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Review

CD45 in Memory and Disease

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Abstract. CD45 (the leukocyte common antigen) is known to function as a tyrosine phosphatase in leukocyte signaling. Biochemical studies indicate that CD45 is involved in the regulation both of T cell receptor-associated kinases and Janus kinases that transmit signals from cytokine receptors. However, the function of the different isoforms of CD45 generated by complex alternative splicing, and indeed the role of the whole extracellular domain of the molecule, remain mysterious. Analysis of CD45 knock-outs and of transgenic mice expressing single CD45 isoforms, as well as the disease associations of human polymorphisms, is providing new insights into CD45 function. Accumulating data from these genetic and biochemical studies promises to elucidate the role of high and low molecular weight isoforms of CD45 in the function of naïve and memory T lymphocytes.

Key words: CD45; cellular signaling; memory T lymphocytes.

Introduction

The leukocyte common CD45 antigen is a transmembrane tyrosine phosphatase expressed only on nucleated hemopoietic cells, where it comprises up to 10% of the cell surface. The phosphatase activity of CD45 antigen is crucial for efficient lymphocyte antigen receptor signal transduction, as has been shown in CD45-negative cell lines, CD45-deficient mice[12, 22] and humans[24, 61]. Both mice and humans lacking CD45 are severely immunodeficient, with very few T cells in the periphery and impaired T and B cell responses. Various members of the Src family of tyrosine kinases have been identified as the main substrates for CD45 phosphatase activity and CD45 has been shown to operate as a positive as well as negative regulator of Src family members[40, 51]. Moreover, new evidence suggests that CD45 can function as a Janus kinase (JAK) phosphatase, negatively regulating cytokine receptor activation involved in the differentiation, proliferation and anti-viral immunity of hemopoietic cells[20]. THOMAS[62], THOMAS and BROWN[63], ALEXANDER[2, 3] and PENNINGER et al.[41] have published recent reviews of these signaling events and made important contributions to our understanding of the substrates and regulation of CD45 phosphatase activity. The present review will therefore focus on current advances in our understanding of the functions of different CD45 isoforms, their relation to immunological memory and turnover of cells, and how abnormalities in CD45 expression are associated with disease.

CD45 Structure

The gene encoding CD45, also known as the protein tyrosine phosphatase receptor-type C gene (*PTPRC*), contains 35 exons and is located on chromosome 1. It

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is close to a region containing several genes of immunological interest, such as the Fc IgG1/IgG2A receptors and the IGFR2 receptor[46], and colocalizes with marker D1S413 on chromosome 1q31–32[17]. Little is known about the regulation of the gene because of its very large size which has been estimated to be >120 kb[18]. We have analyzed[65] 2.7 kb of the 5'flanking region of the human CD45 gene and determined that the only region with strong promoter activity is in the highly conserved first intron of the gene. The promoter activity, which is not tissue restricted, is strongest at the 3'end of intron 1 and the sequence lacks similarities with known promoters and initiators. Five prime RACE identified an alternative exon 1, designated 1a, which, like exon 1b, can be spliced to exon 2, a structure observed in the mouse.

CD45 is a type I transmembrane protein with a large, heavily glycosylated extracellular domain (391–552 amino acids) and an extensive cytoplasmic tail (700 amino acids) containing two domains with phosphatase homology (Fig. 1). The proximal cytoplasmic domain of CD45 has phosphatase activity, whereas the second domain does not, due to changes in critical amino acids necessary for catalytic activity. However, the second domain is required for proper folding and substrate recruitment. CD45 homologues have been identified in various mammals, the chicken, shark and puffer fish *Fugu rubripes*[14, 39]. There is a low sequence similarity between the extracellular CD45 domains of different species, but the cytoplasmic domain is highly conserved, supporting the view that the essential role of CD45 is as a phosphatase required for normal antigen-induced signal transduction.

