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REVIEW

MHC Class II Allosteric Site Drugs: New Immunotherapeutics for Malignant, Infectious and Autoimmune Diseases*

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The discovery of the interactions of the 'Ii-Key' segment of the Ii protein with the major histocmpatibility complex (MHC) Class II allosteric site, which is adjacent to the antigenic peptide-binding site, creates therapeutic opportunities by regulating the antigenic peptide binding to MHC class II molecules. The binding of Ii-Key to the MHC class II allosteric site loosens the hold of the MHC Class II 'clamshell' on antigenic peptides and leads to highly efficient antigenic peptide charging to or releasing from the MHC class II antigenic peptide-binding groove. Ii-Key peptide-induced spilling of bound antigenic peptide, or replacement with inert blockers, leads to 'inert immunosuppression'. High efficient replacement of ambient with vaccine peptides by Ii-Key permits 'active immunosuppression' for antigen-specific control of autoimmune diseases in the absence of cytokines or adjuvants. On the other hand, active immunization against cancer or infectious disease can result from epitope replacement mediated by Ii-Key and accompanied by cytokines or other adjuvants. Finally, linking the Ii-Key peptide through a simple polymethylene bridge to an antigenic sequence vastly increases the potency of MHC Class II peptide vaccines. In summary, the discovery of the MHC class II allosteric site allows one to increase the efficiency of MHC class II-related, antigenic epitope-specific therapy for malignant, infectious, and autoimmune diseases. The focus of this review is on the mechanism and potential clinical use of such novel allosteric site-directed, Ii-key drugs.

INTRODUCTION

Helper T-cell activation by MHC Class II-presented antigens is essential for the immune system to mount an effective and long-lasting response. The key initial step is for the antigen to be acquired and processed by professional antigen presenting cells (APCs) and presented by MHC class II molecules to activate $CD4^+$ T-helper cells. Such T-helper cells in turn activate dendritic cells (DCs) so that they can optimally stimulate the development and potency of cytotoxic T cells. A complete immune response to malignant and infectious diseases is thus effectively induced. MHC Class I molecules pick up antigenic peptides that have been transported into the ER by the transporter of antigenic peptides (TAP) after digestion and selection from cytoplasmic proteins by proteosomes [1, 2]. The MHC Class I/antigenic peptide complexes are recognized by $CD8^+$ cytotoxic T lymphocytes (CTLs). Antigenic peptides cannot be bound to MHC Class II molecules at their synthesis in the ER because the antigenic peptide-binding site is blocked by the Ii protein [3, 4]. Only after transport of MHC Class II/Ii protein complex to a post-Golgi, antigenic peptide-binding compartment [5, 6], is the Ii protein cleaved by proteases, allowing charging by suitably processed antigenic proteins selected from the environment [7–10]. After charging with antigenic peptides, the MHC Class II/peptide-epitope complex travels to the cell surface for presentation to $CD4^+$ T-helper cells.

Two mechanisms have been proposed to explain the function of Ii to enhance the charging of antigenic peptides to MHC class II molecules. Firstly, Ii is digested retaining a segment, CLIP, which binds in the antigenic peptide binding site, MHC class II 'clamshell' [11–14]. DM then removes the CLIP in exchange for an antigenic peptide [15–17]. Secondly, Ii contains a sequence, which bind to an allosteric site, alongside one end of

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the antigenic peptide binding groove. During the staged cleavage, a transient Ii segment containing these sequences is created by protease digestion [9, 10] holding open the antigenic peptide-binding site until an antigenic peptide is bound. That Ii segment has been termed the Ii-Key peptide [18–20]. Ii-Key peptide homologs can spill bound peptides and enhance the charging of antigenic peptides. The allosteric site has been characterized with Ii-Key homologs in peptide binding/release assays with purified, exomembranal HLA-DR1 molecules [21]. Here we focus on the function of the Ii-Key that interacts with the allosteric site of MHC Class II molecules to modulate the efficiency of binding or release of antigenic peptides. The fact that the site of action of the Ii-Key peptide is retained on cell surface MHC Class II molecules allows for accelerated direct charging of Class II molecules with antigenic peptides or direct spilling of a harmful auto-reactive antigenic peptide. This ability to modulate the binding of antigenic peptides to MHC Class II molecules, by targeted interactions at the allosteric site, offers the potential for a new generation of immunotherapeutics.

