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Splicing in immune cells—mechanistic insights and emerging topics

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REVIEW

Abstract

Differential splicing of mRNAs not only enables regulation of gene expression levels, but also ensures a high degree of gene-product diversity. The extent to which splicing of mRNAs is utilized as a mechanism in immune cells has become evident within the last few years. Still, only a few of these mechanisms have been well studied. In this review, we discuss some of the best-understood mechanisms, for instance the differential splicing of *CD45* in T cells, as well as immunoglobulin genes in B cells. Beyond that we provide general mechanistic insights on how, when and where this process takes place and discuss the current knowledge regarding these topics in immune cells. We also highlight some of the reported links to immune-related diseases, genome-wide sequencing studies that revealed thousands of differentially spliced transcripts, as well as splicing studies on immune cells that remain mechanistically not fully understood. We thereby display potential emerging topics for future studies centered on splicing mechanisms in immune cells.

Keywords: alternative splicing, B cells, nuclear bodies, sequencing, T cells

Introduction: splicing of mRNAs, an important layer of gene regulation

The great variations in gene expression in higher eukaryotes do not simply and solely result from enrichment in gene numbers or alleles. Actually, the low number of genes identified (26 564 annotated in the human genome) was rather surprising at the time the human genome was sequenced, with only ~1.1–1.5% being coding sequences (1–3). A eukaryotic gene on average contains 7.8 introns and mRNA processing offers a wide range of possibilities to regulate the expression of the corresponding gene (4). This includes alternative polyA-site usage, leading to different 3′ UTR (untranslated region) lengths, as well as mRNA modifications, such as 5′ capping or m^6A methylation, which influence spatial and temporal regulation of mRNA localization, translation and decay. Another layer of regulation is mediated by alternative splicing (AS), resulting in different usage of exons. This results in dramatic changes in gene expression, since it can lead to changes in protein isoforms. Indeed, RNA-sequencing (RNA-Seq) studies highlight and reveal that ~95% of all human genes undergo AS (5).

Immune cells are repeatedly dividing and differentiating throughout their lifetime and cells of the adaptive immune system represent highly evolved cells in vertebrates. It is therefore not surprising that immune cells utilize AS to ensure high transcript diversity and to regulate gene expression.

In this review, we will give a mechanistic insight of how, when and where splicing takes place in general and in immune cells and we display the underlying mechanisms. We also discuss selected examples of genome-wide sequencing studies, some of the best-understood examples of AS events in immune cells and conclude with links between splicing and immune-related diseases.

Splicing: the question of how, when and where?

The molecular course of splicing

Splicing is mediated by the spliceosome, a multicomponent complex, which is assembled on its target RNA (6). The spliceosome consists of five snRNPs (small nuclear ribonucleoproteins)—protein complexes formed on small nuclear RNAs (snRNAs) named U1, U2, U4, U5 and U6 (6). The assembly of the catalytically active spliceosome starts with the binding of U1 and U2 to the target RNA at its 5′ and 3′ splice site, respectively (complex A), while the binding of U6 to U2 is blocked by its interaction with U4 (7); the spliceosome is inactive at this state, although all five snRNPs are bound to the target RNA (complex B). After the disruption of the pairing of U4–U6, activation occurs upon the release of U1 and U4 from the spliceosome (complex B*) (7). U2 then binds to

the U6 subunit, leading to the formation of the catalytically active spliceosome consisting of U2, U5 and U6 (complex C) (7). This complex brings the splice sites of exons in proximity to each other by forming an intronic loop called lariat, which is then removed from the spliced RNA (6). This process is mediated in part by multiple RNA helicases that destabilize the binding of U1 to RNA (6) and by factors that disrupt the interaction of U4 and U6 (7). The overall course of events is displayed in detail in Fig. 1.

