August 30, 2018

PharmacologyOnLine

Newsletter • 2018 • vol.2 •23-32

TARGETING TOOLS FOR A DNA VACCINE CARRIER, A DESIGN IN PREVENTION OF CANCER

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Abstract

For a successful vaccination, usually dendritic cells play a great role as APC (antigen presenting cell) to activate naïve CD4 T-Cell and CD8 T-Cell, thereby Immunization against disease. Recent decade molecular medicine focuses the investigation of new generation Vaccines against infections and malignant disease. Cancer vaccines are designed to work by activating B cells and killer T cells and directing them to recognize and act against specific types of cancer. Cytotoxic T cells, which are also known as killer T cells, kill infected or abnormal cells by releasing toxic chemicals or by prompting the cells to self-destruct (a process known as apoptosis). However, the world is still lacking effective vaccines kill millions of people every year with Cancer. So there is a demand of the decade in DNA vaccination. Recent efforts toward the development of anticancer vaccines have focused on the generation of tumor-specific CTL responses (Boon et al. 1995, Boon et al. 1997). Cytotoxic T cells recognize peptide antigens on the cell surface of an antigen-presenting cell in association with a class major histocompatibility complex (MHC) molecule. The ability of the peptides to bind to MHC molecules is allele specific, and correlates their immunogenicity (Altuvia et al. J. Mol. Biol. 249. 1995). In this review, the possible targeted tools for DNA vaccination especially MHC-class I mediated pathway were highlighted against the cancer disaster.

Keywords: *DNA vaccines, dendritic cells, Major Histocompatibility Complex, T-cell*

Introduction

Targeting cancer is still now a challenging approach, where Cancer is a dreadful disease of mass of tissues formed as a result of abnormal, excessive, uncontrolled, uncoordinated, autonomous and purposeless proliferation of cells. Cancer is fast becoming a major global health problem. It´s estimated that by 2020 there will be some 15 million new cancer cases a year, the majority of them in developing countries [1]. The worst thing is the cancer metastasis, which is the spread of cancer cell from one part to another part of the body through various stages of cancer. Usually these are a) cell with genetic mutation or chronic inflammation or oncogenic signaling which is the origin of the cancer cell, b) Hyperplasia, c) Dysplasia, d) *in situ* cancer and e) invasive cancer and thereafter f) Intravasation, g) circulation, h) arrest and extravasation and i) proliferation in new place of the body (Fig 1)[2-3] . For an example of breast cancer, which may spread to lung and that can be called metastatic breast cancer to lung.

The Dendritic Cell

The most potent and unique tool is the dendritic cell (DC), which must be targeted properly. They are the most potent antigen presenting cell to the body and able control various components of the total immune system, the unique feature of the DC. The matured DCs are the most important to play their key role. The mature stage (end stage) of DC stimulates and regulates the immune system drastically if proper guided. Especially MHC –Class I presented to CD8 CTL and NK cell as well, and MHC Class II presented to CD4 CTL as well as Cd1d molecule presented to NKT, mediated stimulation are the vital parts to be targeted (Fig 2). The immune tools should be designed accordingly for the respective stimulation. It needs proper differentiation, maturation, activation and polarization [4].

However, the internal tools for the rate limiting steps of antigen processing is bit complicated. The Fig. 3 shows how antigen is processed inside the DC. When varying amounts of a protein are synthesized in the cytosol, a given fraction will be processed to yield peptides bound to MHC class I molecules, following a dose-response curve that eventually may reach saturation levels. CTL that recognize this processed peptide at the cell surface will follow this response; i.e., the more peptide is processed, the more recognition and lysis will be detected [5].It means the processing peptide is the rate limiting step of antigen presentation. Although $CD8^+$ CTL do not recognize intact proteins, they interact with antigens (Ags) on the APC surface as protein fragments bound to MHC class I molecules. The antigenic peptides originate from proteins degraded mainly by the multicatalytic complex proteasome in the cytosol [6] but also by other proteases [7-8]. These peptides are transported by the MHC-encoded transporter TAP to the endoplasmic reticulum (ER), where they may undergo proteolytic processing. Newly synthesized heavy chain/β_2-microglobulin (β_2m)/calreticulin complexes associate with TAP and tapasin before peptide loading. Conformational changes in class I molecules associated with peptide binding are postulated to result in their release from TAP [9]. Finally, the stable complex of heavy chain/β_2m/peptide is transported through the Golgi apparatus to reach the cell surface where it can interact with the TCR of the CTL [10-11].

