Reconstitution of Class I MHC Molecules Expressed in *E. coli* and Complexed with Single Antigenic Peptides

The HLA-Cw3 heavy chain has been expressed at high level as insoluble protein aggregates in *E. coli*. The protein aggregates dissolved in strong denaturant solution were efficiently reconstituted by removal of denaturant in the presence of an HLA-Cw3 binding peptide (FAM) and β2m. The reconstituted HLA- Cw3/FAM protein binds specifically to a p58 natural killer cell inhibitory receptor, a natural ligand. The HLA-A2 molecule has also been reconstituted in complex with either of a peptide from myelin associated glycoprotein (MAG) or a peptide from the GAG protein of human immunodeficiency virus. The HLA-A2/MAG protein crystallized under the identical conditions as HLA-A2 purified from human lymphoblastoid cells. The reconstitution method has yielded an abundant supply of HLA molecules complexed with single antigenic peptides, and may be of general utility in reconstituting any class I MHC molecules. However, the HLA molecules could not be reconstituted either without a peptide or with an irrelevant peptide. Using this property, the reconstitution method could be used to determine whether a peptide is restricted/bound to certain class I MHC molecule. ***(JKMS 1997; 12: 280~5)***

Key Words : *MHC, HLA-A2, HLA-Cw3, Antigenic peptide, NKR, Expression, Refolding, Binding interaction*

Jongsun Kim, Mi-Yeon Kim, Yong Joon Chwae, Jeon Han Park

Department of Microbiology, Yonsei University College of Medicine, Seoul, Korea

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Address for correspondence
Jongsun Kim, Department of Microbiology, Yonsei University College of Medicine, Shinchon-dong, Seodaemoon-gu, Seoul 120-752, Korea
Tel : (02) 361-5277, Fax : (02) 392-7088

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INTRODUCTION

Class I major histocompatibility complex (MHC) molecules are polymorphic cell-surface glycoproteins that consist of two noncovalently associated polypeptide chains, heavy chain and light chain (1, 2). The heavy chain (44 kDa) is encoded within the MHC loci and spans the membrane bilayer (3, 4), while the light chain (12 kDa) is a non-MHC encoded protein called β2-microglobulin (β2m ; ref. 2, 5). In humans, there are three kinds of class I MHC molecules, human leukocyte antigen (HLA) -A, -B and -C (6). Like other conventional MHC molecules, class I MHC molecules present antigenic peptides to T lymphocytes. Presentation of antigenic peptides by class I MHC molecules on virus infected or transformed cell surfaces results in the activation of cytotoxic T lymphocytes and the subsequent lysis of target cells (7).

Like other class I MHC molecules, HLA-C molecules can present antigenic peptides to cytotoxic T lymphocytes, and can elicit alloreactivity (8~10). Recent studies have shown that HLA-C molecules also serve as a recognition element for natural killer cells (11, 12). However, in contrast to HLA-A and -B, HLA-C molecules are weakly expressed on the cell surface (13). This low level expression of HLA-C molecules (10% of HLA-A and -B) may be a result of poor assembly of the molecules (14, 15) or a defective enhancing element at the promoter region (16, 17). The low level expression of HLA-C molecules has hampered the characterization of these molecules and figuring out the functional roles in the immune system.

Here we report the high level expression of the HLA-Cw3 heavy chain in *E. coli* and the efficient reconstitution of the molecule in the presence of an HLA-Cw3 binding peptide. We have also reconstituted the HLA-A2 molecule in complex with either a peptide from myelin associated glycoprotein (MAG) or a peptide from the GAG protein of human immunodeficiency virus (HIV). The reconstitution method has yielded an abundant supply of HLA molecules complexed with single antigenic peptides. High level expression of HLA-A2 heavy chain and β2m in *E. coli* and the efficient refolding of HLA-A2 molecule in the presence of antigenic peptides have been described by Garboczi et al. (18). Recently, the HLA-Cw4 molecule has also been overexpressed in *E. coli* and refolded successfully by using a similar method (19). Our results support that the reconstitution method originally described by Garboczi et al. (18) is of general utility in refolding any class I MHC molecules. The overexpression of HLA molecules will accelerate biochemical, immunological and crystallo-

graphic studies that have been limited by the small amounts of protein available from homozygous human cell lines.

