NLRC5: a key regulator of MHC class I-dependent immune responses

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Abstract | The expression of MHC class I molecules is crucial for the initiation and regulation of adaptive immune responses against pathogens. NOD-, LRR- and CARD-containing 5 (NLRC5) was recently identified as a specific transactivator of MHC class I genes (CITA). NLRC5 and the master regulator for MHC class II genes, class II transactivator (CIITA), interact with similar MHC promoter-bound factors. Here, we provide a broad overview of the molecular mechanisms behind MHC class I transcription and the role of the class I transactivator NLRC5 in MHC class I-dependent immune responses.

The tight regulation of the genes encoding MHC class I, MHC class II and their accessory molecules is crucial for an effective adaptive immune response. Since the identification in 1993 of the class II transactivator (CIITA), which is a member of the NOD-like receptor (NLR) protein family, it has been appreciated that CIITA acts as a master switch for the constitutive and inducible expression of all MHC class II genes and their accessory genes (which encode the invariant chain, HLA-DO and HLA-DM)[1–3]. CIITA was also found to have a role in the regulation of MHC class I gene transcription[4–6]. This notion is supported by the observations that MHC class I promoters are activated by CIITA *in vitro*[4,5,7] and that MHC class I promoter activity is impaired in a CIITA-deficient B cell line[6]. Similarly, activated T cells from an MHC class II-deficient patient with a defect in CIITA had a reduction in MHC class I expression[6]. Furthermore, MHC class I expression was enhanced following the expression of wild-type CIITA in a CIITA-deficient fibroblast cell line[4,6] or in CIITA-deficient tumour cell lines of mouse and human origin[4,7]. However, no reduction in MHC class I expression was observed in CIITA-deficient mice[2,8–10]. The role of CIITA in the regulation of MHC class I expression therefore seems to vary. On one hand, this variability may be related to the use of DNA transfection techniques and the different cell types, tissues, genetic backgrounds and species investigated[4,5,7]. On the other hand, these observations also suggest the involvement of an additional factor in the regulation of MHC class I expression.

The recent discovery that the NLR family member NOD-, LRR- and CARD-containing 5 (NLRC5; also known as NOD27 and CLR16.1) has a crucial role in the *in vivo* and *in vitro* regulation of MHC class I transcription has drastically changed our view[11]. It has been shown that NLRC5 acts as a transactivator of MHC class I genes that specifically associates with and transactivates MHC class I promoters by cooperating with transcription factors that interact with the conserved MHC class I promoter regulatory elements, such as the SXY module[11–14]. Indeed, several studies of NLRC5-deficient mice have independently concluded that NLRC5 is a key factor in the *in vivo* transcriptional regulation of MHC class I genes[10,13,15–17]. Now, it becomes clear that two NLR proteins, NLRC5 and CIITA, transcriptionally regulate the concerted expression of crucial components in the MHC class I and MHC class II antigen-presentation pathways, respectively (FIG. 1).

Here, we discuss the essential role of the NLR family member NLRC5 in the transcriptional regulation of MHC class I genes and highlight the implications for immune responses.

Transcriptional regulation of MHC genes

Proximal promoters of MHC class I and MHC class II genes. MHC class I molecules are constitutively expressed on almost all nucleated cells. By contrast, constitutive expression of MHC class II molecules is tissue specific and restricted to professional antigen-presenting cells (APCs), including dendritic cells (DCs), B cells and macrophages. However, in several non-haematopoietic cell types (for example, fibroblasts, epithelial cells and endothelial cells), which lack constitutive expression of MHC class II molecules, MHC class II expression can be induced following exposure to cytokines, with interferon-γ (IFNγ) being the most potent inducer[18].

The transcription of MHC class I and MHC class II genes is controlled by several conserved *cis*-acting regulatory elements at their proximal promoters (FIG. 2). In detail, enhancer A (which contains nuclear factor-κB (NF-κB)-binding sites), an IFN-stimulated response element (ISRE) and an SXY module (which is comprised of the W/S, X1, X2 and Y boxes) are important for both the constitutive and inducible transcription of MHC class I genes (reviewed in REF. 19). The promoter of the β_2-microglobulin (*B2M*) gene also contains an SXY module, NF-κB-binding sites and an ISRE[6] (FIG. 2). By contrast, the promoters of MHC class II genes contain only an SXY module. Owing to the fact that MHC class II molecules have a more specialized function than MHC class I molecules in the immune system, it has been previously argued that MHC class II genes have evolved in a divergent evolutionary line and have lost the upstream enhancer A and ISRE regulatory modules that are typical of MHC class I genes[5]. This makes MHC class II genes fully dependent on the SXY regulatory promoter module and its interacting factors, whereas the expression of MHC class I genes also depends on the additional *cis*-acting sequence elements described above[5].