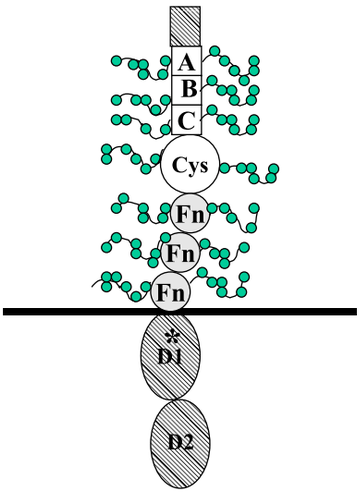

Fig. 1. Schematic organisation of human CD45. Exons that are alternatively spliced are designated A, B and C. O- and N-linked carbohydrates are shown as small circles. The location of the cysteine-rich region is shown by Cys, followed by three fibronectin type III domains (Fn). The two intracytoplasmic phosphatase domains are designated D1 and D2 and the first membrane proximal one with a phosphatase activity is identified by an asterisk

Multiple isforms are generated by alternative splicing of exons 4, 5 and 6 at the N-terminus of the extracellular domain of the molecule[47, 53]. Exons 4, 5 and 6 are also termed A, B and C. Thus, CD45RABC refers to an isoform containing all three variable exons 4, 5 and 6, while CD45R0 refers to the null isoform, in which exons 4, 5 and 6 have been spliced out. The alternatively spliced exons are heavily O-glycosylated and the different CD45 isoforms vary in their glycosylation pattern and size. In addition the glycosylation pattern may be different in different hemopoietic cells. Alternative splicing in the region encoding the O-linked carbohydrate sites is a conserved feature of CD45 from elasmobranchs to mammals, strongly suggesting that changes in glycosylation and the length of the region may be important for the function of the molecule and possible interactions with potential ligands.

The variable exons in the extracellular domain are followed by a cysteine rich domain and three fibronectin type III domains (Fig. 1)[39, 56]. The fibronectin type III domains serve as a platform for N-glycosylation sites, and modeling of the structure of a mutant CD45 associated with severe combined immunodeficiency confirms the importance of the fibronectin-like domains in maintaining CD45 structural integrity[61].

CD45 Ligands

The CD45 extracellular domain has been shown to have an overall rod-like structure[35] and projects from the cell, as well as folds and bends laterally, allowing for possible *trans* and *cis* interactions with potential ligands. The B cell activation and differentiation marker CD22 was first identified as a potential ligand for CD45[52]. However, it was subsequently shown that this interaction is not specific and that CD22 is a lectin which interacts with a wide range of glycoproteins with N-linked sialic acids[50]. The cystein-rich domain of the mannose receptor, which plays a role in antigen uptake and presentation, has also been shown to interact with two low-molecular-weight CD45 isoforms in a carbohydrate-dependent manner[33]. It has been proposed that a soluble form of the mannose receptor can interact with forms of CD45 (among other ligands) present on the cells of secondary lymphoid organs and that this interaction may mediate the delivery of the antigen to follicular areas[34].

The lectin galectin-1, specific for β-galactosides, has been described as interacting with CD45 molecules[16, 42, 55, 68]. Galectin-1, which is expressed by stromal cells in thymus and lymph nodes, may regulate apoptosis during T cell development and maturation. Galectin-1 and galectin-3 can also associate with the antigen receptor complex and co-receptor molecules on T cells and modulate T cell receptor (TCR)-mediated signaling and activation. Galectins are involved in the organization of cell surface glycoproteins, and it is tempting to speculate that CD45 function might be regulated by the interaction of distinct CD45 isoforms with a cell-surface galectin lattice[13].