MATERIALS AND METHODS

Gene Sources and Bacterial Strains

The HLA-Cw3 heavy chain cDNA (HLA-Cw0304, kindly provided by S. Y. Yang, Sloan Kettering) was contained in the eukaryotic expression vector pcDNA3 (Invitrogen). The HLA-Cw3 heavy chain was subcloned into the expression vector pRSETA containing a T7 promoter (Invitrogen) by PCR and expressed in *E. coli*. pHN1+ plasmids containing the HLA-A2 and β2m sequence (kindly provided by D. N. Garboczi and D. C. Wiley, Harvard University) were used to overexpress the HLA-A2 heavy chain and β2m. The *E. coli* strain DH5a was used for DNA manipulation and the BL21 (DE3) plysS was used for HLA-Cw3 heavy chain expression. The *E. coli* strain XA90 F' lacI^q was used for expression of HLA-A2 heavy chain and β2m.

Peptide Synthesis

MAG peptide (amino acids 556-564 of myelin associated glycoprotein) and GAG peptide (amino acid 77~85 of HIV GAG protein) were kindly gifts from D. N. Garboczi and D. C. Wiley (Harvard University). YQF peptide (YQFTGIKKY) and FAM peptide (FAMPNFQTL) restricted to HLA-Cw6 and HLA-Cw3, respectively (20, 21), were synthesized from Bio-Synthesis, Inc. (Lewisville, TX).

Construction of Expression Plasmids

The protein coding region of the entire extracellular domain of HLA-Cw3 heavy chain (amino acids 1~275) was amplified by PCR using *Taq* DNA polymerase with the 5'-oligonucleotide primer ACCCCGCATATGGGC-TCCCACTCCATGAGGTAT and 3'-primer GTTTC-AAAGCTTTAGGTGAGGGGCTCCGGCAGCCC, containing the underlined *Nde*I and *Hind*III restriction sites, respectively. The 5'-primer encoded an N-terminal methionine and the first 7 amino acids of the protein. The stop codon TAA at codon 276 was included in the *Hind*III recognition sites. The amplified DNAs were gel-purified, digested with *Nde*I/*Hind*III, and ligated into pRSETA that had been digested with *Nde*I/*Hind*III and gel-purified. The recombinant DNAs were transformed into *E. coli* DH5α, and clones containing the correct insert were identified by agarose gel electrophoresis. The sequence of the construct was verified by DNA sequencing.

Protein Expression and Preparation of Inclusion Body Proteins

The plasmid (pHLA-sCw3) containing the extracellular domain of HLA-Cw3 heavy chain was transformed into *E. coli* BL21 (DE3) plysS for expression. Six liters of bacteria carrying expression plasmids were grown from a single colony at 37℃ in LB medium containing 100 μg/ml ampicillin and 0.1% glucose, and induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at OD_{600} of 0.6-0.8. Four hours after the induction, bacteria were harvested by centrifugation, and inclusion bodies (insoluble protein aggregates) were isolated as described by Nagai and Thogersen (22), with the modification of a freeze and thaw step after detergent treatment of the bacteria (23). Briefly, the lysate was centrifuged and the pellet was washed four times with the Triton solution (50 mM Tris, pH 8.0, 0.5% Triton X 100, 0.1 M NaCl, 1 mM EDTA) and one time with the Tris solution (50 mM Tris, pH 8.0, 1mM EDTA), and the inclusion body pellet was dissolved in 20 ml of Urea solution (50 mM 2-N-morpholinoethanesulfonic acid (MES, pH 6.5)/8 M urea/1 mM EDTA /1 mM DTT). Insoluble material was precipitated by centrifugation at 15,000 rpm for 30 min at room temperature, and the protein solution was stored at -70℃ in aliquots.