In this review, we are going to focus mainly on AS events in immune cells. AS is controlled by the occurrence of *cis*-regulatory RNA elements, so-called splicing regulatory elements (SREs), which can be distinguished in exonic or intronic splicing enhancers (ESEs or ISEs), as well as exonic or intronic splicing silencers (ESSs or ISSs) that can in turn recruit *trans*-acting proteins such as splicing factors (6). These include members of the serine/arginine-rich (SR) protein family, as well as heterogeneous nuclear ribonucleoproteins (hnRNPs). The combinatorial interplay of *cis*-elements and *trans*-factors then determines inclusion versus exclusion of exons (8).

Splicing—overall a co-transcriptional process

It is widely accepted by now that almost all mRNAs are co-transcriptionally spliced and that RNA-polymerase-II (RNAPII) itself facilitates the recruitment of splicing factors (9). More precisely, when the C-terminal domain (CTD) of RNAPII is phosphorylated at Ser-2, transcriptional elongation takes place and the CTD is able to interact with splicing factors, such as U2AF65 (10, 11). A kinetic coupling model has even been proposed, since splicing reactions are determined by slow transcription elongation rates, enhancing exon inclusion (9). It seems logical to couple transcription with splicing in time and space, but it also raises the question of how this process might be regulated (10). Two studies (in activated macrophages and in activated T cells) highlight that this process is globally delayed after activation and that un-spliced transcripts remain at the chromatin at first (12, 13). Whether this is just a consequence of the new initiation of RNAPII or has a biological reason that is particularly useful for immune cells remains unclear at this stage and interesting to address.

The direct link between transcription and splicing raises the question whether not only RNAPII but in fact transcription factors and transcriptional regulators can directly influence splicing decisions. Indeed, the transcription factor Myb, important in hematopoiesis, was suggested to act as a bifunctional regulator in both transcription and splicing (14). In T cells, the transcription factor Gfi1 has been shown to be of importance in AS of *CD45* by its interaction with the splicing factor U2AF65 (15), whereas the transcription factor FoxO1 regulates splicing of *Ikaros* during immunoglobulin gene rearrangement in pro-B cells (16).

We therefore speculate that potentially many well-known transcriptional regulators of the immune system might play an important role in splicing—functional links that remain so far widely unstudied in immune cells.

Compartmentalization, a potential link between structure and function

The nucleus can mainly be divided into chromatin and interchromatin, for which several sub-nuclear non-membranous compartments have been described (17, 18). Although their link to function remains under debate, nuclear compartments (or nuclear bodies) concentrate proteins and/or RNAs and can represent optimized microenvironments for highly

