# Differentiation-stage Specific Self-Peptides Bound by Major Histocompatibility Complex Class I Molecules

By Paul E. Harris, Floria Lupu, Bian Hong, Elaine F. Reed; and Nicole Suciu-Foca

*From the College of Physicians and Surgeons of Columbia University, Department of Pathology, New York 10032*

## Summary

We have tested the hypothesis that phenotypic changes of development are accompanied by expression of differentiation-stage specific peptides bound to major histocompatibility complex (MHC) class I molecules. The U937 cell line, when cultured in the presence of phorbol myristate acetate (PMA), undergoes differentiation from monoblasts to macrophage-like cells. The high-performance liquid chromatography profile of peptides eluted from purified human histocompatibility leukocyte antigen class I molecules expressed by U937 treated with PMA differs from that obtained from control, untreated U937 cells. Chemical sequencing of eluted peptides identified a peptide derived from cytomegalovirus in both treated and untreated cells. PMA-treated, but not untreated cells, displayed an additional peptide derived from interleukin 1$\beta$. Hence, differentiation-induction of U937 is accompanied by the presentation of at least one differentiation-stage specific peptide. Our results indicate that, similar to viral infection, cellular development and transformation is accompanied by the de novo synthesis of proteins which are processed and presented on MHC class I molecules.

MHC class I molecules present peptides, derived from the processing of intracellular proteins, which are surveyed by CTLs. Under normal conditions, endogenous self peptides presented by MHC class I molecules do not elicit CTL activation (1, 2).

Self peptides bound to MHC class I molecules have been partially characterized and have the following common features: (*a*) they are 8–9 amino acids in length (3, 4); (*b*) they have allele-specific structural motifs (5); and (*c*) although extremely heterogeneous, they derive predominantly from a small set of abundantly expressed intracellular proteins (3, 4). After viral infection, and perhaps transformation, newly synthesized viral and cellular proteins are degraded into peptides in the cytosol, and transported across membranes, into a compartment where they become bound to MHC class I molecules. These complexes are transported to the surface, rendering the cell susceptible to recognition and lysis by specific CTLs (6).

Characterization of peptides eluted from MHC class I molecules, after infection by vesicular stomatitis or influenza virus, has demonstrated that viral protein–derived peptides become bound to class I as viral replication takes over the normal cellular protein biosynthesis (7, 8). Changes in MHC class I–bound peptides which might occur during cellular differentiation and/or transformation, however, have not yet been characterized. The identification of MHC class I–bound peptides associated with specific phases of cellular differentiation and/or transformation would not only further our understanding of the antigenic nature of self, but is also a step towards identifying possible transformation-specific antigens.

To establish whether the set of peptides bound to MHC class I molecules at different stages of cellular differentiation differs, we have used the human monocytic leukemia cell line U937 (9). The phenotype of U937 resembles that of monoblasts. This line is able to form solid tumors in nude mice (10). A unique characteristic of U937 is its reversible maturation block. Culture of these cells in the presence of PMA induces a wide variety of phenotypic changes (11). These changes include the loss of proliferative potential and the acquisition of many effector functions that are characteristic for activated macrophages, such as antibody-dependent cellular cytotoxicity, respiratory burst, and augmented release of IL-1. Because of the phenotypic difference between PMA-treated and untreated U937, this cell line provides an ideal model for studying changes in MHC class I–bound peptides occurring during differentiation. We have purified HLA class I bound–peptides from U937 cells at two different stages of differentiation, i.e., as monoblasts and as PMA-induced macrophage-like cells. The HPLC signatures of peptides eluted from class I molecules obtained from untreated and PMA-treated cells were different. Sequential Edman degradation of peptidic material contained within HPLC peaks yielded two sequences that matched to CMV and IL-1$\beta$ precursor proteins, respectively.

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## Materials and Methods

*Cell Lines and Culture Conditions.* The U937 cell line was obtained from the American Type Culture Collection ([ATCC] Rockville, MD). Cells were cultured in roller bottles using RPMI 1640 media supplemented with 10% FCS, 1% glutamine, and 0.5% gentamycin (all from Gibco, Grand Island, NY), at an initial density of $0.5 \times 10^6$ cells/ml. U937 cells were differentiation induced for 96 h in fresh culture media containing 2 ng/ml PMA (Sigma Immunochemicals, St. Louis, MO). Cell viability after differentiation-induction was >95%. The murine hybridoma producing the mAb W6/32 (12) was obtained from ATCC and cultured in IgG-free media.