At MHC class I promoters, NF-κB interacts with the NF-κB-binding sites of enhancer A, whereas the ISRE is bound by interferon-regulatory factor (IRF) family members[19] (FIG. 2). The X1 box of the SXY module is bound by the regulatory factor X complex (RFX complex), which consists of

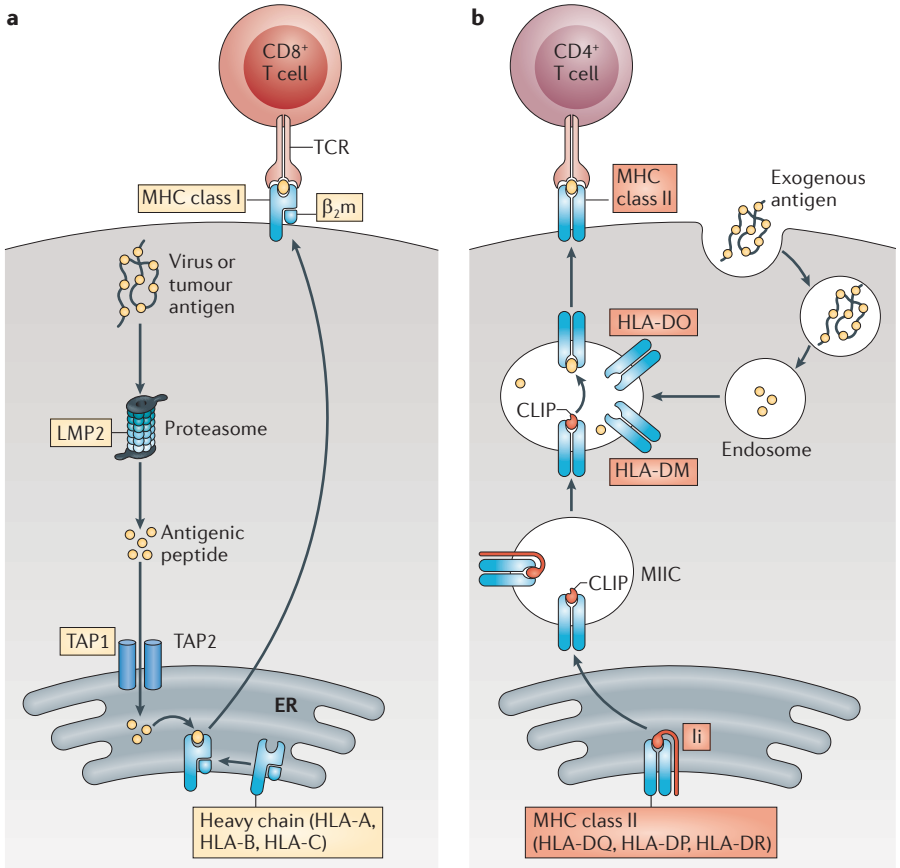

Figure 1 | **The MHC class I and MHC class II antigen-presentation pathways. a** | Intracellular antigens, such as virus or tumour antigens, are processed into peptides by the immunoproteasome, which is composed of multiple subunits, including LMP2. Peptides are transported into the endoplasmic reticulum (ER), where they are loaded into the groove of the MHC class I complex, which is composed of a heavy chain and β_2-microglobulin (β_2m). MHC class I complexes present antigens on the cell surface to $CD8^+$ T cells. Proteins in the MHC class I pathway that are encoded by genes regulated by the MHC class I transactivator NOD-, LRR- and CARD-containing 5 (NLRC5) are shown in yellow boxes. **b** | Antigens from extracellular sources, such as bacterial antigens, are processed by endolysosomal enzymes into peptides. These peptides bind to the groove of the MHC class II complex by displacing the class II-associated invariant chain peptide (CLIP), which is derived from the MHC class II-associated invariant chain (Ii). HLA-DO and HLA-DM regulate the antigen-loading process. The MHC class II complex presents antigens to $CD4^+$ T cells. Proteins in the MHC class II pathway that are encoded by genes regulated by the class II transactivator (CIITA) are shown in red boxes. MIIC, MHC class II compartment; TAP, transporter associated with antigen processing; TCR, T cell receptor.

RFX5, RFX-associated ankyrin-containing protein (RFXANK; also known as RFXB) and RFX-associated protein (RFXAP)[20–24]. Several studies have indicated that the RFX complex comprises a dimer of RFX5 and monomers of RFXAP and RFXANK. The X2 box of the SXY module is bound by cAMP-responsive-element-binding protein 1 (CREB1) and activating transcription factor 1 (ATF1), whereas the Y box of the SXY module is bound by nuclear transcription factor Y (NFY), which consists of the NFYA, NFYB and NFYC subunits. Together, these proteins (that is, the RFX components, CREB1, ATF1 and the NFY subunits), which are ubiquitously and constitutively expressed, assemble on the SXY module of MHC class I and MHC class II promoters to form a multiprotein complex[6,25–27]. RFX is essential for the assembly of this multiprotein complex, as the latter fails to assemble on MHC class I and MHC class II promoters in cell lines derived from patients with an MHC class II deficiency owing to defects in RFX components[6,20]. This in turn results in a lack of constitutive and induced MHC class II gene expression[20] and in reduced constitutive MHC class I gene expression[5,6]. Unexpectedly, however, MHC class I expression is intact in RFX5-deficient mice[28], despite the fact that RFX5 associates with MHC class I promoters in human B cell lines and that RFX5 determines the *in vivo* occupancy of the SXY module in MHC class I promoters[6,25]. We discuss the possible reasons for these unexpected findings in RFX5-deficient mice in more detail below.

In summary, several transcription factors that bind to specific regulatory elements in the MHC class I and MHC class II gene promoters are required for the controlled expression of MHC molecules. However, regulation of MHC expression also requires the presence of the MHC gene transactivators CIITA and NLRC5.