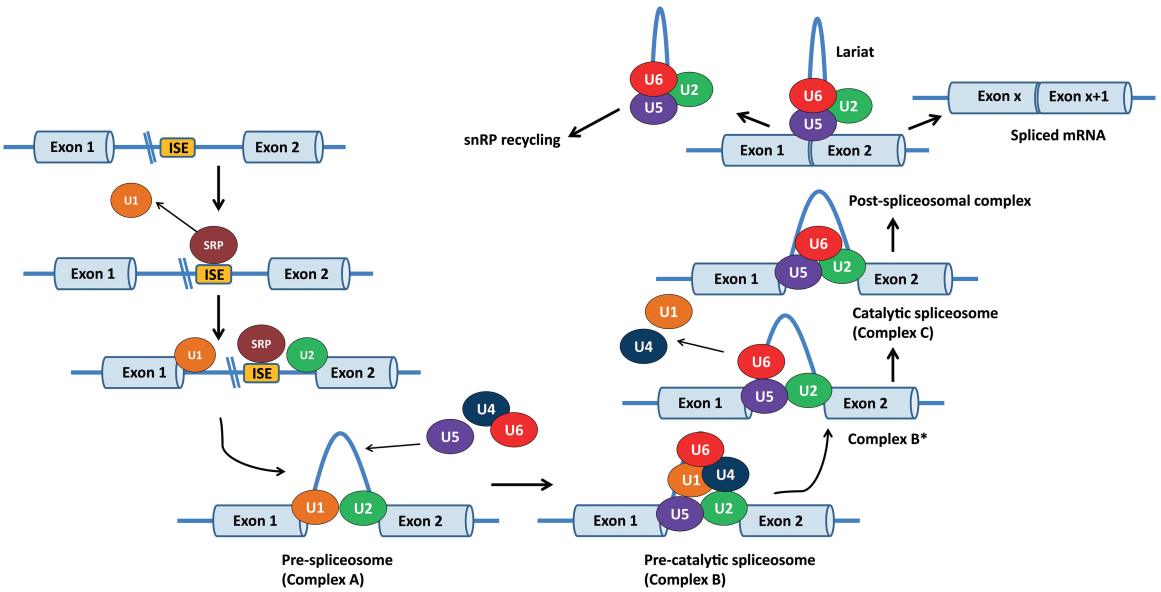


Fig. 1. Schematic view of the mechanism of splicing: a catalytic multistep process is mediated by the spliceosome. Intronic splicing enhancers can recruit *trans*-acting splicing factors like SR proteins, which in turn enhance the binding of the spliceosomal subunits U1 and U2 to the 5′ and 3′ splice site of the target mRNA's exons. Complex A, the pre-spliceosome, then forms by the interaction of the snRNPs U1 and U2 and enhances the binding of the other spliceosomal subunits U4, U5 and U6, representing the pre-catalytic spliceosome (complex B). Complex B in turn is activated by a release of U4 and U1 from the spliceosome (complex B*). Here, the interaction of U2 with U6 leads to a catalytically active spliceosome (complex C). Splice sites of exons are then brought into proximity by the formation of an intronic loop called lariat, which then is removed by the spliceosome, freeing both the spliced mRNA and the spliceosomal subunits that can be recycled to catalyze the next splicing event.

efficient processing of large numbers of RNA transcripts (19, 20). Therefore, these compartments likely play a role in complex cellular processes, for example immune responses (19, 20).

In the following, we will focus on only a few of the well-studied nuclear compartments, in particular nuclear speckles (also named speckles), Cajal bodies (CBs) and paraspeckles, and highlight mechanisms associated with these compartments that might play a role for immune cells, although this still represents a largely undiscovered area in immunology.

Among the most prominent sub-nuclear structures are nuclear speckles. Although many different bodies have been associated with the regulation of splicing, the localization of the splicing machinery seems to be in nuclear speckles. Nuclear speckles consist of 20–50 irregularly shaped granules with varying size that are connected by a thin fibril (perichromatin fibril) that is likely to be the site of co-transcriptional splicing (20). Nuclear speckles have been found to contain RNAPII, mostly with the Ser-2 phosphorylated form of the CTD (21), also in T cells (22). Not only the process of co-transcriptional splicing but also alternative mRNA splicing is believed to take place in nuclear speckles (23). Among others, nuclear speckles contain SRSF1, SRSF2 and NSrp70, which mediate the alternative splice site selection of *CD44*, important for lymphocyte activation (24, 25). The activated serine-468 phosphorylated form of the NF-κB family member p65 also localizes to nuclear speckles, indicating also a role of these structures in the regulation of gene expression in immune responses (26).

Another nuclear compartment is the so-called CB. CBs contain the CB-specific protein coilin and have a diameter of ~0.3–1.0 μm (17, 27, 28). Although previously suggested, splicing does not occur within CBs (28). Instead, CBs concentrate components important for snRNP biogenesis and turnover (18, 29). Furthermore, CBs are associated with immune cell function: the human CB component hCINAP is able to negatively regulate NF-κB signaling in response to TNF stimulation (30). However, CBs can only be identified in a limited number of cell types (17, 27, 28).

