MHC–Ig Dimers: The next Frontier in Transplantation Immunology

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Review Article

MHC-Ig Dimers: The next Frontier in Transplantation Immunology?

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Abstract

Organ transplantation offers hope to a variety of patients with terminal functioning organs and tissues. This is currently made possible by administration of general immunosuppressive drugs. To date, inducing tolerance to allografts has become the holy grail of every transplantation immunologist. In effect, there has been a great deal of research aiming to come up with alternative therapeutic mechanisms that would allow one to do away with or reduce the amount of general immunosuppressive drugs used either at the induction, maintenance, or both phases. It has been shown in recent years that MHC-Ig dimers can induce suppression of alloresponsive T cells in a donor specific fashion. Consequently, MHC-Ig fusion proteins are currently forming the next field for exploitation which is great progress in transplantation immunology since the discovery of the first immunosuppressive drugs in terms of inducing tolerance to transplanted organs and tissues. This review aims to discuss this progress.

Keywords: MHC-Ig dimer, immunosuppressive drugs, graft rejection, transplant tolerance.

1. Introduction

Transplantation has become one of the most important medical therapies today. However, the major drawback is rejection of the tissues by the host's immune system. The targets in transplantation rejection are alloantigens by T cells. The major alloantigens are MHC molecules. During the process of transplantation, it is important to closely match the donor MHC molecules and those of the recipient. This can be achieved perfectly only in identical twins or inbred animals. Close relatives will bring the match very close. However, even in the case of close matching, minute differences still exist due to the highly polymorphic nature of the MHC molecules and this provides a continuous source of alloantigens to the recipient's alloreactive T cells which will lead to rejection soon or later. This is to say, closely matched individuals will only extend the survival time of the graft but rejection is inevitable at some point. Minor histocompatibility antigens also influence the outcome of transplants.

Due to the growing importance of organ transplantation as a therapeutic procedure, a lot of studies have been done to elucidate the mechanisms involved in the rejection process. These have led to a great deal of understanding of this art and as a result, more and more immunosuppressive drugs have been discovered. These drugs have made it possible for transplantation without precise matching of tissues thereby relying less on natural mechanisms of tolerance such as those associated with blood transfusion and maternal alloantigen exposure. As a result, organizations such as the national Organ Procurement and Transplantation Network in the United States only require typing for HLA-A, B and DR antigens [1]. However, it should not be forgotten that the better the matching of HLA antigens between donor and recipient the better the chance of survival of a given organ [2]. A major concern in the use of immunosuppressive drugs is the susceptibility to infections rendered to the transplant recipient due to the non-specific action of these agents. Coupled to this is the problem of cost of attaining the drugs which together bring about a serious issue of patient incompliance [3]. Therefore, attaining allograft transplantation without or with minimal use of the currently available general immunosuppressive drugs

resulting in complete graft tolerance is the holy grail of every transplant immunologist today. However, the future seems to be bright for these grail seekers. There is a new approach that has been shown to confer antigen specific suppression of alloresponses – the use of MHC-Ig dimers. Although still in its infancy and a lot more is needs to be done, MHC-Ig dimers offer some hope, a ray of light if you will, at the end of the tunnel. This review aims to highlight what might be in store for transplantation immunology in future. In the process, we will try to answer the question of whether MHC-Ig dimers have a chance to be the next frontier in transplantation immunology and therefore redefine the use of immunosuppressive drugs as we know them today.

We begin by discussing the mechanisms of action of MHC-Ig dimers. Then we will look at how

three classical molecules in the adaptive immune system currently play a role in the construction and functioning of MHC-Ig dimers. Lastly, we discuss MHC-Ig dimers from their inception to what we think they may contribute to transplantation immunology in the future.

2. How MHC-Ig Dimers Work

To understand how these molecules work in transplantation immunology, we will first have to understand the mechanisms that lead to allograft rejection. T cells of a given recipient recognize alloantigens and initiate alloresponses in a process called allorecognition. Allorecognition takes place through two important mechanisms, viz; direct and indirect pathways (Figure 1).

