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Soluble MHC class I complexes for targeted immunotherapy

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Abstract

Major histocompatibility complexes (MHC) have been used for more than two decades in clinical and pre-clinical approaches of tumor immunotherapy. They have been proven efficient for detecting anti-tumor-specific T cells when utilized as soluble multimers, immobilized on cells or artificial structures such as artificial antigenpresenting cells (aAPC) and have been shown to generate effective anti-tumor responses. In this review we summarize the use of soluble MHC class I complexes in tumor vaccination studies, highlighting the different strategies and their contradicting results. In summary, we believe that soluble MHC class I molecules represent an exciting tool with great potential to impact the understanding and development of immunotherapeutic approaches on many levels from monitoring to treatment.

Keywords

MHC-class I; T cells; vaccination; tumor; immunotherapy

Introduction

MHC class I and class II molecules have been evolutionarily optimized for the presentation of a variety of peptide antigens (Ag's), which are then recognized by T cell receptors (TCR) present on the surface of T cells. To date over thirty unique TCR-MHC class I and over

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Conflict of interest

A.S. and C.S. have nothing to disclose. Under a licensing agreement between NexImmune and the Johns Hopkins University, J.P.S. and M.O. are entitled to shares of royalty received by the University on sales of aAPC products described in this article. They also own NexImmune stock, which is subject to certain restrictions under University policy. Dr. Schneck is a member of the company's Board of Directors and Scientific Advisory Board. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.

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twenty unique TCR-MHC class II complex structures have been described in detail that result in effective CD8⁺ and CD4⁺ T cell stimulation, respectively, when loaded with corresponding Ag's [1].

While both MHC class I and II have been utilized for immunotherapy approaches, this review will focus on the use of MHC class I molecules. MHC class I molecules have been used in many multimeric formulations, from dimeric to dodecameric (reviewed in [2] and [3]), for detection and analysis of antigen-specific CD8⁺ T cell responses. Specifically, after immobilization on artificial structures such as paramagnetic beads, liposomes or biodegradable backbones of different sizes and shapes, MHC class I molecules have been used to efficiently stimulate or modulate antigen-specific CD8⁺ T cell responses *in vitro* and *in vivo* (reviewed in [4–7]). More recently MHC class I molecules have also been utilized for redirection of antigenspecific CD8⁺ T cells to tumor cells, which they would otherwise not recognize [8–13]. Furthermore, MHC class I molecules have been utilized to deplete antigenspecific CD8⁺ T cells. In these cases, they were conjugated to alpha emitting epitopes or toxins, or they were immobilized onto a surface of a paramagnetic or biodegradable bead together with a death inducing second signal such as an anti-Fas monoclonal antibody (mAb) (reviewed in [14] and [15–18]). Also noteworthy is that Yoshida et al. show in an elegant way how one can use the significant technological improvements in mass spectrometry based immunopeptidomics and *in silico* methodologies for the identification of tumor-antigens by utilizing transferred secreted human MHC class I molecules presenting peptides derived from intracellular proteins, which are then further processed and used for vaccination [19].

Finally, soluble MHC class I molecules have been used in various vaccination protocols resulting in a wide range of contradicting outcomes, reaching from tolerance induction to induction of anti-tumor responses [20–28]. Here, we will discuss possible reasons and explanations for either favorable or unfavorable immunotherapeutic soluble peptide-MHC-class I complex (pMC) based vaccination strategies. This work wants to contribute to the claim of Hu et al. that the full toolbox of immunotherapeutic approaches to cancer therapy has to be revisited to unleash the immune system's full potential [29].

Possible mechanism of action for soluble pMC

If a T cell engages a pMC (signal 1), it depends on the presence and type of the costimulatory signal (signal 2) if it results in activation, anergy or depletion. While immobilized pMC's have been described to activate naïve CD8⁺ T cells *in vitro* by a CD28/B7 and LFA-1/ICAM-1 independent mechanism [21] following the “strengthof-signal” hypothesis [30,31], activation is also influenced by an adjuvant induced pro-inflammatory environment in combination with co-stimulatory signals provided by professional antigen-presenting cells (APC) or activated T cells (**Fig. 1A**).