CIITA: the master regulator of MHC class II gene expression. The transcription of MHC class II genes absolutely requires the presence of the co-activator CIITA. CIITA is recruited to the promoters of MHC class II genes, where it interacts with various components of the multiprotein transcription factor complex to form an enhanceosome. The CIITA enhanceosome consists of RFX, CREB1–ATF1 and NFY, which all bind to the SXY module of the MHC class II gene promoter, and CIITA[25,27,29] (FIG. 3a). The multiple interactions of CIITA with the SXY module-associated factors have a reciprocal stabilizing effect that contributes to promoter occupancy and enhances the binding of CIITA[25]. In addition, CIITA interacts with and acts as a platform for the recruitment of many transcriptional co-activators and their associated complexes to modulate the activity and stability of the enhanceosome, and to modify and remodel the chromatin architecture surrounding MHC promoters[3,18,19].

The chromatin-modifying factors that are recruited to MHC promoters by CIITA include histone acetyltransferases, histone deacetylases and histone methyltransferases. CIITA also interacts with the ATPase SUG1 (also known as PSMC5; a component of the 19S proteasome cap complex) and the ATP-dependent chromatin-remodelling factor BRG1 (reviewed in REFS 3,18,19) (FIG. 3a). Besides their role in chromatin remodelling, several studies have shown that the recruited acetyltransferases and deacetylases acetylate or deacetylate lysine residues in CIITA, thereby regulating the protein interactions and the nuclear localization of CIITA[3,18,19].

In APCs, the constitutive expression of CIITA confers constitutive MHC class II expression. By contrast, in non-haematopoietic cells, the expression of CIITA can be induced by IFNγ. This IFNγ-induced expression of CIITA can be downregulated by several cytokines, including transforming growth factor-β (TGFβ) and interleukin-10 (IL-10)[30]. The transcriptional regulation of *CIITA* is

controlled by three independent promoter units that can be individually activated in a cell type- and stimulus-specific manner[31]. Each of these promoters controls the transcription of a unique first exon, resulting in the production of three CIITA isoforms that differ at their amino terminus (FIG. 3b).

CIITA is also recruited to the factors assembled on the SXY module in MHC class I and *B2M* promoters[25,29]. Whereas the recruitment of CIITA is essential for MHC class II expression, it has an ancillary role in the expression of MHC class I genes, in particular in the IFNγ-induced activation of MHC class I genes[4]. The exact role of CIITA in the constitutive expression of MHC class I genes was unclear, especially given that MHC class I genes are constitutively expressed in almost all nucleated cells, whereas CIITA expression is mainly restricted to professional APCs. This realization led to the identification of NLRC5 as a specific transactivator of MHC class I genes in both humans and mice.

NLRC5: a novel MHC class I transactivator

NLRC5 structure, expression and localization. NLRC5 has a tripartite domain structure similar to that of other NLR proteins[11,32–34] (FIG. 3b). NLRC5 contains an N-terminal caspase activation and recruitment domain (CARD), a centrally located nucleotide-binding domain (NBD) and carboxy-terminal leucine-rich repeats (LRRs). The CARD at the N-terminus consists of repeated alpha helices, but it is structurally distinct from other CARDs and may thus be referred to as an atypical CARD[35]. The NBD contains a Walker A motif (also known as a P-loop; a nucleoside triphosphate (NTP) binding site) and a Walker B motif (an NTP hydrolysis site), which are crucial for the subcellular localization and function of NLRC5. In CIITA, the NBD is bound by GTP; however, it has not yet been determined whether the NBD of NLRC5 binds to ATP or GTP. The long LRR domain of NLRC5 renders it one of the largest NLR proteins, comprising 1,855 amino acids[34] (FIG. 3b). NLRC5 also contains a nuclear localization signal (NLS), which controls its subcellular distribution (see below). Although NLRC5 and CIITA do not have a high level of sequence similarity (FIG. 3b), a phylogenetic analysis of the CARD-containing NLRs revealed that among all NLR proteins NLRC5 is the one most closely related to CIITA[11,34]. This is underscored by a similar analysis of the NBD and LRR domains of all known NLRs, which also showed that CIITA and NLRC5 are closely related[34].