Figure 1: Direct and Indirect allorecognition mechanisms
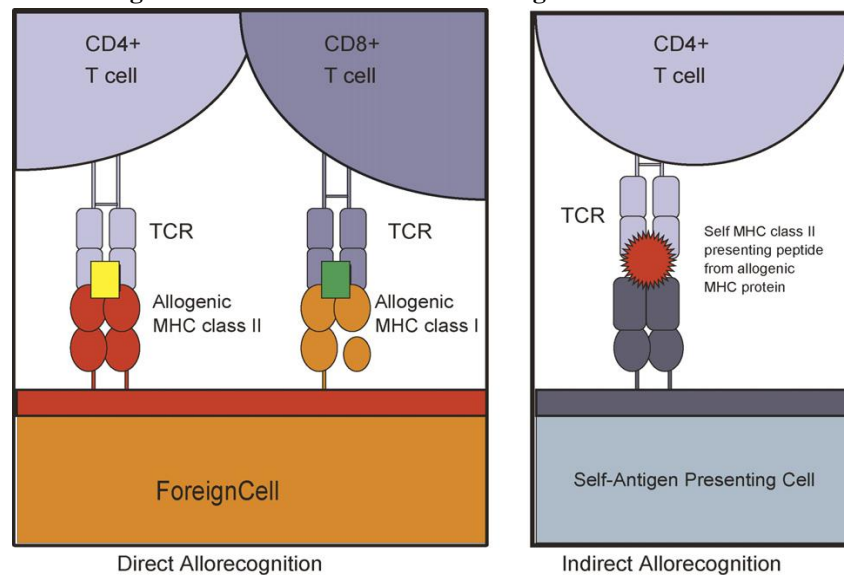
This diagram is adopted from the work by Archbold *et al*[4]

In the direct pathway of allorecognition, allo-antigen specific recipient T cells recognize and interact with either MHC class I or II molecules on the surface of donor cells through their (recipient T cells) TCRs. Alloantigen specific recipient T cell clones are then activated and proliferate.. They then undergo differentiation to become effector T cells which (depending on the phenotype; either CD8^{+} or CD4^{+}) go on to attack the graft or provide help to other cell types that then destroy the graft. The indirect pathway of allorecognition differs in that, here, recipient APCs process and present alloantigens (mostly donor MHC but also donor minor histocompatibility molecules) to the recipient's allo-antigen specific CD4^{+} T cells. Like in the direct pathway, the allo-antigen specific CD4^{+} T cell clones get activated, undergo proliferation, and differentiate to become effector T cells which then provide help to other cell types that go on to destroy the graft or

secrete molecules that lead to destruction of the graft through a variety of mechanisms (depending on the secreted molecules) which will not be discussed here.

In both direct and indirect mechanisms of allorecognition, there is need for transduction of signal 1 and 2 where the first comes from the interaction between the MHC and TCR molecules while the second comes from co-stimulatory molecules. In the case of MHC-Ig dimers, the MHC sub-units interact with the TCR of the allo-antigen specific T cells. Because they are dimeric in structure – containing a valence of two – the affinity is high enough to allow for their long enough interaction with the TCR thereby providing signal 1. Due to the absence of signal 2, the T cells become anergic thereby abrogating the response.

MHC-Ig dimers function through the direct mechanism of allorecognition since they are produced in vitro as soluble molecules (Fig. 2).

Consequently, they are useful in abrogating acute rejection. There is experimental evidence showing that the vast majority of alloreactive T cells react to alloantigens presented by the direct pathway. This is supported by the realization that the frequency of cells engaged in the indirect pathway of recognition is approximately 100-fold lower than that of cells engaged in direct recognition [5]. As a result, the direct pathway accounts for >90% of acute skin graft rejection in a variety of mice models [6][7] In fact, the direct pathway has classically been considered the major driving force in alloresponses as reviewed by Womer *et al*[8].

