Tolerogenic property of MHC class I and class II molecules: lessons from a gene therapy approach

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1. ABSTRACT

Conditions allowing the development of a stable state of hematopoietic chimerism, i.e., the coexistence of foreign and recipient hematopoietic cells in the same individual, remain the most effective means to produce immune tolerance to antigens expressed by foreign cells/grafts. However, heavy immunosuppression is required to achieve chimerism and as such has limited clinical application. MHC antigens being the primary targets of immune responses, we pioneered an innovative approach in which foreign MHC genes were first introduced in bone marrow cells of recipients of subsequent grafts expressing the same MHC antigens. This strategy produced MHC class I (MHC I) or class II (MHC II) molecular chimerism, did not require heavy conditioning, and enabled long-term transplant acceptance in rodents as well as large animals. However, recent developments have indicated, that although the outcomes of gene transfer were similar, the mechanism of tolerance induction by MHC I and MHC II gene transfer were markedly different. This review examines the tolerance mechanism arising after MHC gene transfer to infer that MHC II gene therapy is clinically more relevant since the transfer of a single gene enables broad tolerance to all graft antigens via the production of MHC II peptides for selective activation of regulatory T cells (T-regs).

2. INTRODUCTION

The introduction of foreign protein antigens in bone marrow cells, via gene transfer, has proven to be an efficient means to produce stable molecular chimera which subsequently developed immune tolerance (permanent failure to respond to a defined antigen) to the introduced proteins. Tolerance to cell surface as well as intracellular protein antigens have been reported using gene transfer strategies (1-5). In all these cases, gene transfer led to the absence of response specifically to the introduced molecules while maintaining normal immune reactivity toward third-party antigens (6, 7). This suggested that the initial step of the tolerance mechanism resided in the recognition of the introduced genes/proteins.

MHC I and II proteins that are exposed on grafted tissues/cells, have long been recognized as the primary transplantation antigens (reviewed in (8)). As such, they have been of particular interest for gene therapy strategies of bone marrow cells because expression of donor-type MHC antigens in/on recipient bone marrow cells, could promote unresponsiveness (temporary failure to respond to defined antigens) and possibly tolerance to subsequent donor tissue grafts. When tried using rodent (6, 7) and large animal models of transplantation (9, 10), MHC gene therapy delivered the expected outcomes: MHC-

Table 1. Basic features of MHC I and MHC II gene therapy protocols

Features	MHC I	Reference	MHC II	Reference
Transgene (Tg) expression	persistent	7	transient	9
T cell response to Tg antigen before transplantation	no	6,7	yes	9
T cell response to Tg antigen after transplantation	no		no	
Linked tolerance	no	6	yes	9
Mechanism	deletion?	11	regulation	12,41

specific unresponsiveness to the transferred products resulting in long-term acceptance of transplants expressing the ectopic gene product(s). However, a closer examination of the tolerance mechanisms involved suggested that MHC II gene therapy may have decisive advantages over MHC I gene transfer for the induction of immune tolerance to allografts for both experimental and clinical transplantation.

The present study intends to delineate the characteristics of MHC I- and II-induced tolerance and identify the structural and functional causes of these differences. This analysis also brings new insights on the role of MHC II molecules and their derived peptides in the control of T cell responses to foreign antigens.

3. FEATURES OF MHC CLASS I- AND CLASS II-INDUCED TOLERANCE

Retroviral transfer of a single graft-type MHC I or MHC II molecule in bone marrow of recipients of subsequent vascularized grafts (skin, heart or kidney) inhibited acute and chronic cellular rejection episodes and fostered indefinite graft survival and donor-specific tolerance. Although operationally similar, MHC I and II-induced tolerance exhibited distinct characteristics which may provide clues to their mechanism. As summarized in Table 1, persistent donor MHC I gene expression was required to maintain tolerance, even in the presence of surviving transplants that also expressed the same MHC I. The need for sustained MHC I expression in this model suggested that graft MHC I molecules were acting as targets for graft rejection whereas transferred MHC I were, in essence, inducers of T cell unresponsiveness to graft antigens. The fact that anti-donor MHC I responses were inactivated in MHC I recipients (before and after grafting) corroborated this view (Table 1). Importantly, MHC I-induced tolerance was specific to the transferred product since transgene recipients accepted grafts carrying the same MHC I but rejected transplants that expressed this MHC I together with other MHC antigens (no linked tolerance) (6).