Additionally, in the nucleus 5–20 paraspeckles, named due to their localization adjacent to nuclear speckles (31) and with a similar size as CBs, can be found in most cell types, including primary and transformed cells but not in stem cells (32). The function of these structures was initially described as sites of RNA editing and subsequent RNA retention (18). Adenosine to inosine (A-to-I) editing can lead to altered splice sites or SREs and therefore could influence AS (18). Therefore, we speculate that paraspeckles are of importance in medullary thymic epithelial cells (mTECs) for antigen presentation. RNA processing and RNA editing in these cells is an important mechanism to minimize self-recognition by expanding the diversity of the self-antigen repertoire to almost 85% of the entire coding genome (33). Paraspeckles contain PSP1 (paraspeckle protein 1), p54/nrb (Nono), the splicing factor SFPQ (PSF) and probably also TDP-43, which associate around the long non-coding RNA (lncRNA) *NEAT1* (31, 34, 35), for all of which an association with immune cell function has been described. SFPQ binds to exon 4 of *CD45* in stimulated T cells and suppresses its inclusion, which is prevented by the binding of TRAP150 to SFPQ in resting cells (36). Moreover, ~40 T-cell AS events that were sensitive to SFPQ knockdown could be identified (36). These included splicing of the transcription factor LEF1, LRR1 or the splicing factor PRPF3, which in turn might influence splicing in a re-stimulation-dependent manner (36).

Interestingly, SFPQ acts as a repressor at the promoter of cytokines, for example IL-8, likely as a complex with Nono, again highlighting the link between regulation of splicing and transcription (37). SFPQ has also been shown to be associated with acute myeloid leukemia (AML) (38, 39), whereas *NEAT1* expression is also induced by influenza and HSV-1 infection (37) and by a combinatorial stimulation with LPS and IFN-γ (35), suggesting a role in the regulation of immune responses for paraspeckle kinetics.

Recently, a novel nuclear compartment induced upon LPS treatment of macrophages and bone marrow-derived dendritic cells (BMDCs) was suggested. This compartment seems to be involved in the mRNA processing of IL-6 and IL-10 and therefore was named the IL-6 and IL-10 splicing activating compartment (InSAC) (40, 41). Here, spliceosomal components of the CB are recruited to InSACs by TDP-43 (40). However, the question remains about whether this body-like structure could be related to paraspeckles, as TDP-43 previously had been described to also localize to paraspeckles (31).

A schematic overview of the described nuclear compartments and their so-far described functions within immune cells is shown in Fig. 2.

Splicing in immune cells—links to differentiation and function

To provide an overview of the splicing literature in immune cells, we decided to discuss selected genome-wide studies, the best-understood splicing mechanisms in immune cells and end with immune-related diseases that have been connected to mutations in the process of splicing. Here, we are focusing on macrophages and dendritic cells, as well as T and B cells. The reviewed topics in these cells are illustrated in Fig. 3.

Genome-wide observations of AS in immune cells

The ImmGen Consortium revealed by sequencing that AS is pervasive, as ~60% of genes show different AS isoforms in T or B cells, with ~70% of these AS events linked to lineage differentiation, and could identify 7599 previously unreported isoforms (42).

Earlier studies using microarrays showed that 10–15% of the profiled alternative exons were regulated on the exon inclusion level during T-cell activation (43). Using RNA-seq profiling, Martinez *et al*. (44) could also identify 178 exons in 168 genes with changes in exon inclusion upon stimulation of the TCR and an enrichment of functional annotations related to the immune response, such as *CD45*, *Fyn*, *CELF2*, *TRAF3*, *BRD8* and *TRIM*. Changes in AS are in part due to stimulation of the TCR (1319 genes with AS), but mostly through co-stimulation via CD28 (1575 genes with AS upon TCR/CD28 activation) (45), connecting the regulation of splicing with T-cell function. One possible facilitator of this effect is the splicing regulator hnRNPLL, which is dependent on CD28 signaling (45).