Fig. 2: Secretion of MHC-Ig dimers
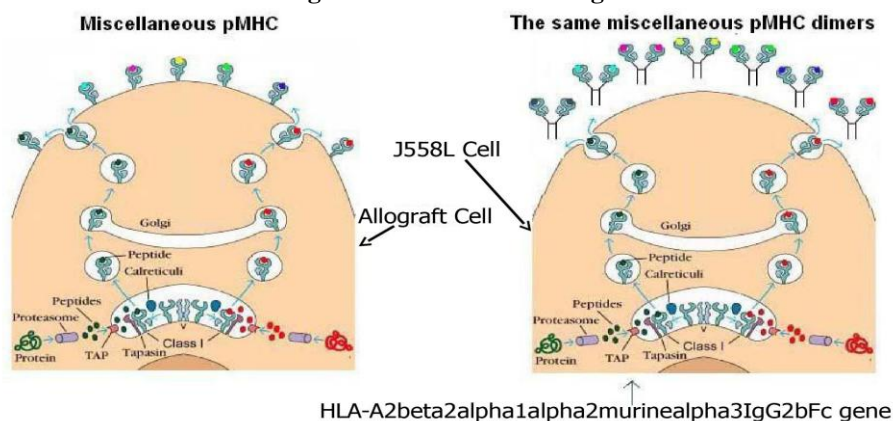
The left hand side cell shows how a normal donor cell would process its peptides and load them to its MHC molecules. The right hand side cell shows how MHC-Ig dimers are secreted by transfected cell lines in our laboratory. We chose the J558L cell line since it is of similar origin to the donor so that the synthesized dimer molecules are loaded naturally by similar peptides as they would in the donor cells.

There is also evidence that allorecognition through the direct pathway may actually inhibit allograft rejection at least later in the course of rejection, through inhibition of direct alloresponses [9-11]. Therefore, preventing acute rejection in the initial stages of transplantation is paramount.

It should be noted that the indirect pathway of allorecognition, though accounting for a small percentage of all the T cell alloresponses, is paramount to direct allorecognition due to its ability to perpetuate chronic rejection. As long as the graft is present, donor APCs will process and present alloantigens to T cells hence perpetuation of chronic rejection unless, of course, other forces act in the opposite. The question is how do we surmount chronic rejection? It has been shown that targeting donor MHC peptides to dendritic cells [12] or administering them either intrathymically [13], orally [14], or as donor peptide-pulsed recipient APCs [15] induces tolerance through the indirect pathway of allorecognition. Could MHC-Ig dimers – being chimeric molecules containing donor MHC molecules – act in similar fashion? Could MHC-Ig dimers – especially those of class II in origin – by any chance interact with alloantigen specific recipient APCs and therefore influence in some way how indirect allorecognition takes place? Could they, as they induce tolerance through the direct pathway of allorecognition, at the same time direct other cell types to change phenotype and become suppressor cells and therefore augment donor specific hyporesponsiveness? These are the questions that we need to actively seek solutions for. So far, there is evidence showing that HLA-A2 dimer induces $CD8^{low}CD28^-$ T cells with suppressive functions in vitro [16].

One great advantage of using MHC-Ig dimers is that their construction does not necessarily require a live donor. Another advantage is that they do not pose any graft versus host reactions. However, there are still major problems that exist in the use of MHC-Ig dimer technology. One is that there are many different alloantigens that are sources of alloreactions and currently most studies have only dealt with one alloantigen at a time. We don't know if, in the case that there are two or more mismatches, many different MHC-Ig dimers specific for different alloantigens can be used at the same time and what would be the effects to such usage. Further, it must be noted that time is of great importance in treating patients. But the process of getting a working dimer can be time consuming. For highly experienced personnel, it can take up to two months from the time genes of interest have been identified if everything runs smoothly. Because the dimers must be tailor-made for every patient, as far as current knowledge is concerned, there is no way they can be pre-made and stockpiled. Another problem experienced in many laboratories today is the purification of the dimers to get a pure product. This can be circumvented for

example by concentrating the supernatant using Polyethylene Glycol (PEG) 20000 which may work ok or maybe even better because it removes the need to load the dimers with peptides. However, in this case, the dimers must have been produced in a system that allows for natural loading of the specific peptides of interest.

3. Ig, MHC, and TCR Molecules

3.1. Immunoglobulin (Ig) molecules

In construction of the MHC-Ig dimers, the carboxyl terminal of the MHC is joined to the amino terminal of the Fc region. This allows for branching from the hinge region thereby allowing the formation of the dimeric structure. The importance of this is seen in the increased affinity of the dimers as compared to monomers when they interact with the TCRs on the surface of T cells. The Fc region of antibodies is what forms a scaffold in the construction of MHC-Ig dimers.