MHC Class I allele is also called Human leucocyte antigen or Allele HLA. Major histocompatibility complex-I (MHC I) is a fundamental protein usually found on the plasma membrane. It helps immune cells to fight against bacterial and virus. MHC I is composed of two chains: a light chain (β-macroglobulin; β2m) and a heavy chain (α chain with 3 domains). The α-chain genes in case of murine have different haplotypes, dependent on the strain of mice. H-$2K^b$/ H-$2D^b$ is the gene for α chain in C57 BL/6 mice [12].

In 1968 the WHO nomenclature committee for factors of HLA system first disclosed representing the major histocompatability complex in human, known as Human Leucocyte Antigen. HLA-A, HLA-B were proposed by them. It was mentioned that HL-A2 became HLA-A2, HL-A7 became HLA-A7 and HL-A8 became HLA-A8. These are the alleles of HLA and representing human MHC genes. A further modification was done as HLA-A*02:101 and HLA-A*02:102 as serological subtypes.

There are 6 potential pockets designated A through F in HLA groove could be treated as potential targeting tool for a DNA vaccine. In the absence of foreign antigens, MHC Class I molecules are occupied by hetergenous peptides derived from

self-proteins. Bound peptides usually display a narrow lenghth distribution encompassing 8-10 or 11 amino acids. Peptide binding groove and define an allelle-specific microenvironment for bound peptides, could be treated as potential tool of a DNA vaccine to be designed.

HLA Class I loci are highly polymorphic: 118 alleles have been identified for HLA-B locus, 59 for the HLA-A locus and 36 for the HLA-C locus. All of the MHC Class I structures analyzed to date show a closed peptide binding groove able to retain the free NH_2 and COOH-groups at the ends of the peptide. Only tightly bound high-affinity peptides have a chance to reach the cell surface and trigger an immune response. In HLA-B40, the predominant amino acid found in position 2 of bound peptides is negatively charged glutamic acid. In HLA-B27, the predominant amino acid at position two is positively charged arginine. In each example, the appropriately countercharged amino acid can be found at the bottom of the B pocket [13].

There are various approaches to predict about the affinity-binding between peptide and MHC molecule, which is the rate limiting step of DNA vaccination. In Table 1, various peptide sequence with their affinity binding position were given against respective diseases. This is known as Altuvia approach for peptide HLA binding prediction.

An Approach of Hydropathy Index

The hydropathy index of an amino acid is a number representing the hydrophobic or hydrophilic properties of its side chain. The larger the positive number is, the more hydrophobic the amino acid. The most hydrophobic amino acids are isoleucine (+ 4.5) and valine (+ 4.2). The most hydrophilic ones are arginine (-4.5) and lysine (-3.9) [17-18].

Sette-Altuvia approach and hydropathy index

PEPTIDE epitope	Peptide sequence		$IC_{50\%}$	Peptide rank potential	∑[H
HIV-1 gpl20 1-861	TLTSCNTS	GP	294 nM	262 (31)	+2.5
Influenza A matrix 1-252	GILGFVFT	MT	6 nM	1(1)	+20.4
HIV-1 Rtase 1-848	ILKEPVH	RT	242 nM	35(5)	+4.1
HTLV-I TAX 1-353	LLFGYPVY	TX	11 nM	5(2)	14.2
Ova 257-264	SIINFEKL	SL8			+3.9

The above Proposed hypothesis for MHC Class I targeted binding and thereby DNA vaccine targeting tools as well as protein vaccine could be as follows on the basis of discussion narrated in the references [14] to [18]:

- Toward the end of the pocket, Met 45 of α_1 helix is surrounded by Ala 24, Gly 26, Val 34, and Val 67, and, therefore, the pocket is hydrophobic in nature (Fig 4 and Fig 5).
- In HLA-B40 , the predominant amino acid found in position 2 of bound peptides is negatively charged glutamic acid. In HLA-B27, the predominant amino acid at position two is positively charged arginine.
- Preferential binding to TAP of peptides that carry hydrophobic residues in the third (P3) residue from the N-terminus Peptide length (hydrophobic) Position carrying anchoring aminoacids. Especial preferance for non-anchoring aminoacid lysine /arginine or glutamic/aspartic acid. Only tightly bound high-affinity peptides have a chance to reach the cell surface
- Over all structural similarity an epitope as efficient targeting tool for DNA vaccination, which is MHC class I restricted should have a hetero hydrophobic amino acid chain $[H_{p(+)}]_{7\text{-}9} \geq 3$ with an ionic amino acid (K/R/D/E) at position 2 as well as a hydrophobic amino acid at position 3 from the N-terminal/C-terminal of the epitope peptide.

