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Review/Praca poglądowa

Peptide-based immunotherapy in multiple myeloma

Immunoterapia peptydowa w leczeniu chorych na szpiczaka plazmocytowego

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ABSTRACT

The modifications of the immune system with immunomodulatory drugs or monoclonal antibodies are still insufficient to obtain long-lasting complete remissions in most of the multiple myeloma patients (MM). Peptide-based immunotherapy combined with an autologous hematopoietic stem cell transplantation, immunomodulatory drugs or monoclonal antibodies might represent novel therapeutic strategies, that might retain immune control on disease development and progression resulting in prolonged overall survival. New epitopes derived from tumor-associated antigens (TAA), that are able to induce strong and long-term immune response against MM cells are under investigation. Peptide modifications or targeting multiple epitopes on MM cells could efficiently induce stronger immune response in comparison with single and natural antigens. In this work we have summarized the results of latest studies regarding characterization of TAA that could be used as targets for peptide-based immunotherapy as well as clinical trials utilizing peptide immunotherapy in MM.

Introduction

Multiple myeloma (MM) is a hematological B-cell malignancy, characterized by proliferation and accumulation of monoclonal plasma cells [1]. In many cases MM develops from premalignant stage named monoclonal gammopathy of undetermined significance (MGUS), characterized by presence of monoclonal protein lower than 30 g/L, with an absence of other clinical manifestations [2]. The rate of progression from MGUS to MM is about 0.5–1% per year [3]. More progressive and heterogeneous stage observed between MGUS and MM is asymptomatic multiple myeloma (AMM). It consists a subset of patients with biological

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features of premalignancy that will require therapy in a certain time while others won't be treated for years [4]. In total, the risk of transformation to malignant disease in the first 5 years after diagnosis is relatively high, about 10% per year [5]. Patients with AMM, are not routinely qualified to therapy, but updated diagnostic criteria allow to treat patients earlier once AMM met SLIM CRAB diagnostic criteria for early myeloma [6]. The introduction of novel treatment modalities including lenalidomide, bortezomib as well as recently pomalidomide and carfilzomib (in US only) followed by active participation of MM patients in clinical trials utilizing new drugs such as monoclonal antibodies (daratumumab, elotuzumab, SAR650984), next generation proteasome inhibitors or histone deacetylase inhibitors prolonged overall survival (OS) to 6–8 years, especially among younger patients [1, 7, 8]. However, MM remains an incurable disease and discovery of the novel treatment options is still required. Experimental treatment using peptide-based immunotherapy profits from the identification of specific tumor-associated antigens (TAA), tumor-specific antigens (TSA) or myeloma-specific transcription factors [9]. There are many benefits of inducing antitumor immune responses using peptide immunotherapy, including safety, low toxicity and easy monitoring [10]. The exact place of peptide immunotherapy is not yet determined, but it preferably should be introduced for treatment of early stages MM patients or at remission stage after conventional therapy when immune system function is not seriously impaired or under reconstitution. Identification of premalignant stages of disease as well as remissions when patients do not require standard therapy, open a therapeutic window for experimental treatment. The following work summarizes the current status of peptide-based immunotherapeutic approaches for MM.

Tumor associated antigens

MAGE-A3

Melanoma-associated antigen A3 (MAGE-A3) is one of the most promising cancer/testis antigen utilized in cancer immunotherapy. It was identified on many tumors [11–13], including hematological malignancies [14–16], such as MM. The expression of MAGE-A3 is correlated with the frequency of proliferating malignant cells [17]. MAGE-A3 has ability to inhibit apoptosis through repression of BAX protein by inhibition of p53-dependent up-regulation and maintenance of survivin expression [18]. It also appears that the MAGE-A3 determines the resistance to chemotherapy [19]. The presence of MAGE-A3 on tumor cells is associated with worse prognosis and prolonged survival of tumor cells [20]. Recent studies of Moreno-Bost et al. [21] revealed that epigenetic modulation using 5-azacitidine combined with histone deacetylase inhibitor MGCD0103 could induce MAGE-A3 mRNA as well as protein expression, resulting in enhanced MAGE-A3-specific cytotoxic T lymphocytes (CTL) recognition. Hobo et al. [22] demonstrated that dendritic cells (DCs) pulsed with MAGE-A3 combined with survivin and B-cell maturation antigen (BCMA) are capable to stimulate TAA-specific T-cell responses in MM patients. Moreover, the application of these TAA-specific DCs in combination with other immunotherapeutic strategies such as co-inhibitory signaling blockade may further boost antimyeloma immunity after autologous stem-cell transplantation (ASCT). Rapoport et al. [23] showed a high frequency of vaccine-specific T-cell responses using above mentioned combined therapy. The transplantation of costimulated autologous T cells with Poly-ICLC/GM-CSF-primed MAGE-A3 vaccine resulted in 74% 2-year OS and 56% 2-year event-free survival among the group of sixteen patients.