3.2. MHC molecules

Under normal physiological conditions, MHC molecules are responsible for protecting the individual from infections. In fact, it is now clear that alloresponses (through the indirect mechanism of allorecognition) in transplantation occur in an indistinguishable manner to responses against viral infections. The only difference is that in viral infections, only the cells infected will be destroyed where as in transplantation, the whole organ or tissue will be destroyed because every one of the cells expresses alloantigens. This shows that as long as the organ is present, chronic rejection will take place unless other mechanisms are applied to halt it.

Class I MHC molecules present bound peptides to CD8$^+$ T cells while class II MHC molecules present bound peptides to CD4$^+$ T cells. Due to their ability to interact with the TCR on T cells, the MHC molecules are used in the formation of the MHC-Ig fusion proteins.

3.3. TCR molecules

TCRs are either associated with CD4 molecules or CD8 molecules which form their co-receptors. They recognize foreign antigens such as those in infections or those in allograft transplantation presented by MHCs on the surface of nucleated cells or APCs thereby mounting an immune response against them. The recognition takes place through receptor-ligand (pMHC/TCR) and adjuvant molecule interactions, which include those of co-receptor molecules CD4 or CD8, co-stimulatory molecules, and adhesion molecules. The intrinsic ability of TCRs to interact with MHC molecules allows for the proper functioning of the MHC-Ig dimers thereby allowing them (the MHC-Ig dimers) to function as tools for modulating immune responses.

4. MHC-Ig Fusion proteins

Soluble MHC (sMHC) molecules were first shown to exist in healthy humans more than 40 years ago when Charlton and Zmijewski [17] and van Rood *et al* [18] independently found that serum MHC class I antigens were able to block anti-HLA-A2 antibodies. About twenty years later, these sMHC molecules were implicated in disease after they were shown to inhibit allospecific T cell responses [19]. Interest grew in the scientific community to see if the effects of these molecules could be experimentally modulated to surmount rejection. After many studies (well reviewed by Zavazava [20]), it became clear that these molecules have a therapeutic potential in transplantation immunology. Meanwhile, however, Sumimoto and Kamada [21] published results of their work in 1991 showing that continuous infusion of donor-derived sMHC antigens after transplantation failed to prevent rejection. This may have led to a notion that soluble monovalent class I molecules alone are unable to effectively inhibit T-cell responses in vitro or in vivo. This was supported by experimental results later showing that in the presence of low doses of cyclosporine, donor derived monomeric sMHC molecules prolonged graft survival of fully allogeneic histoincompatible Lewis (RT1.Al) cardiac allografts in euthymic and thymectomized DA (RT1.Aa) recipient rats [22]. The knowledge that Ig molecules are Y-shaped in nature thereby endowed with the intrinsic ability to form dimers when fused together and that they had previously been successfully fused with other different molecules [23-25], coupled with the failure of monomeric sMHC to effectively abrogate alloreactive T cell responses as had been shown in previous experiments might have led to the idea to engineer the first MHC dimers [26]. A few years later, Altman and Davis in Stanford, USA, and their collaborators in Oxford, UK, introduced the first MHC class I tetrameric complex technique in 1996 [27]. The work by Dal Porto *et al* [26] in conjunction to that by Altman and Davis [27] gave rise to the birth of MHC-Ig multimer fusion proteins which were later shown to be useful in many different applications such as analyzing phenotypes of antigen specific T lymphocytes and distinguishing them on the basis of their antigenic specificity[27,28]. Since then, many different kinds of MHC-Ig multimers have been made with different uses stemming from the same principle of MHC-TCR interactions. The principle of the technique is that peptide/MHC complexes, as TCR ligands, can be used to interact

with T cells on a particular antigen specific manner leading to expansion or suppression of these cells or, where labels are employed, lead to identification and subsequent isolation of these cell types.

Thus far, in the preceding paragraph, we have given a glimpse of a chronological order of events that might have led to the birth of MHC-Ig fusion molecules. It is now easy to see that MHC-Ig fusion proteins are proteins consisting of MHC and Ig molecules fused together to form dimers or higher valencies. They are usually expressed in eukaryotic cells thereby removing the need to refold them after production. At the present, there are MHC-Ig dimers of MHC class I and II in origin, MHC-Ig tetramers, pentamers, hexamers, octamers, streptamers, and dextramers that have been engineered (Table 1). As we mentioned earlier, MHC-Ig fusion proteins have been shown to have a myriad of uses, evidence for which will be highlighted in the next sub-sections.