Reconstitution of HLA-Cw3 and HLA-A2 with Single Antigenic Peptides

Refolding and complex formation of the two denatured HLA-Cw3 subunits, together with an HLA-Cw3 specific peptide, FAM (FAMPNFQTL), was performed by a dilution method in the presence of a reduced/oxidized glutathione redox buffer (18). Inclusion body proteins of heavy and light chains of HLA-Cw3 and the antigenic peptide were diluted into 200 ml of 0.1 M Tris (pH 8.0)/0.4 M L-Arginine/2 mM EDTA/5 mM reduced glutathione/0.5 mM oxidized glutathione/0.5 mM phenylmethylsulfonyl fluoride (PMSF). The final concentrations of the heavy chain, β2m, and the peptide were 31 μg/ml (1 μM), 24 μg/ml (2 μM), and 10 μg/ml (10 μM), respectively. The refolding mixture was incubated at 10℃ for 48-72 hr. HLA-A2 was reconstituted by the same method in the presence of either the MAG peptide or GAG peptide.

Purification of Refolded Protein

The 200 ml of refolding mixture was concentrated with a Centriprep-30 and a Centricon-30 to a volume

of 0.5~1 ml. The concentrated protein was subjected to a Sephacryl S-200 gel-filtration column (Pharmacia) equilibrated in 20 mM Tris (pH 7.5). The fractions were collected in 1.5 ml Eppendorf tubes, and were analyzed by SDS PAGE under non-reducing conditions. The fractions containing the correctly folded heavy and light chains together were pooled and concentrated with a Centricon-30 to a volume of 250 μl.

Native Gel Shift Assay

The soluble p58 NK inhibitory receptor, NKR-K7 and HLA-Cw3 were mixed with equal molarities and incubated for 2~4 hr at 4℃. Protein concentration was determined by Bradford dye binding method. The reaction mixture was then assayed on a 15% nondenaturing acrylamide gel in 0.3 M Tris (pH 8.5). Protein samples were neither heated nor reduced. The native gel running buffer contained 24.8 mM Tris/192 mM glycine.

RESULTS

HLA-Cw3 heavy chain expression and purification

The HLA-Cw3 heavy chain (HLA-Cw0304) was expressed in *E. coli*. To create polypeptide of the extracellular domain (31 kDa) of the HLA-Cw3 heavy chain, translation stop codons were placed at amino acid residue 276 using PCR. The polypeptide was expressed at high level in *E. coli* (Fig. 1), and appeared from preliminary experiments to be insoluble. The inclusion body protein was purified by taking advantage of its insolubility in aqueous and detergent solution. Lysing the bacterial cells by freeze and thaw method with lysozyme and detergents and washing the inclusion bodies several times with Triton solution yielded highly purified proteins up to 20~30 mg per liter of bacterial culture. The purified inclusion body protein was over 80% pure as determined on SDS gels (Fig. 1).