Mathematically it may be expressed as follows:

$$\sum_{i=0}^{n=7\text{-}9}[H_{p(+)}]\text{-}X_{(Hp+)}\text{ - (K/R) -}NH_2 \text{ or,}$$

$$\sum_{i=0}^{n=7\text{-}9}[H_{p(+)}]\text{-}X_{(Hp+)}\text{-(D/E) –COOH and,}$$

$$\sum_{i=0}^{n=7\text{-}9}[H_{p(+)}] \geq 3$$

Epitopes derived from the MART-1/Melan-A protein for clinical vaccines [19]

Two overlapping epitopes spanning amino acid residues 26 through 35 are of particular interest: numerous clinical studies have been performed using variants of the MART-1 26–35 decamer, although only the 27–35 nonamer has been found on the surface of targeted melanoma cells. Here, we show that the 26–35 and 27–35 peptides adopt strikingly different conformations when bound to HLA-A2.

The epitopes spanning amino acid residues 26–35 and 27–35 from the MART-1/Melan-A protein, highly expressed in melanoma cells, provide a prime example of T cell recognition of multiple peptides and the use of peptide variants designed to elicit altered immunological responses. Initial studies identified the 27–35 nonamer (AAGIGILTV, referred to as AAG; see Table 2) as the immunodominant epitope of the MART-1 protein,10,11 although the 26–35 decamer (EAAGIGILTV, referred to as the EAA decamer), was also found to be recognized by MART-1-reactive T cells. Heterogeneity is preferred to homogeniety in respect of HLA binding.

In conclusion it is clear that Peptide-MHC Class I molecule binding processing can be the rate-limiting step for $CD8^+$ T-cell stimulation. There are many approaches about the peptide-MHC binding prediction and here one simple approach was explained on the basis of hydropathy index as the following formula, where $[H_{p(+)}]_{7\text{-}9} \geq 3$

$$\sum_{i=0}^{n=7\text{-}9} [H_{p(+)}]\text{-}X_{(Hp+)}\text{-}(K/R/D/E)\text{-}NH_{2/COOH}$$

"*An epitope as efficient targeting tool for DNA vaccination, which is MHC class I restricted should have a hetero hydrophobic amino acid chain* $[H_{p(+)}]_{7\text{-}9} \geq 3$ *with an ionic amino acid (K/R/D/E) at position 2 as well as a hydrophobic amino acid at position 3 from the N-terminal or C-terminal of the epitope peptide*". In Malignant melonoma, AAGIGILTV or ELAIGILTV might be a potential tool for designing DNA vaccination.

Acknowledgement

I would like to thank Prof. Harashima Hideyoshi, Faculty of pharmaceutical Scinces, Hokkaido University, Sapporo, Hokkaido, Japan, for giving the facilities to present a part of this review in his place and also financial help.

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Table 1

Peptides and proteins used in analysis

Sequence	Code in sequence bank	Peptide	Position	Abbreviation	PDB[a] file
HIV-1 gp120 1-861	env_hv1br	TLTSCNTSV	197–205	GP	1HHG
Influenza A matrix 1-252	vmt1_iapue	GILGFVFTL	58–66	MT	1HHI
HIV-1 RTase 1-848[b]	pol_hv1h2	ILKEPVHGV	309–317	RT	1HHJ
HTLV-1 TAX 1-353	I03562.gb_vi[c]	LLFGYPVYV	11–19	TX	1HHK
HBV ayw core 1-183	cora_hpbvy	FLPSDFFPSV	18–27	HBV	1HHH

[a] Coordinate file of peptide–HLA-A2.1 complex from the Protein Data Bank (PDB; Bernstein *et al.*, 1977).
[b] Peptide numbering in the sequence file is 464 to 472, the sequence ranges from residue 156 to 1003.
[c] Protein sequence translated from Genbank.

