C (rs3130062), as well as haplotypes composed of promoter SNPs, from IkBLp*01 to IkBLp*05. The first study carried out by Okamoto et al identified IKBL as a candidate risk locus for rheumatoid arthritis (RA), in which the −62T allele conferred the susceptibility. Subsequent study conducted by different group using independent samples supported that the −62T allele was associated with RA, but the other SNPs in close linkage disequilibrium (LD) with the −62T may also shape the susceptibility to RA. Another autoimmune disease, systemic lupus erythematosus (SLE), was also reported to be associated with SNPs of IKBL. The −62A and +738C alleles showed decreased and increased odds risk for SLE, respectively, while the −62A+738T haplotype was found to decrease the risk. Furthermore, +738C allele in an ancestral haplotype 7.1 was reported to confer a resistance to multiple sclerosis (MS). The associations with IKBL were also reported for other autoimmune diseases; Graves disease (susceptibility with −62A) and type I diabetes (T1D) (resistance with IkBLp*03 haplotype).

Genetic variations of IKBL are also associated with series of chronic inflammatory diseases. A meta-analysis in Japanese populations revealed that −262G and −62T were the candidate loci for susceptibility to ulcerative colitis, although another European group additionally reported an association with +738C. In addition, the associations were found for other inflammatory diseases such as chronic Chagas cardiomyopathy (susceptibility with −262A and −62A alleles, and −262A–62A haplotype), Takayasu arteritis (TA) (susceptibility with IkBLp*03 haplotype), and chronic thromboembolic pulmonary hypertension (susceptibility with IkBLp*03 haplotype). These lines of evidence strongly suggested the involvement of IKBL in autoimmune and/or inflammatory diseases. However, the molecular function of IKBL, as well as the molecular basis underlying the pathogenesis of these immune-related diseases, remained largely unknown.

**Molecular function of IkBL**

Evidence has mounted that SNPs in the promoter region of IKBL influence the expression of IKBL. Shibata et al. have reported that the promoter SNPs consist of five different haplotypes, IkBLp*01 to IkBLp*05, which conferred different transcriptional activities of IKBL. Interestingly, IkBLp*01 and p*03, which showed the lowest and highest promoter activities, were associated with the susceptibility to RA and TA, respectively. Furthermore, the −62 position was predicted to be a binding site for 6EF1, USF1 and E47 transcription factors, and the −62 SNP was indeed demonstrated to affect the binding of these transcription factors, which was supposed to have an impact on the expression of IKBL. Taken these observations into account, it could be speculated that the association between IKBL with immune-related diseases may attribute to the altered expression of IkBL.

Overexpression and/or knockdown of IKBL were reported for investigating the functional role of IkBL in the context of immune regulation. First, the role of IkBL in IKK-IkB-NF-κB signaling pathway was examined. Inflammatory signal-induced phosphorylation of IkB leads to its degradation, releasing NF-κB dimer to translocate into nucleus and to initiate transcription. As compared with the members of IkB family, such as IkBa and IkBβ, which are central molecules in the inflammatory signaling, the amino acids sequences of IkBL showed only a limited homology.
IκB and immune-related diseases

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In addition, IκB did not show any transactivation activity (our unpublished observation).

We and others investigated the intracellular localization of IκB. It was found that EGFP-tagged IκB localized within nuclear speckles, the punctuate staining pattern under microscope, which are known as typical localization pattern of RNA splicing factors, such as serine/arginine rich (SR) proteins, as evidenced by the co-localization of IκB and a SR protein, SC35 (Figure 1). In addition, immunoprecipitation assay revealed that IκB bound RNA. These lines of evidence implied that IκB might participate in the processing of RNA. Transcribed pre-mRNA undergoes post-transcriptional splicing, categorized into constitutive and alternative splicing. Depending on the cis-regulatory elements and splicing-related factors, splicing events discriminate introns from pre-mRNAs and combine exons to form mature RNA transcripts in the constitutive splicing. On the other hand, the alternative splicing is an important mechanism in the post-transcriptional control of gene function in eukaryotes, in which target exons in pre-mRNAs could be either excluded or included depending on specific cellular contexts.