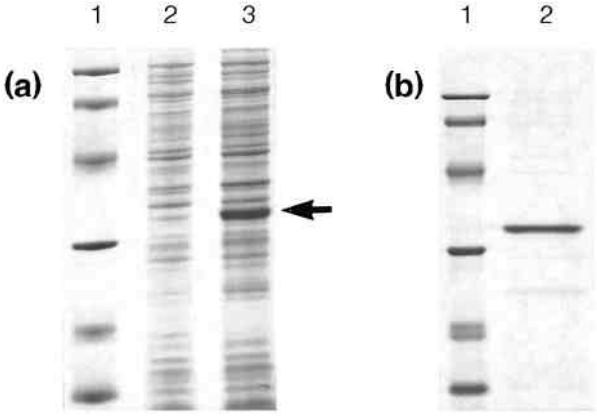

Fig. 1. (a) SDS PAGE of the expressed HLA-Cw3 heavy chain in *E. coli*. Bacterial cells were lysed with the SDS loading buffer, and the samples were loaded on a SDS gel. Lanes : 1, size marker proteins (97, 66, 45, 31, 21.5 and 14.5 kDa from the top); 2, lysate from cells with plasmid expressing amino acids 1-275 of the heavy chain, not induced; 3, lysate from cells with plasmid expressing amino acids 1-275, induced by 0.5 mM IPTG. (b) SDS PAGE of purified inclusion body protein of HLA-Cw3 heavy chain. Lanes : 1, size marker proteins ; 2, inclusion body protein of HLA-Cw3 heavy chain. Gels were stained with Coomassie blue R-250.

Reconstitution of class I MHC molecules with single antigenic peptides

The extracellular domain of the HLA-Cw3 heavy chain (amino acids 1~275) and β2m were reconstituted by a dilution method (18) in the presence of an HLA-Cw3 binding peptide, FAM. The refolded protein was purified on a gel-filtration column. The typical gel-filtration chromatography profile exhibits three major peaks. The first peak was eluted at the excluded volume of the column (fraction numbers 3~7 in Fig. 2) and the second peak was eluted with a retention volume corresponding to 40 kDa (fraction numbers 10~12). The third peak was eluted with a retention volume corresponding to 15 kDa (fraction numbers 18~20). SDS PAGE analysis of the peak fractions (Fig. 2) reveals that the first peak consists of aggregated heavy chain, the second peak consists of correctly folded heavy chain and β2m, and the third peak consists of correctly folded β2m. HLA-Cw3 was reconstituted with a final efficiency of ~10% in the presence of an HLA-Cw3 binding peptide, but was not reconstituted at all either without a peptide or with

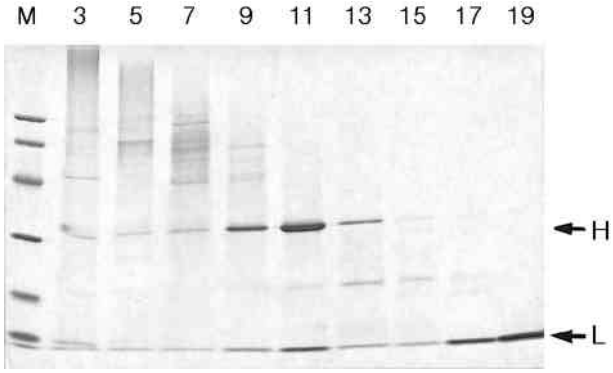

Fig. 2. Nonreducing SDS PAGE of fractions from Sephacryl S-200 gel-filtration chromatography of HLA-Cw3 reconstitution mixture. Lanes : M, size marker proteins (same as fig. 1) ; 3-19, fraction number. Fractions were collected in 1.5 ml Eppendorf tubes, and the gel was stained with Coomassie blue R-250. The heavy and light chains of HLA-Cw3 are marked H and L, respectively.

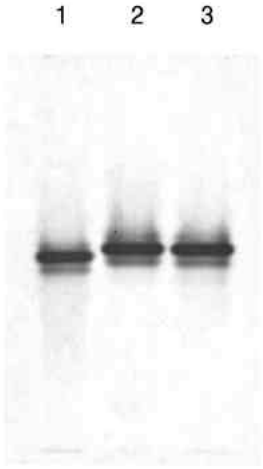

Fig. 3. Nondenaturing PAGE of reconstituted class I MHC molecules. Lanes : 1, HLA-Cw3/FAM ; 2, HLA- A2/MAG ; 3, HLA-A2/GAG. Proteins were detected with Coomassie blue R-250.

an irrelevant peptide.

HLA-A2 was also reconstituted by the same method in the presence of the MAG peptide and GAG peptide, respectively. The gel-filtration chromatography profile of HLA-A2 was similar to that of HLA-Cw3. Interestingly, however, the HLA-A2 reconstitution was more efficient than that of HLA-Cw3. The final yield of HLA-A2 reconstitution was 15~20%.