Heat shock proteins

Heat shock proteins (HSPs) belong to chaperones family responsible for the proper folding and stabilization of protein structure, protecting against unexpected molecule aggregation and consequently function loss [24]. Increased mRNA and protein levels in the most of MM primary cells in comparison with normal cells were detected, indicating this protein as promising myeloma-associated antigen [25]. Li et al. [26] identified HSP-specific CD8+ CTL in MM patients. Afterward, 2 highly immunogenic peptides HSP90AA1$\alpha_{670–678}$ (ALLSSGFSL) and HSPB1$_{27–35}$ (RLFDQAFGL) able to induce functional peptide-specific CTL with ability to lyse HLA-A*0201-restricted MM cells were selected. Moreover, T cells directed against HSP-derived epitopes decreased tumor burden in mice, demonstrating potential effectiveness of peptide vaccine based on HSP epitopes.

CS-1

CS1 (also known as CRACC, CD319 and SLAMF7) is a tumor-associated antigen characterized by high frequency (over 95%) of expression on plasma cells of MM patients [27]. The antigen is presented on different cell subsets, such as natural killers (NK), NK-like T cells, CD8+ T cells, activated monocytes and DCs but the level of its expression is significantly lower than on plasma cells [28]. Targeting CS1 using a humanized monoclonal IgG antibody, elotuzumab, in combination with lenalidomide and dexamethasone gave enthusiastic results, manifesting a 92% overall response rate and median progression-free survival of 26.9 months [29]. The CS1 antigen provides also a unique target for the development of peptide-based immunotherapy. Bae et al. [30, 31] identified novel immunogenic HLA-A2-restricted CS1$_{239–247}$ (SLFVLGLFL) peptide, which induced CS1-specific CTL against MM cells. The immune response to MM cells was demonstrated by antigen-specific cytotoxicity, proliferation, degranulation and interferon-γ production. In preclinical study Bae et al. [32] used CS1 as a component of a multiepitope cocktail to enhance cytotoxic T-cell response against MM cells.

HM1.24

HM1.24 (also known as BST2 or CD317) is a protein characterized by high frequency of expression on more than 90% primary MM cells, as well as myeloma cancer stem cells [33, 34]. The antigen is involved in the pathogenesis of MM by the activation of the NFκB pathway [35]. Recent studies proved HM1.24 ability to induce specific cytotoxic immune

response of CD8+ [36, 37] as well as long-term immune response of CD4+ cells [38]. The fusion of proteins consisting of a HM1.24-specific single-chain fragment variable (scFv) and a truncated variant of *Pseudomonas aeruginosa* exotoxin A lacking the receptor binding domain I (ETA') generate the novel immunotoxin HM1.24-ETA'. The recombinant immunotoxin is biologically active and inhibits the proliferation of HM1.24-expressing myeloma cell lines L363, RPMI-8226, JK-6 and the IL-6-dependent plasmacytoma cell line INA-6. Additionally, HM1.24-ETA' is able to induce the apoptosis of JK-6 and L363 cells. Moreover, HM1.24-ETA' does not exhibit cytotoxicity against CD317-positive nonmalignant monocytes and human umbilical vein endothelial cells [39]. The preclinical experiments proved also the high efficiency of humanized anti-HM1.24 monoclonal antibodies (AHMs) expressed by inducing marked antibody-dependent cellular cytotoxicity (ADCC) and consequently resulted in lysis of myeloma cells and their progenitors [40].

