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# REGULATION OF CLASS II MAJOR HISTOCOMPATIBILITY COMPLEX GENES: RELATION TO MULTIPLE SCLEROSIS

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## Introduction.

Susceptibility to certain autoimmune diseases is highly correlated with specific polymorphic residues in molecules encoded by the class II region of the major histocompatibility complex (MHC) [42]. Therefore, sequence analysis of the protein-coding regions of class II MHC genes has been of considerable importance in clarifying mechanisms of autoimmunity [41].

It has also been proposed that aberrant expression of class II MHC genes may predispose to autoimmunity [41]. Class II MHC molecules have a highly restricted tissue distribution, being normally expressed only on B lymphocytes, macrophages, dendritic cells and activated T lymphocytes [14]. Ectopic expression of class II MHC molecules has been demonstrated in a variety of organ-specific autoimmune states, including thyroid disease, insulin-dependent diabetes mellitus and multiple sclerosis (MS) (reviewed in [32, 43]). *In vitro,* the presence of class II MHC molecules is sufficient for effective antigen presentation (reviewed in [13]); therefore it has been speculated that ectopic class II MHC expression could facilitate local immune responses. Understanding the regulation of class II MHC expression may therefore provide insight into the pathogenesis of organ-specific autoimmunity. Furthermore, the regulation of this well-characterized family of genes is a model for analysing tissue-specific and inducible gene expression. In this review, I will focus primarily on the regulation of transcription of human class II MHC genes.

## Overview of regulation of class II MHC expression.

Class II MHC structures are α/β heterodimers; both subunits are integral membrane glycoproteins. In human cells, at least three types of class II MHC molecules, HLA-DR, HLA-DQ and HLA-DP, are expressed. Polymorphic genes within the MHC on chromosome six encode the individual α- and β-subunits. Induction of each heterodimeric class II MHC structure involves the coordinate transcription of both subunit genes. A further requirement for constitutive or induced expression may be the presence of a third glycoprotein, the invariant chain, which associates intracellularly with class II MHC α- and β-subunits [7]. The potential role of the invariant chain was recently discussed, within the context of a comprehensive review of post-translational processing of class II MHC antigens [7].

There is a spectrum of class II MHC expression at the level of the individual cell. Along this spectrum, four general phenotypic states can be distinguished. Constitutive class II MHC expression at a high level of cell surface density is observed in B lymphocytes and activated human T lymphocytes. Macrophages and dendritic cells constitutively express class II MHC molecules at lower cell surface density and can be

induced to enhanced expression. A wide variety of parenchymal cells, on which class II MHC molecules are normally undetectable, can be induced to class II MHC expression both in tissue-culture and *in vivo* [6]. Finally, class II MHC molecules cannot be induced on a minority of cell types, including human fœtal neurons in tissue culture [27]. The detailed characterization of mechanisms which permit this degree of flexibility in class II MHC gene expression would be of considerable interest.

In several laboratories including our own, it has been documented that expression of class II MHC molecules is directly correlated with the abundance of mRNA encoding class II MHC products (reviewed in [17, 11, 34]). Therefore, processes affecting the steadystate levels of these mRNA are crucially important in regulating the expression of class II MHC molecules. It is likely that a major contribution to increased steadystate levels of class II MHC mRNA in cells exposed to interferon-γ results from increased transcription of the cognate genes [4].

### *Cis*-regulatory sequences.

The accurate and efficient transcription of eukaryotic genes requires regulatory DNA sequence elements designated as promoters; for genes transcribed by RNA polymerase II, these elements are located 5' of the mRNA-cap site. Sequence analysis of 5'-flanking regions of numerous class II MHC genes has revealed several conserved sequences, which by virtue of their conservation are candidate regulatory elements (reviewed in [17, 18, 30]). In particular, two short DNA segments, which have been designated class II MHC X and Y boxes, have been identified in the promoters of all human class II MHC genes (reviewed in [17, 40]). Homologous sequence elements are present in the promoters of murine class II MHC genes as well [26]. In the human DR-α promoter region, the X box is located at −108 to −95 relative to the mRNA-cap site; the Y box is located downstream at −74 to −61 [17]. The Y box contains contains an inverted form of the sequence CCAAT. This sequence element is a general transcriptional activator, and it has been proposed that the Y box exerts its function in part by associating with the universal CCAAT-binding transcription factor [9, 10]. Boss and Strominger demonstrated the relevance of the conserved X and Y boxes for expression of a transfected DQ-β gene in human fibroblasts [5]. Plasmids containing DQ-β genes truncated progressively within 5'-flanking sequences were assayed in stably transfected cells for their ability to direct the synthesis of DQ-β mRNA [5]. Deletions encroaching upon the X- and Y-box sequences resulted in decreased basal DQ-β expression, suggesting that these sequence elements were positive regulators of class II MHC expression. Deletions which eliminated the upstream X box and portions of the spacer between the X and Y boxes directed the synthesis of suprabasal DQ-β mRNA, suggesting that a negative regulatory element could be present in the spacer [5].

An additional conserved DNA sequence has been identified in all human class II MHC promoters analysed to date [17]. This putative control element is homologous to the octamer sequence, which was first characterized in the promoters of immunoglobulin genes and has subsequently been identified in promoters of numerous eukaryotic genes [37, 39]. Whereas octamer sequences occur in a fixed position upstream of all immunoglobulin genes, their location in class II MHC genes is not conserved, and their relevance for class II MHC expression has not been addressed by deletion analysis.