Characterization of reconstituted class I MHC molecules

The reconstituted and purified class I MHC molecules were analyzed by non-denaturing PAGE (Fig. 3) and SDS PAGE (Fig. 4a, b, c) under reducing and non-reducing conditions. The reconstituted MHC molecules behaved like a normal globular protein and appeared as one band on a native gel and two bands (heavy chain and β 2m) on a SDS gel with appropriate mobility. The MHC molecules were very soluble and stable for at least six months at 4℃, while the HLA molecules reconstituted without peptide or with irrelevant peptide were not stable and disappeared when the molecules were collected and concentrated.

Crystallization of HLA-A2 : MAG peptide complex

Vapor diffusion crystallization experiments using hanging drops were done with HLA molecules, and the reconstituted HLA-A2 : MAG peptide complex crystallized under the buffer conditions of 0.1 M MES (pH 6.5)/15% polyethylene glycol (PEG) 8,000, indicating that the HLA-A2 protein is correctly folded. The crystallization condition is identical to the previously reported one for HLA-A2 from homozygous human cell lines (24). Thin, overlapping plate crystals were grown within 2~3 days, with the occasional formation of single crystals. SDS PAGE analysis of the washed crystals revealed that the heavy chain and β 2m are present in the crystals (data not shown).

Native gel shift assay

Earlier studies indicate that the p58 natural killer cell inhibitory receptor (NKR) is a natural ligand for HLA-C molecules (25). To verify that the reconstituted HLA-Cw3 molecule has native conformation, we decided to check whether it can form a complex with p58 NKR. p58 NKR was expressed in *E. coli* and refolded by a dilution method in the presence of a reduced/oxidized glutathione redox buffer (Kim et al., manuscript in preparation). The direct binding interaction between the HLA-Cw3 and a soluble form of p58 NKR was observed using native gel shift assay, as had been successfully used to detect the direct binding interaction

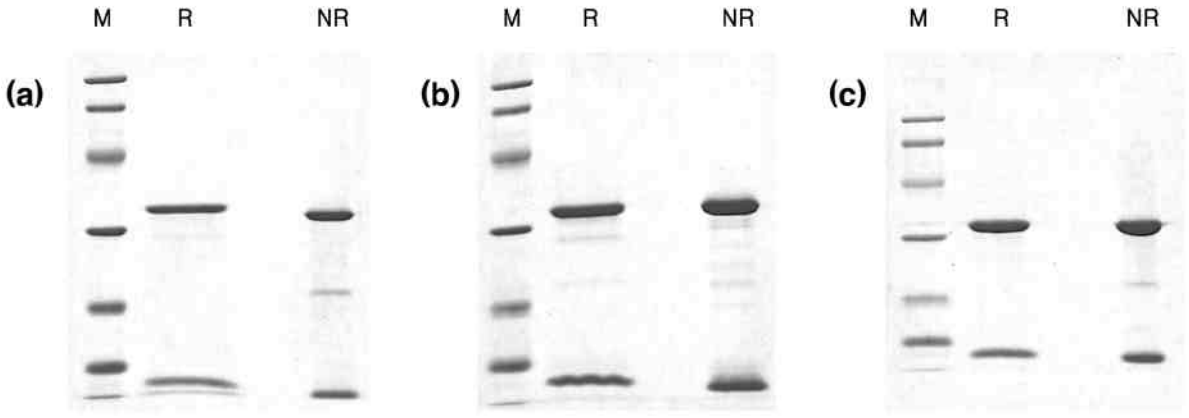

Fig. 4. SDS PAGE of reconstituted class I MHC molecules under reducing and nonreducing conditions. (a) HLA-Cw3/FAM, (b) HLA-A2/MAG and (c) HLA-A2/GAG. Lanes : M, size marker proteins (same as fig. 1) ; R, reducing conditions ; NR, nonreducing conditions.