Mucine 1

Mucine 1 (MUC1) is a type I transmembrane glycoprotein, overexpressed in carcinomas [41, 42] and hematological malignancies [43, 44]. The frequency of expression on MM cells is 92% indicating this protein as an excellent target for immunotherapy [45]. Moreover, Choi et al. [46] confirmed presence of MUC1-specific CTL in peripheral blood as well as in bone marrow of MM patients. The most recent studies were focused on modifications on MUC1 antigen. Rossmann et al. [47] proved the potential effectiveness of immunotherapy with the use of tecemotide, which is a liposomal antigen-specific cancer immunotherapeutic agent targeting MUC1, consisted of non-glycosylated MUC1 lipopeptide (BLP_{25}) and monophosphoryl lipid A immunoadjuvant in a liposomal (L) delivery system. The immunization with tecemotide resulted in the induction of a MUC1-specific immune response, involving CD4+ and CD8+ T cells, in 47% of patients.

HLA-DOβ

B cell specific molecule (HLA-DOβ) is a novel, promising antigen, showed high expression in MM cells. Kang et al. [48] identified the two the most immunogenic HLA-A*0201 restricted epitopes HLA-$DOB_{232–240}$ (FLLGLIFLL) and HLA-$DOB_{185–193}$ (VMLEMTPEL). HLA-$DOB_{232–240}$ is capable to induce specific CTL from peripheral blood mononuclear cells in response to peptide stimulation in 4 out of 5 MM patients. These findings suggested that HLA-DOβ derived epitopes could represent novel, highly immunogenic targets for peptide-based immunotherapy. All of described TAAs in MM are characterized in Table I.

Myeloma-specific multiepitope vaccines

Since MM cells express multiple TAA epitopes recognized by T cells and some of the epitopes could be lost or re-expressed at different times, many trials utilizing vaccines against multiple TAA epitopes are undertaken in order to determine whether multiple peptide therapy proves to be more effective than a vaccine against a single epitope [30, 32, 49]. Design of an immunotherapeutic approach to induce broad CTL responses specific to multiple antigens may overcome problems caused by the mechanisms of neoplastic cells to escape from immunosurveillance, including (i) antigen mutation or deletion by tumor cells; and (ii) the variation of the appropriate T-cell repertoire in the patient. Recent research of Bae et al. [32] demonstrated that a cocktail of HLA-A2-specific peptides, heteroclitic XBP1 $US_{184–192}$, heteroclitic XBP1 $SP_{367–375}$, native $CD138_{260–268}$ and native $CS1_{239–247}$ could elicit multipeptide specific CTLs (MP-CTL) in MM patients *ex vivo*. MP-CTL exhibited mainly memory T-cell immunophenotype CD45RO+ and multifunctional activity with restriction of HLA-A2 against MM cells. Simultaneous stimulation with the peptide mixture did not interfere with the induction of MP-CTL, proving the functionality of these cells.

Clinical trials

Definition of the therapeutic targets described above allows initiating trials in patients with MM. Hobo et al. [22] described results of the clinical trial on peptide immunotherapy in 12 patients with Salmon-Durie stage II or III MM [50], treated with induction chemotherapy and high-dose melphalan. Patients were vaccinated three times with

Table I – Novel targets for peptide-based immunotherapy in multiple myeloma
Tabela I – Nowe cele dla immunoterapii na bazie peptydów w szpiczaku mnogim

Antigen	Peptide sequence	Normal expression	MM expression	Humoral response	T-cell response	Ref.
MAGE-A3	IMPKAGLLI	Testis	25–80%	+	+	[21, 58, 59]
	ELVHFLLLK				+	
	VIFSKASSSLQL				–	
	KISGGPHIS				–	
HSPs	ALLSSGFSL	All	Overexpressed	Not tested	+	[26, 60]
	RLFDQAFGL				+	
CS1	SLFVLGLFL	NK cells	>90%	Not tested	+	[28, 31]
HM1.24	LLLGIGILV	Terminally differentiated B cells	>90%	+	+	[61]
MUC1	STAPPVHNV	Glandular epithelium	92%	+	+	[45, 62]
HLA-Doβ	FLLGLIFLL	B-lineage cells, subset of	>20 fold	Not tested	+	[48]
	VMLEMTPEL	thymic medullary epithelium	expression		–	