To clarify the role of IκB, we made an effort to investigate its function in the alternative splicing. Because abnormal alternative splicing in several immune-related genes was reported to link with autoimmune diseases including MS, SLE and T1D, mini-gene of CD45, CD72 and CTLA4 were designed and constructed to be tested for the alternative splicing in the context of IκB function. It was found that knockdown of IκB promoted the exon exclusion, whereas overexpression of IκB counteracted the exon skipping. On the other hand, IκB affected the alternative splicing of Influenza A virus M gene. These results for the first time demonstrated that IκB played role as a regulator of alternative splicing in the immunity and infection (Figure 2).

Molecular mechanism of IκB in the alternative splicing

We further asked the molecular mechanism of IκB-mediated regulation of alternative splicing. By yeast two hybrid screening, IκB was found to interact with CDC-like kinase 1 (CLK1), a well-known factor to regulate the alternative splicing by phosphorylating SR proteins. The effects of CLK1 in the alternative splicing of immune-related genes were found to counteract IκB, leading to a hypothesis that IκB may interfere with the kinase activity of CLK1. However, IκB did not affect the CLK1-induced phosphorylation of SR protein. Furthermore, kinase activity of CLK1 was dispensable for the alternative splicing. These results have suggested that IκB and CLK1 regulate the alternative splicing by a novel mechanism distinct from the CLK1-dependent phosphorylation (Figure 2).

Our works contribute to understanding the function of IκB. However, there are several topics to be discussed. First, CLK1, as the interacting partner of IκB, may serve as a clue to investigate the mechanism of IκB-mediated splicing.

In addition, EGFP-tagged IκB construct was transfected into HeLa cells. The transfected cells were immunostained by anti-SC35 antibody followed by AlexaFluor 568-conjugated secondary antibody. (a) EGFP signals (green) representing the localization of IκB, (b) localization of SC35 (red), and (c) merged image of left and middle images. Scale bar; 5 μm.
alternative splicing. We found that the N-terminal regulatory domain of CLK1 played an important role in the alternative splicing\textsuperscript{15}, but no definite function was deciphered for the N-terminal domain of CLK1. Second, it is well known that phosphorylation of SR proteins has significant impacts on the RNA splicing\textsuperscript{23}. Albeit that I\textsubscript{k}BL did not affect the phosphorylation of ASF/SF2, it should be considered that I\textsubscript{k}BL might affect the phosphorylation status of other splicing factors. In addition, SR proteins interacting with I\textsubscript{k}BL may not limit to ASF/SF2. Third, given that the regulation of alternative splicing by I\textsubscript{k}BL is independent from the kinase activity of CLK1, the exact mechanism for the involvement of I\textsubscript{k}BL in the alternative splicing remains elusive. I\textsubscript{k}BL was found to associate with the RNA recognition motifs (RRMs) of ASF/SF2 (Figure 2), implying that I\textsubscript{k}BL would interfere with the RNA binding of SR proteins. On the other hand, it was reported that RRM2 of ASF/SF2 mediated autoregulation in their expression\textsuperscript{24}. The fact that I\textsubscript{k}BL associates with RRMs of ASF/SF2 suggests that I\textsubscript{k}BL might control the expression of ASF/SF2 or other SR proteins. Fourth, a fundamental issue still remains to be uncovered; that is, how I\textsubscript{k}BL is induced and where it is expressed in the context of immune-related diseases. It was found that the expression of \textit{IKBL} was relatively low in human tissues and organs, although the overexpression and knockdown assays demonstrated that altered expression of \textit{IKBL} could affect the alternative splicing events. Indeed, the expression of \textit{IKBL} was inhibited by activation stimuli with PMA to affect the alternative splicing in an established human T cell line\textsuperscript{15}. It is worth to assess whether stimulations of primary immune cells would change the \textit{IKBL} expression.