**Table 1: Comparison between different MHC-Ig fusion proteins; monomers, dimers, tetramers, and others** [29-32]

MHC-Ig fusion proteins	Valence	Affinity	Expression system	Steric hindrance	Potential usefulness in transplantation
Monomers	1	Low	Eukaryotic/Bacterial	None	None at the moment
Dimers	2	Moderate	Eukaryotic/Bacterial	None	Very high (can used for tolerance induction)
Tetramers	4	High	Bacterial for monomer production followed by in vitro folding of monomers to form polymers	Weak (upto 3 of the 4 bind /cell)	Very high (can used for detection of T cells)
Other (higher than tetramers)	Higher than 4	Higher	Bacterial for monomer production followed by in vitro folding of monomers to form polymers	Strong (several do not access binding sites on any one given cell)	May be used for detection of antigen specific T cells (more useful in other immunological applications for diagnosis and follow-up studies)

4.1. MHC class I – Ig dimers

These are MHC-Ig dimers of class I in origin (Figure 3). It has been shown that *in vitro*, a single, soluble peptide/MHC complex has a low affinity for its corresponding TCR, resulting in a weakly bound complex with a rapid dissociation rate [33]. MHC-Ig multimers (dimers included), however, can greatly enhance the avidity of the binding to TCRs due to the increase in valency and consequent receptor cross-linking leading to a stable interaction [34,35].

Fig. 3: A diagrammatic representation of MHCI-Ig dimers.

HLA-A2-beta2m-alpha1alpha2-mousealpha3-IgG2bFc dimer
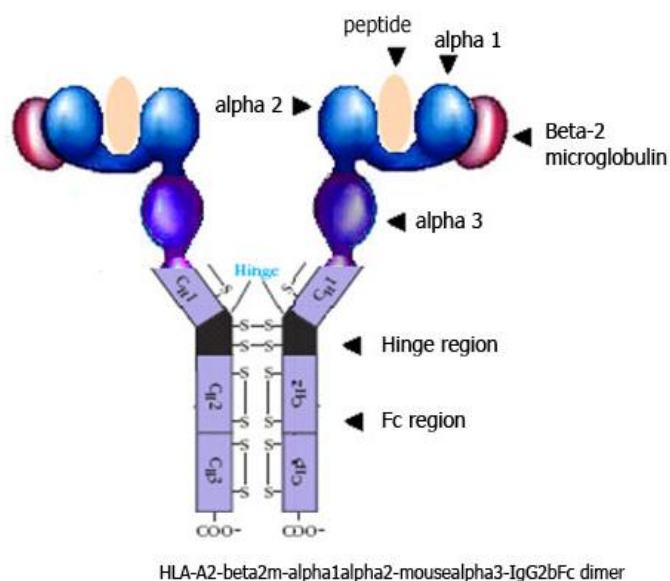
This particular molecule is a schematic representation of a dimer that we engineered in our laboratory. Here, the β-2 microglobulin molecule is covalently linked to the α-1 domain of the HLA-A2 molecule. The α-3 and Fc regions originate from a murine animal model.

MHC-Ig dimers of MHC class I in origin are divalent fusion proteins containing the extracellular domains of a class I MHC molecule fused to the constant domains of IgG molecules. They can either be non-covalently or covalently linked to β2-microglobulin molecules as we have engineered in our laboratory. Being MHC-Ig fusion proteins that are multimeric in structure, they have a higher valence than monomers and therefore have increased affinity and avidity for their ligands.

