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When the Lymphocyte Loses Its Clothes

Minireview

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The type II bare lymphocyte syndrome (BLS) or major histocompatibility complex class II (MHCII) deficiency is a severe combined immunodeficiency (SCID) that is characterized by the absence of constitutive and inducible expression of MHCII determinants on immune cells. Four complementation groups of BLS have been defined, and they result from mutations in DNA-bound activators and the coactivator for MHCII transcription. Recently, all complementation groups of BLS patients have been accounted for. Studies of the syndrome and specific mutations reveal important lessons for the genetics of the immune response.

BLS is an autosomal recessive disease, where patients fail to express MHCII determinants on their immune cells (reviewed in Reith and Mach, 2001; Ting and Trowsdale, 2002). As a consequence, thymic education and peripheral T cell help are compromised. All these manifestations can be explained by the absence of antigen processing and presentation (APP) via MHCII molecules (reviewed in Klein et al., 1993; Villard et al., 2001). Because hematopoiesis is not affected, BLS patients have normal numbers of circulating B and T cells, but the $CD4^+$ T cell counts are reduced. Nevertheless, they are unable to mount immune responses to foreign antigens, as manifested by absent delayed type hypersensitivity skin tests, decreased capacity to proliferate in allogeneic mixed lymphocyte reactions, panhypogammaglobulinemia, and recurrent viral, bacterial, fungal, and protozoan infections. One-third of the patients also have reduced $CD8^+$ T cell counts, which could be due to reduced levels of MHCI determinants on some BLS cells and/or to the lack of T cell help. Most patients die in early childhood from severe malabsorption with failure to thrive. An unusual clinical feature of BLS is sclerosing cholangitis, which is not found in other SCID (reviewed in Klein et al., 1993).

In BLS patients, transcription factors that bind MHCII promoters, rather than the genes themselves, are mutated. Therefore, BLS is a prototypic disease of gene regulation. On the basis of fusion experiments using transformed B cells from patients and several in vitro-generated B cell lines, four complementation groups (CG) of BLS were defined; i.e., four different genes bear mutations that display autosomal recessive phenotypes. Whereas the three subunits of the regulatory factor X (RFX), namely RFXANK/B (RFX containing ankyrin repeats) (Masternak et al., 1998; Nagarajan et al., 1999), RFX5 (fifth member of the RFX family) (Steimle et al., 1995), and RFXAP (RFX-associated protein) (Durand et al., 1997) do not function properly in CG B, C, and D, respectively, the class II transactivator (CIITA) is mutated in CG A (Figure 1) (Steimle et al., 1993). All four proteins are required for MHCII transcription. Most mutations occur in splice sites that cause frameshifts, which result in severely truncated or absent proteins, providing an easy explanation for no transcription and surface expression of MHCII determinants (reviewed in Reith and Mach, 2001). However, in other cases, these transcription factors carry subtle single point mutations that can result in milder symptoms. Recently, structure-based studies highlighted underlying mechanisms that connected several of these mutations with the resulting immunodeficiency (Nekrep et al., 2001). One of them elucidated the nature of the putative fifth CG of BLS (Nekrep et al., 2002). What follows are several lessons for studies of complex genetic diseases.

Biochemistry Can Err

More than a decade ago, the expression of MHCII genes was known to require several protein complexes (reviewed in Reith and Mach, 2001; Ting and Trowsdale, 2002), but their specific subunits had not been identified. The earliest efforts to characterize proteins that bind conserved upstream sequences (CUS) in their compact promoters (Figure 1) employed in vitro assays that identified numerous DNA-protein interactions. However, the "acid test" for the relevance of these complexes, individual subunits of which were still unknown, was never met; i.e., they were unaltered in cell lines derived from BLS patients. Antisense experiments, which reduced the expression of the X1 box binding protein RFX1 or the X2 box binding protein XBP-1 had modest effects on MHCII transcription (Reith et al., 1990). However, the introduction of their genes did not restore MHCII transcription in BLS cells. Later, RFX1, also known as the methylated DNA binding protein (MDBP), became an important negative transcription factor for the collagen (Col1A2) promoter, among others (Sengupta et al., 2002). XBP-1, a member of the cAMP responsive element binding protein/activating transcription factor (CREB/ATF) family, actually mediates responses to ER stress (Calfon et al., 2002). Nevertheless, its genetic inactivation in the germline or blastocyst of the Rag-deficient mouse leads to absent hepatocyte develop-