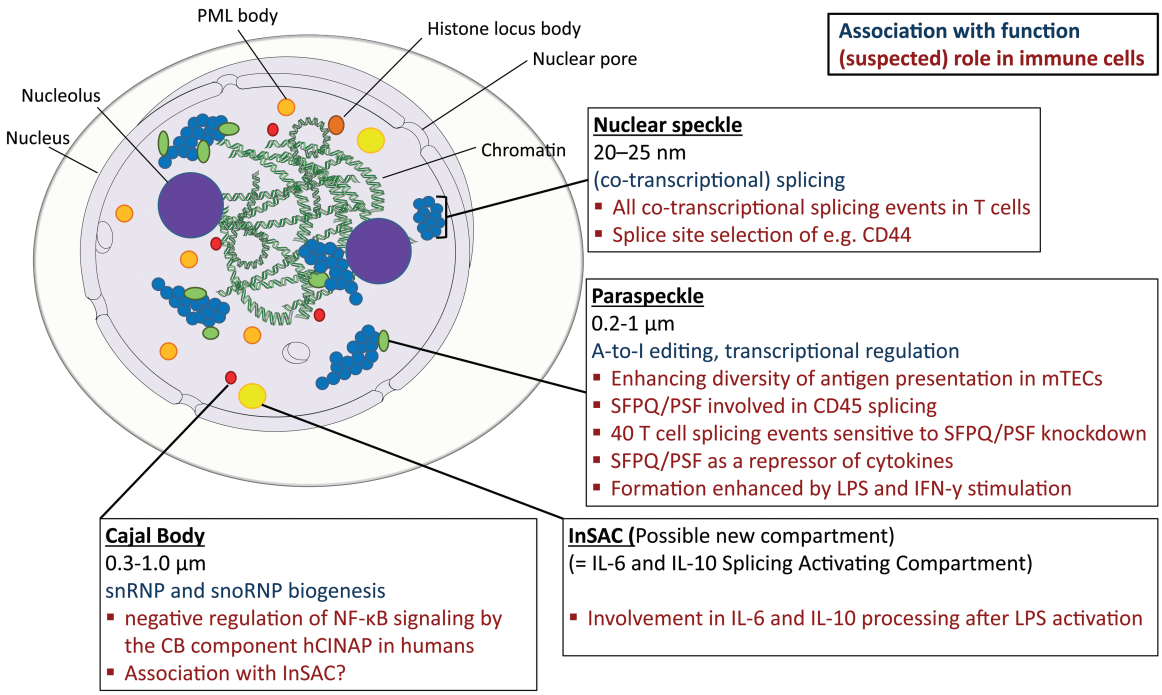


Fig. 2. Schematic drawing of nuclear compartments and their proposed molecular functions within immune cells. The interchromatin contains several body-like structures, including nuclear speckles (blue), paraspeckles (green), InSACs (yellow) and CBs (red). Indicated in black text is the size of each compartment, as well as its proposed function (blue) and a possible link for immune cell differentiation or function (red). Nuclear speckles seem to represent the location of co-transcriptional as well as AS in all cell types, including immune cells (20, 22, 23, 26). CBs are the location of snRNP and snoRNP biogenesis (18, 29) and have been associated with the negative regulation of NF-κB signaling (30), whereas A-to-I editing is proposed to take place in paraspeckles (18), which might be of particular necessity in mTECs to decrease self-recognition (33). Paraspeckle formation has been shown to be induced by LPS and IFN-γ stimulation and paraspeckle components are involved in transcriptional regulation and splicing decisions in immune cells (35–39). The stimulation with LPS also leads to the localization and processing of IL-6 and IL-10 in InSACs (40, 41). PML, promyelocytic leukemia protein; snoRNP, small nucleolar ribonucleoprotein.

The extent to which AS is utilized in B cells has also become evident lately, as >90% of genes with multiple exons were shown to be spliced alternatively with one isoform being predominantly expressed (shown for 20 766 genes and 67 453 of their isoforms) (46). In mouse BMDCs, different splicing patterns could be linked to maturity states, which are also heterogeneous between single cells upon LPS stimulation and might reflect functional differences (47).

In general, a variety of studies implicate that AS is particularly important in complex systems, where information is processed differently depending on time and context, such as axon guidance (48, 49) or in the immune system (5, 50).

Various specific splicing mechanisms and splice variants in immune cells have been associated with an altered function. In the following paragraphs, we would like to reflect on a few of the most prominent AS events that have been addressed in the innate and adaptive immune systems.

AS in innate immune cells

A role of splicing in the control of innate immunity can be demonstrated by the splicing factor SF3A1 that regulates splicing of genes involved in TLR signaling pathways in macrophages (51). The inhibition of SF3A1, for example, leads to a decreased production of positive regulators of TLR signaling, including IRAK1 and CD14, as well as IKKβ and an increased production of the negative regulators sTLR4 and Rab7b, highlighting the importance of splicing in the regulation of an immune response in innate immune signaling (51).