THERAPEUTIC OPPORTUNITIES WITH MHC CLASS II-PRESENTED ANTIGENIC PEPTIDES

Two immunotherapy strategies focus on the control of antigen binding to the MHC Class II antigenic peptide binding groove [22–24]. The first focuses on the identification and use of MHC Class II-presented antigenic peptides to induce either an active immune response or antigen-specific tolerance. The second entails blocking the antigenic peptide-binding groove to prevent or stop the binding of antigens that result in a harmful immune response. Numerous, clinically relevant antigenic peptides (epitopes) have been identified. Some antigenic epitopes have been discovered after expressing the fragments of structural genes in an APCs that is subsequently screened with a T-cell line [25]. Other antigenic epitopes have been identified by acid elution of immunopurified MHC Class II molecules followed by HPLC and tandem mass spectrometry, with or without T-cell line monitoring of fractions [26]. Yet other antigenic epitopes have been discovered after the synthesis and testing of libraries of overlapping 15 amino acid peptides of target antigenic proteins [27]. From these data, consensus motifs for both MHC class I- and II-restricted epitopes have been proposed [28].

While some peptides of potential clinical importance have been identified and specific immune responses have been observed in patients treated with those peptides, good therapeutic efficacy has not been obtained. The main obstacles appear to be [1]: relatively modest stability of MHC Class II antigenic peptide complexes does not allow an easy replacement of a designed antigenic peptide (or a blocker) with bound ambient peptides to form a new antigenic/MHC class II complex and [2] quick clearance of peptide *in vivo*. To overcome these effects, antigenic peptides (or binding site blockers) would have to be given at saturating concentrations, which is impractical for clinical use.

By enhancing binding of the antigenic epitopes in the peptide-binding groove of MHC Class II molecules, Ii-Key compounds, alone or in hybrids, opens the door the to discovery and clinical exploitation of many MHC Class II-presented epitopes, in a wide range of disease applications.

DISCOVERY OF THE MHC CLASS II ALLOSTERIC SITE

In studying the molecular mechanisms of several potentially immunoregulatory Ii peptides, Adams *et al*. found that coincubation of the Ii77–92 peptide (termed Ii-Key) greatly enhanced the presentation of certain antigenic peptides to their respective murine T hybridomas [18, 19]. The response of a T hybridomas recognizing the pigeon cytochrome C81-104 increased up to 40 times baseline with limiting doses of antigenic peptide presented on a syngeneic APC-cell line. In a study of 160 systematically varied homologs of the original Ii-Key peptide, the active core of Ii-Key was found to be Ii77–83 (LRMKLPK) [19]. A minimal fragment was also found, Ii77–80 (LRMK), which had half-maximal activity relative to the Ii-Key core segment. The linear distance in the Ii protein sequence from this fragment to the region of Ii protein binding in the antigenic peptide-binding groove (Ii 90–105, with M90 being in the P1 site [29]); indicates that the Ii-Key core interacts at an allosteric site relative to the antigenic peptide binding groove.

Using purified soluble exomembranal HLA-DR1, Xu *et al*. found that Ii-Key peptides varied according to extension of the C-terminus from the Ii-Key core segment, in terms of exchange or release of antigenic peptide binding. Specifically, differences were noted in the requirement for exogenous free antigenic peptide to facilitate the exchange mediated by a given Ii-Key sequence [21]. Certain Ii-Key peptides with long C-terminal extensions from the 7 amino acid core LRMKLPK effectively release indicator, bound antigenic peptide without unlabelled peptide in the solution. However, the seven amino acid core Ii-Key peptide and homologs extending only a few amino acids at the C-terminus, released bound indicator peptide only when unlabelled antigenic peptide was present in the supernatant. Additional competitive binding experiments with biotinylated Ii-Key peptides or antigenic peptides helped to define the spatial relationship of the allosteric site with respect to the antigenic peptide binding site [21]. The allosteric site is located just a few amino acids away from the end of the groove holding the N-terminus of the antigenic peptide. These mechanistic studies support the view that Ii-key spillers can replace autoantigenic peptides with ambient peptides having sequences capable of binding in the antigenic peptide-binding site. It also supports the view that long C-terminal extensions of the Ii-Key peptide may reach into the antigenic peptide binding site to dislodge autoantigenic peptides.