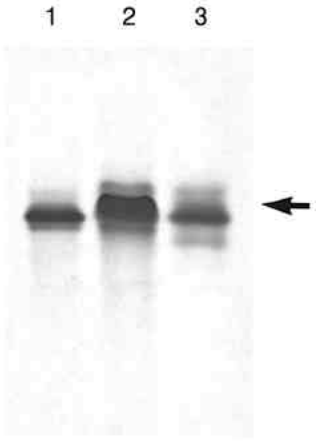

Fig. 5. Native gel shift assay reveals that the reconstituted HLA-Cw3 molecule forms a complex with the p58 NKR-K7. Lanes: 1, HLA-Cw3/FAM ; 2, p58 NKR-K7 + HLA-Cw3/FAM ; 3, p58 NKR-K7. The shifted complex band is marked with an arrow.

of the HLA-A2/TAX and a T-cell antigen receptor (TCR), and of superantigen and a TCR β-chain (23, 26). The HLA-Cw3 formed a complex with p58 NKR-K7 and the complex appeared as a prominent shifted band that migrated at a position, distinct from either HLA-Cw3 or NKR-K7 alone (Fig. 5). When the HLA-Cw3 and p58 NKR-K7 were mixed at a 1:1 molar ratio, the HLA-Cw3 and NKR-K7 protein were completely shifted into the complex band, indicating that the binding stoichiometry is 1:1. However, no shifted band appeared when the p58 NKR-K7 was mixed with HLA-A2/MAG or HLA-A2/GAG (data not shown), indicating that the interaction between the HLA-Cw3 and p58 NKR-K7 is highly specific.

DISCUSSION

We have overexpressed the HLA-Cw3 heavy chain in *E. coli* as insoluble protein aggregates (inclusion bodies). After dissolving the inclusion body proteins in strong denaturants (8 M urea), we have been able to reconstitute the HLA-Cw3 heavy chain and β2m under dilute conditions by the removal of denaturant in the presence of an HLA-Cw3 binding peptide. We have also reconstituted the HLA-A2 heavy chain and β2m in the presence of the MAG peptide and GAG peptide restricted to HLA-A2. The reconstituted HLA proteins appear as a sharp peak on gel-filtration chromatography at an elution volume consistent with the expected molecular weight of the complex. Analysis of the complex by SDS PAGE reveals that it is composed of the HLA heavy chain and β2m. The reconstituted HLA proteins appear as one band on a nondenaturing polyacrylamide gel and SDS gel under reducing and nonreducing conditions indicating that the HLA heavy chain and β2m are correctly refolded and reconstituted. The reconstituted HLA proteins are very soluble and stable for at least 6 months at 4℃.

The reconstitution method has yielded an abundant supply of HLA molecules complexed with single antigenic peptides. Interestingly, however, the reconstitution of HLA-Cw3 molecule was less efficient (about 2 fold) than that of HLA-A2. This may provide a possible explanation why the expression level of HLA-C molecules is much lower than that of HLA-A or -B molecules on the cell surface (13). However, since the expression level of HLA-C on the cell surface is about 10% of HLA-A or -B, poor assembly of HLA-C molecules observed in this study does not completely account for the weak expression of HLA-C molecules. The defective enhancing element found at the 5' promoter of the HLA-C genes (16, 17) could be a more important factor for the low expression level of HLA-C molecules on the cell surface.

The reconstitution method, originally described by Garboczi et al. (18), has been successfully used to reconstitute the HLA-A2, HLA-Aw68 and HLA-B27 (18, 27, 28). We have also used the same method to reconstitute the HLA-Cw3 with the FAM peptide and the HLA-A2 with the MAG peptide and GAG peptide. These results suggest that the reconstitution method may be of general utility in refolding any class I MHC molecules.