In striking contrast, the induction of transplantation tolerance by MHC II gene therapy did not require lasting transgene expression: indeed, transgene transcription faded out in peripheral blood cells between 4-6 weeks after organ transplantation. Mixed lymphocyte reactions performed with host responder T cells and donor stimulator cells, in the presence of blocking MHC antibodies, showed that T cell responses to transferred MHC II were intact before grafting ((9); Table 2). This result appeared different from that obtained with MHC I therapy which showed

that transgene expression led to thymic deletion of cognate high affinity TCR bearing T lymphocytes (11). More importantly, we also demonstrated that hosts of a single donor MHC II gene did not reject grafts expressing this allogeneic MHC conjointly with other MHC antigens (linked antigens), a phenomenon which has been referred to as linked or spreading tolerance (9, 12).

From these findings, we inferred that shared MHC II molecules, derived from the transgene as well as the vascularized graft, primarily acted through a regulatory mechanism which inactivated the overall anti-graft response. Results from recent experiments in a murine model of MHC II gene therapy that induced tolerance to fully allogeneic cardiac allografts, lend support to the regulatory role of MHC II products. In this model, MHC II IAb-induced tolerance was exclusively mediated by CD4+, CD25+ T-regs isolated from long-term tolerant CBA (H-2k) animals and could be adoptively transferred to naïve CBA recipients of C57BL/6 heart grafts (IAb+). Furthermore, our data suggested that an intracellular form of the transferred IAb , likely IAb peptides loaded on MHC IIk, was involved in T-reg activation which was required for suppression and ensuing tolerance.

Collectively, these data indicated that the mechanism of tolerance induction was substantially different between MHC I and MHC II transfers; i.e., elimination/inactivation of the cognate effector T cells for MHC I and down-modulation of the whole anti-graft T cell response for MHC II. Given that ectopic MHC II molecules were not expressed at the surface of transduced bone marrow derived antigen presenting cells (APCs), we hypothesized that the primary cause for the regulatory property of MHC II molecules resided in their ability to present their own peptides to allow T-reg differentiation and activation. If correct, this hypothesis will predict that peptide cargos of MHC II surface dimers would predominantly consist of MHC II peptides whereas MHC I molecules would rather bind to a full array of self peptides.

4. MHC CLASS I POORLY PRESENT THEIR OWN PEPTIDES

Over the past two decades numerous studies have established the amino acid sequence of dominant peptides presented by MHC molecules (13-16). A large variety of self peptides was characterized from acidic eluates of antigen-presenting cells and other cell types, among which many MHC-derived peptides were detected. We have re-examined the peptide elution tables from the SYFPEITHI

MHC peptides for down-regulation of T cell responses

Table 2. Summary table of MHC peptides detected in MHC grooves of human (top) and murine (bottom) MHC molecules