*HLA Class I Typing.* The HLA class I phenotype of U937 cells was determined serologically by complement-dependent cytotoxicity using a panel of alloantisera and mAbs obtained from One Lambda, Inc. (Canoga Park, CA) as previously described (13).

*Immunofluorescence Cytofluorometry.* The expression of A and B locus molecules was determined, as previously described, by immunofluorescence flow cytometry (14, 15) using mAbs specific for HLA-A3 (GAP.A3) and HLA-Bw6 (SFR8.B6), both obtained from ATCC. The expression of HLA-B18 was ascertained using the HLA-B18 specific alloantiserum (COL. 5233) (14).

*Reagents.* The mAb W6/32 was obtained from culture supernatants and purified by protein A affinity chromatography with reagents supplied by Bio-Rad (Richmond, CA). Cyanogen bromide-activated Sepharose-4B, purified normal mouse IgG, aprotinin, tosyl lysyl chloromethyl ketone (TLCK),[1] tosyl prolyl chloromethyl ketone (TPCK), and iodoacetamide were obtained from Sigma Immunochemicals, pepstatin A, leupeptin, and PMSF were obtained from Boehringer Mannheim (San Diego, CA). All HPLC solvents, TFA, NP-40, and the bicinchonnic acid (BCA) protein assay kit were obtained from Pierce (Rockford, IL). Tran $^{35}$S-label, 1,000 Ci/mmol, and [$^3$H]TdR was obtained from ICN Biochemicals, Inc. (Costa Mesa, CA). All other reagents were of the highest commercially available quality.

*Assays of Differentiation Induction.* Biochemical assays for the expression of lysozyme, acid and alkaline phosphatase, and quantitation of [$^3$H]TdR incorporation for measuring proliferation, were performed as previously described (16, 17).

*Immunoprecipitation Analysis of Class I Expression.* U937 cells ($5.0 \times 10^6$) were cultured in the presence or absence of PMA (2 ng/ml) for 96 h. During the last 12 h of culture, the complete RPMI 1640 medium was replaced with methionine-free RPMI 1640 supplemented with 5% dialyzed FCS and 100 $\mu$Ci [$^{35}$S]methionine–[$^{35}$S]cysteine (Tran $^{35}$S-label). $^{125}$I cell surface labeling was performed as previously described (18). Cells ($5 \times 10^6$) were harvested, washed once in PBS, and lysed in 5 ml of cold lysis buffer (10 mM Tris, pH 7.8, 150 mM NaCl, 1.0% (wt/vol) NP-40, 2 mM EDTA, 2 mM PMSF). All subsequent steps were carried out at 4°C. The lysates were incubated for 15 min, then centrifuged for 5 min at 15,000 *g*. The lysates were precleared for 1 h each in ethanolamine–Sepharose-4B, followed by normal mouse IgG coupled to Sepharose-4B. HLA Class I molecules were immunoprecipitated from the lysate for 1 h using an excess (60 $\mu$g) of W6/32 mAb coupled to Sepharose-4B. The immunoprecipitate was washed in 30 vol lysis buffer, 30 vol lysis buffer supplemented to 500 mM NaCl, 30 vol lysis buffer, and 20 vol 10 mM Tris-HCl, pH 7.8. Class I molecules were eluted from mAb–Sepharose by incubation for 5 min at 95°C in Laemmli sample buffer with $\beta$-ME. The samples were then analyzed by SDS-PAGE on a 17.5% gel. The gel was dried and an autoradiogram prepared after overnight exposure at −70°C.