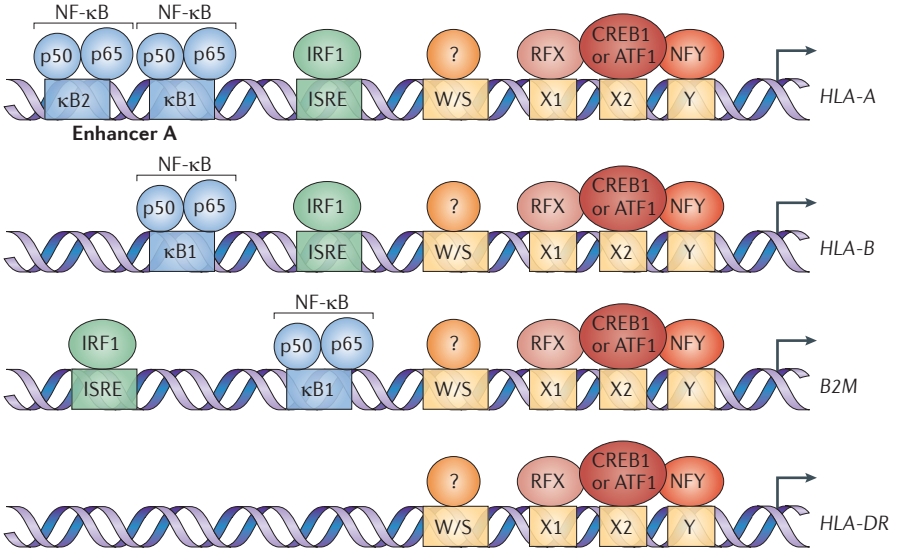

Figure 2 | ***Cis*-regulatory elements in the proximal promoters of MHC class I and MHC class II genes.** The SXY module (which comprises the W/S, X1, X2 and Y boxes) is conserved in the promoters of MHC class I and MHC class II genes. MHC class I promoters also contain an enhancer A element, which contains nuclear factor-κB (NF-κB)-binding sites, and an interferon-stimulated response element (ISRE). The X1 box is bound by the regulatory factor X (RFX) complex, which comprises RFX5, RFX-associated ankyrin-containing protein (RFXANK) and RFX-associated protein (RFXAP). The X2 box is bound by cAMP-responsive-element-binding protein 1 (CREB1) and activating transcription factor 1 (ATF1), and the Y box is bound by nuclear transcription factor Y (NFY). The factor(s) that interact with the W/S box are still poorly defined. The NF-κB-binding sites (κB1 and κB2) of enhancer A are bound by NF-κB, and the ISRE is bound by interferon-regulatory factor (IRF) family members, such as IRF1. In the promoter of the *HLA-B* locus, additional binding sites for upstream stimulatory factor 1 (USF1) and USF2, and for the transcription factor SP1, can also be found[19] (not shown). *B2M*, β_2-microglobulin.

In contrast to the more restricted expression pattern of CIITA, NLRC5 is constitutively expressed in various tissues in both humans and mice. However, NLRC5 is most highly expressed in haematopoietic cells[32,34]. In particular, the highest expression levels of NLRC5 can be found in lymphocytes (specifically, $CD4^+$ T cells, $CD8^+$ T cells, $CD19^+$ B cells, natural killer (NK) cells and natural killer T (NKT) cells), whereas $CD14^+$ cells (namely, monocytes) and $CD11b^+$ splenic myeloid cells have intermediate expression levels[13,33].

Similarly to CIITA expression, the expression of NLRC5 can be efficiently induced by IFNγ through the activation of signal transducer and activator of transcription 1 (STAT1) in both haematopoietic and non-haematopoietic cells (FIG. 3c). This is in line with the notion that STAT1-deficient cells are unable to induce the expression of NLRC5 (REFS 13,16). Moreover, NLRC5 expression is modestly activated by type I IFNs (specifically, IFNβ) as well as by polyinosinic–polycytidylic acid (polyI:C), virus infection and lipopolysaccharide (LPS), which can induce type I IFNs through the TRIF (TIR domain-containing adaptor protein inducing IFNβ)-dependent pathway downstream of Toll-like receptor 4 (TLR4)[11,13,16,32–34].

Whereas most NLR proteins are localized in the cytoplasm, NLRC5 is found in both the cytoplasm and the nucleus[11,13,14,34]. CIITA — which also has a heterogeneous distribution in the steady state — shuttles between the nucleus and the cytosol via importin-α-mediated nuclear import and exportin 1 (also known as CRM1)-dependent nuclear export[36–38]. Similarly to CIITA, NLRC5 is trapped in the nucleus following treatment with the exportin 1 inhibitor leptomycin B, which indicates that the subcellular trafficking of CIITA and NLRC5 may be mediated through similar mechanisms[11,14,34]. NLRC5 contains a bipartite NLS between the CARD and NBD, and point mutations within the NLS prevent the import of NLRC5 into the nucleus[11,39] (FIG. 3b). Moreover, NLRC5 molecules with mutations in the Walker A motif of the NBD cannot translocate into the nucleus, suggesting that NTP binding at the Walker A motif is required for possible conformational changes that allow active import of NLRC5 into the nucleus[11,14,39] (FIG. 3c).