MHC class I					MHC II				
Human									
Allele (a)	pI/total (b)	Peptide from; position	Poly (c)	Self (d)	Allele	pII/total	Peptide from; position	Poly	Self
HLA-A*01	0/12				HLA-DQ2	1/12	DQα; 1-31	Y	s
HLA-A*201	2/35	HLA-E; -22	N		HLA-DQ7	2/14	DQβ; 43-55	Y	s
		HLA-A; -20	N				DRα; 57-72	N	
HLA-A*2402	0/2				HLA-DQ8	2/10	DQα; 75-87	Y	s
HLA-A*03	0/6						DRβ1; 65-79	Y	
HLA-A*6801	0/23				HLA DQ9	1/1	DRβ1; 65-79	Y	
HLA-B*0702	1/13	HLA-C; -20	N		HLA-DR1				
HLA-B*08	0/12				*0101	1/9	DRβ1; 22-35	Y	s
HLA-B*1510	0/15				*0301	0/10			
HLA-B*2701	1/53	HLAB27;146	N	s	*0401	2/22	DQβ; 24-38	Y	
HLA-B*4402	0/8						DRβ1; 95-107	N	s
HLA-B*5101	0/28				*0402	2/13	DRα; 238-252	N	s
							DRβ; 220-237	N	s
					*0404	1/13	DRα; 54-73	N	s
					*0405	0/13			
					*0701	4/14	DRα; 3 peptides	N	s
							DQβ; 179-208	N	
					*1101	1/15	DRβ1; 22-35	Y	s
					*1301	2/7	DQβ1; 21-38	Y	
							; 123-140	N	
					*1302	3/11	DRβ1; 43-60	Y	
							DQβ1; 43-60	Y	s
							; 53-70	Y	
					*1501	2/11	DRα; 45-68	N	s
							DQβ; 43-58	Y	
Mouse									
H-2Kb	0/19				H-2 IAb	3/28	Aβ; 55-71	Y	s
H-2Kd	0/8						Aβ; 75-89	Y	s
H-2Kk	1/15	H-2 Dk; 88					Eα; 52-68	N	s
H-2 Dd	0/15				H-2 IAd	1/8	Eα; 52-68	N	s
H-2Ld	0/5				H-2 IAk	4/8	Aβ; 3 peptides	Y	s
							Eβ; 33-47	Y	s

The analysis surveyed 508 peptides eluted from either MHC I or MHC II molecules that have been referenced in the SYFPETHI database (17). Peptides derived from proteins of the culture medium, such as serum albumin, alpha1-antitrypsin or transferrin were excluded from the study. (a) MHC allele analyzed. (b) number of MHC I or MHC II peptides /total number of peptides identified. (c) peptide derived from a polymorphic region of the MHC sequences: Yes, No. (d) peptide derived from self MHC molecules (s).

database (17) with a particular focus on the presentation of MHC-derived peptides by either murine or human MHC I or MHC II surface heterodimers.

A total of 269 peptides that have been described as primary binders of MHC I were surveyed. Our analysis indicates that MHC-derived peptides represent a minority among the array of self peptides that are able to bind to MHC I molecules as only 8% of the 269 peptides analyzed were of MHC origin. A closer examination of this peptide fraction reveals that less than 2% of peptides were derived from MHC I alpha chains suggesting that molecular complexes made of MHC I molecules binding self MHC I peptides (pI/MHC I complexes) are minor entities displayed on APCs. Interestingly, the vast majority of pI sequences encompassed leader sequences of MHC I alpha chain (Table 2, left column). For example, the human HLA-E isotype presents a 9-mer polypeptide cleaved from the leader sequence of HLA-E that starts at position -22, a feature that it shares with its murine homolog, Qa1 (not shown). Likewise, the bulk of murine H-2 molecules examined is not loaded with MHC I self peptides, with the exception of H-2 K^k that binds to a H-2 D^k-derived peptide. It is striking that the only pI that is not from the leader

sequence and engages the groove of the MHC I molecules from which it derives, is that of HLA-B27, a molecule which has been associated with susceptibility to autoimmune pathology in humans.

Thus, presentation of self MHC peptides by MHC I molecules is a rare event which primarily involves pI derived from conserved leader or framework sequences.

5. MHC CLASS II PRIMARILY PRESENT THEIR ALLELIC IDENTITY

A similar study was then conducted on 239 peptides eluted from MHC II grooves. In contrast to the presentation of MHC peptides by MHC I heterodimers, up to 30% of the peptide load of MHC II surface molecules is derived from MHC molecules. The contribution of MHC II for peptide production was dominant (60%) over that of MHC I, resulting in 18% of peptide cargo being of MHC II origin. Both the alpha and beta chains from all of the murine and human MHC II isotypes but two (HLA-DR1*0301 and DR1*0405) produced peptides that fit into the MHC II grooves. Approximately 60% of presented MHC II peptides (pII) encompassed allelic sequences that,

in most instances, were cleaved from self alpha or beta polypeptide chains.