*Immunoaffinity Purification of Class I Molecules and Elution of Bound Peptides.* U937 cells were cultured to a final yield of $10^{10}$ cells in the presence or absence of PMA (2 ng/ml). PMA-treated cells became adherent to the roller bottle surface and were harvested in cold PBS containing 2 mM PMSF by gentle scraping. Cells were pelleted and lysed in 150 ml lysis buffer containing the following: 2 mM PMSF, 5 $\mu$g/ml aprotinin, 1 $\mu$M pepstatin, 1 $\mu$M leupeptin, 20 $\mu$g/ml TPCK, 20 $\mu$g/ml TLCK. All subsequent steps were performed at 4°C. Lysates were incubated on ice for 30 min, centrifuged for 1 h at 28,000 *g*, and filtered through a 0.22-$\mu$ nylon filter. The filtered lysates were incubated with 10 ml ethanolamine–Sepharose-4B for 2 h, and then with 5 ml normal mouse IgG (30 mg) coupled to Sepharose-4B for 2 h. The precleared lysate was diluted to 200 ml with lysis buffer and incubated with W6/32 (25 mg) coupled to Sepharose-4B for 6 h. The immunoprecipitate was washed in 40 vol lysis buffer supplemented to 500 mM NaCl, 40 vol lysis buffer, and 20 vol 10 mM Tris-HCl, 0.5% octylglucoside, pH 7.8. HLA class I molecules were eluted from mAb–Sepharose by a method adapted from Falk et al. (5), in three 5-ml washes, 15 min each, with 0.1% TFA. The washes were pooled and lyophilized. The lyophilized material was resuspended in 1 ml of an aqueous solution of 0.1% TFA (vol/vol) and the protein concentration determined by BCA assay. Aliquots containing 1 $\mu$g of protein were analyzed by SDS-PAGE and silver staining. The final yields of class I molecules were 212 and 184 $\mu$g, from the untreated and PMA-treated cells, respectively. Equal masses of protein (180 $\mu$g) from each of the eluates were incubated for 3 min at 100°C, then centrifuged through a 10,000-dalton ultrafiltration cartridge (Centricon 10; Amicon, Danvers, MA). The low molecular weight (<10,000 dalton) fraction was lyophilized and stored at −70°C.

*HPLC Purification and Sequencing of Eluted Peptides.* The peptide fraction was analyzed by reverse-phase HPLC using a C18 column (Vydac, Hesperia, CA; 2.1 × 250 mm) and Waters Associates (Waltham, MA) equipment. The gradient was created using a solution (A) of $H_2O$/0.1% TFA in combination with solution (B) of acetonitrile/0.1% TFA at a flow rate of 0.250 ml/min. The low molecular weight fraction was reconstituted in solution A and applied to the column. 1-min fractions were collected from a linear 1-h gradient running from 5–60% solution B. From the absorbance trace at 215 nm of the eluate, the single fractions containing a given peak were selected for microsequencing by $NH_2$-terminal Edman degradation using a protein sequencer (model 477A; Applied Biosystems, Inc., Foster City, CA).

*PCR Analysis of IL-1$\beta$ Gene Expression and CMV Infection.* Expression of IL-1$\beta$ was determined by reverse PCR. Total RNA was obtained from PMA treated (as described above) and untreated U937 cells and reverse-transcribed into cDNA. The cDNAs were analyzed for the presence of $\beta$-actin and IL-1$\beta$ message using specific primers in the presence of [$^{32}$P]dCTP in a PCR using the conditions described by the manufacturer (Clontech, Palo Alto). Amplification products were analyzed by agarose gel electrophoresis and autoradiography. The incorporated cpm were determined by scintillation counting. PCR analysis of CMV immediate early (IE) and late antigen (LA) gene products were performed as previously described (19).

[1] *Abbreviations used in this paper:* BCA, bicinchonnic acid; ER, endoplasmic reticulum; IE, immediate early; LA, late antigen; MCF, mean channel fluorescence; PTH, phenylthiohydantoin; TLCK, tosyl lysyl chloromethylketone; TPCK, tosyl prolyl chloromethylketone.

## Results

*Differentiation-Induction of U937 Cells.* The phenotype of uninduced U937 cells resembles monoblasts. U937 cells grow in suspension, express low levels of Fc receptor and chemotactic peptide receptor, and lack or express low amounts of many of the lysosomal enzymes present in monocytes and macrophages. After incubation with PMA, and a variety of other inducing agents, U937 cells are induced towards macrophage-like differentiation which is characterized by adherence, loss of proliferative potential, upregulated or de novo expression of a variety of cell surface antigens, and the acquisition of microbiocidal activity (11). To document that PMA treatment of U937 cells induces differentiation, we first assayed the proliferative potential of PMA-treated and untreated cells by [$^3$H]TdR incorporation. PMA-treated U937 cells incorporated <1% of the amount of [$^3$H]TdR incorporated by untreated cells, demonstrating inhibition of proliferation. Determination of the levels of three lysosomal enzymes showed that PMA treatment induced increased expression of acid phosphatase, alkaline phosphatase, and lysozyme, all consistent with a macrophage-like phenotype (16) (Table 1).