A second mechanism is the direct stimulation of a TCR by Fcγ-receptor (Fcγ-R) bound pMC on the surface of an APC. It has been demonstrated that antibody Fc functionalized pMC's can bind via Fcγ-RI and Fcγ-RIII onto professional antigenpresenting cells which enables them to activate antigen-specific T cell responses. This can be further enhanced through maturation of the APC with immune-stimulatory agents, such as lipo-poly-

saccharide (LPS) [24], Poly (I:C) [32], and anti-CD40 mAb [23,33,34]. These agents cause up-regulation of Fc γ -R and expression of costimulatory molecules. Thus mature APC can present more pMC and provide better co-stimulation, which results in enhanced T cell stimulation, activation and proliferation (**Fig. 1B**).

Finally, pMC can be internalized by APC such as immature DC, by a mechanism called phagocytosis and/or pinocytosis. This initial uptake of pMC's initiates a process described as cross-presentation. After internalization the pMC can be exported into the DC cytosol and processed by the proteasome. Subsequently, the dissociated peptide of a pMC will then be loaded onto host MHC class I molecules (reviewed in [35,36]). Alternatively, the pMC will be degraded into various peptides in the phagosome and directly loaded onto MHC class I molecules resulting in the presentation of multiple antigenic peptides, all different from the original loaded peptide of the pMC (reviewed in [37]). Though nature and extent of the immune response will depend on the DC phenotype (**Fig. 1C**).

While all three mechanisms may contribute to the generation of a pMC induced immune response, to date it is not clear as to which extent each mechanism contributes to the formation of this response.

However, it has been demonstrated that cross-presentation cannot be the only mechanism, as vaccination of TCR transgenic 2C mice that are of H2^b background with soluble QL9-peptide loaded L^d-Ig molecules initiated a 2C T cell response. While 2C cells recognize QL9-peptide in the context of an L^d-MHC molecule in an allo-response, the QL9-peptide cannot be presented on the host K^b-MHC molecules. Therefore, the 2C response must be induced by one of the other two mechanisms [23]. Furthermore, it could be demonstrated that anti-CD40 mAb induced maturation of APC prior to pMC immunization was crucial for an effective immune response. Administration of anti-CD40 mAb together with or after pMC administration did not result in formation of a robust immune response, which further supports that the pMC is presented on the cell surface and not internalized [23].

Context of Stimulation

In general, to ensure a proper CD8⁺ T cells response upon re-stimulation a costimulatory signal during the initial stimulation is required. Furthermore, while primary CD8⁺ T cell responses can be independent of CD4⁺ T cell, dependent on antigen levels and presence of danger signals, the generation of a long-lived functional memory CD8⁺ T cell response requires sufficient CD4⁺ T cell help (reviewed in [38]).

Recently we demonstrated that pre-treatment with anti-CD40 mAb prior to pMC immunization initiated and significantly increased formation of a long-lived memory CD8⁺ T cell response *in vivo* [23]. This is in line with findings demonstrating that CD40/CD40L signaling between CD4⁺ T cells and APC renders the later one into a stimulatory cell for efficient CD8⁺ T cell priming [39]. Therefore, application of antiCD40 mAb leads to APC maturation and effective co-stimulation along with pMC resulting in a robust memory CD8⁺ T cell response. Interestingly, Goldstein et al. demonstrated efficient activation of CD44⁺ naïve CD8⁺ T cell by stimulation with immobilized pMC's independent of CD28/B7 and LFA-1/ICAM-1 signaling. This *in vitro* data was in line with the assumption that generation

of a primary T cell response does not require an obligatory co-stimulation but whether these T cells developed a memory phenotype was not investigated [21,22]. Another study from Carey and colleagues, co-applying LPS and pMC demonstrated effective CD8⁺ T cell priming leading to prolonged survival after *in vivo* HSV-1 challenge [24]. Similarly, Sakita et al. and Maile et al. could demonstrate the formation of an *in vivo* anti-pMC T cell response but neither study provided any data regarding the T cell phenotype [25,26]. Furthermore, both studies utilized pMC isolated from *E.coli* inclusion bodies that might bear the risk of LPS contamination or non-specific immune stimulation by a different glycosylation pattern of the pMC [40].

In summary, we feel that more complete, comparative studies are necessary to answer the question as to what extent pMC immunization requires co-stimulation and in what form. However, there is already clear evidence that the addition of proper costimulation enhances pMC induced T cell response [20,24] and the formation of a long lived memory T cell population [23]. Thus, co-stimulation represents a crucial factor for pMC induced cytotoxic T cell responses.