Several conserved elements in class II MHC gene promoters have been analysed with regard to their potential significance for tissue-specific or interferon-γ-inducible expression. Promoter elements extending approximately 200 nucleotides upstream of the cap site are sufficient for both tissue-specific and interferon-γ-inducible expression of transfected genes in several cell types, including human glioblastoma cells

[3, 36]. For the expression of the DQ-β gene in fibroblasts, deletion to −128, within an element designated the W box, abolishes interferon-γ inducibility, but elevates basal expression levels, suggesting that the W box may contain an interferon-γ-inducible negative regulatory element [5]. Another putative regulatory element, termed the Z box, is similarly located upstream of the X box in the DR-α-gene promoter [40].

### *Trans*-acting factors.

The mechanism by which individual DNA elements provide promoter and enhancer function involves association with specific putative transcription factors. It was recently demonstrated that interferon-γ-induced transcription of DR-α genes in HeLa cells requires *de novo* protein synthesis [4]. This observation suggested that the synthesis of a transcription factor or production of an activity capable of modifying a preexisting factor is essential for the induction of class II MHC expression by interferon-γ in HeLa cells [4]. This requirement was specific for class II MHC gene transcription, since expression of class I MHC genes was insensitive to inhibition of protein synthesis [4].

Several lines of additional evidence indicate that *trans*-regulatory factors, at least some of which are encoded outside the MHC, are essential for both constitutive and induced class II MHC expression. A particularly convincing demonstration has come from analysis of the severe combined immunodeficiency (SCID) phenotype, in which cells fail to express class II MHC antigens or their transcripts, and cannot be induced to express class II MHC molecules by interferon-γ, but contain invariant-chain transcripts [31]. The SCID phenotype has been mapped genetically outside the MHC [31]. In an elegant series of experiments, Reith and coworkers assayed nuclear extracts of B lymphocytes from SCID patients and normal controls for activities which bound DR-α putative regulatory sequences [35]. Whereas control nuclear extracts contained potential transcription factors which bound X box, Y box and immunoglobulin-gene-related octamer sequences, several SCID extracts exhibited a striking deficiency of the X-box-binding activity, indicating its centrality in this phenotype [35].

It has also been documented that nuclear extracts of Raji cells (a B-lymphoma-derived human line), contain activities which exhibit sequence-specific association with DNA fragments containing W-box, X-box and Y-box sequences [28]. The specificity of this binding was demonstrated by gel retardation, DNase-I-footprinting and methylation interference analyses [28]. No differences were observed, however, between class-II-MHC-expressing Raji cells and class-II-MHC-negative variants, indicating that the Raji cell nuclear factors which associate with these sequence elements are not sufficient for expression of class II MHC genes [28].

The recent isolation by Liou and coworkers of cDNA clones corresponding to potential X-box-binding *trans*-regulatory factors will facilitate investigation of their physiologic role in governing class II MHC expression [20]. Surprisingly, two clones identified by screening a lambda-phage expression library with labelled oligodeoxynucleotides were distinct, although both encoded X-box-binding activities [20]. Furthermore, the binding activity was specific for the murine class II A-α promoter, in that fragments containing E-α X-box sequences were not bound [20].

### Other modulators of class II MHC expression.

It is unclear how the interaction of interferon-γ with its receptor results in transcription of specific genes. The successful cloning of the human receptor for interferon-γ by Aguet and coworkers should facilitate rapid progress in characterization of this transmembrane-signaling event [1].

In addition to the prototypic inducer, interferon-γ, a great diversity of other influences have been demonstrated to affect class II MHC expression. Other positive modulators of class II MHC expression include murine coronavirus particles, which specifically induce class II MHC expression in rat astrocytes, but not in microglia [23]. Simian immunodeficiency virus induces class II MHC expression in infected tissue culture cells [16]. Tumour necrosis factor can synergize with interferon-γ or measles virus infection for induction of class II MHC expression [25, 33]. B-cell stimulation factor type 1 (BSF-1) enhances expression of class II MHC on resting B lymphocytes; intriguingly, interferon-γ appears to downregulate this induction [29].

Varied additional influences downregulate class II MHC expression. These include immune complexes and α-fœtoprotein, as well as hormones such as interferons-α/β, norepinephrine, prostaglandins, corticosteroids and transforming growth factor $\beta_1$ [2, 8, 11, 12, 15, 19, 21, 38, 44]. Although almost no information is available about the mechanism of action of these agents, it has been demonstrated in several cases that decreased expression of class II MHC molecules is accompanied by decreased abundance of the cognate mRNA [8, 11].

## Conclusions.

The impression which arises from these studies of regulation of class II MHC transcription is one of formidable complexity, which is apparent in several respects: regulation involves multiple *cis*-regulatory DNA elements and a diversity of *trans*-acting protein factors, which may function differently in different cell types and in response to varying physiologic stimuli. Finally, the individual class II MHC molecules, DR, DQ and DP, are differentially expressed, both in cells constitutively class-II-MHC-positive and under certain conditions of induction [17].

In addition to the already evident complexity of class II MHC regulation at the single-cell level, there is clearly genetic variability in control of class II MHC expression in populations of organisms. Furthermore, these variations may be specific to cell types or tissues. Most relevant for MS, it has been demonstrated that inducibility of class II MHC on astrocytes by interferon-γ and by the coronavirus JHM correlates with susceptibility to experimental autoimmune encephalomyelitis (EAE) [22, 24]. Several testable hypotheses about the relationship of regulation of class II MHC expression to MS may be formulated.

1) Susceptibility to MS may correlate with a generalized (non-tissue-specific) abnormality of regulation of MHC expression; this abnormality could be isotype-limited.

2) Predisposition for developing MS may correlate with a genetically-determined or -acquired class-II-MHC-hyperinducibility, which is expressed only in neuroepithelial cells.

3) MS susceptibility could correlate with physiologic abnormalities in regard to one of the hormones implicated in regulation of class II MHC expression.

The rapid acquisition of knowledge about the regulation of class II MHC expression should permit these issues to be addressed in the near future.

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