*Expression of HLA Class I Molecules after Differentiation-Induction.* Malignant transformation is often accompanied by changes in the level of MHC class I expression (20). Such changes may result in quantitative and/or qualitative differences between peptides bound to the MHC molecules expressed by normal and malignant cells. Hence, we determined whether differentiation-induction of U937 cells affects the expression of class I molecules. We first compared the total amounts of class I molecules in PMA-treated and untreated cells by metabolic labeling and immunoprecipitation of HLA class I molecules with mAb W6/32. The mAb W6/32 recognizes all mature HLA-A, B, and C heavy chain-$\beta_2$-microglobulin heterodimers (21). We found that the total amount of class I expressed did not change after differentiation induction by PMA treatment (Fig. 1). This observation was corroborated by cell surface labeling of $10^6$ cells with $^{125}$I and immunoprecipitation of HLA class I molecules with mAb W6/32. Bands of equal intensity were obtained from PMA-treated and untreated cells (data not shown).

**Table 1.** *PMA-induced Differentiation of U937 Cells*

| | Control | PMA (2 ng/ml) |
|---|---|---|
| Adherence* | <5% | >90% |
| [$^3$H]TdR incorporation | 98,631 cpm‡ | 271 cpm |
| Acid phosphatase | 3.0 U§ | 8.0 U |
| Alkaline phosphatase | 24.0 U | 60.0 U |
| Lysozyme | 5.2 U | 11.2 U |

* U937 cells normally grow in suspension. After differentiation induction cells become adherent to substratum.
‡ Mean cpm, 5 × $10^4$ cells from triplicate cultures.
§ 1 U equals Δ absorbance × $10^3$/5 min/$10^5$ cells.

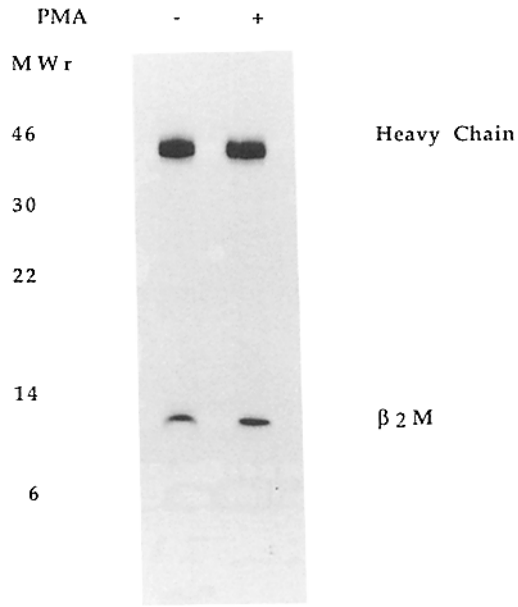


**Figure 1.** Expression of HLA class I molecules by differentiation-induced and uninduced U937 cells. PMA-treated and untreated U937 cells were metabolically labeled with [$^{35}$S]methionine. Detergent lysates of labeled cells were immunoprecipitated with mAb W6/32. The immunoprecipitate was analyzed by SDS-PAGE on a 17.5% acrylamide gel and prepared for autoradiography. The autoradiogram is from an overnight exposure at −70°C.

To determine whether the relative amounts of HLA-A and HLA-B locus proteins change after differentiation-induction, we analyzed the level of expression of the individual HLA-A and -B antigens by immunofluorescence flow cytometry. The HLA class I phenotype of U937 is HLA-A3, B5(51), B18, Cw1, Cw7, Bw4, Bw6. Testing of the cells with HLA-A3 and Bw6 specific mAbs, and with an alloantiserum specific for HLA-B18, showed that the mean channel of fluorescence (MCF) remained unchanged after PMA induction of U937 cells (Fig. 2). This indicates that differentiation induction of U937 did not alter the expression of HLA class I molecules.

*Comparison of HLA Class I Bound Peptides from Normal and Differentiation-Induced U937 Cells.* To determine whether the set of MHC class I bound peptides expressed by transformed monoblastic cells differs from that displayed by differentiation induced more mature (i.e., macrophage-like) U937 cells, we purified MHC class I bound peptides from PMA-treated and untreated U937 cells.