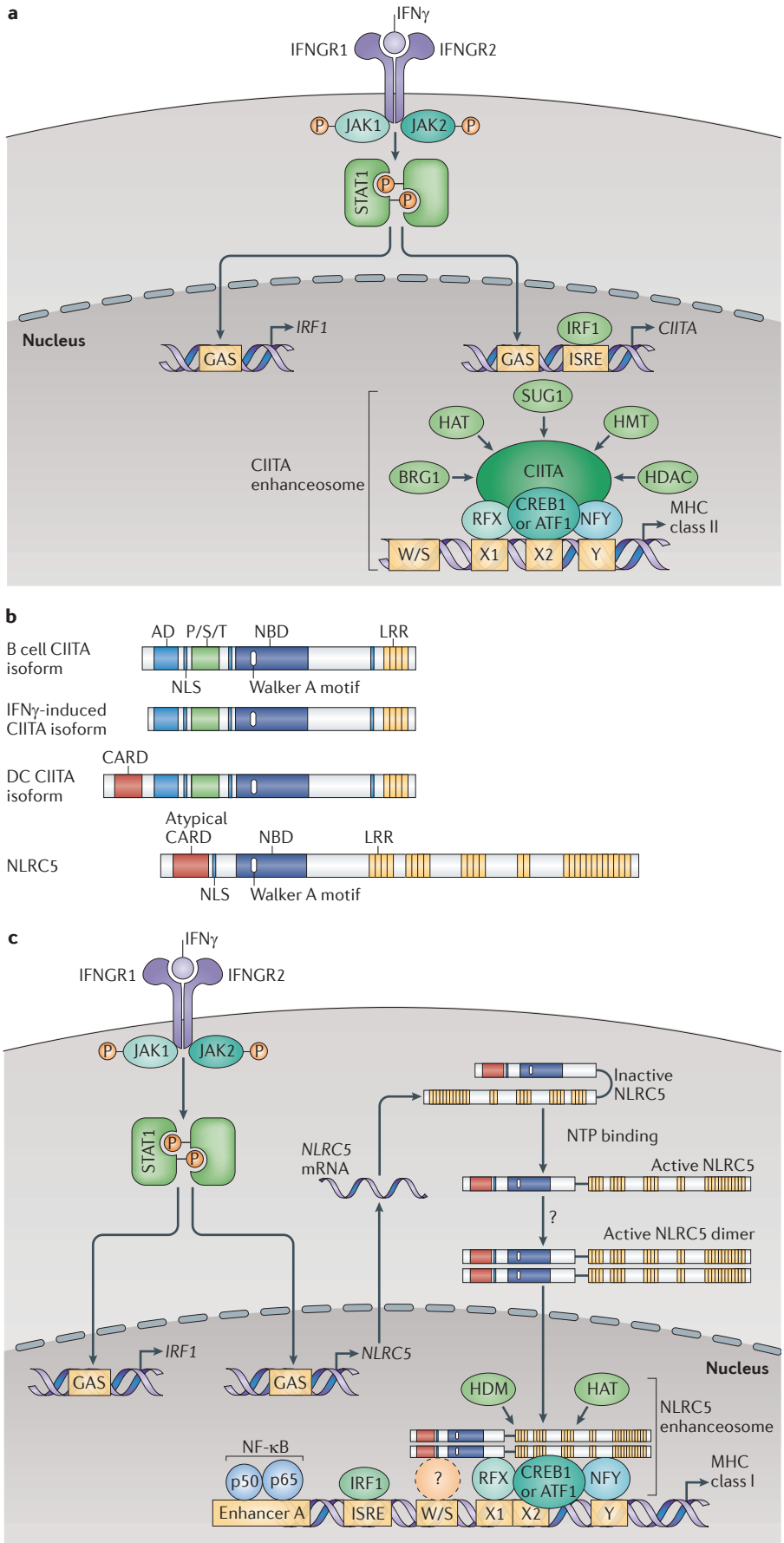

Figure 3 | **MHC class I and MHC class II gene transactivation.** **a** | MHC class II gene expression is mediated by the class II transactivator (CIITA). One isoform of CIITA is induced by an active signal transducer and activator of transcription 1 (STAT1) homodimer (which is induced by IFNγ stimulation via Janus kinase 1 (JAK1) and JAK2) and the transcription factor IFN-regulatory factor 1 (IRF1) following interferon-γ (IFNγ) stimulation. CIITA activates MHC class II genes by interacting with a multiprotein transcription factor complex — comprised of regulatory factor X (RFX) components, cAMP-responsive-element-binding protein 1 (CREB1), activating transcription factor 1 (ATF1) and nuclear transcription factor Y (NFY) — that is assembled on the SXY module in MHC class II promoters. The binding of CIITA to this multiprotein complex forms an enhanceosome, which acts as a platform for the recruitment of other proteins to MHC class II promoters. Such proteins include histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs), SUG1 (a component of the19S proteasome cap complex) and BRG1. These factors collectively further regulate the transcription of MHC class II genes. **b** | The structures of the CIITA isoforms that are expressed in B cells, in response to IFNγ stimulation and in dendritic cells (DCs) are shown, together with the structure of NOD-, LRR- and CARD-containing 5 (NLRC5). Note that the caspase activation and recruitment domain (CARD) at the amino-terminus of NLRC5 is structurally distinct from the CARDs of other NOD-like receptor (NLR) proteins and may thus be referred to as an atypical CARD[35]. **c** | A model of NLRC5-mediated MHC class I gene expression is shown. NLRC5 expression can be induced by activated STAT1 following IFNγ stimulation. This is mediated through the binding of phosphorylated STAT1 homodimers to the *NLRC5* promoter, probably at the two predicted STAT-binding sites. The *NLRC5* promoter also contains a putative IFN-stimulated response element (ISRE), which may facilitate transactivation by IRF1. Following its synthesis, the NLRC5 protein may change conformation into an active form through NTP binding and/or NTP hydrolysis and is imported into the nucleus. Similarly to CIITA, NLRC5 might form an active dimer complex, although this remains to be determined. NLRC5 functions as an MHC class I transactivator (CITA) and forms an enhanceosome with transcription factors (such as the RFX complex) at the MHC class I promoter to induce MHC class I gene expression. HATs and histone demethylases (HDMs) cooperate with NLRC5 to regulate gene expression. Other transcription factors — such as IRF1 and nuclear factor-κB (NF-κB) — further regulate MHC class I promoter activity through the ISRE and the NF-κB-binding sites of enhancer A, respectively. AD, acidic domain; GAS, IFNγ-activated site; LRR, leucine-rich repeat; NBD, nucleotide-binding domain; NLS, nuclear localization signal; P/S/T, proline/serine/threonine-rich domain.