Alternatively, the CD45 extracellular domain may interact in *cis* with molecules on the same cell surface. It has been shown that various CD45 isoforms associate with other molecules, including Thy1, CD2, LFA1, the TCR complex and CD4. For example, reconstitution of a murine CD45 negative cell line with different single CD45 isoforms suggested that the CD45R0 isoform promoted greater IL-2 secretion upon engagement of the TCR with the cognate MHC peptide compared with other isoforms[38]. Co-capping experiments in these cells revealed preferential CD4-CD45R0 association. However, more detailed capping and co-immunoprecipitation studies indicated a basal association of CD45R0 with the TCR-independent of CD4 expression and suggested that co-capping of CD4 with CD45R0 was mediated by this prior CD45R0-TCR association[27]. Nevertheless, CD4-CD45 association has also been shown in primary $CD4^+$ T cells[10, 37].

Homodimeric forms of CD45 have been also described[57] and it has been suggested that homodimerisation of CD45 may lead to inhibition of its tyrosine phosphatase activity[32]. The crystal structure of the receptor-type protein tyrosine phosphatase α (RPTPα) membrane proximal D1 phosphatase domain indicates that this domain forms a symmetrical dimer in which the catalytic site of one molecule is blocked by specific contacts with a helix-turn-helix wedge from the other[9]. This wedge is conserved within the RPTPs, including CD45. Mutation of the putative inhibitory wedge domain of CD45 in a mouse line resulted in autoimmunity, consistent with the loss of inhibition of CD45 phosphatase activity[32]. It has been hypothesized that the CD45R0 isoform might homodimerise more easily and, consequently, be subject to negative regulation by the inhibitory wedge. In contrast, the extarcellular domain of $CD45RA^+$ isoforms is heavily O-glycosylated and carries a strong negative charge, which could form an electrostatic barrier to homodimerisation.

CD45 Isoforms and Memory

CD45 can exist as at least 8 different isoforms, generated by alternative splicing, ranging in size from 180 to 220 kDa molecular weight[2]. The splice factor SR protein SC35 has been shown to control the removal of alternatively spliced exons[69]. The expression of different CD45 isoforms depends on the state of activation and differentiation of hemopoietic cells, and in humans, mice, rats and sheep it has been used to distinguish subsets of T cells with distinct functional properties[1, 26, 29, 44]. B lymphocytes express the high-molecular-weight isoform CD45RABC of 220 kDa (also called B220). Immature $CD4^+CD8^+$ thymocytes mainly express the low-molecular-weight isoforms, whereas mature $CD4^+$ and $CD8^+$ thymocytes and peripheral T cells can express multiple isoforms.

Expression of different isoforms also changes during T cell activation. In man, two major subsets of T lymphocytes, expressing high-molecular-weight isoforms containing the A exon ("CD45RA cells") or the l80 kDa isoform ("CD45R0 cells"), have been characterized and termed naïve and memory cells. While these terms are an oversimplification, the two subsets do have very different properties. In the CD4 subset, the majority of CD45RA T cells are small resting cells with long telomeres that divide only rarely, while CD45R0 cells show evidence of activation, have shorter telomeres and divide relatively frequently[30, 36]. The TCR repertoire of CD45RA cells is extremely diverse, but the CD45R0 subset contains a much smaller number of expanded clones. The question of reversion of CD45 phenotype from CD45R0 to CD45RA has been much discussed. MICHIE et al.[36] proposed that it might occur on the basis of mathematical modeling of data on lymphocyte lifespan. However, under conditions allowing the generation of responses to antigens previously not encountered (primary responses) from $CD4^+$ $CD45RA^+$ T cells, the same cells did not respond to the recall antigen tetanus toxoid[71]. In contrast, $CD4^+CD45RA^+$ cells from tetanus immune donors can respond to tetanus toxoid when provided with additional co-stimuli through ligation of CD28[43], but in this case the responding cells may be low-affinity naïve cells that were not stimulated by *in vivo* exposure to tetanus toxoid alone.