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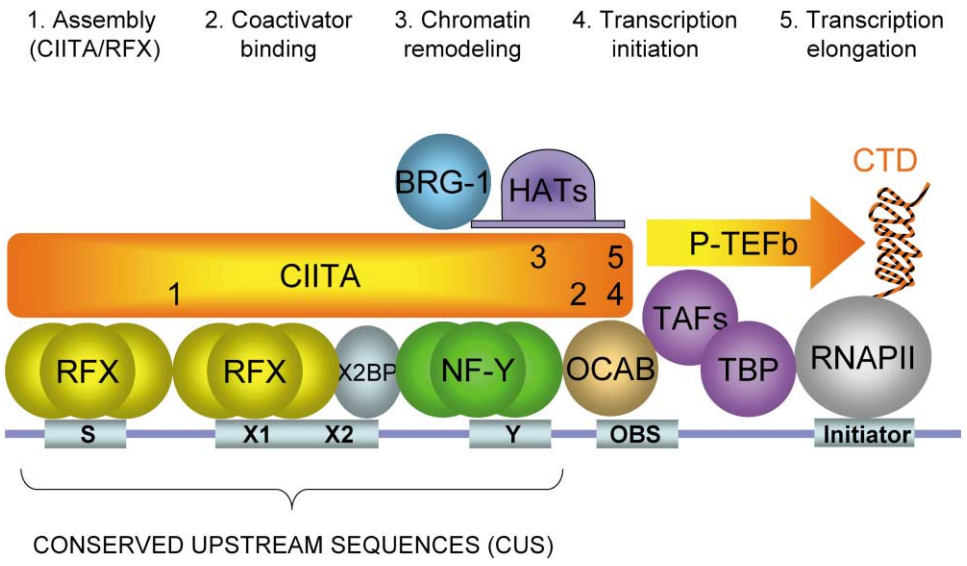

Figure 1. *cis*-Acting Sequences and *trans*-Acting Factors on MHCII Promoters

Conserved upstream sequences (CUS) in the distal promoter contain S, X, and Y boxes. The X box is subdivided further into X1 and X2 boxes. Whereas the spacing between S and X boxes is invariant, X and Y boxes can be moved by single helical turns. In the DRA gene, the octamer binding site (OBS) and Initiator form the core or proximal promoter. RFX, which contains RFXANK/B, RFXAP, and RFX5, binds S and X1 boxes. X2 box binding protein (X2BP) binds the X2 box. NF-Y, which contains NF-YA, NF-YB, and NF-YC, binds the Y box. Octamer binding proteins bind OBS. In turn, they recruit the B cell octamer binding protein 1/Octamer binding factor 1/Oct coactivator from B cells (Bob1/OBF-1/OCAB, here OCAB). The initiator binds general transcription factors and TAFs. It positions RNA polymerase II (RNAPII) correctly for the initation of transcription. CIITA is recruited to the MHCII enhanceosome, which consists of RFX, X2BP, NF-Y on CUS. It binds histone acetyl transferases (HATs, CBP/p300, and P-CAF) and SWI/SNF complexes (via BRG-1) that remodel chromatin, GTFs, OCAB, and TAFs that recruit RNAPII, and P-TEFb that phosphorylates its C-terminal domain (CTD) and facilitates the elongation of MHCII transcription. Steps in the transcription process are given above the drawing.

ment (Reimold et al., 2000) or impaired plasma cell differentiation (Reimold et al., 2001), respectively.

Eventually, efforts to purify the correct CUS binding complexes were successful. However, it required the genetic identification of RFX5, which rescued the expression of MHCII determinants in CG C cells (Steimle et al., 1995). Although CIITA was also cloned using genetic complementation in CG A cells (Steimle et al., 1993), RFXANK/B (Masternak et al., 1998; Nagarajan et al., 1999), and RFXAP (Durand et al., 1997) could then be isolated biochemically. To date, these three subunits of RFX and CIITA not only account for all CGs of BLS but represent the only gene-specific regulatory factors for MHCII promoters.