A model for regulating the binding of the antigenic peptides by Ii-Key compounds at the allosteric site on MHC Class II molecules is presented in Fig. 1. Binding of a ligand to the allosteric site can induce a conformational change in the

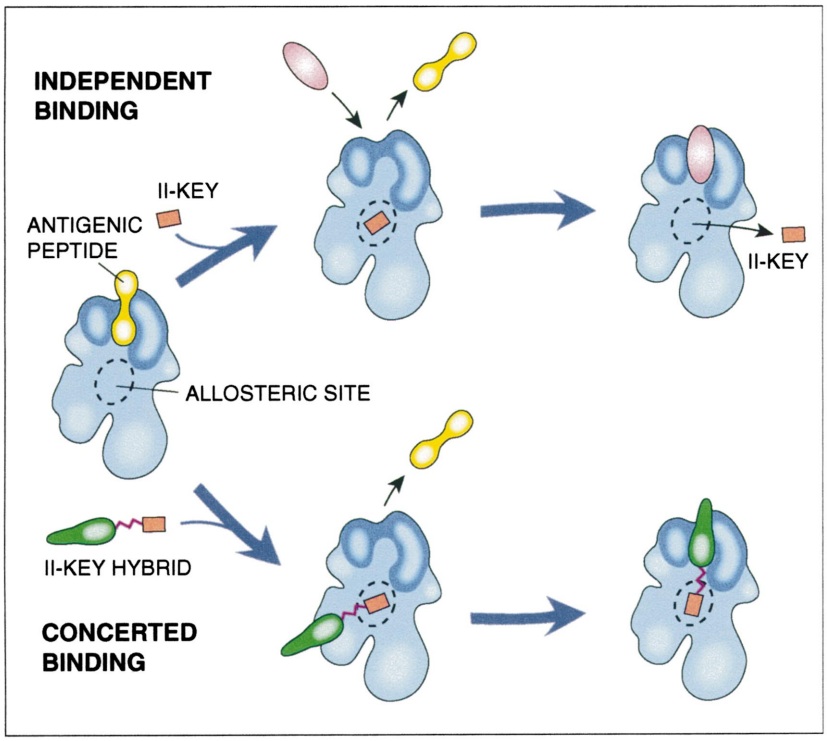

Fig. 1. The Ii-Key allosteric site ligand has different immunological activities depending upon whether it binds independently of major histocompatibility (MHC) class II restricted peptide epitopes or whether it is covalently attached to the epitope. When it binds alone, it causes 'spilling' of any antigen occupying the antigenic peptide binding groove (e.g. auto-antigens) and subsequent re-charging with ambient peptides. When hybridized to a therapeutic antigen (e.g. from an infectious agent or a tumour-associated antigen) the Ii-Key/antigenic peptide hybrid replaces the endogenously bound antigen with the therapeutic antigen directly. This diagram illustrates the allosteric site laying alongside the end of the antigenic binding site holding the N-terminal end of the antigenic peptide.

antigenic peptide-binding groove such that it adopts a more accessible conformation. The allosteric-site ligand, with a lesser binding affinity than the antigenic peptide for the antigenic peptide-binding groove [21], dissociates to allow stabilization of the MHC Class II/epitope complex ('the clamshell closes').

II-KEY/ANTIGENIC PEPTIDE HYBRIDS

In order to increase the potency of the Ii-Key segment on antigenic peptide binding and to enable the use the allosteric site-directed therapeutics for *in vivo* use, the Ii-Key core was physically linked to an antigenic epitope by different methods. A systematic series of hybrids was synthesized and tested in an *in vitro* T-cell hybridoma stimulation assay (Fig. 2 [30]). In the hybrids, the Ii-Key core (LRMK) was joined to the antigenic epitope of pigeon cytochrome C (PGCC81-104). The spacers joining Ii-Key and PGCC81-104 were either a simple polymethylene ($-CH_2-$) bridge or the natural sequence of the Ii protein extending from the C-terminus of LRMK. While hybrids with either type of bridge were effective, it is important to note that even the simplest polymethylene chain linker suffices. Some of these hybrids enhanced presentation of an antigenic epitope up to 250 times the baseline stimulation observed using the free antigenic peptide.