The situation is rather more complex among CD8 T cells. Although it is clear that naïve CD8 T cells express the high-molecular-weight isoform "CD45RA" phenotype, activated or memory CD8 cells have a variable CD45 phenotype[19]. During acute EBV infection, most CD8 T cells become CD45R0 positive and this population contains many large antigen specific ex-

panded clones[31]. Following resolution of the acute illness and a return to a normal lymphocyte count, many of the expanded clones can now be detected in both the CD45R0 and CD45RA populations. Nevertheless, although there is a reversion to expression of CD45RA expression, these antigen-experienced CD45RA cells can be distinguished from naïve CD45RA cells by other phenotypic markers, such as CD27, CD28, CCR7 or CD11b. It is not yet clear whether these $CD8^+$ $CD45RA^+$ primed cells represent terminally differentiated effector cells or a non-dividing CD8 memory population.

In spite of this understanding of the function of these T cell subsets, the mechanisms linking the expression of different isoforms to T cell function and the regulation of CD45 activity remain unclear.

CD45 and Disease

Recently, we identified a homozygous 6 bp deletion in the gene encoding CD45 in a Kurdish infant from consanguineous parents, which resulted in a lack of surface CD45 expression and severe combined immunodeficiency (SCID)[61]. The 6 bp deletion in exon 11 of the CD45 gene resulted in a loss of Glu339 and Tyr340 in the first fibronectin type III module of the extracellular domain. Molecular modeling suggests that Tyr340 is crucial for the structural integrity of the fibronectin type III module and, thus, for the CD45 protein (Fig. 2). Another SCID patient lacking CD45 expression has also been reported in whom two separate genetic abnormalities, distinct from that reported by us, were shown to be associated with lack of CD45 expression in the patient's cells[24]. These studies provide evidence