Assembly of MHCII Enhanceosome and Transcriptosome

Essential complexes for MHCII transcription include the nuclear factor Y (NF-Y), which consists of NF-YA, NF-YB, and NF-YC that bind the Y box, oligomers of RFX that bind X1 and S boxes, X2 box binding proteins (X2BP: CREB or activated protein 1, AP-1) that bind the X2 box, and CIITA (Figures 1 and 2) (reviewed in Reith and Mach, 2001; Ting and Trowsdale, 2002). S, X1, X2, and Y boxes are contained in CUS. They are present in the promoters of structural (α and β chains of DR, DO, DP, and DQ isotypes) as well as MHCII-associated DMA, DMB, and invariant chain (Ii) genes. Proximal MHCII promoters lack a typical TATA box but contain initiator sequences (Inr) (reviewed in Reith and Mach, 2001; Ting and Trowsdale, 2002) (Figure 1).

While RFX and NF-Y are indispensable for all MHCII promoters, X2BP and other transcriptional activators can be isotype specific (reviewed in Reith and Mach, 2001; Ting and Trowsdale, 2002). RFX5, which contains a winged-helix DNA binding domain (DBD) (Gajiwala et al., 2000), forms oligomers (Jabrane-Ferrat et al., 2002) and binds preassembled RFXANK/B and RFXAP (Nekrep et al., 2000) (Figure 2). RFX and NF-Y then form the enhanceosome (Figure 2). The assembly begins off DNA but is strengthened by DNA-protein interactions. Individual DBDs contact CUS on the same side of the double helix, which explains the constrained topology between S, X, and Y boxes (Figure 2). By selecting against and for RFX1 and RFX5, respectively, NF-Y specifies and stabilizes the final enhanceosome (Fontes et al., 1997). CIITA is a coactivator that does not bind DNA but interacts with this transcriptional platform via multiple subunits of RFX and NF-Y (Ting and Trowsdale, 2002). Together, they form the transcriptosome (Ting and Trowsdale, 2002) (Figure 2). The posttranslational modification of CIITA by phosphorylation leads to its oligomerization, accumulation, and increased activity on MHCII promoters (Figure 2) (Tosi et al., 2002). Other modifications include acetylation and ubiquitylation, which could modulate its retention in the nucleus and stability, respectively. X2BP contributes to the formation of the enhanceosome (Moreno et al., 1999). Thus, it is not surprising that the absence of any subunit of RFX leads to unoccupied, bare MHCII promoters in in vivo footprinting studies (CG B, C, and D). In sharp contrast, they are occupied in CG A, which lacks CIITA (Kara and Glimcher, 1991).

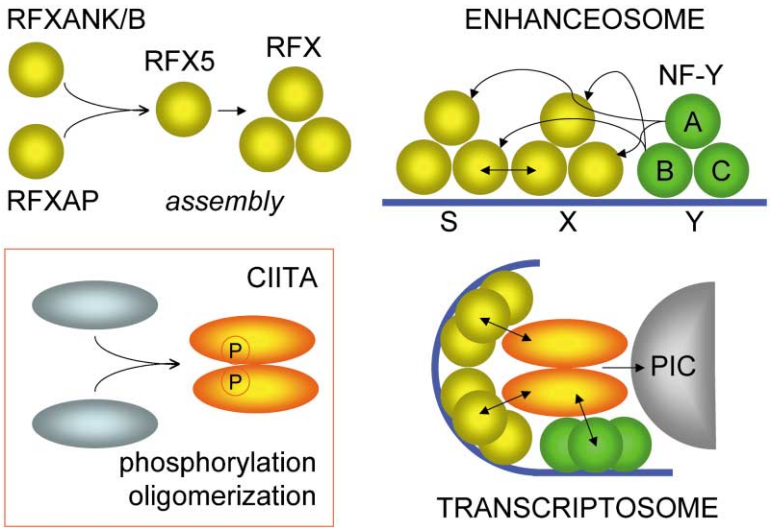

Figure 2. The Assembly of the MHCII Enhanceosome and Transcriptosome

Via its ankyrin repeats, RFXANK/B binds RFXAP and nucleates the RFX complex. RFX5 is recruited to this combinatorial surface and forms oligomers. RFX binds S and X boxes and NF-Y the Y box. They form the MHCII enhanceosome, which is strengthened by X2BP (not presented). CIITA, which is expressed from four separate and differentially regulated promoters, is modified posttranslationally (here by phosphorylation), which leads to its oligomerization and retention on MHCII promoters. It helps to form the MHCII transcriptosome, which contains the preinitiation complex (PIC).