NLRC5 is an MHC class I gene transactivator. The identification of NLRC5 as a class I transactivator was achieved through genome-wide gene expression profiling of stable human cell lines expressing wild-type or mutant NLRC5 (REF. 11). NLRC5 expression induced both classical MHC class I genes (that is, *HLA-A*, *HLA-B* and *HLA-C*) and the non-classical MHC class I gene *HLA-E*. NLRC5 also upregulated the expression of the MHC class I accessory genes *B2M*, *LMP2* (also known as *PSMB9*) and transporter associated with antigen processing 1 (*TAP1*)[11] (FIG. 1). Strikingly, NLRC5 had no effect on the expression of the MHC class II locus and its accessory genes[11]. Moreover, upregulation of NLRC5 was crucial for the efficient induction of MHC class I gene expression by IFNγ[11] (FIG. 3c). This was underscored by the observation that small interfering RNA (siRNA)-mediated knockdown of NLRC5 expression impaired the IFNγ-induced upregulation of MHC class I expression but did not affect the upregulation of MHC class II expression[11]. Interestingly, there is a small IFNγ-mediated increase in MHC class I expression in NLRC5-deficient cells, which could be achieved through the direct binding of IRF1 to the ISRE in the proximal MHC class I promoter[10,13,15] (FIG. 3c). At the moment, little is known about the transcriptional regulation of *NLRC5* and its modulation by cytokines other than IFNγ and IFNβ.

Using genetically modified mice, several laboratories unanimously demonstrated that NLRC5 has a crucial role in the constitutive and inducible expression of MHC class I genes *in vivo*[10,13,15–17]. In NLRC5-deficient mice, the constitutive expression of the classical murine MHC class I genes (namely, *H2-K* and *H2-D*) was substantially impaired in T cells, NK cells and NKT cells. The expression of these genes was also reduced in B cells, albeit to a lesser extent, and was mildly decreased in macrophages and DCs[10,13,15–17]. The high expression levels of CIITA may substitute for the lack of NLRC5 in these APCs. This would explain why NLRC5-deficient APCs have higher levels of MHC class I expression than NLRC5-deficient T cells and NK cells, which lack endogenous CIITA expression.

Interestingly, the expression of non-classical MHC class I genes (namely, *H2-M3*, *H2-Qa1* and *Tla*) was also reduced in NLRC5-deficient mice. In agreement with observations in human cell lines, NLRC5 was also found to be required for the constitutive expression of *B2m*, *Tap1* and *Lmp2* in mice[10,15,17]. The expression of MHC class II genes (namely, *H2-A* and *H2-E*) and the MHC class II-related gene *H2-O*, on the other hand, was intact in NLRC5-deficient mice, confirming that NLRC5 is required solely for the expression of MHC class I genes and their accessory genes[10,15–17].

Unlike β_2m-deficient mice, in which the number of $CD8^+$ T cells is severely reduced, the number of $CD8^+$ T cells is only mildly reduced in the spleen, liver and lymph nodes of NLRC5-deficient mice[13,17]. However, NLRC5-deficient cells have an impaired ability to induce antigen-specific $CD8^+$ T cell activation, as evidenced by reduced IFNγ production and diminished cytolytic activity[13,15,17]. As a consequence, NLRC5-deficient mice are susceptible to infections that require $CD8^+$ T cell responses. Following the infection of NLRC5-deficient mice with the intracellular bacterium *Listeria monocytogenes*, $CD8^+$ T cells are poorly activated and the mice harbour substantially increased bacterial loads in the liver and spleen compared with control mice[15,17]. Together, all of these recent findings indicate the central role of NLRC5 in immune responses through the regulation of MHC class I expression.

Mechanism of NLRC5-mediated MHC class I gene activation. As the expression of NLRC5 correlates with that of MHC class I molecules both in the steady state and following stimulation in various tissues and cells, it seems that the expression level of NLRC5 is a crucial determining factor in NLRC5-mediated MHC class I gene transcription[11,14]. In addition, the nuclear localization of NLRC5 (which is controlled by the Walker A and NLS motifs) is required for the regulation of MHC class I transcription (FIG. 3c). Interestingly, although appending virus-derived artificial NLS motifs to a Walker A mutant of NLRC5 can rescue its lack of nuclear import, the nuclear Walker A mutant is still unable to activate MHC class I promoters. This indicates that the NBD of NLRC5 is essential both for its nuclear import and for the transactivation of MHC class I genes[39].

NLRC5 specifically associates with the promoters of MHC class I genes *in vitro* and *in vivo*[11,13,14]. As NLRC5 itself does not possess a DNA-binding domain, nuclear NLRC5 needs to cooperate with the multiprotein complex comprising RFX, CREB1–ATF1 and NFY that is assembled on the SXY module in the MHC class I promoter[6] to exert its transactivation activity. Indeed, by using reporter assays to measure the activity of mutated versions of the MHC class I promoter with various substitutions and deletions, it was shown that the X1 and X2 boxes are required for NLRC5-mediated MHC class I promoter activation[12,14]. Activation of MHC class I promoters by NLRC5 also requires the presence of the W/S box and its interacting factors[12]. By contrast, although the W/S box is required for the recruitment of CIITA to MHC class II promoters[40,41], CIITA-mediated transactivation of MHC class I promoters is independent of the W/S box[12]. These observations suggest that, despite the highly similar architecture of the SXY module and its interacting factors in MHC class I and class II promoters, differences exist in the requirements for the various promoter elements for NLRC5- and CIITA-mediated transactivation of MHC class I and class II genes. These differences remain to be identified, but one could speculate that the additional upstream MHC class I promoter elements, such as the ISRE, may have a role in the NLRC5-mediated induction of MHC class I transcription (FIG. 2). Another possible explanation is that there might be an additional binding partner of the SXY module in MHC class I promoters that determines NLRC5 specificity.