Table 2. MART-$1_{26/27-35}$-based peptides

MART-1 residue number / Peptide position	26 P0	27 P1	28 P2	29 P3	30 P4	31 P5	32 P6	33 P7	34 P8	35 P9	HLA-A2 binding affinity relative to AAG[a]	$\sum[H_{p(+)}]$
Native AAG nonamer		A	A	G	I	G	I	L	T	V	1	**+19.1**
Modified ALG nonamer		A	L	G	I	G	I	L	T	V	40	**+21.1**
Modified LAG nonamer		L	A	G	I	G	I	L	T	V	1	**+21.1**
Native EAA decamer	E	A	A	G	I	G	I	L	T	V	4	**+15.6**
Modified ELA decamer	E	L	A	G	I	G	I	L	T	V	9	**+17.6**

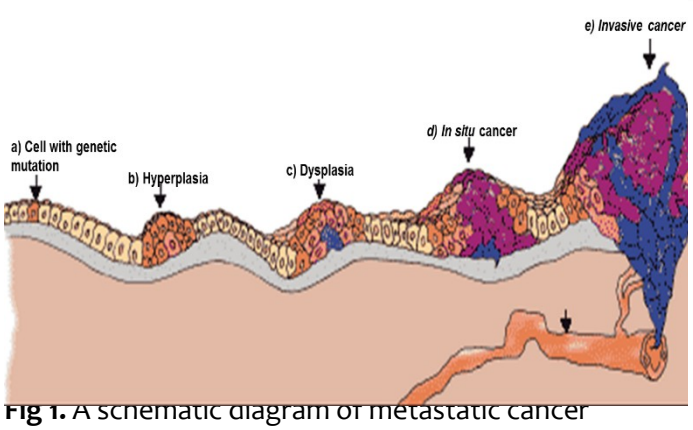
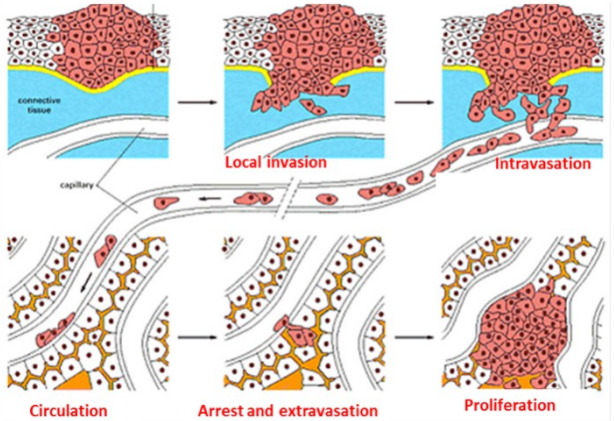


Fig 1. A schematic diagram of metastatic cancer development.