MHC class I molecules were purified by immunoaffinity chromatography from U937 monoblasts and macrophage-like cells (Fig. 3). Peptides, bound to equal masses of MHC class I molecules, were released by denaturation and purified by ultrafiltration and reverse-phase HPLC. Several absorbance peaks at 215 nm were observed in the peptide pools obtained from PMA-treated and untreated cells (Fig. 4). Most peaks were common to PMA-treated and untreated cells (peaks 1–4), though in certain instances quantitative differences could be seen (peaks 1 and 2). Some peaks, eluting between 30 and

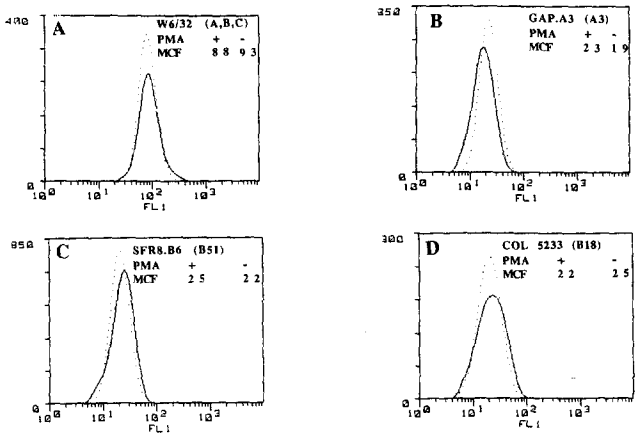


**Figure 2.** Cell surface expression of HLA-A3, Bw6, and B18 molecules on differentiation-induced and uninduced U937 cells. PMA-treated and untreated U937 cells were reacted with allele-specific mAbs or alloantisera followed by FITC-labeled anti-mouse or anti-human Ig. The fluorescence intensity (mean channel of fluorescence) of the cell populations was measured by flow cytometry. (- - - - -) Uninduced U937. (·····) Differentiation-induced U937. (*A*) HLA class I expression using mAb W632 (reacts with all HLA A, B, and C molecules). (*B*) HLA-A3 expression using mAb GAP.A3. (*C*) HLA Bw6 (an epitope of HLA-B51) expression using mAb SFR8.B6. (*D*) HLA-B18 expression using Col 5322 alloantiserum.

35 min, appeared to be unique to untreated cells, yet no sequence information could be obtained from them. Eight HPLC fractions eluting between 16 and 29 min were submitted for sequential $NH_2$-terminal Edman degradation. One of these fractions, peak 2 from non PMA-treated U937, gave no sequence information. Full confidence sequence information of peptides were obtained from three fractions of the non PMA-treated group (peaks 1, 3, and 4) and from four fractions of the PMA-treated group (peaks 1–4). We were able to match two of the sequences to their known precursors. The approximate yields of each of the sequenced peptides are shown in Table 2.

From peak 1 we obtained the amino acid sequence SEY-

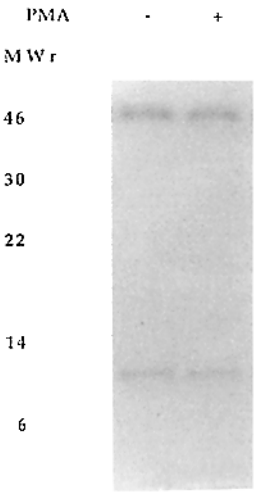


**Figure 3.** SDS-PAGE analysis of HLA class I molecules purified from U937 cells. HLA class I molecules were purified by immunoaffinity chromatography using the mAb W6/32 from detergent lysates of $10^{10}$ cells. HLA class I molecules were eluted from the affinity matrix in dilute TFA and a 1-$\mu$g aliquot was analyzed by SDS-PAGE on a 17.5% acrylamide gel followed by silver staining.