One prominent example for AS in innate signaling is the isoform of MyD88, MyD88s. This isoform has been shown to regulate the extent of inflammatory cytokine production in murine macrophages, therefore limiting the innate immune activation downstream of TLR signaling (52, 53). A connection between splicing factors and innate immune responses could be shown by the regulation of MyD88s by Eftud2 (52). Also, in the context of TLR4 signaling, SF3A1, SF3A2, SF3A3 or SF3B1 are essential for the production of the long isoform of MyD88, since their knockdown leads to an increase of the short isoform, and therefore an inhibition of MyD88 signaling (53). In humans, MyD88s has been proposed to be associated with sepsis (54), indicating that this is an important mechanism in humans to dampen immune responses.

For TLR4, a soluble isoform with a 144-bp insertion between exons 2 and 3, which leads to a premature stop codon, has been described that is induced upon LPS treatment (55). This isoform differs in its function from the membrane-bound isoform as it inhibits the production of TNF-α and NF-κB signaling in a mouse macrophage cell line, therefore acting as a negative feedback mechanism to limit an excessive LPS response (55).

The generation of soluble isoforms of membrane receptors indeed is a mechanism of splicing that is frequently utilized by immune cells, e.g. for IL-4R (56), IL-5R (57) or IL-6R (58)

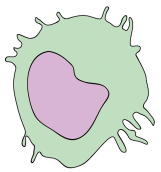
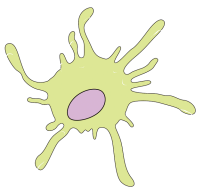
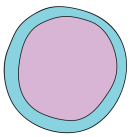
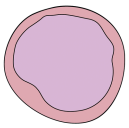
	Genome-wide analysis	Described examples	Disease association
 Macrophages	▪ 791 AS events SF3A1 sensitive [51] ▪ 81 AS events LPS sensitive [51] ▪ Unspliced transcripts remain at chromatin [12]	TLR4 [55] MyD88 [52–54] IL-6 [40, 41] IL-10 [40, 41]	▪ AML and SFPQ [38, 39] ▪ AML and U2AF1 [97]
 Dendritic cells	▪ link between maturity states and splicing patterns [47] ▪ widespread variability in AS events on a single-cell level upon LPS stimulation [47]	MyD88 [52–54] IL-6 [40, 41] IL-10 [40, 41]	▪ AML and SFPQ [38, 39] ▪ AML and U2AF1 [97]
 B cells	▪ More than 90% of multi-exon genes alternatively spliced [46]	IgM/IgD [59–63] IgM [64–67] IgE [68–72]	▪ CLL and SF3B1 [93]
 T cells	▪ 1,319 AS events upon ligation of the TCR [45] ▪ 1,575 AS events upon TCR and CD28 stimulation [45] ▪ Enrichment of AS in genes related to immune response [44] ▪ Unspliced transcripts remain at chromatin [13]	CD44 [81–83] CD45 [80, 84–89] MALT1 [77] FOXP3 [78, 79]	▪ Multiple sclerosis [89] ▪ Type-1 Diabetes [101]

Fig. 3. Overview of reviewed genome-wide studies, examples of AS and disease association of splicing in immune cells. Global sequencing studies (indicated in brackets) revealed the occurrence of differentially spliced transcripts in macrophages, dendritic cells, B cells and T cells. Additionally, we have described mechanisms for AS in genes important for immune cell differentiation and function, e.g. *CD45* in T cells or IgM/IgD in B cells. Associations between splicing defects or mutations in splicing factors and the onset or progression of immune-related disease, e.g. SF3B1 and AML are indicated for the different cell types.

and represents another layer of regulation of the immune response. This mechanism is also utilized in B cells and will be discussed in the following paragraph.

Splicing mechanisms of importance for T- and B-cell function

There are three main examples for AS in the generation of immunoglobulins in B cells. However, the outcome of these AS events significantly differs, leading to a class switch, to changing between cell-bound and secreted isoforms or to increasing transcript diversity.