Metastasis

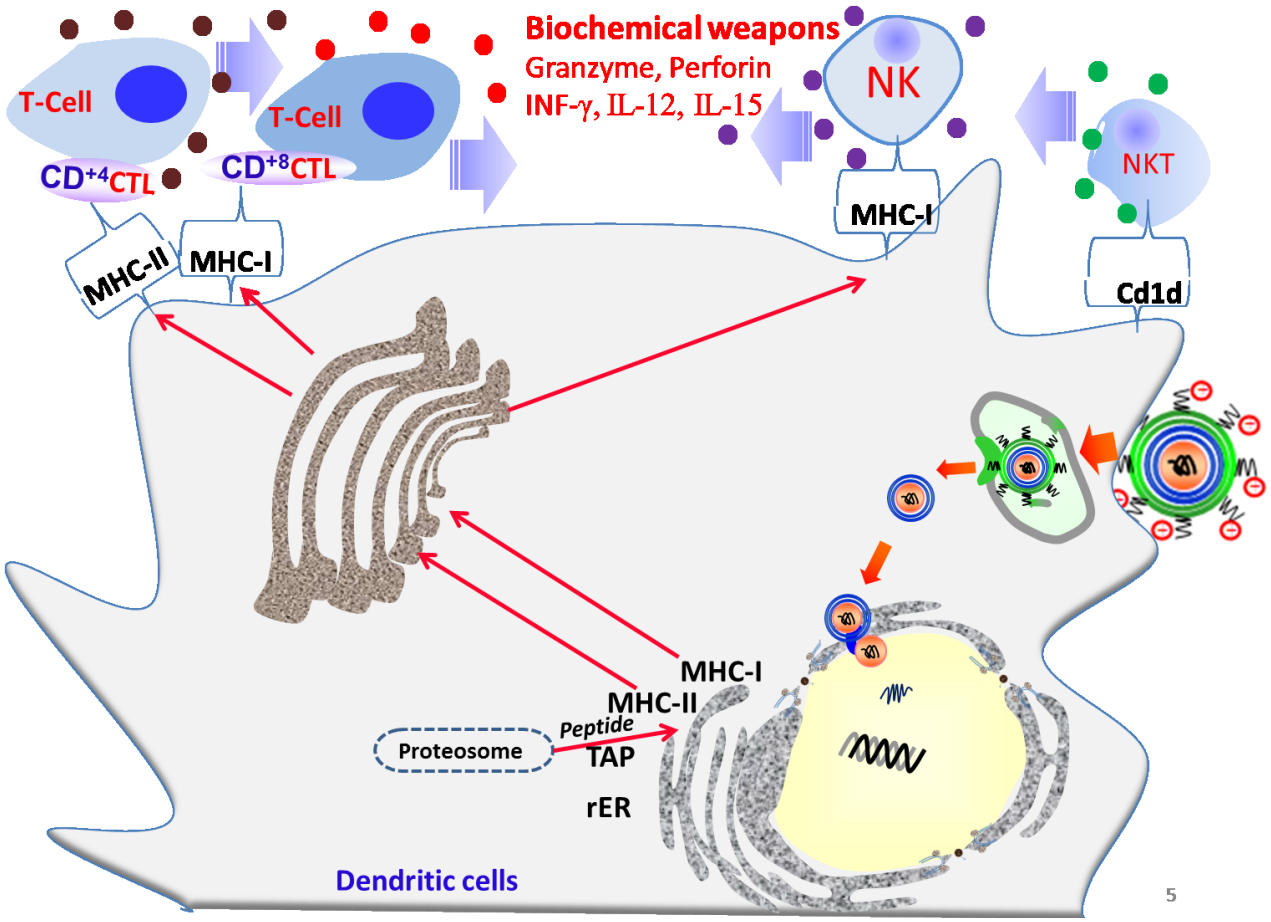


Fig 2. The ways how dendritic cells work as detective soldier of the body and presents potentially antigens of interest to the respective immune cells, especially CTLs, NK and NKT cells.