CIITA is a transcriptional integrator (Fontes et al., 1999). Chromatin remodeling complexes, basal transcription factors, and proteins involved in the elongation of transcription bind CIITA (Figure 1). Histone acetylation and SWI/SNF complexes modulate the chromatin structure at MHCII promoters. Indeed, X2BP, NF-Y, and CIITA recruit several histone acetyltransferases, and CIITA has been reported to possess intrinsic acetyltransferase activity (reviewed in Ting and Trowsdale, 2002). Changes in acetylation of histones H3 and H4 have also been correlated with MHCII transcription (Beresford and Boss, 2001). By binding the brahma related gene-1 (BRG-1), CIITA also recruits the chromatin remodeling SWI/SNF complex (Mudhasani and Fontes, 2002). Furthermore, CIITA binds components of the general transcription apparatus, including TFIIB, TFIIH, $hTAF_{II}32$, and $hTAF_{II}70$ and the B cell octamer binding protein 1/Octamer binding factor 1/Oct coactivator from B cells (Bob1/OBF-1/OCAB) (reviewed in Reith and Mach, 2001; Ting and Trowsdale, 2002). Finally, the recruitment of P-TEFb leads to the efficient elongation of transcription (Kanazawa et al., 2000) (Figure 1).

Single Amino Acid Substitutions in Proteins Offer Structural Clues

The MHCII enhanceosome cannot form in the absence of any subunit of RFX, and promoters remain unoccupied in vivo (Masternak et al., 2000). Thus, in a typical BLS, MHCII genes are not transcribed. However, there are several exceptions to this rule, and representative patients fall into one of two broadly defined groups. In the first, patients' immune cells express residual surface MHCII determinants. In the second, patients' cells show a discordant expression of MHCII genes, where only certain genes are active. Since their immune systems retain some APP, both groups of patients experience a milder course of disease. In this atypical BLS, genes coding for transcription factors contain single point mutations. Mutant proteins retain most of their functional domains. By modeling these domains based on homologous modules with known crystal structure, it is possible to visualize the location of the point mutation that causes the disease. Therefore, the three-dimensional structure is a powerful method for site-directed binding studies and better evaluation of BLS. Let us look more closely at some of them.

RFXANK/B is mutated in CG B that contains the largest number of BLS patients. The severity of BLS can be correlated with levels of residual MHCII determinants on patients' immune cells. Their levels can be increased by exogenous CIITA, e.g., in patients FZA and EBA (Nagarajan et al., 2000). Thus, CIITA stabilizes the existing enhanceosome and ameliorates the BLS defect. In FZA patient, RFXANK/B carries the point mutation L195P. Although the corresponding mRNA is expressed at wild-type levels, less than 1% of MHCII determinants are expressed on the surface of the patient's cells (Nagarajan et al., 2000). Insight into the structure of ankyrin repeats in RFXANK/B elucidated the mechanism of this mutation (Figure 3) (Nekrep et al., 2001). Furthermore, it led to alanine mutageneses of exposed variable residues that mapped the interacting surfaces with RFXAP and CIITA. For example, β-hairpin loops in the three N-terminal ankyrin repeats and one helix in the third ankyrin groove are required for the binding between RFXANK/B and RFXAP, which nucleates RFX. Thus, the mutation L195P is likely to break the inner helix in the third ankyrin repeat and block the formation of the RFX complex (Figure 3).

Several cases of discordant expression of MHCII genes were described in BLS. Immortalized Clone13 B cells lack functional CIITA and express DQ but not DR or DP isotypes (Douhan et al., 1997), and revertants of mutant 6.1.6 B cells express only DR and DP isotypes (Levine and Pious, 1984). Moreover, exogenous CIITA increased the expression of only the DR isotype on fibroblasts from patient SSI from CG C (Peijnenburg et al., 1999). This discordant expression might reflect different promoter strengths of the genes in the MHCII locus (Masternak and Reith, 2002; Nekrep et al., 2002). Indeed, different binding affinities for different MHCII promoters were demonstrated for RFX and X2BP (Hasegawa and Boss, 1991).