In a study using RFX-deficient cell lines, it was demonstrated that NLRC5 can cooperate with the RFX complex (FIG. 3c). In particular, NLRC5 associates with RFXANK via its ankyrin repeats[12]. NLRC5 also synergizes with ATF1, which binds to the X2 box, to activate MHC class I promoters. Therefore, NLRC5 interacts and cooperates with the multiprotein complex assembled on the SXY module in the proximal MHC class I promoter to form the NLRC5 enhanceosome and induce MHC class I transcription. As both CIITA and NLRC5 bind to the same multiprotein complex[11,13,25,29], one could argue that they compete for interactions with the same binding sites. This is underscored by the observation that dominant-negative mutant forms of CIITA indeed seem to compete with NLRC5, thereby preventing MHC class I promoter activation[14]. The opposite was also demonstrated, in that the presence of CIITA had an additive effect on NLRC5-dependent MHC class I expression[14]. These observations could explain why CIITA-deficient mice do not show a large reduction in MHC class I expression, particularly in the lymphoid and myeloid compartments that were investigated, as these cells highly express NLRC5.

NLRC5, like CIITA, also acts as a platform for histone-modifying enzymes that regulate chromatin dynamics[10,12]. *In vitro*, NLRC5 functions synergistically with histone acetyltransferases to activate MHC class I transcription[12], and *in vivo* NLRC5 was also shown to facilitate the modification of the methylation status of histone H3 lysine 27 at the proximal promoter of *H2-K1* (REF. 10) (FIG. 3c). NLRC5 is therefore an important factor that regulates the constitutive and inducible expression of MHC class I genes. This function is mediated through the interaction of NLRC5 with the factors bound to the conserved SXY module in MHC class I proximal promoters.

Open questions on CIITA and NLRC5 function. There remain several issues to be addressed with regard to the role of the enhanceosome complex that contains RFX and NLRC5 and/or CIITA in the regulation of MHC class I expression. In RFX-deficient cell lines derived from patients with an MHC class II deficiency, a reduction in MHC class I expression is observed[5,6]. This finding reveals the importance of the RFX complex in the expression of MHC class I genes[5,6]. The residual expression of MHC class I molecules may be due to possible functional redundancy among RFX proteins. Even in the absence of one of the components of the RFX complex, NLRC5 may still be able to form a functional (but not complete) NLRC5 enhanceosome that drives sufficient transcription of MHC class I genes. This could be achieved through multiple synergistic interactions with the other components of the SXY module-binding multiprotein complex, as in the case of CIITA[25,29]. This would also explain why there is residual MHC class II expression in RFX5-deficient mice. In line with this notion is the observation that expression of exogenous CIITA in an RFX5-deficient fibroblast cell line results in a partial rescue of MHC class II expression[42].

It is also tempting to speculate that NLRC5 and CIITA form a functional partnership for MHC class I expression to provide the optimal antigen-presenting capacity of APCs. In non-immune cells, only NLRC5 is constitutively expressed, but inflammatory conditions that upregulate NLRC5 and induce CIITA would also result in a strong increase in MHC class I expression. It would therefore be of high interest to evaluate the combined role of NLRC5 and CIITA in MHC class I expression by generating double knock-out mice.

With regard to the MHC class I accessory genes that are upregulated by NLRC5, only *B2M* contains a functional SXY module in its proximal promoter[6]. Thus, it remains to be established whether the expression of *TAP1* and *LMP2* is regulated by the direct binding of NLRC5 or indirectly through regulatory elements similar to those in the proximal promoters of MHC class I genes and *B2M*. Furthermore, it is unclear why NLRC5 upregulates only the expression of *TAP1* and *LMP2* and not that of the *TAP2*, *LMP7* and tapasin genes, the products of which are also crucial for the processing of antigens in the MHC class I pathway and for peptide transport into the endoplasmic reticulum. This is contrary to what is observed for CIITA, which also induces the expression of the other components involved in the MHC class II antigen-presentation pathway (FIG. 1).

A role for NLRC5 in innate immunity? As NLRC5 belongs to the NLR protein family, early efforts were devoted to investigating its possible function in the innate immune system. In particular, NLRC5 was shown to be a negative regulator of TLR4 signalling and retinoic acid-inducible gene I signalling (RIG-I signalling) via its direct interactions with IκB kinase-α (IKKα) and IKKβ, and RIG-I, respectively[43]. In agreement with this, knockdown of NLRC5 expression in a murine macrophage cell line resulted in enhanced responses to LPS stimulation[34]. However, other groups showed that the silencing of NLRC5 expression decreases the production of type I IFNs in response to viral infection, suggesting a contradictory antiviral function for NLRC5 (REFS 32,33). NLRC5 has also been proposed to be an activator and component of the inflammasome, a protein complex that activates caspase 1 for pro-inflammatory cytokine maturation[44].

Glossary

BRG1
The ATPase BRG1 is the catalytic subunit of the human SWI/SNF complex that is needed for nucleosome remodelling to provide a more open chromatin structure. This open structure facilitates the interaction of transcription factors with their cognate binding sites in gene regulatory elements to promote transcription.