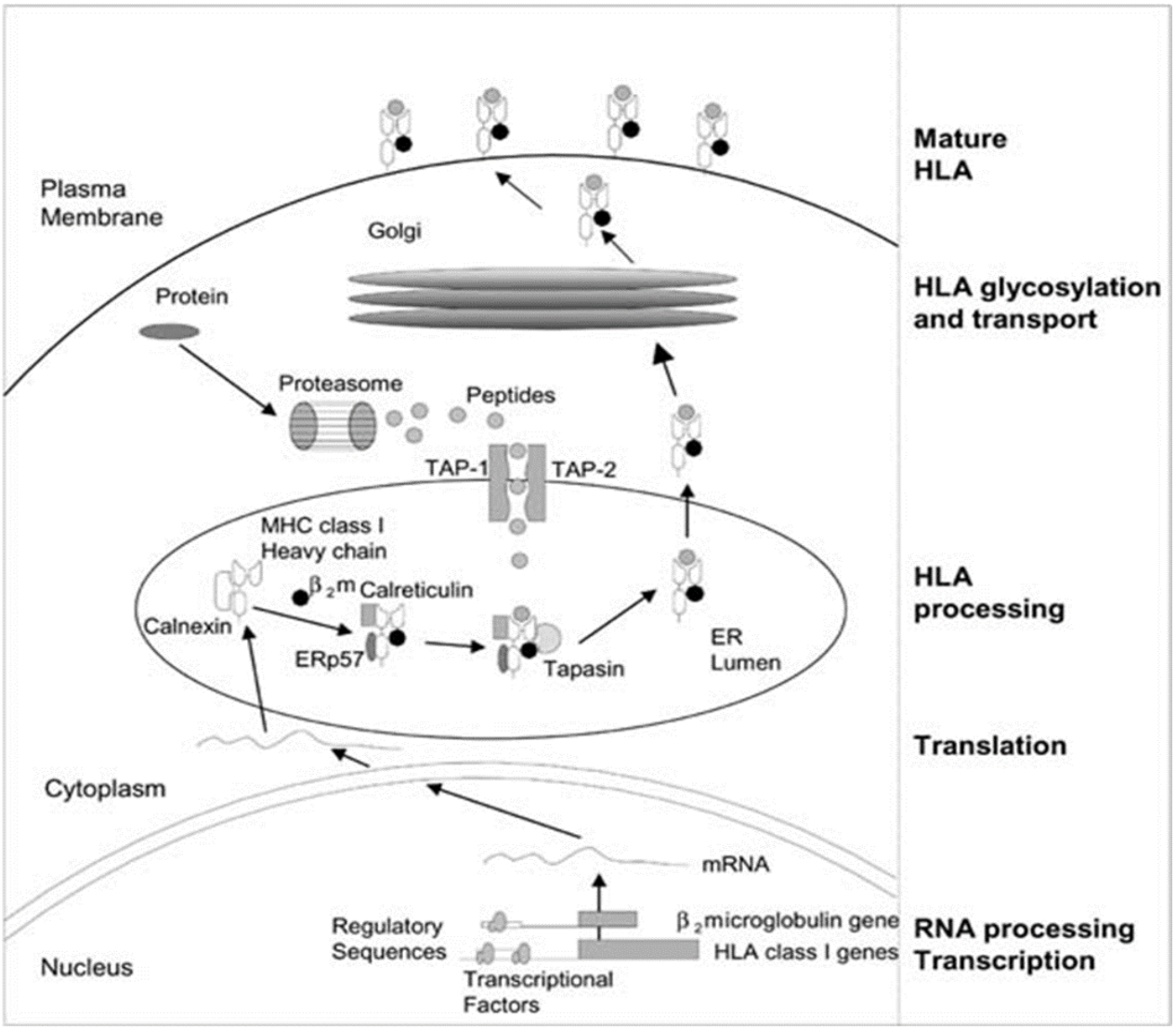


Fig 3. Peptide-MHC molecule binding processing, the rate-limiting step of antigen presentation

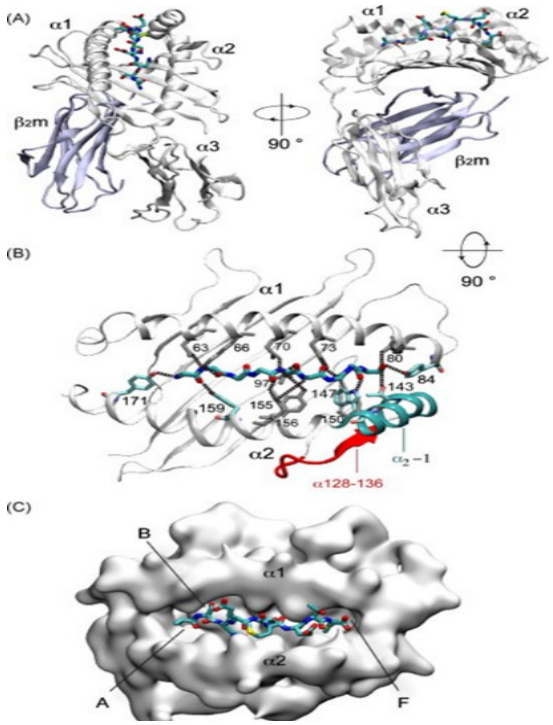


Fig 4. MHC class I (HLA) play a key role in the immune system and autoimmunity:
(A) shows H-2D^b (α chain in grey, β_2m chain in blue) in complex with ASNENMETM an epitope restricted to (blue stick model, pdb 1HOC) Heavy chain α_1, α_2 and α_3 with small subunit β_2m (micro globulin).

(B) View from the top into the class I peptide binding groove. Conserved side chain residues that form hydrogen bonds with main chain atoms of the bound peptide are blue, polymorphic side chains are gray. Hydrogen bonds are indicated by dotted lines. The tapasin binding region α128-136 is in red, and the α_2-1 helix is in cyan.

(C) The main class I pockets A, B, and F are shown
The main anchor residues for class I are often the second and the last side chain from the N-terminus of the peptide, which fit into the B and F pockets. The α_1 and α_2 domains of the heavy chain form two a-helices resting on a sheet of eight β-strands, forming a groove were peptides are brought to rest

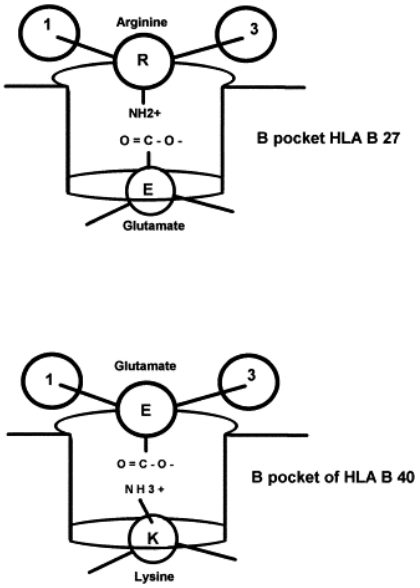


Fig 5. Potential pockets in HLA groove