HLA-A2 and -Cw3 molecules can be successfully reconstituted in the presence of correct peptides. However, the HLA molecules can not be reconstituted either without a peptide or with an irrelevant peptide. Garboczi et al. found that the HLA complexes formed without peptide are not stable *in vitro* (18). Such presumed "empty" HLA molecules appear early in a time course of refolding, but disappear by 24 hr when the complexes are collected and concentrated. These findings are reminiscent of the antigen presenting mechanism to the class I MHC molecules *in vivo*. Class I MHC molecules are complexed with calnexin in the cell before they are loaded with antigenic peptides (29). Therefore, it is possible to speculate that the calnexin is introduced to stabilize the short-lived "empty" HLA molecules in the cell.

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REFERENCES

1. Cresswell P, Turner MJ, Strominger JL. *Papain-solubilized HLA antigens from cultured human lymphocytes contain two peptide fragments. Proc Natl Acad Sci USA 1973; 70:*

1603-07.

2. Peterson PA, Rask L, Lindblom JB. *Highly purified papain-solubilized HLA antigens contain β2-microglobulin. Proc Natl Acad Sci USA 1974; 71 : 35-9.*
3. Orr HT, Lopez de Castro JA, Parham P, Ploegh HL, Strominger JL. *Comparison of amino acid sequences of two human histocompatibility antigens, HLA-A2 and ALA-B7: Location of putative alloantigenic sites. Proc Natl Acad Sci USA 1979; 76 : 5839-42.*
4. Malissen M, Malissen B, Jordan BR. *Exon/intron organization and complete nucleotide sequence of an HLA gene. Proc Natl Acad Sci USA 1982; 79 : 893-7.*
5. Grey HM, Kubo RT, Colon SM, Poulik MD, Cresswell P, Springer T, Turner M, Strominger JL. *The small subunit of HLA-A antigens is β2-microbulin. J Exp Med 1973; 138 : 1608-12.*
6. Klein J. *Natural History of the Major Histocompatibility Complex, J. Wiley & Sons, New York. 1986.*
7. Townsend A, Bodmer H. *Antigen recognition by class I-restricted T lymphocytes. Annu Rev Immunol 1989; 7 : 601-24.*
8. Schendel DJ, Reinhardt C, Nelson PJ, Maget B, Pullen L, Bornkamm GW, Steinle A. *Cytotoxic T lymphocytes show HLA-C restricted recognition of EBV-bearing cells and allorecognition of class I molecules presenting self-peptides. J Immunol 1992; 149 : 2406-14.*
9. Johnson RP, Trocha A, Buchanan TM, Walker BD. *Recognition of a highly conserved region of human immunodeficiency virus Type 1 gp120 by an HLA-Cw4 restricted cytotoxic T-lymphocyte clone. J Virol 1993; 67 : 438-45.*
10. Littaua RA, Oldstone MB, Takeda A, Debouck C, Wong JT, Tuazon CU, Moss B, Kievits F, Ennis FA. *An HLA-C restricted CD8 cytotoxic T-lymphocyte clone recognizes a highly conserved epitope on human immunodeficiency virus Type 1 gag. J Virol 1991; 65 : 4051-6.*
11. Ciccone E, Pende D, Viale O, Than A, Di Donato C, Orengo AM, Biassoni R, Verdinai S, Amoroso A, Moretta A. *Involvement of HLA class I alleles in natural killer specific functions. J Exp Med 1992; 176 : 963-71.*
12. Colonna M, Brooks EG, Falco M, Ferrara GB, Strominger JL. *Generation of allospecific natural killer cells by stimulation across a polymorphism of HLA-C. Science 1993; 260 : 1121-4.*
13. Snary D, Barnstable CJ, Bodmer WF, Crumpton MJ. *Molecular structure of human histocompatibility antigens: the HLA-C series. Eur J Immunol 1977; 8 : 580-5.*