In a soluble form, MHC-Ig dimers of class I in origin have been shown to suppress proliferation of antigen specific T cells. O'Herrin *et al* showed that these molecules can induce modulation of surface TCR expression and inhibit T cell cytotoxicity activity at nanomolar concentration in vitro and inhibit alloreactive T cell responses in vivo [36]. Zhong *et al* [37] found that sHLA-G dimer inhibits T-cell alloresponse at nanomole concentration. They found that the dimer was more effective than sHLA-G monomer, and also up-regulated inhibitory receptor Ig-like transcript 2 on alloreactive CD8^{+} T cells. The study by Dal Porto *et al* [26] generated and characterized a soluble divalent class I MHC molecule, H-2Kb/IgG, which was seen to inhibit the lysis of target cells by alloreactive, H-2K[b] specific T-cell clones and bulk cultures at nanomolar concentrations. Direct binding assays showed that the chimeric protein was bound to an alloreactive T-cell clone with high affinity. It has been shown that like monoclonal antibodies (mAbs) directed against the TCR/CD3 complex, soluble divalent peptide-MHC molecules are capable of delivering a signal through the TCR and result in transduction of a unique signal that leads to immune dysfunction and tolerance induction. In addition, Weng *et al* [38] have shown that Tyr/HLA-A2 dimer inhibits alloreactive CTL responses in a peptide-specific manner. In a different study Weng *et al* [38] showed that alloreactive T cells (HLA-A2-ve CTL-T2/Tyr) are peptide-specific, thereby demonstrating that the alloreactive T cells can recognize a peptide-MHC ligand in the same fashion as the nominal Ag-specific T cell lines (HLA-A2+ve CTL-T2/LMP) do. This is supported by other studies showing that TCRs interact with allogeneic MHC in a manner that is almost indistinguishable from conventional recognition of antigenic peptide presented by self-MHC [39]. Combining this knowledge with the knowledge that alloreactive cytotoxic T cells attack allografts in transplantation, we can design MHC-Ig dimers of class I origin to suppress those specific T cells that are reactive to the graft thereby prevent rejection.

In a membrane or surface bound form, MHC-Ig dimers have been shown to activate antigen specific T cells. Carey *et al* [40] have shown that divalent MHC-Ig fusion proteins activate T cells in vitro and in vivo. They employed immobilized dimers in their *in vitro* studies which allowed for TCR cross-linking while they utilized LPS to induce CD4^{+} T cells and up-regulate co-stimulatory molecules in their *in vivo* studies. Rickert *et al* [41] also showed that a HLA-B7-Ig fusion protein was able to stimulate alloreactive T cells to proliferate and produce IL-2 and IFN-γ. The expression of activation markers CD25 and CD69 was also up-regulated in their experiments. They too immobilized the dimer which they had coated on plates. These experiments show that immobilization of the dimer leads to an increase in cross-linkages and due to an increase in valence and thereby avidity conferred by the dimeric nature of the molecules, MHC-Ig dimers function to transduce stimulatory signals. This allows for studying membrane interactions and lead to greater understanding of the molecules and signals involved in cell signaling.

Fusion proteins of MHC-Ig dimers allow for the interaction of these molecules with Fc receptor (FcR) on the surface of certain cell types thereby allowing for studying immunological responses in the context of antigen presentation. This is exemplified by Weng *et al* [42] who showed that a divalent HLA-A2/IgG1-Fc molecule (HLA-A2 dimer) constructed by fusing the extracellular domains of HLA-A2 with the constant domains of human IgG1 (from our laboratory) was able to load onto monocytes via interaction of the Fc and FcR. This interaction led to the expansion of antigen specific CD8^{+} T cells in co-cultures which could in turn kill melanoma cells that expressed an identical molecule as that loaded on the dimer. A similar molecule was shown to have the ability to attach TCR ligands to the cells bearing FcRI, such as Monocytes [43]. Other similar strategies have been reported to generate nominal, single epitope-specific CTLs [40, 44]. In addition, Carey *et al* [40] explored the feasibility of these strategies to generate allo-restricted, peptide specific CTLs. After in vitro co-culture, dimer-loaded HLA-A2-ve monocytes were seen to promote autologous PBLs proliferation, and the expanded CD8^{+} T cells showed peptide/HLA-A2-specific cytotoxicity. This ability to promote proliferation of specific cell types allows MHC-Ig dimers to be useful in the fight against leukemia versus host disease and other forms of cancer where the expanded cells can target the cancer cells and destroy them.

Horowitz *et al* [45] developed a novel system, incorporating immobilized HLA-A2-Ig dimeric proteins that are loaded with nonapeptides in order to stimulate antigen-specific CD8^{+} T cells in

the absence of APC. This system is based on the dimeric protein technology first described by Greten *et al* [46]. Their assay permitted them to determine the level of functional response, i.e. cytokine / chemokine production, of antigen-specific CD8^{+} T cells in an HLA-A2-restricted manner. The assay enables unlimited flexibility in which HLA-A2 restricted peptides can be used and since it circumvents the use of APC, interpretation of the results is less biased. Further, this assay requires a limited number of CD8^{+} T cells, making it relevant for the examination of tissue compartments, where sample size may be small, such as in mucosal sites.