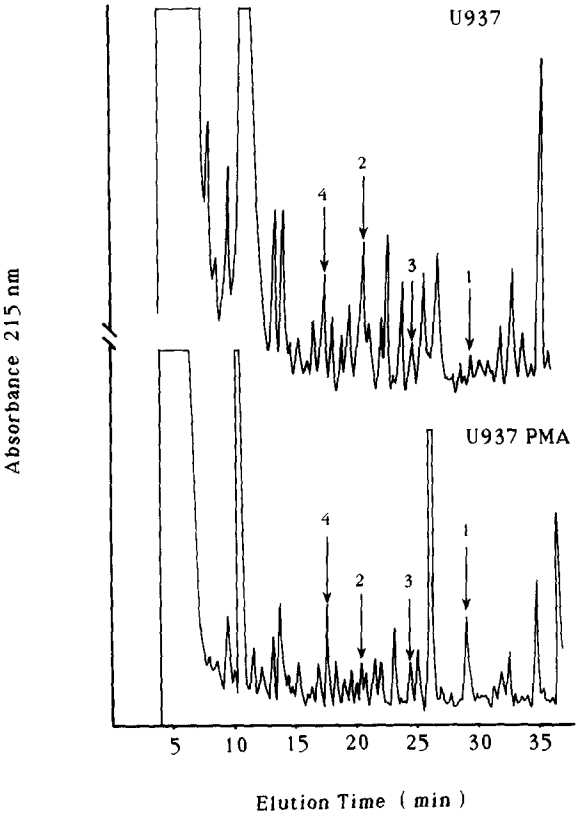


**Figure 4.** Reverse phase HPLC separation of MHC class I bound peptides, isolated from U937 cells. (*Top*) U937 cells. (*Bottom*) Differentiation-induced (PMA-treated) U937. HLA class I molecules were purified by immunoaffinity chromatography. Peptides bound to class I were released by heat denaturation in dilute TFA, and purified by ultrafiltration. Peptide mixture was separated on a C18 column (250 × 2.1 mm) using a 5–60% acetonitrile (0.1% TFA vol/vol) gradient run over 1 h at a flow rate of 0.25 ml/min. 1-min fractions were collected.

**Table 2.** *HLA Class I Bound Peptides Isolated from U937 Cells*

| Peak | U937 | Yield | Sequence | Precursor |
|---|---|---|---|---|
| | | *pmol* | | |
| 1 | PMA | 6.11* | SEYRVKEYK‡ | CMV |
| 1 | Non-treated | 3.45 | SEYRVKEYK | |
| 2 | PMA | 3.57 | SVDPKNYPK | IL-1$\beta$ |
| 2 | Non-treated | <0.30 | | |
| 2 | PMA | 0.56 | ALYGVAA | Unknown |
| 2 | Non-treated | <0.3 | | |
| 3 | PMA | 2.13 | KVYPSXAS | Unknown |
| 3 | Non-treated | 2.82 | KVYPSXAS | |
| 4 | PMA | 3.56 | XIXKVGNQK | Unknown |
| 4 | Non-treated | 4.18 | AVPASXKV | Unknown |

* Yield of first PTH-amino acid derivative of the sequence.
‡ Standard IUPAC amino acid abbreviations are used. X denotes an unidentifiable PTH-amino acid derivative.

RVKEYK. A search of nucleic acid and protein sequence data banks matched this sequence to HXLF1, a predicted transmembrane glycoprotein coded in the short unique sequence region of the CMV strain AD169 genome (10). The data base sequence, SEYRVEYSE, matches the identified peptide at seven out of nine residues (SEVRV-E-Y). By comparison to a standard phenylthiohydantoin (PTH) amino acid derivative, the estimated yield of the CMV peptide obtained from induced cells was 6.11 pmol. Uninduced cells yielded 3.45 pmol of this peptide. Since the total amount of HLA class I molecules from which the pool of peptides was obtained was ~4,000 pmols, it appears that this peptide occupied ~0.1% of the HLA class I binding site. This is a conservative estimate, however, since it is based on all the MHC class I molecules expressed by U937 cells, rather than on the specific allelic product to which the peptide was bound.

In peak 2 (fraction 20) from PMA-induced U937 cells, we obtained two unambiguous amino acid sequences: a major sequence, SVDPKNYPK, and a minor sequence, ALYGYAA. Sequencing of the corresponding peak (fraction 20) and of two adjacent fractions (nos. 19 and 21) from the uninduced U937 cells showed neither of these peptides. A search of nucleic acid and protein sequence data banks matched the major sequence, SVDPKNYPK, to the human IL-1$\beta$ protein (100% homology) (22). The yield of the peptide was 3.5 pmols, suggesting about 0.1% occupancy of the HLA class I binding sites by the IL-1$\beta$ peptide. The HPLC elution characteristics of a synthetic peptide with the sequence SVDPKNYPK was determined using conditions identical to those under which the natural peptides were isolated. Both synthetic and natural peptides eluted in the 20–21 min range (data not shown). The IL-1$\beta$ peptide shares with the CMV peptide a serine residue in the first position and a lysine residue in the 9th position, suggesting an allele-specific motif in the structure of these HLA class I bound peptides. The yield of the PTH amino acid derivatives of the minor sequence (ALYGVAA) dropped below the level of detection after the 7th cycle of Edman degradation, preventing further analysis, and this peptide showed no sequence homology to any proteins contained in the data bases.