14. Gussow D, Rein RS, Meijer I, de Hoog W, Seemann GH, Hochstenbach FM, Ploegh HL. *Isolation, expression, and the primary structure of HLA-Cw1 and HLA-Cw2 genes: evolutionary aspects. Immunogenetics 1987; 25 : 313-22.*
15. Neefjes JJ, Ploegh HL. *Allele and locus-specific differences in cell surface expression and the association of HLA class I heavy chain with β2-microglobulin. Eur J Immunol 1988; 18 : 801-10.*
16. Cereb N, Yang SY. *The regulatory complex of HLA class I promotors exhibits locus-specific conservation with limited allelic variation. J Immunol 1994; 152 : 3873-83.*
17. Park JH, Kim JD, Kim SJ. *Nuclear protein binding patterns in the 5'-upstream regulatory elements of HLA class I genes. Yonsei Med J 1994; 35 : 295-307.*
18. Garboczi DN, Hung DT, Wiley DC. *HLA-A2-peptide complexes : Refolding and crystallization of molecules expressed in E. coli and complexed with single antigenic peptides. Proc Natl Acad Sci USA 1992; 89 : 3429-33.*
19. Fan QR, Garboczi DN, Winter CC, Wagtmann N, Long EO, Wiley DC. *Direct binding of a soluble natural killer cell inhibitory receptor to a soluble human leukocyte antigen-Cw4 class I MHC molecule. Proc Natl Acad Sci USA 1996; 93 : 7178-83.*
20. Falk K, Rotzschke O, Grahovac B, Schendel D, Stevanovic S, Gnau V, Jung G, Strominger JL, Rammensee HG. *Allele-specific peptide ligand motifs of HLA-C molecules. Proc Natl Acad Sci USA 1993; 90 : 12005-9.*
21. Sidney J, del Guercio MF, Southwood S, Engelhard VH, Appella E, Rammensee HG, Falk K, Rotzschke O, Takiguchi M, Kubo RT, Grey HM, Sette A. *Several HLA alleles share overlapping peptide specificities. J Immunol 1995; 154 : 247-59.*
22. Nagai K, Thogersen HC. *Synthesis and sequence-specific proteolysis of hybrid proteins produced in Escherichia coli. Methods Enzymol 1987; 153 : 461-81.*
23. Garboczi DN, Utz U, Ghosh P, Seth A, Kim J, van Tienhoven E, Biddison WE, Wiley DC. *Assembly, specific binding, and crystallization of a human TCR αβ with an antigenic Tax peptide from human T lymphotropic virus Type 1 and the class I MHC molecule HLA-A2. J Immunol 1996; 157 : 5403-10.*
24. Bjorkman PJ, Strominger JL, Wiley DC. *Crystallization and x-ray diffraction studies on the histocompatibility antigens HLA-A2 and HLA-A28 from human cell membranes. J Mol Biol 1985; 186 : 205-10.*
25. Moretta A, Vitale M, Bottino C, Orengo AM, Morelli L, Augugliaro R, Barbaresi M, Ciccone E, Moretta L. *P58 molecules as putative receptors for MHC class I molecules in human natural killer cells. J Exp Med 1993; 178 : 597-604.*
26. Kim J, van Tienhoven E, Garboczi DN, Utz U, Kim MY, Wiley DC. *Specific binding of a soluble recombinant β-chain T-cell antigen receptor to toxic shock syndrome toxin-1, a superantigen. Mol Cells 1996; 6 : 590-6.*
27. Madden DR, Gorga JC, Strominger JL, Wiley DC. *The three-dimensional structure of HLA-B27 at 2.1 A resolution suggests a general mechanism for tight peptide binding to MHC. Cell 1992; 70 : 1035-48.*
28. Silver ML, Parker KC, Wiley DC. *Reconstitution by MHC-restricted peptides of HLA-A2 heavy chain with β2-microglobulin, in vitro. Nature 1991; 350 : 619-22.*
29. Jackson MR, Cohen-Doyle MF, Peterson PA, Williams DB. *Regulation of MHC class I transport by the molecular chaperone, Calnexin (p88, IP90). Science 1994; 263 : 384-7.*