One surprise during these studies has been the finding that tethering Ii-Key to an antigenic epitope does not lead to auto-ejection of the vaccine epitope, but instead to its replacement of endogenous antigenic peptide. *A priori* one might have expected the Ii-Key function would auto-eject the tethered antigenic epitope. Seeing that does not occur, leads to the view that the Ii-Key function induces lability of the endogenously bound antigenic peptide, which is replaced by the antigenic epitope of the hybrid. Once inserted, the binding energy of the antigenic epitope in the MHC Class II groove far exceeds that of the Ii-Key motif in its site. Consequently, we hypothesize the that binding of the antigenic peptide in its site pulls the Ii-Key motif from its site. The fact that simple linkers work, when their linear distance is less than the proposed distance of the Ii-Key site from the P1 site of the antigenic peptide binding groove, supports this multistep view of hybrids binding and settling in.

OTHER EVIDENCE FOR THE ALLOSTERIC SITE CONCEPT

Given our studies with the Ii-Key peptides and hybrids, previous studies on activities of 'second peptides' on binding of antigenic peptides to MHC Class II molecules can be seen in a new light. Feng and Lai showed that a low affinity peptide with hydrophobic and cationic side chains (i.e. characteristics similar to those seen in Ii-Key peptides) enhanced activation of a T-cell clone by the indicator antigen [31]. Similar observations have been made with other peptides [32]. Tampe and McConnell showed in energy transfer studies between respective labels on two antigenic peptides, that two peptides could bind simultaneously in close proximity on MHC Class II molecules [33]. Such second peptides enhanced on rate for binding of an antigenic peptide [34, 35]. While their interpretation was that both peptides occupied the antigenic peptide-binding site, the

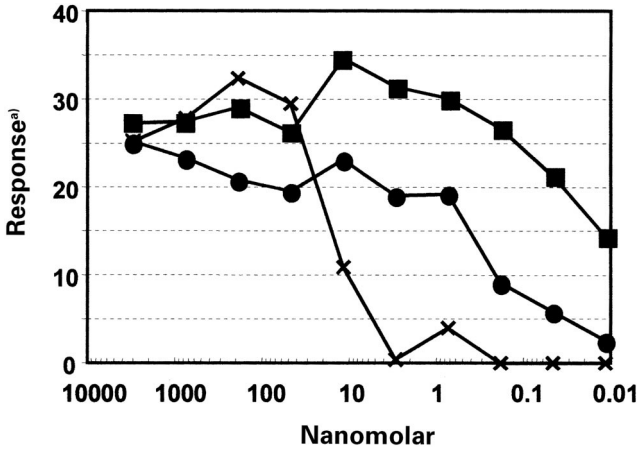

Fig. 2. Enhanced antigenic response to Ii-Key/antigen hybrids as measured by cytokine-release and tritiated thymidine uptake. The antigens are: the pigeon cytochrome C epitope IALKQATAK (×); LRMK-LPKS-IALKQATAK (●); LRMK-*ava*-IALKQATAK (■) [*ava* = epsiolon amino-valeric acid]. Response is measured by the effect of supernatant cytokines from the APC/T hybridoma culture with antigenic peptides or Ii-Key-peptide hybrids, on tritiated thymidine uptake in a second culture of H-2 cells (^{3}H-Tdr uptake, CPMx10^{-3}).

possibility exists that one also extended into the Ii-Key site. In studies with the Ii-derived CLIP, extremely N-terminal portions of certain long homologs of the CLIP peptides appear to control peptide charging at the antigenic peptide-binding groove. Urban and Strominger found that the lability of CLIP binding in MHC Class II molecules under acidic conditions depended upon the presence of N-terminal amino acid residues from positions 81–89 [36]. Kropshofer *et al*. [37] also determined that at pH 5.8, characteristic of endosomes, CLIP Ii90–105 dissociates rapidly from HLA-DR3 but only when the N-terminal segment L^{81}PKPPKPVS^{89} is present. These results indicated that the N-terminus of CLIP, specifically residues 81–89 of Ii, has an allosteric effect on the binding of CLIP Ii90–105 and may bind to MHC Class II molecules somewhere outside of the antigenic peptide-binding groove. Similarly, Stumptner and Benaroch [38] under different experimental conditions showed that the Ii81–90 sequence loosens the association of CLIP81-104 with MHC Class II molecules. X-ray crystallographic studies of HLA-DR1/CLIP complexes [29] have shown that Ii91–104 occupies the entire groove, with M91 binding in the first, hydrophobic pocket of the groove (the 'P1 site'). The binding site of further N-terminal residues was not defined. These N-terminal residues presumably could act at the Ii-Key allosteric site. Finally, in the context of hybrid structures between antigenic epitopes and segments of the Ii protein immediately N-terminal to the M92 residue occupying the P1 site, Hess *et al*. demonstrated immunological activity *in vivo* using Ii86–90 covalently linked to a tetanus toxoid antigen [39]. Recently, Bartnes *et al*. have shown that the N-terminal elongation of an antigenic peptide beyond the first primary anchor improves its binding to the MHC class II molecules [40]. This observation is very similar to the allosteric phenomenon.