It has been shown that MHC-Ig dimers of class I in origin can be employed in analytical studies to determine certain immunological responses. Because they can bind antigen specific T cells with high sensitivity and specificity, they provide a highly valuable tool for detection of antigen specific T cells [47]. This can happen when these complexes are tagged with fluorescent dyes thereby allowing individual T cells to be identified by flow cytometry analysis. If the specific a T cell is co-tagged with different fluorescent-conjugated antibodies that are specific for cell surface markers (such as cytokines or chemokines) the phenotype of the cell can be elucidated simultaneously. For example, it was shown that a Lewis-derived rat MHC class I dimer, RT1.Al-Fc, could induce graft tolerance to donor-type cardiac allografts and also allowed quantification of alloreactive T cells among splenocytes in cardiac allograft recipient Lewis rats [48]. Hu *et al* [49] produced a recombinant; empty HLA-A2 dimer by expressing the α-1, α-2, and α-3-domains of HLA-A2 linked to the β2-microglobulin (β2-m) human-IgG1-Fc fusion protein in *Drosophila* S2 cells. The empty dimer was stable at room temperature, and could be readily loaded with any HLA-A2-binding peptide. When used in combination with intracellular cytokine staining, the peptide/dimer complex provided both a quantification and functional characterization of peptide-specific T cells. It was thought that the novel HLA-A2-restricted dimer complexes would enhance the immunologic monitoring of vaccine trials for infectious and malignant diseases in HLA-A2+ patients and provide a valuable tool to improve vaccine design which was later shown to be the case [50].

Taken together, these studies show that the MHC-Ig dimers of class I origin could be used to modulate antigen specific alloreactive T cell immune responses and at the same time, track these responses through labels. Under such observation, we can see that MHC-Ig dimers of class I origin can be used to

promote CTL expansion which can be used to eliminate malignant cells. Most importantly, they can be used to inhibit alloresponsive T cells and therefore play a crucial role in inhibiting organ and/or tissue transplant rejection. However, as we have discussed earlier, these molecules act through the direct pathway of allorecognition and therefore only the acute rejection can be suppressed. Despite this, studies based on transplantation rejection using these dimers are highly promising. This is due to the unique characteristic of the mechanism of action of these molecules which offer a more targeted inhibition rather than that seen in currently used general immunosuppressive drugs. These molecules are therefore emerging as the "magic bullets" so to speak, in allo-immunosuppression.

4.2. MHC Class II – Ig dimers

These are MHC-Ig dimers of class II in origin (Figure 4). They have been constructed [50, 51] and shown to activate antigen specific T cells [32,50,52]. They have also been implicated in antigen specific induction of anergy to CD4^{+} T cells which play crucial roles in a number of autoimmune diseases and therefore it has been possible to apply these types of dimers in studies to combat autoimmune diseases among them type 1 diabetes and multiple sclerosis [53-56]. Karabekian *et al* showed that an engineered 161–180/IA[r]/Ig dimer, produced in insect cells, binds to a uveitogenic 161–180-peptide specific T-cell line and lead to antigen specific T cell proliferation or anergy depending on the presence or absence of co-stimulation [57].
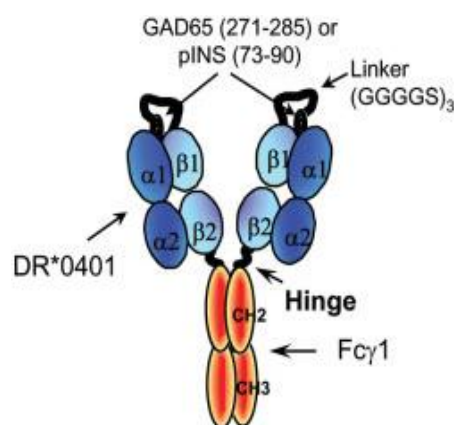

Fig. 4: A diagrammatic representation of MHCII-Ig dimers. This diagram is adopted from the work by Preda and others [54].

