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Binding of prostate-specific membrane antigen to dendritic cells: a critical step in vaccine preparation

Stefano Garetto[1], Federico Sizzano[2], Davide Brusa[1], Alessandro Tizzani[3], Fabio Malavasi[4] and Lina Matera[1]

[1]Laboratory of Tumor Immunology, Department of Internal Medicine, University of Turin, Turin, Italy, [2]Laboratory of Transplantation Immunology, San Giovanni Battista Hospital, Turin, Italy, [3]Urology Department, University of Torino, Torino, Italy, and [4]Department of Genetics, Biology and Biochemistry and CeRMS, University of Turin, Turin, Italy

Background aims

Dendritic cell (DC)-based vaccines hold promise as a safe therapy for prostate cancer (PCa), and prostate-specific membrane antigen (PSMA) fulfils the requirements for a tumor-associated antigen (TAA) to be clinically effective. We evaluated the actual binding of selected HLA-A2-restricted PSMA peptides to HLA class I molecules on ex vivo*-generated mature (m) DC.*

Methods

mDC were generated from peripheral monocytes of HLA-A2 normal donors. The PSMA peptides $PSMA_{711}$ (ALFDIESKV), $PSMA_{27}$ (VLAGGFFLL) and $PSMA_{663}$ (MMNDQLMFL) were selected based on computer-assisted prediction programs, documented CTL epitope activity or previous use in clinical trials. The model cell line T2 and the clinical grade ($CD83^+$ $CCR7^+$) mDC were pulsed with fluorescein (FL)-conjugated peptides and an anti-HLA-A2 monoclonal antibody (MAb) and analyzed.

Results

Flow cytometry analysis showed best binding efficiency to be by $PSMA_{27}$. Confocal microscopy confirmed coincident fluorescence emission of HLA-A2 MAb and FL–$PSMA_{27}$. Virtual co-localization of $PSMA_{27}$ and HLA class I molecules was supported further by fluorescence resonance energy transfer (FRET) analysis. The clinical relevance of our findings has to be validated in vivo.

Conclusions

The present report is the first to score selected PSMA peptides based on their detectable binding to mDC. It identifies $PSMA_{27}$ as the choice candidate among other PSMA peptides and it should be included in developing DC vaccine protocols for HLA-A2 PCa patients.

Keywords

Antigenic peptides, dendritic cells, prostate cancer, prostate-specific membrane antigen, vaccines.

Introduction

The description of tumor-associated antigens (TAA) [1] has provided the basis for tumor-targeted immunotherapy. TAA are presented by dendritic cells (DC), which express the co-stimulatory molecules necessary to trigger naive CD8 cytotoxic T lymphocytes (CTL) [2].

Although androgen ablation represents an effective treatment modality for recurrent disease, a significant number of prostate cancer (PCa) patients develop androgen-independent PCa, poorly responsive to traditional therapies. Therefore, effective novel therapeutic approaches are needed and immunotherapy is a good candidate [3]. Because of the abundance of TAA and a limited availability of autologous tumor for DC loading, PCa can be considered an attractive target for DC TAA-based vaccine immunotherapy [4].

In theory, TAA indispensable for tumor survival and propagation should be the first choice in designing a vaccine protocol. Prostate-specific membrane antigen (PSMA) is a type II membrane glycoprotein of 110 kDa

Correspondence to: **Lina Matera**, Department of Internal Medicine, University of Turin, Turin, Italy. E-mail: lina.matera@unito.it

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with ectoenzymatic (glutamate carboxypeptidase II and folate hydrolase) activities [5]. Increases in both expression and enzymatic activities of PSMA in aggressive prostate tumors [6–8] imply that PSMA provides a selective unique advantage to cells expressing it, thereby contributing to the development and progression of prostate cancer [9–11]. In addition, DC transfected with an adenovirus coding for PSMA acquire the potential of cross-priming PCa-specific CTL [12]. PSMA thus fulfils the requirement for a TAA to elicit an efficient anti-PCa CTL response. The fact that PSMA is expressed on the neovasculature of most of the solid tumors, but not in that of normal tissues [13,14], further strengthens its importance as TAA.