Perhaps the most intriguing case of discordant expression of MHCII genes was found in twins Ken and Ker, where only promoters facing the centromere on the

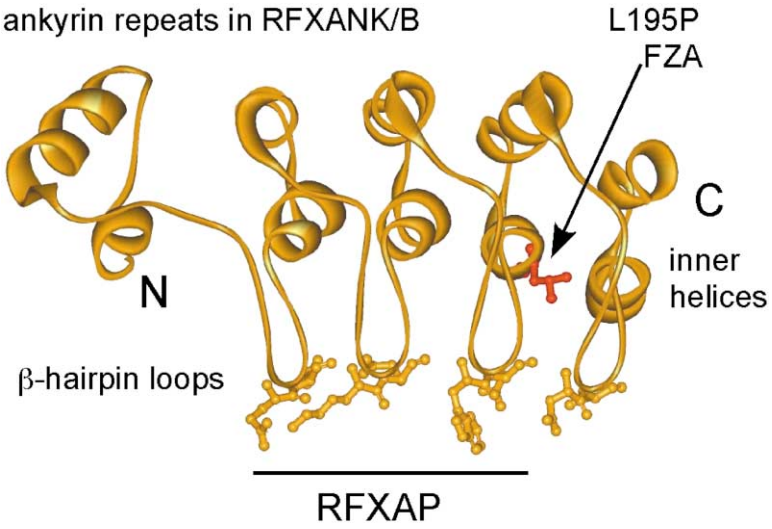

Figure 3. A Model of Ankyrin Repeats in RFXANK/B (from Positions 88 to 243)

β-hairpin loops and inner helices in ankyrin repeats mediate the interaction between RFXANK/B and RFXAP. The critical leucine at position 195 (red residue) is located in the first (inner) helix of the third ankyrin repeat (see arrow). Its mutation to proline in patient FZA of CG B is likely to break that helix and destabilize the structure of the ankyrin repeats.

short arm of chromosome 6 are expressed (Figure 4) (Douhan et al., 1996). This finding suggested a chromatin remodeling defect, possibly in a putative locus organizing region. Moreover, because of fusions with cells form other CGs, Ken and Ker were placed into the fifth CG (CG E) of BLS. However, they contain a point mutation in the winged-helix DBD of RFX5 (Figure 5) (Nekrep et al., 2002). Indeed, the wild-type RFX5 protein restored the binding of RFX to DNA, transcription of previously silent genes and expression of MHCII determinants on the surface of affected B cells. The crystal structure of the DBD of RFX1 (Gajiwala et al., 2000) bound to a symmetrical X box DNA was used as a matrix for modeling the three-dimensional structure of the DBD of RFX5, which then explained the mechanism of this mutation (Nekrep et al., 2002). Indeed, the arginine at position 149 in RFX5 is one of nine residues that form a direct contact with DNA (Gajiwala et al., 2000). Its mutation to glutamine, which is found in Ken and Ker, leads to the loss of a positive charge in the DNA binding interface and the loss of several hydrogen bonds that are required for DNA-protein interactions (Nekrep et al., 2002). As a consequence, one major contact point that RFX5 makes with DNA is lost and binding of RFX to DNA is markedly reduced or does not occur at all (Figure 5). The general picture of the four CGs of BLS is now complete.

Immunological Lessons

BLS is a relatively rare hereditary SCID that is found in families with high degree of consanguinity (reviewed in Klein et al., 1993; Villard et al., 2001; Reith and Mach, 2001). While severe BLS represents complete SCID, in less severe cases, residual expression of MHCII determinants on certain immune cells enables some thymic education and peripheral immune responses. BLS is often fatal and requires bone marrow transplantation. Milder forms of this disease require repeated immunoglobulin injections and rapid treatment of infections. In them, the function of cytolytic lymphocytes also remains intact.