Dosage, timing and route of administration

The timing of pMC administration is another crucial factor for the generation of either a protective anti-tumor or a suppressive T cell response to prolong transplant survival. In a rat model, Fried et al. demonstrated prolonged cardiac graft survival when animals were immunized with pMC daily for 14 days [27] and O'Herrin et al. described inhibition of allo-reactivity in mice through four immunizations with pMC [28]. In a comparative approach Maile et al. demonstrated that a single dose treatment with HY peptide loaded D^b tetramers resulted in graft rejection whereas multiple injections, each with the same amount of the single dose treatment HY peptide, slowed down the rejection [26]. In the absence of co-stimulation, these experiments indicate that multiple pMC immunizations can result in the formation of a suppressive T cell response rather than the formation of a cytotoxic T cell response by single dose administration of pMC.

In contrast, the study of Savage et al. using the same animal model but a different HY peptide, showed 5 fold expansion of peptide specific T cells after triple immunization with HY loaded D^b tetramers on day 0, 1 and 3 without additional co-stimulation [20]. One possible explanation for these findings is the previous immunization of female B6 mice with male B6 splenocytes several weeks before HY loaded D^b tetramer immunization. This suggested the induction of a baseline HY-specific T cell response could provide co-stimulation during HY loaded D^b tetramer immunization, a mechanism similar to the one that has been described by Paine et al. in the human system [41]. The study of Savage et al. did not provide direct co-stimulation along with multiple pMC immunizations, however, it is the only one priming an *in vivo* HYspecific T cell response prior to pMC immunization. All other studies utilizing multiple pMC applications neither provided co-stimulation nor *in vivo* priming [26– 28], suggesting that in the absence of co-stimulation the fate of immunization outcome (activation or anergy) is determined by pMC immunization timing. However, pMC immunization along with co-stimulations seems to result in the formation of an effective antigen-specific T cell response independent of timing.

Furthermore, it has been demonstrated that the formation of a cytotoxic T cell response could be further increased by a booster injection. To this purpose Schuurhuis et al. and our group demonstrated that a second booster injection or a tumor challenge should be applied between day 14 and 30 after initial immunization, providing enough time to induce a robust T cell response and the formation of a protective memory response [23,42].

Many studies have been performed utilizing pMC's in a range from 0.5 – 1000 µg/mouse either applied in a single dose or in multiple steps. In general there is no correlation between amount of pMC applied and efficacy of the pMC-specific T cell response induced. Only two studies provided titration data suggesting that one time administration of high amounts of pMC resulted in a more robust pMC-specific T cell response formation *in vivo* [23,25]. Thus, dosage of pMC's can vary and influence the strength of a pMC-specific T cell response but it seems not to determine the outcome of the immunization in terms of T cell activation or anergy induction. In addition, several of the discussed studies have analyzed the impact of the route of pMC administration i.p.[20,24,26–28] or s.c. [23,25] and concluded that the demonstrated effects of pMC immunization are independent of the route of administration.

Conclusion

Soluble pMC immunization has proven to induce a strong anti-tumor and anti-viral response when applied under pro-inflammatory conditions or with proper costimulation and with the correct timing. At the same time pMC immunization has the potential to be applied for tolerance induction to a specific Ag, facilitating the possibility of transplant protection when utilized without co-stimulus and in multistep immunization protocols. Furthermore, in light of recent progress in identification of tumor neo-Ag's [19],[43–45], pMC based immunization could turn into an new approach of highly personalized cancer immunotherapy. Thus, we believe that the data gathered on soluble pMC immunization justifies further investigation for possible application in various immunological disorders. We hope this review highlights the importance and provokes further effort in this intriguing field of research.

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List of abbreviations:

MHC	major histocompatibility complex
mAb	monoclonal antibody
APC	antigen presenting cell
aAPC	artificial antigen presenting cell
DC	dendritic cell

Ag's	antigens
pMC	peptide-MHC-class I complex
TCR	T cell

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Figure 1: Schematic of possible mechanisms of action for soluble peptide MHC class I complexes (pMC). (A) Direct interaction of soluble pMC with T cells. (B) Interaction of T cells with Fc γ receptor bound pMC's on professional antigenpresenting cells (APC). (C) Recognition of pMC-antigens as a result of antigen crosspresentation. In general the nature and extent of the induced T cell response will be dependent on a pro-inflammatory environment and co-stimulation provided by APC.