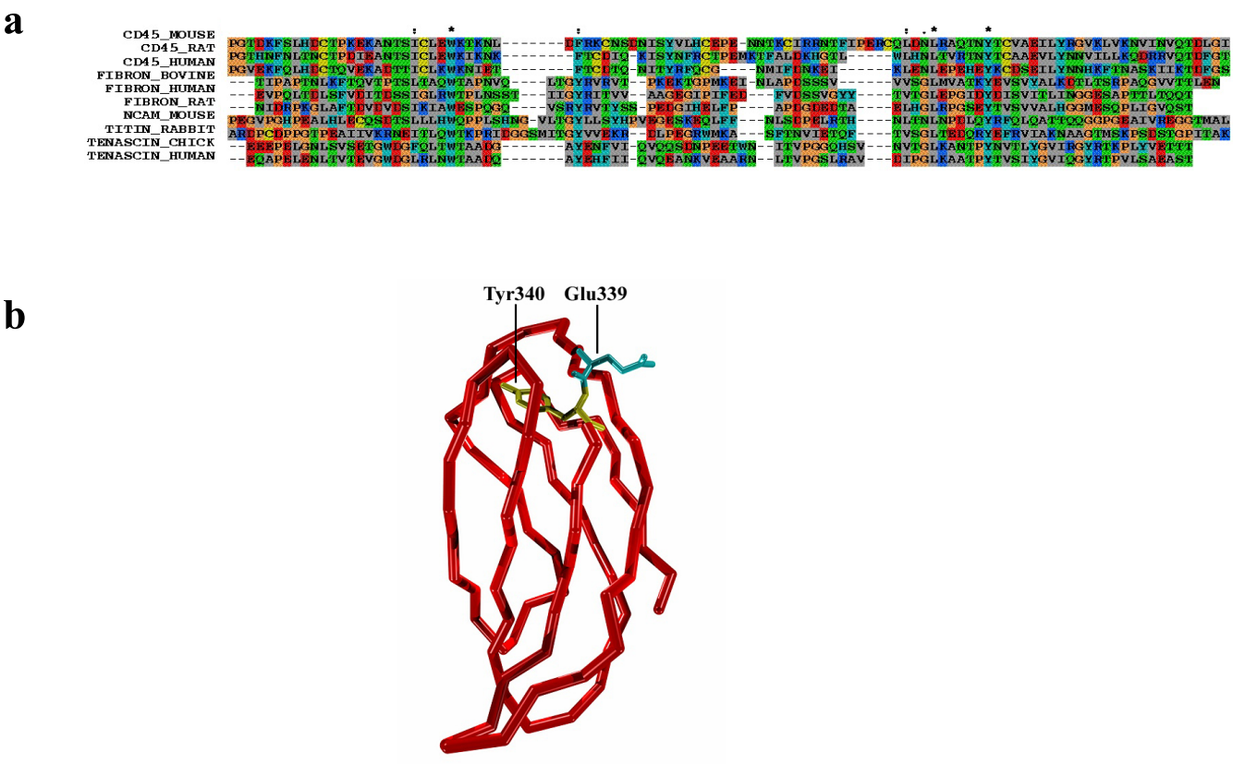

Fig. 2. A deletion in the gene encoding CD45 in a patient with SCID. **A** – sequence alignment of vertebrate CD45 proteins and a number of representative fibronectin type III module sequences. An asterisk marks the deleted Tyr340 identified in a SCID patient. The Tyr340 is highly conserved in all CD45 sequences (human, rat, mouse, chicken, shark and puffer fish) and the equivalent tyrosine is conserved in known fibronectin sequences, **B** – the positions of the deleted residues, glutamic acid 339 (Glu339) and Tyr340, are shown on the Cα trace of the modeled fibronectin type III module. The model suggests that this tyrosine makes an important contribution to maintenance of the packed core of the fibronectin structure and deletion of this residue will seriously destabilize the fibronectin module, leading to unfolding and intracellular degradation

for the importance of CD45 in immune function in humans and show that abnormalities in CD45 expression are a cause of SCID in humans and should be included in the investigation of SCID patients and other patients with unexplained immunodeficiency.

In contrast, a mouse model has been described in which cells expressing a constitutively active form of CD45 developed a severe autoimmune nephritis with antibody production, resulting in death[32]. Similarly, mice expressing a non-oncogenic level of a constitutively active form of the tyrosine kinase LckY505F developed aggressive thymic lymphomas on a CD45$^{-/-}$ background, which implies that CD45 may function as a tumor suppressor in this experimental system[5]. Administration of antibodies to the CD45RB isoform can induce long-term engraftment and donor-specific tolerance to murine renal and islet allografts[4, 25], and it has been suggested that anti-CD45RB acts through mechanisms that include CTLA-4 upregulation[15]. In a mouse model of Alzheimer's disease, modulation of CD45 isoforms can activate microglia, while in human patients with Alzheimer's disease, CD45 expression is up-regulated on microglial cells and crosslinking CD45 can supress CD40 ligand-induced and β-amyloid peptide--induced microglia activation[8, 58, 59].

All of these studies suggest that CD45 is a potent candidate target for immune intervention and that its modulation may affect autoimmunity, cancer and transplantation.

C77G Polymorphism and Abnormal CD45 Splicing

In addition to the association of SCID with lack of CD45 surface expression, abnormalities of CD45 splicing have been recognized in humans[48, 49]. A C to G transversion in the fourth exon of CD45 (exon A C77G) has been shown to prevent normal splicing of the N-terminal region of the gene[64, 72]. This C77G mutation does not change the amino acid sequence but prevents splicing by disrupting a strong exonic silencer[28]. Activated or memory lymphocytes in these individuals continue to express both high- and low--molecular-weight CD45 isoforms, in contrast to the normal pattern of low-molecular-weight isoform expression (Fig. 3). Expression of the C77G polymorphism has recently been shown to be associated with development of multiple sclerosis (MS) in some families in 3 of 4 independent case-control studies in Ger-