AS plays a role during class switch from IgM to IgD in B cells. Although the principle of allelic exclusion applies in B cells, IgM and IgD can be expressed simultaneously by a differential splicing of one transcript containing the *VH*, as well as the *Cμ* and *Cδ* domains (coding for IgM and IgD, respectively) (59), where the inclusion of either *Cμ* or *Cδ* leads to the production of IgM or IgD in the same cell (60, 61). One possible regulator of this process is the zinc finger protein 318 (Zfp318) (62). Zfp318, which contains a U1-type zinc finger domain, was found to decrease IgD and increase IgM levels on follicular B cells when being alternatively spliced (63). This emphasizes a role of Zfp318 in the regulation of the splicing of the *VH*, *Cμ* and *Cδ* precursor and therefore a specific role in balancing the IgD and IgM output.

The second mechanism of AS in B cells involves the generation of a secreted form of IgM upon differentiation (64). Whereas the membrane-bound form of IgM is produced in immature and mature B cells (65), upon differentiation the secretory form is expressed (66). This is tightly regulated by both splicing and cleavage-polyadenylation at an alternative poly(A) site at the 3′ end of the *μ* pre-mRNA (65). Whereas the binding of cleavage-stimulatory factors (e.g. CstF64K) leads to the production of secretory IgM, a binding of the

spliceosomal protein U1A inhibits the binding of cleavage factors and enhances the production of membrane-bound IgM (64). The U1A amount associated with the U1RNA and therefore available to inhibit the secretory poly(A) site is increased in differentiated cells (67), indicating a link between the formation of the spliceosomal subunit U1snRNP and differentiation in B cells.

AS can also increase the transcript diversity of IgE. The IgE exists in various isoforms, both secreted and membrane-bound (68–70). The production of the different IgE isoforms has been shown to be dependent on different stimuli and to follow a developmental profile (71), highlighting a differentiation-related regulation of the splice-variant expression (72).

One example of a potent regulator for these AS events and splicing in general in B cells is the RNA-binding protein HuR (ELAVL1) (73, 74). Whereas a loss of HuR resulted in a defective class-switching and led to B-cell death, the importance of HuR-mediated RNA processing additionally was highlighted by the HuR-dependent splicing of hundreds of transcripts (74), emphasizing the importance of tightly regulated splicing mechanisms in the differentiation and activation of B cells.

There are a couple of examples for the relevance of AS in the regulation and differentiation of T cells. Here, we want to focus on selected examples important for T-cell signaling, the transcription factor FoxP3, as well as the most prominent examples CD44 and CD45.

T-cell signaling can be influenced by AS variants of RelA (p65) that lead to a diminished DNA-binding ability and therefore represent non-functional dimers negatively regulating NF-κB signaling (75), or by AS of the deubiquitinating enzyme CYLD (76). T-cell activation additionally is supported by the proper splicing of *MALT1* into *MALT1A* by the inclusion of exon 7 upon TCR activation (77). MALT1A facilitates the recruitment of TRAF6 and therefore enhances downstream signaling, promoting an optimal T-cell activation. Naive T cells express almost exclusively MALT1B since the splicing factor hnRNP U in these cells suppresses the inclusion of exon 7 and therefore negatively regulates T-cell activation (77).

For FOXP3, the master regulator of Treg function, three splice variants have been described to date, including the full-length version (FOXP3fl), an isoform lacking exon 2 (FOXP3Δ2) as well as an isoform lacking both exon 2 and 7 (FOXP3Δ2Δ7) (78). The expression of the isoform FOXP3Δ2Δ7 has been shown to be induced by IL-1β (79) and to inhibit the function of FOXP3fl in a dominant negative manner since it is itself not able to repress NFAT-mediated or NF-κB-mediated gene transcription and therefore impairs the suppressive function of Tregs (78). FOXP3Δ2Δ7 not only inhibits Treg function, but it also favors the differentiation into T_h17 cells *in vitro* (79), linking splicing with differentiation of T-cell subsets.