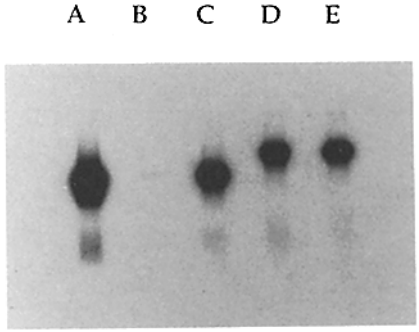


**Figure 5.** Expression of IL-1$\beta$ mRNA in differentiation-induced and -uninduced U937 cells. Total RNA was obtained from PMA-treated and untreated U937 cells and reverse-transcribed into cDNA. The cDNAs were analyzed for the presence of $\beta$-actin and IL-1$\beta$ message using specific primers in the presence of radiolabeled nucleotides in a PCR. Amplifications products were analyzed by agarose gel electrophoresis and autoradiography. The autoradiogram is from a 2-h exposure at room temperature. (*Lane A*). IL-1 $\beta$ primers and control cDNA from LPS-treated macrophages. (Lane *B*) IL-1$\beta$ primers, uninduced U937. (Lane *C*) IL-1$\beta$ primers, differentiation-induced U937. (Lane *D*) $\beta$-actin primers, uninduced U937. (Lane *E*) $\beta$-actin primers, differentiation-induced U937.

The presence or absence of a given peptide, bound to MHC molecules, probably depends on the level of expression of the corresponding protein and gene. To determine whether the absence of the IL-1$\beta$ peptide on uninduced U937 cells was due to quantitative differences in the level of IL-1$\beta$ expression, we measured the amount of IL-1$\beta$ messenger RNA by quantitative reverse PCR. RNA was extracted from induced and uninduced U937, reverse transcribed, and equal amounts of cDNA amplified in the presence of appropriate primers and [$^{32}$P]dNTPs. Analysis of the amplified material showed that differentiated U937 cells strongly expressed IL-1$\beta$ mRNA. Uninduced cells, however, expressed at least 50 times less of this message (Fig. 5).

Peak 3 of both PMA-treated and untreated U937 cells yielded an identical peptide which could not be matched to a precursor.

Peak 4 of PMA-treated U937 yielded a peptide whose sequence was XIXKVGNQK. The corresponding peak from non PMA-treated U937 contained a peptide with a different sequence: AVPASXKV. Neither of these two peptides matched known sequences.

## Discussion

The recent development of methods for analyzing endogenous MHC bound peptides from cell lines or tissues, by HPLC profiles and amino acid sequence analysis of individual peptides, allows the characterization of transformation and differentiation-specific peptides bound to MHC molecules. Such studies are central to our understanding of the ability of the immune response to discriminate between self and nonself antigens and may permit the identification of neoantigens associated with malignant transformation.

In this report we describe for the first time an endogenously processed differentiation-stage specific peptide derived from a cytokine bound to MHC class I molecules.

The human monoblastoid leukemia cell line U937 is a model in which differentiation of precursor cells can be studied. After PMA treatment, U937 cells undergo morphological and functional changes which result in the acquisition of a macrophage-like phenotype. We have compared the HPLC profile and the sequence of prominent peaks of HLA class I associated peptides eluted from PMA-treated and untreated U937 cells.

One of the self peptides identified appears to derive from IL-1$\beta$. IL-1$\beta$ is a cytokine with a wide variety of effects on cells involved in inflammation, immune responses, and hematopoiesis. Although IL-1$\beta$ is predominantly secreted by activated monocytes/macrophages, a great variety of other cells, including endothelial, dendritic, smooth muscle, etc., have also been shown to secrete IL-1$\beta$ (23). IL-1$\beta$ is unusual among most secreted proteins in that the IL-1$\beta$ precursor protein lacks the typical signal sequence which directs vectorial

transport across the endoplasmic reticulum (ER) (24). In peripheral blood monocytes, IL-1β is synthesized as a 35-kD precursor protein. Upon activation with LPS, IL-1β is processed in the cytoplasm to its biologically active 17-kD form and secreted via a brefeldin A and monensin (inhibitors of vesicular traffic at the ER and Golgi level, respectively) insensitive pathway (25). IL-1β biosynthesis in PMA-induced U937 cells appears to be similar to that in peripheral blood monocytes. IL-1β is not detectable in supernatants from U937 control cells, whether assayed by bioactivity or RIA (26). Northern blot analysis also demonstrates that IL-1β messenger RNA is not present in U937 control cells that have not been treated with PMA. 48 h after (PMA) induction, however, IL-1β message becomes detectable and increases up to 16 d thereafter (27). Western blot analyses of IL-1β protein expression in PMA-treated U937 cell lysates and culture supernatants show the accumulation of large amounts of the 35-kD precursor protein within the cells and its secretion into the extracellular compartment (25). Culture supernatants also contain the 17-kD form of IL-1β (25).