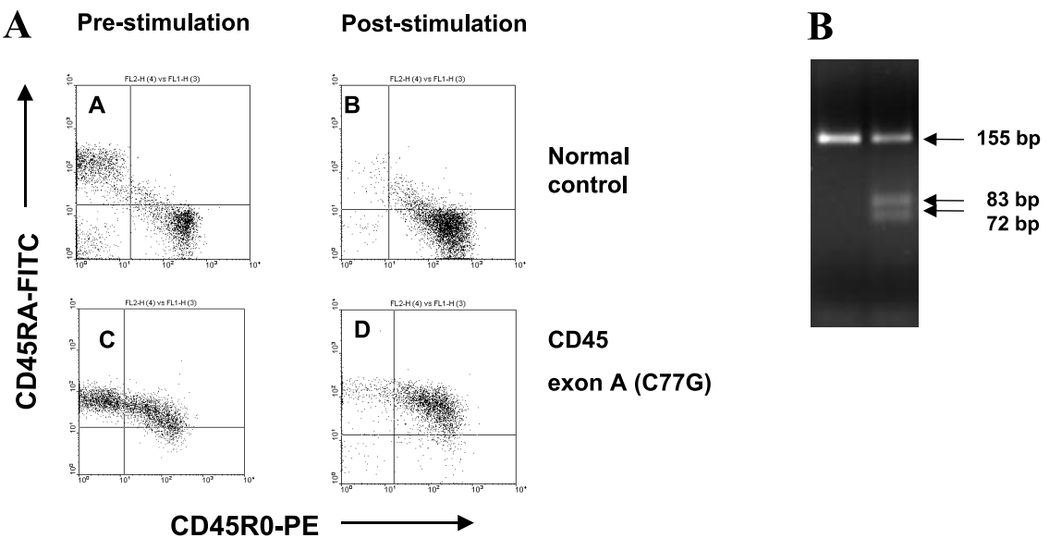

Fig. 3. Abnormal CD45 splicing. **A** – flow cytometric analysis of variant CD45 splicing. Expression of CD45 isoforms in human peripheral T cells pre- and post-stimulation. PBMC were stimulated with 1 μg/ml PHA and on day 0 and day 10 stained with isoform specific CD45R0-PE and CD45RA-FITC together with CD3-APC antibodies. Analysis was performed on gated CD3$^+$ cells. The normal pattern of CD45 splicing is characterized by loss of CD45RA and gain in CD45R0 expression after activation (A and B). Variant CD45 splicing can be identified by the absence of the CD45RA-negative population and the T cells remain CD45RA/R0 double positive after activation (C and D), **B** – identification of C77G mutation. PCR analysis for detection of variant C77G allele was performed on genomic DNA with primers on either side of the mutation, amplifying a fragment of 155 bp. The C77G transition introduces a new restriction site for Msp I, which cleaves the mutant PCR product into two fragments of 72 and 83 bp. The presence of an undigested band of 155 bp indicates the presence of the wild-type allele

many[21]. However, we have analyzed the allele frequency in a large number of MS patients, patients with common variable immunodeficiency (CVID) and IgA deficiency (IgAD), and over 1000 controls from Sweden and the UK. In this study we found no difference in the frequency of the C77G allele between patients with these disorders, with a strong autoimmune component in etiology, and controls[66]. Similarly, BARCELLOS et al.[7] did not find any association of C77G with MS in an extensive family-based and case-control study in the USA.

We have recently shown an increased frequency of the C77G variant allele in HIV-1 positive individuals in the UK[60]. We analysed 197 HIV-1 patients and identified 11 individuals with the variant allele (allele frequency of 2.6%) compared with 4 out of 236 healthy donors (allele frequency 0.85%, p=0.03 using the two-tailed Fisher's test). Furthermore, two patients exhibiting the variant C77G allele and aberrant CD45 splicing with hemophagocytic lymphohistiocytosis and erythrocytic hemophagocytosis (HLH syndrome) have been described[11, 67]. Recently, we also identified a patient with CVID and a history of prolonged excretion of poliovirus who also exhibited abnormal CD45 splicing and the C77G polymorphism (manuscript submitted). All of these studies suggest that abnormal CD45 splicing may be associated with impaired anti-viral responses or clearance of the virus.