As reported in other studies (18, 19), this analysis confirms that MHC II molecules do "present themselves" as peptides that are cleaved from their own alpha and beta polypeptide chains. It also emphasizes that self presentation of pII is a prevalent function of MHC II molecules. It is conceivable that the overall proportion of 18% of pII found in peptide eluates of generic APCs may in fact be higher on discreet APC subsets such as immature dendritic cells which seem dedicated to pII processing and presentation (20, 21). It is also remarkable that the majority (60%) of the pII/MHC II complexes include peptides which cover polymorphic stretches of allelic sequences derived from the hypervariable regions of either MHC II chains. The cause of preferential presentation of allelic MHC II determinants remains puzzling given that gene allelism has no real meaning within a given mouse strain or human individual. However, MHC II polymorphic regions being mainly exposed on MHC II heterodimers, it is conceivable that presentation of polymorphic pII is the result of determinant capture of accessible MHC II sequences by empty MHC II molecules found on immature/resting APCs (22, 23).

The propensity of MHC II molecules to present themselves as peptides, suggests that pII/MHC II complexes are associated with a specific function. As mentioned above, intracellular MHC II chains from donor origin, likely in the form of peptides, induced T-reg mediated transplantation tolerance. Could this indicate that pII/MHC II contribute to the emergence of T-regs for subsequent down-modulation of T cell reactivity?

6. MHC II GENE THERAPY FOR SPREADING TOLERANCE: ROLE OF MHC PEPTIDE/MHC II COMPLEXES IN PROMOTING T-REG SUPPRESSION

Several data are in agreement with a preferential pII presentation by MHC II molecules. Indeed, MHC II molecules have been shown to be the targets for their own proteolysis as pII together with peptides from apolipoprotein E, cystatin-c, transferrin receptor and invariant chain are prevalent entities presented by APCs (24, 25). Binding of self pII to MHC II heterodimers was also documented in several instances in both human and mouse (26-28). In vivo, pII have been reported as dominant peptides among the full array of peptides presented by MHC II: 9.3% of splenic and 1.1% of thymic MHC II molecules are occupied by the $A\beta^k$ peptide 37-51 (29). Similarly, 10-15% of the MHC II expressed on peripheral B lymphocytes, dendritic cells and macrophages of IA^b, IE^+ mice, present a single $IE\alpha56-73$ peptide detected by the YA-e mAb (30, 31). More importantly, pII/MHC II complexes have been shown to be recognized in vivo by self T cells to elicit immune responses (32). These data strongly suggest that MHC II presentation of self pII is dominant over the presentation of other peptides and leads to surface complexes detected by self T cells.

The role of MHC II in T-regs activation has also been documented in various models. As they belong to the

conventional $CD4^+$ subset, naturally occurring T-reg differentiation and function have been strongly associated with recognition of MHC II molecules (33). T-reg thymic differentiation occurs on MHC II$^+$ thymic epithelium (34) and appears to be specific to a more limited set of self peptides/MHC II complexes than that presented to conventional $CD4^+$ T cells (35). The nature of the peptides which are recognized by T-reg TCR remains, however, a matter of debate. Some evidence would lend support to a key contribution of self pII in this specificity. For example, our recent data indicated that expression of intracellular MHC II IAb products, which are likely IAb-derived peptides, generated T-regs which conferred spreading tolerance to IAb$^+$ allogeneic grafts (12). In our miniature swine transplantation model we also demonstrated that purified $CD4^+$, $CD25^+$, Foxp3$^+$ T-regs (with suppressive function) recognized self pII presented by self dendritic cells (DCs) as seen by spectratyping analysis of T-reg TCR selection. Such a TCR selection was not observed with conventional $CD4^+$ T effector cells (36).

Studies on the role of T-regs in autoimmunity models have, likewise, provided evidence on the role of pII on T-reg emergence and function. Immunization of NOD mice with pII covering polymorphic regions of the β chain led to T-reg activation (37). In human and murine models of rheumatoid arthritis, it was shown that presentation of self DRβ or IEβ peptides by DQ or IA prevented the development of the disease (38). Zanelli et al. provided direct evidence of pII binding to self MHC II molecules (39). More recently, it was observed in a transplantation model that host DCs expressing pII/MHC II complexes promoted the generation of CCR4$^+$, CD4$^+$, CD25$^+$ Foxp3$^+$ T-regs that mediated allograft tolerance (40).