Enhanceosome
A multiprotein complex containing transcription factors, co-activators and additional proteins that binds to regulatory regions in genes (such as proximal promoters and/or enhancers) to accelerate gene transcription.

Histone acetyltransferases
Enzymes that mediate the addition of an acetyl group to lysine residues that are located at the N-termini of histones. Histone acetylation facilitates transcription.

Histone deacetylases
Enzymes that remove the acetyl groups from lysine residues that are located at the N-termini of histones. In general, decreased levels of histone acetylation are associated with the repression of gene expression. The balance of histone acetylation is maintained by the interplay between histone deacetylases and histone acetyltransferases.

Histone demethylases
Enzymes that remove the methyl groups from modified lysine residues in histones.

Histone methyltransferases
Enzymes that catalyse the transfer of methyl groups to lysine and/or arginine residues in histones. Depending on the residue that is methylated, this histone modification is associated with either gene repression or gene activation.

Inflammasome
A large multiprotein complex composed of an NLR protein, the adaptor protein ASC and caspase 1. Inflammasomes contribute to the secretion of IL-1β and IL-18 by activating caspase 1.

MHC class II deficiency
(Also known as bare lymphocyte syndrome (BLS)). A severe combined immune deficiency disease recognized by the lack of MHC class II molecule expression owing to defects in CIITA (type II BLS) or in RFX components (namely RFXBANK, RFXAP or RFX5; type III BLS).

NOD-like receptor
(NLR; also known as a nucleotide-binding domain, leucine-rich repeat-containing protein). A member of a diverse family of cytosolic pattern-recognition molecules that are involved in the innate immune sensing of pathogens and inflammatory responses.

Nucleotide-binding domain
(NBD; also known as a nucleotide-binding oligomerization domain). A domain that is crucial for the function of NLR proteins. The NBD induces the oligomerization or dimerization of proteins following the binding and hydrolysis of ATP or GTP.

Retinoic acid-inducible gene I signalling
(RIG-I signalling). A signalling pathway that is activated by the interaction of viral RNA with the receptor RIG-I. Through the adaptor protein MAVS (also known as IPS1, VISA and CARDIF), these signals activate the transcription factors IRF3 and IRF7, leading to the production of type I interferons.

Regulatory factor X complex
(RFX complex). RFX drives the assembly of the multiprotein complex on the SXY module of MHC gene promoters. RFX components fail to assemble this complex if genetic defects of RFX genes exist, and this results in rare hereditary immunodeficiency diseases (type III bare lymphocyte syndrome), characterized by the absence of MHC class II expression associated with reduced levels of MHC class I expression.

SUG1
The ATPase SUG1 is a component of the regulatory 19S proteasome cap complex and appears to be important for regulating histone H3 acetylation at MHC proximal promoters. Furthermore, SUG1 is also required for recruiting CBP and CIITA to MHC proximal promoters.

Contrary to the proposed function of NLRC5 in innate immunity, cells derived from NLRC5-deficient mice can respond to the stimulation of TLR2, TLR4 or TLR9 and to bacterial challenge without any defects[10,13,15,17,45]. Wild-type and NLRC5-deficient cells produce similar levels of IFNβ and pro-inflammatory cytokines following challenge with RNA viruses, DNA viruses, intracellular bacteria, B-form DNA (poly(dA:dT)) or polyI:C[10,13,17,45]. Moreover, activators of the NOD-, LRR- and pyrin domain-containing 3 (NLRP3), NLRC4 and absent in melanoma 2 (AIM2) inflammasomes can induce comparable levels of inflammasome activation in NLRC5-deficient and wild-type cells, as evidenced by normal caspase 1 activation and IL-1β secretion[45]. Overall, NLRC5 seems to display innate immune functions in cells with modulated NLRC5 expression. However, most of the data from cells derived from NLRC5-deficient mice indicate that NLRC5 does not have a significant role in TLR signalling, antiviral innate immune responses or inflammasome activation (reviewed in REF. 46).

Conclusion and perspectives

Since the discovery of CIITA and of the common regulatory promoter elements in MHC class I and MHC class II genes, it had been postulated that a similar protein specifically regulates MHC class I genes. Therefore, it is not surprising that there are several structural and functional similarities between CIITA and NLRC5. They both belong to the CARD-containing NLR subfamily and they have a closer phylogenetic relationship with each other than with any other NLR proteins[11,47]. Moreover, both molecules contain NLSs and can translocate into the nucleus (REFS 1,36–39). The expression of both CIITA and NLRC5 is highly inducible following the activation of STAT1 in response to IFNγ stimulation[9,11,32,48–51]. In addition, the NTP-binding motifs in NLRC5 and CIITA are required for the transactivation of MHC class I and MHC class II genes, respectively[11,39,52,53]. Despite the lack of a DNA-binding domain, both NLRC5 and CIITA can transactivate MHC genes by forming enhanceosomes through their association with promoter-assembled factors, including the RFX proteins and CREB1–ATF1 (REFS 4,7,11). Strikingly, both NLRC5 and CIITA orchestrate the concerted expression of sets of functionally related genes that are crucial for antigen presentation (FIG. 1).

Given the key role of MHC class I molecules in the immune system, the discovery of NLRC5 as an important regulator of MHC class I gene expression should facilitate our progress in treating infectious diseases and tumours, and in developing vaccines against pathogens and cancers.

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Competing interests statement
The authors declare no competing financial interests.

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