Although most studies using MHC-Ig dimers of class II in origin have looked at autoimmune disease, these studies have also suggested that these dimers may have a role to play in transplantation immunology as well. It has been shown that they can induce antigen specific helper T

cell energy both *in-vivo* and *in-vitro* [36, 55, 58, 61]. As MHC-Ig dimers, these molecules also act through the direct pathway of allorecognition. Whether they also have an influence on the indirect pathway of allorecognition or not is a question that still needs to be answered. It is thought that these class II molecules can induce expansion of other cell types with suppressive functions.

4.3. MHC tetramers

These are molecules consisting of MHC molecules of either class I or II in origin. They can be fused to Ig molecules and multimerized using protein A thereby forming MHC-Ig tetramers. Alternatively, they can be fused to an enzymatic biotinylation site (Bir) followed by fluorochrome conjugated streptavidin coupling to form MHC-Bir tetramers [27,40,59]. Those of MHC class I in origin interact with CD8^{+} T cells while those of MHC class II in origin interact with CD4^{+} T cells thereby allowing for the identification and subsequent isolation and/or characterization of antigen specific CD8^{+} or CD4^{+} T cells respectively. They can also be used to modulate immune responses in an antigen specific manner. For example, soluble MHC class I tetramers have been shown to contain the ability to identify scarce alloreactive T cell populations and also directly modulate Ag specific T cell responses in an *in vivo* model by Maile and Frelinger *et al* [60]. These T cell responses could be blocked using anti-CD8 antibodies thereby showing that the responding T cells were CD8^{+}. What makes this model interesting is that they used wild-type mice where a highly immunogenic epitope for H-2D[b] restricted antigen was loaded to the tetramer to form H-2D[b] tetramer. Injection of this tetramer primed B6 female mice but this response was reversed when multiple injections were administered resulting in survival of male skin grafts. This is thought to have been mediated through anergy or activation induced cell death.

4.4. Other multimers of Higher Valence than Tetramers

Here we refer to those with five monomers or higher joined together. They are pentamers, hexamers, octamers, streptamers, and dextramers. These multimers function to identify specific T cells through labels that are attached to them where flow cytometry can be used for analysis. Discussion of these molecules is however beyond the scope of this review.

5. Prospects in Transplantation Immunology

In just about two decades since its inception, the MHC-Ig dimer and MHC tetramer technology has grown tremendously. It is now used in many areas of immunology which include but not limited to transplantation immunology, tumor immunology, autoimmune diseases, and infectious diseases. It is no doubt however, that transplantation immunology is the biggest beneficiary so far. As interest grows in the scientific community, we see this technology playing a more crucial role in combating transplantation rejection in future. This is because of the current situation where we have general immunosuppressive drugs that suppress the immune system indiscriminately whereas MHC-Ig dimers act on a donor antigen in a specific manner by suppressing alloreactive T cells. What makes this technology even more appealing is that it acts as a double-edged sword as it can be used to identify and monitor antigen specific T cells as well. We envisage that in future, MHC-Ig dimers and tetramers technology will materialize the much anticipated ability to confer tolerance in organ transplantation.

6. Concluding Remarks

Transplant rejection is the drawback to organ transplantation. This rejection occurs due to the fact that the immune system sees the graft as foreign and therefore fights to eliminate it. This is expected since the system emerged evolutionarily to protect the host from invaders aided by the same molecules that are the driving force in transplantation rejection; the MHC molecules. Thus far, transplantation has been carried out under the help of general immunosuppressive agents. This helps to extend the survival period of the graft and improve somewhat the quality of life of certain patients. The problem here is that these agents are not specific and as such suppress the immune system without discrimination. This leads to susceptibility to infections and other drug related side effects to the graft recipient. There has been a great deal of studies on soluble MHC molecules in the last few decades which eventually gave rise to the technology of MHC-Ig fusion protein multimers and MHC tetramers. These molecules have proved to have a lot of uses including but not limited to (one) modulating immune cell responses and (two) acting as tools for analysis of immune responses. Of particular interest are the MHC-Ig fusion protein dimers made of MHC molecules fused to the Fc region of immunoglobulins. These molecules have been shown to inhibit alloresponses in a peptide specific manner by interacting with alloreactive T cells. We, therefore, see these molecules as the promising "magic bullets" in suppressing alloresponses and therefore dominating the next frontier of tolerance induction in transplantation immunology.

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