Hence, the view of self pII/MHC II complexes, that we named T-Lo complexes (41), as being major determinants of T-reg specificity, is compatible with T-reg features observed in transplantation and autoimmunity models. We are presently assessing the role of T-Lo in the differentiation and suppressive functions of T-regs using our murine and porcine transplantation models.

7. CONCLUSIONS AND FUTURE PERSPECTIVES

The transfer of either a single graft-type MHC I or MHC II transgene conveyed tolerance to vascularized grafts that were matched to the transgene. However, the mechanism of tolerance was markedly different and notable in that the introduced MHC I behaved as any other foreign antigen introduced in recipient hematopoietic system by gene transfer; i.e., deletional (central) tolerance was induced to grafts that expressed the transgenic but not additional antigens. The failure of a single MHC I gene transfer to promote tolerance to allografts severely compromises the potential of this strategy for clinical applications since one would have to introduce the entire panel of alloantigens in recipients with the hope to prevent alloreactive T cell responses and graft rejection.

Importantly and in contrast to MHC I gene transfer, the efficacy with which MHC II gene therapy

generated spreading tolerance to allografts in both rodent and preclinical large animal models has established the proof of concept of this approach. We suggest that it is through activation of T-regs by presenting their own peptides that MHC II insures another critical function of immunity: the down-modulation of effector T cell reactivity and ensuing spreading tolerance. This property would be specific to MHC II molecules and possibly to some of their derived pII, which would have an important potential for clinical application. At this point it is conceivable to envision MHC II gene therapy as a means to restore deficient T-reg functions and/or to activate naturally occurring T-reg cells when needed. Major hurdles have still to be overcome in order to bring this promising approach to the bedside. These include the generation of safer MHC II delivery systems, the identification of the most appropriate hematopoietic cells to serve as recipients of foreign MHC II gene(s) and the optimal timing for transgene expression.

The enthusiasm generated by the success of initial gene therapy protocols in correcting genetic defects in patients (1, 2), has been dampened by recent reports of serious complications resulting from oncogene activation by inserted murine proviral genomes (42). Although recombinant retroviruses have no real match in comparison to other expression systems in achieving sustained physiological levels of transgenic products, it is clear that progress has to be made to improve vector safety. A new generation of "disable" lentiviruses is presently under development in several laboratories which have already generated exciting data concerning ectopic gene expression and safety of insertion. Such vectors contain multiple safety features such as deleted UTR to generate self-inactivated LTRs, altered potential initiation codons, replacement of the viral polyadenylation/termination signal by exogenous homologs and insertion of chromatin isolators to prevent the oncogenic risk of insertional mutagenesis (4, 43). Hopefully, these new expression systems will achieve better expression levels in safe conditions with limited side effects.

Due to their ability to present peptides in either tolerogenic or immunogenic conditions (44), bone marrow-derived DCs have been considered as a primary target for gene therapy (45, 46). However, one should not ignore other potential cell targets such as T cells which have been involved in deletional tolerance to alloantigens (47). Timing of MHC II transgene expression will also be critical to insure optimal effects. Our data suggested that donor MHC II expression was required at time of transplantation but transgene expression was dispensable at later time points when the graft was fully accepted (9).

These later aspects lead to exciting perspectives in which a short pulse of tolerogenic pII, delivered at the right time and in the right cells will provide conditions for tolerance to allogeneic grafts.

This review proposes that it is because of the propensity of MHC II to present self-derived peptides which, in turn, generate pII-specific T-regs, that MHC II gene therapy offers an overwhelming advantage over MHC I in the control of T cell reactivity to foreign (graft) or self (autoimmunity) antigens. Thus, we believe that MHC II gene therapy has great potential for clinical applications since mild conditioning of recipients should suffice to achieve transient transgene expression. Hopes are now focused on better delivery systems for targeting the right hematopoietic cell type at the right time with the appropriate MHC II peptides.

8. ACKNOWLEDGEMENTS

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Abbreviations: MHC I: major histocompatibility complex antigen I; MHC II: major histocompatibility complex antigen II; T-regs: regulatory T cells; PI: MHC I-derived peptides; PII: MHC II-derived peptides. Tg antigen: transgenic antigen

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