MHC CLASS II ALLOSTERIC SITE-DIRECTED IMMUNOTHERAPY

The allosteric site on MHC Class II molecules, at which Ii protein-derived peptides and peptidomimetics act to alter the binding at the antigenic peptide binding site, is an important

Table 1. Proposed applications for MHC Class II allosteric site directed therapeutics

Allosteric ligand function	Therapeutic mechanism	Disease target
Ii-Key as a Spiller	Releasing autoantigenic peptides from MHC Class II molecules	Autoimmune diseases
Ii-Key as blocker-enhancer	Enhancing the binding of a blocking (nonantigenic) peptide in the MHC Class II antigenic peptide binding site	Autoimmune diseases
Ii-Key as vaccine peptide enhancer	Enhancing the binding of an antigenic peptide in the MHC Class II antigenic peptide binding	Vaccine for cancer, infectious disease Tolerogen for allergy, autoimmune diseases, transplantation
Ii-Key/blocker hybrid	Enhancing the binding of a blocking (nonantigenic) peptide in the MHC Class II antigenic peptide binding site	Autoimmune diseases
Ii-Key/antigenic epitope hybrid	Enhancing the binding of an antigenic epitope in the MHC Class II antigenic peptide binding	Vaccine for cancer, infectious disease Tolerogen for allergy, autoimmune diseases, transplantation

target for the design of drugs useful in a variety of indications (Table 1). Compounds acting at this site can be used to induce the release of autoantigenic or allergic peptides, to be replaced either by ambient peptides or antigenic-site blockers. In addition, Ii-Key drugs can promote the binding of vaccine peptides. Such Ii-Key containing constructs can induce a Th1 response when administered in combination with inflammatory cytokines or adjuvants. In the absence of Th1-inducing signals, but perhaps with Th2-inducing cytokines, specific suppression can be induced to certain vaccine epitopes, for example those from inflammatory autoantigens.

We are pursuing these concepts in the context of peptide vaccines for melanoma gp100 [41], HER-2/neu [42] in these two cancers (in the presence of inflammatory cytokines or adjuvants). To suppress immune responses following Th2 activation (in the absence of inflammatory cytokines), MBP models of EAE (toward MS) are being tested. For example, incorporating the hMBP90-102 epitope, which shows the activity in suppressing some indicators of MS in immunized patients [43], might be combined with an Ii-Key for administration under an immunosuppressive regimen for the control of MS.

CONCLUDING REMARKS

The Ii-Key allosteric site has been well defined by the structure-activity relationship studies with many compounds. These studies have entailed both functional antigen presentation assays using murine T hybridomas as well as biophysical peptide binding and release assays using purified human MHC Class II molecules. The discovery of this allosteric site provides a powerful means to overcome problems associated with the therapeutic use of MHC Class II-presented antigenic peptides. Furthermore, studies have suggested that the activity of Ii-Key compounds may be modulated by C-terminal extensions relative to the Ii-Key core. Such extensions may either block the antigenic peptide-binding site in an inert manner or facilitate directly the insertion of vaccine peptides. Because Ii-Key-hybridized vaccine peptide hybrids can be introduced to the immune system in very low doses without inflammation, such hybrids might effectively induce tolerance. Conversely, the concomitant use of inflammatory cytokines would be expected to result in potent active immunization. In short, a wide range of clinically useful therapeutics may be generated from further development of Ii-Key-based compounds.

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Author Queries

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