The T-cell epitope potential of PSMA HLA-A2 peptides derived from computer-assisted prediction programs [15–17] has been tested *in vitro* and *in vivo*, based on their ability to recruit CTL and to be recognized as CTL targets. The first clinical trial on PCa patients used HLA-A2-restricted $PSMA_4$ LLHETDSAV and $PSMA_{711}$ ALFDIESKV peptides, with clinical responses reported in only a few cases [18]. Two other protocols adopted $PSMA_4$ LLHETDSAV in combination with peptides from other TAA [19,20]. This approach led to a $PSMA_4$ LLHETDSAV CTL response in blood [19] and transient decline of the levels of circulating PSA [20]. A more recent study, relying on $PSMA_4$ LLHETDSAV used as a single peptide, did not show any significant clinical advantage [21]. $PSMA_4$ LLHETDSAV failed to elicit, in an *in vitro* sensitization system [22], a CTL response when presented by mature (m) DC. $PSMA_{711}$ ALFDIESKV induced a CTL response against peptide-pulsed target cells but not against the PSMA-positive prostate cancer cell line LNCaP. In contrast, the peptide $PSMA_{27}$ VLAGGFFLL, which was not used in any of the clinical trials, was able to both induce peptide-specific CTL and kill the LNCaP cell line. Another peptide not yet included in clinical trials has been shown to recruit CTL *in vitro* and be recognized by CTL. DC transfected with a PSMA plasmid induced CTL that specifically recognized targets loaded with the $PSMA_{663}$ MMNDQLMFL, as well as the naturally PSMA-expressing LNCaP cell line [23]. In addition, $PSMA_{663}$ MMNDQLMFL presented by DC generated a CTL response against LNCaP cells.

While the TAA epitope dictates the specificity of the CTL response, its magnitude relies upon the number of HLA class I molecules expressed by the antigen presenting cells. Immature (i) DC generated from blood monocytes progress to mDC in the presence of cytokines that mimic a pro-inflammatory environment. Terminal maturation of DC entails the appearance of surface molecules such as CD83 and the chemokine receptor CCR7, while HLA class I molecules are significantly up-regulated [1]. Because of the expression of the chemokine receptor CCR7, mDC rapidly migrate to the lymph node after intradermal injection and, because of the high expression of HLA class I, they present optimal numbers of HLA class I–peptide complexes to naive CD8.

Whereas the mature phenotype is a well-established quality parameter in the production of clinical-grade DC, the density of HLA–peptide complexes expressed by mDC has not yet been evaluated in detail. By combining flow cytometry and microscopy analyzes, we assessed the ability of $PSMA_{711}$ ALFDIESKV, $PSMA_{27}$ VLAGGFFLL and $PSMA_{663}$ MMNDQLMFL peptides to form complexes with HLA-A2. Data obtained on the TAP-defective cell line T2 were exploited to verify the binding and persistence of the best binder peptide to clinical-grade monocyte-derived HLA-$A2^+$ mDC.

Methods

Reagents

Human recombinant granulocyte–macrophage colony-stimulating factor (GM-CSF) and prostaglandin E^2 (PGE_2) were from Sigma-Aldrich (Milan, Italy). Interleukin (IL)-4, IL-1β, IL-6 and Tumor Necrosis Factor (TNF)-α were from Peprotech (Rocky Hill, NJ, USA) and IL-2 was from Chiron (Milan, Italy).

Peptides

Peptides VLAGGFFLL ($PSMA_{27}$), ALFDIESKV ($PSMA_{711}$), MMNDQLMFL ($PSMA_{663}$) and GDLVYVNYA ($PSMA_{172}$) (80–90% purity) were from INBIOS (Naples, Italy). Peptides were conjugated to fluorescein (FL) by means of LC (8-amino-3,6-dioxa-octanoic acid), a linker that allows mobility of the two structures. FL peptides were dissolved in 0.1% trifluoroacetic acid H_2O and stocked at –20°C.

Cell lines

The HLA-$A2^+$ TAP-deficient cell line T2 [24], and the HLA-$A2^-$ leukemia human cell line K562 [25], were grown in RPMI-1640 medium (Sigma-Aldrich) containing 10% fetal calf serum (FCS; Life Technologies Ltd, Paisley, UK), 1 µg/mL L-glutamine and 100 mg/mL streptomycin, hereafter referred as complete medium (CM).

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