Although the exact function of individual CD45 isoforms remains unknown, expression of different isoforms does alter TCR-mediated signal transduction. Studies with transgenic mice support the idea that expression of only a high-molecular-weight isoform compromises immune function, and these mice cannot generate cytotoxic T cell responses or neutralising antibodies after viral infection[23]. Furthermore, the recent demonstration that CD45 can dephosphorylate the JAK shows that CD45 plays an important role in the control of cell proliferation, differentiation and anti-viral activities mediated by cytokines such as IL-3, IL-4, EPO and IFN-α[20]. Thorough functional studies have not been performed on (C77G) heterozygous individuals, but by analogy with the transgenic mice, it is likely that abnormal CD45 splicing is associated with immunodeficiency[11, 67], autoimmunity[21] or impaired anti-viral responses[60].

The frequency of the C77G transversion in different normal populations is less clear. We have analyzed healthy blood donors from the UK and found a frequency of the allele of 0.85% compared with 0.16% or less in healthy donors in Germany[21]. In North America and Sweden the frequency of C77G allele has been shown to be higher (in the region of 1.4 to 1.8%)[21, 66]. We further analyzed the distribution of the variant C77G allele in different African and Asian populations and found striking variations in frequency (manuscript submitted). It is interesting that the highest frequency was found in some Central Asian populations. Further analysis of populations with a high frequency of the C77G variant will be very informative as to the role of the mutation. The high frequency of the C77G allele in Central Asia may also suggest that the variant arose there, although given the historic mixing of eastern and western Eurasians in this region, the C77G mutation may have originated in Europe and been spread by subsequent migrations of European populations into Asia[70].

Comparative genomics suggests that the CD45 locus is a site of strong selection in other vertebrates. For instance, three divergent allelic families of CD45 have been described in cattle in Africa based on the pattern of CD45R0 reactivity[6]. These studies show that the CD45 locus in cattle has been subject to strong natural selection, suggested to be pathogen driven[6]. CD45 allelic variants have also been identified in the mouse[45], rat[54] and puffer fish[14]. Given this extensive diversity in other vertebrates, it is surprising that C77G is the only polymorphism repeatedly identified thus far in humans, that it exhibits a restricted ethnic distribution, and that it is relatively rare. Thus, it is likely that this relative monomorphism of CD45 either reflects a lack of appropriate population sampling or a peculiarity of the human immune system and should be investigated by more extensive research.

Conclusions

CD45 is clearly essential for normal lymphocyte signaling, as KO mice or humans lacking CD45 are severely immunodeficient[12, 22, 24, 61]. Lesser degrees of abnormal CD45 expression are associated with excessive immunoreactivity[32] or poor immune function, as in transgenic mice expressing the high-molecular-weight isoform[23]. As yet, the data suggesting that the C77G point mutation is a determinant of disease susceptibility or severity remain to be substantiated. Nevertheless, taken together, the data from mice and humans indicate that alterations in CD45 profoundly affect immune function.

The problem remains to understand the function of the extracellular domain and its complex alternative splicing. While high-molecular-weight isoforms might be expected to dimerise poorly and should therefore be constitutively active, in fact, cells expressing only a high-molecular-weight isoform signal poorly[23]. In

contrast, those expressing CD45R0, which should dimerise and be inhibited, actually signal very well. These paradoxical results suggest that there is a long way to go in understanding CD45. Better understanding of cell surface *cis* interactions of each isoform and *trans* ligands on other cells or extracellular matrix may help in making progress. In the meanwhile, although CD45 may in the long term offer an attractive target for immunomodulatory strategies, in the short term it is more likely to continue to be used as a marker for identifying and separating lymphocyte subpopulations.

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