Perhaps the most important lesson from BLS is that there are at least four essential, i.e., nonredundant and interconnected genes that are dedicated solely to trigger APP by the MHCII compartment. Since CIITA is expressed from up to four distinct promoters only in thymic epithelial cells, professional APCs, and mature B cells, CIITA is the master switch of this process. As RFX is found in all cells, it is also the only protein that must be introduced into somatic cells to render them APCs (Reith

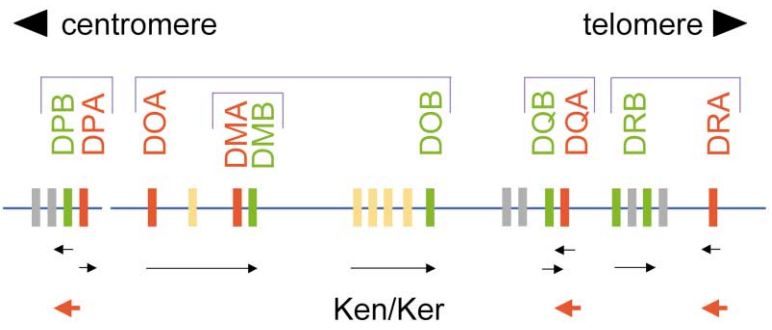

Figure 4. The Organization of MHCII Genes on the Short Arm of the Human Chromosome 6, Direction of Their Transcription, and Active Promoters in Ken and Ker Cells

Three structural and polymorphic heterodimers are transcribed from promoters that point in opposite directions (DRA and DRB, DQA and DQB, DPA and DPB, short black arrows). Monomorphic DOA and DOB as well as DMA and DMB heterodimers are also transcribed, but their promoters face the telomere (long black arrows). Genes for α and β chains are depicted as red and green bars, respectively. Several DRB, DQ, and DP pseudogenes are also found (gray bars). Orange bars depict MHCII-unrelated genes. In Ken and Ker cells, only promoters facing the centromere are transcribed (red arrows); thus, no productive MHCII heterodimers form.

and Mach, 2001). Caveats include the posttranslational modification of CIITA and the presence of appropriate proteases to produce antigenic peptides. This knowledge is of importance for the future manipulation of the immune response, how it can be increased in immunodeficiencies and attenuated in autoimmune diseases. Finally, genetic approaches with wild-type subunits of RFX and CIITA offer a better alternative to bone marrow transplantation for the cure of BLS.

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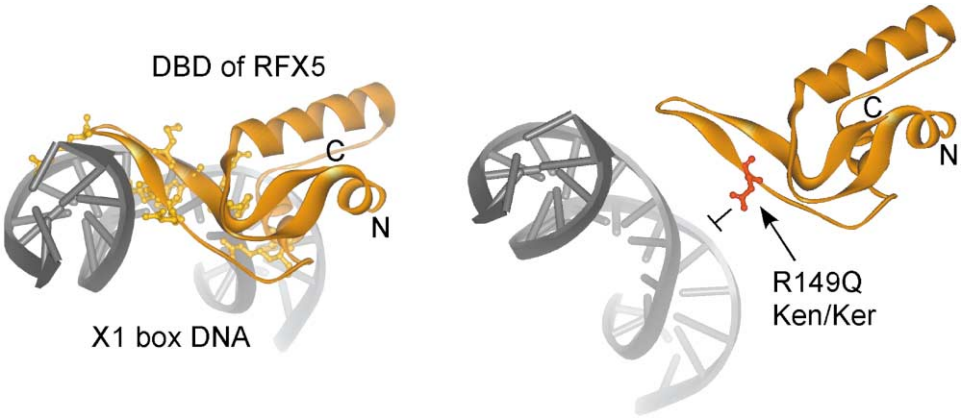

Figure 5. A Model of the Wild-Type and Mutant Winged-Helix DNA Binding Domains (DBD) from RFX5 On and Off DNA

In the wild-type RFX5 protein, nine residues mediate direct intermolecular contacts to nucleotides of the palindromic X1 box (left panel). In Ken and Ker, a critical arginine residue at position 149, which forms the interface with DNA, is mutated to glutamine. As a consequence, the binding of RFX to DNA is markedly reduced or does not occur at all (right panel).