However, the best-studied examples for the importance of splicing in T cells remain the splicing of CD44 and most importantly the regulation of the eight different isoforms of the *PTPRC* (*CD45*) gene due to the variable use of exons 4, 5 and 6 during T-cell differentiation [reviewed by Trowbridge and Thomas (80)].

The cell-surface glycoprotein CD44 whose variant CD44v is involved in both lymphocyte activation and metastasis (81) is regulated by the splicing regulator Sam68 (82). Sam68 is induced upon the Ras–Raf–MEK–ERK signaling cascade, but not the p38 MAPK pathway (83) and *CD44* therefore is a good example for a splicing event that is regulated in response to extracellular cues like activation (82).

The isoforms of CD45, also named RA/RB/RC/RO, are expressed in different patterns in functionally distinct T-cell populations (both in CD4 and CD8 subsets) and the patterns change upon activation of naive T cells (80). The expression of the RA or RB isoforms is associated with a naive cell state while memory cells lose expression of these isoforms, but are RO positive (84). Since inclusion patterns are tissue specific, tissue-specific splicing factors like members of the SR protein family of splicing factors, whose expression pattern also changes during T-cell activation, are candidates of the AS regulation (85). In a short-hairpin RNA interference screen, Oberdoerffer *et al*. (86) identified hnRNPLL to bind *CD45* transcripts and induce alternative *CD45* splicing upon activation. Additionally, Sc35 (87) and other hnRNPs, namely hnRNPL, PTB and hnRNP E2, have been found to regulate the variable exon 4 of *CD45* (CD45RA) (88).

Importantly, alterations in the CD45 splicing pattern in activated T cells were associated with multiple sclerosis (89), highlighting the functional importance of a proper control of CD45 splicing.

Indeed, quite a few immune-related diseases are associated with defects in splicing processes, which we are going to highlight in the next section.

Immune-related diseases—links to splicing

The link between certain diseases and RNA-processing regions has been extensively debated (90, 91) since a deregulation of splicing and specific isoforms has been described as a common mechanism in cancer cells, specifically in the epithelial–mesenchymal transition and metastasis (92).

Quite a few studies also reported a link between mutations in splicing factors themselves and different types of cancers of the immune system, e.g. myelodysplasia or leukemia (93, 94). Two splicing machinery components, SF3B1 and U2AF1, have been described as frequently mutated in myelodysplastic syndromes (MDS), occurring with ~20 and 9%, respectively (95–97), while in the MDS sub-group defined by ring sideroblasts—erythroblasts with iron-loaded mitochondria—up to 75% of patients showed mutations in *SF3B1* (96). In another study, in 16 out of 29 analyzed patients with myelodysplasia, components of the splicing machinery were mutated, with *U2AF35*, *ZRSR2* and *SRSF2* being the most commonly mutated (98). More extensive studies with 105 individuals who suffered from chronic lymphocytic leukemia (CLL) pinpointed recurring mutations in the key spliceosome component SF3B1 (93). Mutations of U2AF1 also lead to an increased risk of the progression from MDS to AML but show no difference in the overall survival (97), which might be due to the oncogenic potential of a number of U2AF1 target genes (99).

Additionally, autoimmune diseases have been correlated with altered splicing. Significant differences in alternative isoforms could be detected in psoriasis (100), in type 1 diabetes where AS has been detected both in pancreatic β cells and T cells (101) and in the previously mentioned example of CD45 in the context of multiple sclerosis (89).

Conclusions

In this review, we highlight the importance of the temporal and spatial regulation of splicing mechanisms and the relevance for immune cell function and differentiation. Although some selected splicing mechanisms are extremely well studied, this field is still in its infancy. Not only do thousands of differentially spliced mRNAs revealed by RNA-Seq reports remain unstudied for immune cell function, but also the role of nuclear compartments as well as underlying splicing mechanisms that are causative for immune-related diseases remain poorly understood. We propose that splicing in immune cells will be a flourishing topic in the field of molecular immunology in the future, displaying a great deal of potential new discoveries and insights.

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