**Figure 2** Involvement of I\textsubscript{k}BL in the Clk1-mediated alternative splicing

Clk1-mediated alternative splicing process is schematically represented. (a) In the absence of I\textsubscript{k}BL, pre-mRNA binds RRM domain of ASF/SF2 and undergoes splicing mediated by heterogeneous nuclear ribonucleoprotein (hnRNP) such as hnRNPL, hnRNPLL and FOX1. Clk1 usually enhances the splicing process by phosphorylating RS domain of ASF/SF2. In the Clk1-mediated phosphorylation process by its kinase domain, N-terminal domain of Clk1 binds RRM domain of ASF/SF2. The process may result in skipping exons of human immune-related genes including CD45, CD72 and CTLA4 as well as an influenza virus M gene, which might lead to accelerated inflammation and increased viral replication. It should be noted that the alternative splicing process of these genes could be found in the absence of kinase function. (b) In the presence of I\textsubscript{k}BL, Clk1-mediated alternative splicing is attenuated. I\textsubscript{k}BL binds both RRM domain of ASF/SF2 and N-terminal domain of Clk1. Clk1-mediated phosphorylation of RS domain of ASF/SF2 is not inhibited by I\textsubscript{k}BL. The attenuated splicing process may result in the inclusion of exons, leading to prolonged inflammation and attenuated viral replication.
**IkBα and Diseases**

We have demonstrated that IkBα might regulate the immune system via modulating alternative splicing of immune-related genes, which coincides with the notion that the disturbance of alternative splicing in immune-related genes would link with autoimmune diseases\(^{17-19}\). However, functional evidence for that the pathogenesis of immune-related diseases is attributable to the deregulation of alternative splicing is still lacking. Even though splice variants of CD45, CD72 and CTLA4 have been suggested to regulate the function of B and T cells\(^{23-27}\), further studies illustrating the causal relationship between the alternative splicing and diseases are required. Furthermore, IkBα appears to control a large variety of alternative splicing, but the mechanisms controlling the gene-specificity are waiting to be identified, and a comprehensive analysis of target genes is particularly essential. For this purpose, next generation sequencing could be applied for exploring the RNAs regulated by IkBα in cells involved in the immune regulation, appended with the information of exact interacting sites or motifs. These results will not only propose the characteristics of IkBα-interacting RNAs, but also provide an overview to which extent IkBα is involved in the alternative splicing of immune-related genes.

In order to investigate the role of IkBα in the autoimmune and inflammatory diseases, IKBL-knockout (KO) mice will undoubtedly be required. On the other hand, it was reported that IKBL-transgenic (Tg) mice show resistance to collagen-induced arthritis, an experimental model for RA\(^{20}\). It is worth trying to apply IKBL-KO or -Tg mice into other models of immune-related diseases such as myelin-induced experimental autoimmune encephalomyelitis, a model of MS. Besides, examining the alternative splicing of target genes in IKBL-KO or -Tg mice will be valuable for establishing the link between the alternative splicing and immune-related diseases.

**IKBα** also regulates the alternative splicing of influenza A virus M gene\(^{29}\). Given that inhibition of the synthesis of M2 variant accounts for decreased virus titer\(^{29}\), IKBα provides us with an insight into the host-dependent control of viral replication. It also suggests that IkBα, as well as splicing factors, would be useful to prevent viral infection by modulating alternative splicing of viral genes. Beside of influenza A virus M gene, genes of other virus are known to undergo alternative splicing in infected cells, such as tat, rev genes of human immunodeficiency virus (HIV)\(^{30}\).

Whether IkBα affects expressivity of HIV genes and lead to an impact on virus replication will be an attractive issue for investigation.

**Conclusion remark**

Acknowledging to genetic association studies, IKBL was identified to be a candidate gene involved in the immune regulation. Albeit several issues remain to be clarified, recent studies have suggested that IkBα modulates the alternative splicing in both human and viral genes. These observations led to further understanding about the function of HLA region in the immune system and in the pathogenesis of immune-related diseases. In the future, as an excellent achievement of biomedical research, we expect IkBα as a potential target of therapeutic strategy in clinical treatments.

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IkBL and immune-related diseases

IkBL mapped within the HLA region is a novel regulator of alternative splicing involved in the pathogenesis of immune-related diseases

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