intravenous and intradermal vaccines of mature monocyte-derived DCs, pulsed with keyhole limpet hemocyanin (KLH) and electroporated with MAGE3, survivin or B-cell maturation antigen (BCMA) mRNA, at biweekly intervals. Vaccination was well tolerated and no severe toxicities were observed during the treatment period. All patients developed strong anti-KLH T-cell responses, but no generation of KLH antibodies were observed. In 2 patients, vaccine-specific T cells were detected in delayed-type hypersensitivity biopsies. In one patient, MAGE3-specific CD4+ and CD8+ T cells, and CD3+ T cells reactive against BCMA and survivin were found. In the other patient, low numbers of MAGE3 and BCMA-reactive CD8+ T cells were detected. These findings illustrate that TAA-mRNA-electroporated mature DCs are capable of inducing TAA-T-cell responses in MM patients and the application of these TAA DCs in combination with other immunotherapeutic strategies such as co-inhibitory signaling blockade may further boost antimyeloma immunity after ASCT.

Cohen et al. [51, 52] applied recombinant MAGE-A3 contained full length MAGE-A3 combined with *H. influenzae*-derived protein D mixed with AS15 immunostimulant. Thirteen MM patients received first vaccine before ASCT, and 7 doses after ASCT, resulted in development of antibody production and Th1 CD4 T-cell responses, without CD8 responses in all patients. The one year after ASCT, 5 patients showed complete remission and very good partial response was observed in 3 patients, with a median follow-up of 19 months. Noteworthy, the loss of MAGE-A3 expression in relapsing patients was observed.

The autologous anticancer vaccine containing DCs loaded with Id-protein was performed by Zahradova et al. [53]. The group of 12 MM patients who relapsed or were in stable measurable disease received a total of 6 vaccine doses intradermally in monthly intervals. Immune responses measured by Enzyme-Linked ImmunoSpot (ELISpot) assay were noted in 3 patients and delayed-type hypersensitivity (DTH) skin test for Id-protein was positive in 8 patients. During the follow-up the disease remained stable in 7 patients. Although, no direct cytolytic effect of vaccination has been observed in this study, these results suggest that the vaccination could stabilize the disease in the part of patients and vaccination remains a promising therapeutical modality.

Rosenblatt et al. [54] presented results of vaccination following ASCT in 24 patients. The aim of the immunotherapy was to target minimal residual disease by evoking a polyclonal response directed against multiple antigens, including those unique to a particular patient. Patients received three post-transplant vaccinations given every 4 weeks containing fusions of DCs with MM cells. Authors observed that the increase in CD4+ and CD8+ myeloma-specific T cells, observed after ASCT, was significantly expanded following post-transplant vaccination. All evaluable patients demonstrated at least a two-fold expansion of myeloma-specific CD4+ and/or CD8+ T cells. Seventy eight percent of patients achieved the best result of complete response or very good partial response. The study demonstrated that potent anti-tumor immune responses and elimination of post-transplant residual disease could be achieved with an autologous tumor vaccine administered in the early post-transplant period.

Carmon et al. [55] design long synthetic peptide vaccine (ImMucin) containing signal peptide domain capable to strongly bind T- and B-cell epitopes [56, 57]. Fifteen MM patients with expression of MUC1 and residual or progressive disease following ASCT were vaccinated 6 or 12 times at biweekly intervals. Although vaccine induced antibodies and specific T-cell responses in all of treated patients, 10 out of 15 patients showed progressive disease after 2 years.

Conclusions

This review showed that peptide-based immunotherapy might be effective treatment alternative for exclusive group of MM patients. Modifications of peptide targets, multi-peptide vaccines or the use of peptides in combination with immunomodulatory drugs or epigenetic modulators might augment effectiveness giving hope for longer survival of MM patients. Moreover, anti-tumor immune responses and elimination of post-transplant residual disease could be achieved with an autologous tumor vaccine administered in the early post-transplant period, without induction the increased risk of secondary malignancies or significant toxicity. Finally, peptide-based therapies could represent the one of the promising treatment strategies for patients with MM in early stage: ''high-risk'' MGUS and ''high-risk'' AMM patients, which could induce immune system to eradicate tumor cells, before clinical symptoms will develop and disease progress.

Authors' contributions/Wkład autorów

According to order.

Conflict of interest/Konflikt interesu

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Ethics/Etyka

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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