Our results corroborate these studies by demonstrating that uninduced U937 cells express a minimal amount IL-1β mRNA and that differentiation-induction affects an increase in IL-1β mRNA expression. That an increase in IL-1β mRNA expression is accompanied by the appearance of the IL-1β peptide bound to MHC molecules, suggests that the de novo transcription of differentiation-stage specific genes can result in the presentation of the corresponding peptides by MHC molecules.

The finding that an IL-1β-derived peptide binds to MHC class I molecules is consistent with the current view on MHC class I antigen presentation of endogenously processed proteins (28). The IL-1β peptide sequence, $S^{200}$ VDPKNYPK$^{208}$, is contained within the biologically active 17-kD form of IL-1β ($A^{117}$-$S^{269}$) adjacent to two lysine residues, which have been identified as potential proteolytic cleavage sites (24), suggesting that a tryptic protease is involved in its processing. Since differentiation induction by PMA results in the accumulation of a cytoplasmic pool of IL-1β precursor protein within U937 cells, our data suggest that this protein becomes accessible to proteosomes and transport associated proteins, and enters the endogenous pathway of antigen presentation. These processing events may account for the formation of an IL-1β peptide–MHC class I complex.

The fact that developmentally regulated self proteins, such as receptors (29–31) and cytokines are processed and presented by MHC molecules provides important clues about the way in which the TCR repertoire is shaped. It is apparent that, to avoid autoimmunity, TCR reacting with cytokine and cytokine receptor-peptides should be subjected to deletion during intrathymic development and that such deletion requires recognition of the corresponding peptides on self MHC. This hypothesis is substantiated by the present observation that an IL-1β–derived peptide binds to MHC class I molecules, and by previous studies showing that peptides derived from the transferrin receptor (29) as well as MHC proteins (29–31) can bind to MHC class II molecules. In situ hybridization studies, which have revealed the intrathymic expression of IL-1β at the junction between the cortex and the medulla, support the concept that IL-1β–specific TCR can be deleted within the thymus (32).

While the IL-1β peptide was displayed only by the HLA molecules of induced U937 cells, a peptide derived from a CMV structural protein was found on both induced and uninduced U937 cells. Given the absence of inclusion bodies in U937 cells, this finding was unexpected. However, the fact that U937 cells are infected with CMV was substantiated by PCR analysis of U937 cDNA using a CMV-specific probe for the IE antigen (Harris, P., unpublished results). The relative amount of CMV structural peptide was greater in PMA-treated cells. This is consistent with the observation that differentiation-induction of U937 removes an early block to herpes simplex virus 1 replication (33).

The CMV peptide which we found matches the data base sequence at seven out of nine residues. Since human CMV is a heterogeneous family, comprising thousands of strains that can be identified by restriction mapping analysis (34), the peptide sequence obtained is probably derived from an uncharacterized strain. Our data are reminiscent of those reported by Rudensky et al. (31) who found peptides derived from the envelope protein gp70 of murine leukemia virus AKV and/or Rad LV bound to MHC class II molecules (I-$A^b$, I-$E^b$). PCR analysis of cDNA derived total mRNA of the murine cell line confirmed the fact that the line contained a latent viral infection, as is also the case of U937 grown in our laboratory. Lastly, although structural similarity exists between the IL-1β and CMV peptides and the IL-1β and CMV precursor proteins, respectively, it is not excluded that these MHC bound peptides derive from other uncharacterized precursors.

The characterization of differentiation-stage specific peptides should further our understanding of the molecular basis of self-non-self discrimination. Malignant transformation is presumably accompanied by alteration in the antigenic profile of MHC bound peptides. It is expected that such cells may acquire the expression of neoantigens, while also losing the capacity to present peptides corresponding to proteins that they cease producing. The identification of these peptides and other MHC bound self peptides from cells at different stages of differentiation remains a challenge for the future.

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Address correspondence to Dr. Paul E. Harris, College of Physicians and Surgeons of Columbia University, Department of Pathology, 630 West 168th Street, P & S 14-403, New York, NY 10032.

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