Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium

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ABSTRACT Conventional major histocompatibility complex (MHC) class I genes encode molecules that present intracellular peptide antigens to T cells. They are ubiquitously expressed and regulated by interferon γ . Two highly divergent human MHC class I genes, *MICA* and *MICB*, are regulated by promoter heat shock elements similar to those of *HSP70* genes. *MICA* encodes a cell surface glycoprotein, which is not associated with β_2 -microglobulin, is conformationally stable independent of conventional class I peptide ligands, and almost exclusively expressed in gastrointestinal epithelium. Thus, this MHC class I molecule may function as an indicator of cell stress and may be recognized by a subset of gut mucosal T cells in an unusual interaction.

Major histocompatibility complex (MHC) class I molecules are ubiquitously expressed heterodimers of a class I heavy chain and β_2 -microglobulin (β_2m), which display peptides derived from proteins degraded in the cytosol on the cell surface and thus facilitate the recognition of intracellular antigens by circulating cytotoxic CD8⁺ T cells with $\alpha\beta$ T-cell receptors (1, 2). Class I related molecules have variously altered functions, including the presentation of lipid and lipoglycan compounds to CD4[−]8[−] T cells by human CD1b encoded outside the MHC (3, 4). Moreover, so-called nonclassical MHC class I molecules in the mouse selectively present bacterial peptides (5, 6), are recognized by T cells with $\gamma\delta$ T-cell receptors in the absence of detectable bound peptides (7–10), or are mainly expressed in intestinal epithelium where they may interact with a subset of the resident T cells (11, 12). Although the precise functions of some of these molecules remain to be defined, they are conceptually appreciated as potential components of a first-line immune defense (13, 14). However, functionally corresponding genes and molecules have not been found in other mammals and humans. Conversely, two highly divergent human MHC class I genes closely linked to *HLA-B*, *MICA*, and *MICB* are conserved in most if not all mammals but seem to be missing in the mouse (15, 16). These genes are transcribed in epithelial cell lines but not in B cells, T cells, and monocytes and are not induced by interferon γ . Their translation products share an exceptionally low average of 27% identical amino acids in the extracellular $\alpha 1\alpha 2\alpha 3$ domains with human and mouse MHC class I sequences, include multiple sites for potential N-linked glycosylation, and lack all of the residues implicated in the binding of CD8 (15, 16).

The unusual characteristics of the *MICA* and *MICB* genes and the evidence for diverse functions of class I related molecules in antigen presentation and T-cell recognition led us to investigate the expression, subunit composition, and tissue distribution of *MICA*. This report describes the generation of specific monoclonal antibodies (mAbs) and their use in immunochemical experiments with normal and transfected mu-

tant cell lines, as well as in immunohistology stainings. The results show that *MICA* is a highly glycosylated membrane-anchored cell surface protein that is not associated with β_2m and is conformationally stable in the absence of conventional MHC class I peptide ligands. In accord with previous concepts, the almost exclusive expression of *MICA* in gastrointestinal epithelium combined with the intriguing transcriptional regulation of the *MICA* gene by a promoter heat shock element implies that this MHC class I molecule may function as a ligand for a subset of T cells in the intestinal intraepithelial lymphocyte compartment. This putative interaction is probably unrelated to conventional MHC class I antigen presentation and may serve a specialized T-cell immune surveillance function.

MATERIALS AND METHODS

DNA Clones and Constructs, Cell Lines, and Transfections.

The cDNA hybrid constructs were made in consecutive steps using the primer overlap-extension technique and PCR (17), with *MICA* and K^b and D^b cDNA templates (18). Error-free cDNA hybrids were identified by sequencing, subcloned into RSV.5neo, and stably transfected into C1R cells (19) by electroporation as described (20). Using the same procedure and conditions, lymphoblastoid cell line mutants Daudi (21) and 5.2.4 (22) were transfected with *MICA* cDNA in RSV.5neo and stable transfectants selected with G418 (GIBCO; 0.5 and 0.1 mg/ml, respectively). Mouse LTK fibroblasts were transfected with cosmid M32A (23) using lipofectin (BRL) and selected with G418 (1 mg/ml). In all transfection experiments, stable positive isolates were identified by indirect immunofluorescence stainings with specific mAbs purified from ascites or from hybridoma supernatants and flow cytometry using a FACScan (Becton Dickinson) and, when necessary, cloned by limiting dilution. *MICA*-D^b and -K^b hybrid molecules on C1R transfectant cells were detected with mAbs 28-14-8 (anti-D^b $\alpha 3$) and Y3 (anti-K^b $\alpha 1\alpha 2$), respectively (24, 25). The HT29 colon carcinoma and U373 astrocytoma cell lines were from the American Type Culture Collection. Cell lines were grown in RPMI medium supplemented with 10% fetal calf serum, 10 mM Hepes, 2 mM glutamine, and antibiotics.

Production of mAbs. RBF/DnJ mice (The Jackson Laboratory) were injected intraperitoneally three times at weekly intervals with 10⁸ C1R cells expressing *MICA* mRNA after stable transfection with *MICA* cDNA in RSV.5neo. After a final boost immunization, isolated splenocytes in suspension were fused with P3-X63Ag8.653 myeloma cells (26) by standard polyethylene glycol treatment (27). Hybridomas were grown in RPMI media with 10% of each CPSR-3 heat-

Abbreviation: MHC, major histocompatibility complex; β_2m , β_2 -microglobulin.

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inactivated serum replacement (Sigma) and Hybridoma Enhancing Supplement (Sigma) in 96-well plates under hypoxanthine aminopterin thymidine selection on irradiated MRC-5 feeder cells (27). Supernatants were differentially screened for specific reactivity with C1R-MICA cells versus untransfected C1R cells by indirect immunofluorescence and flow cytometry and hybridomas from positive wells were subcloned twice. The isolated mAbs 56, 83, and 2C10 are of the IgG2a, IgG1, and IgG3 isotypes, respectively.

Labeling, Immunoprecipitation, and Detection of MICA. For surface labeling, washed cells in phosphate-buffered saline (PBS) were biotinylated with Sulfo-NHS-LC-biotin (Pierce) (100 μ g/ml) for 30 min at 4°C and reactions quenched by addition of 25 mM lysine. Cells ($1-3 \times 10^7$) were lysed in 1 ml lysis buffer (1% Triton X-100/50 mM Tris-OH, pH 7.4/150 mM NaCl/5 mM EDTA/5 mM iodoacetamide/protease inhibitors). Protein in cleared supernatants was quantitated with a MicroBCA kit (Pierce) and lysates were precleared using Ultralink-Protein A/G beads (Pierce). MICA was precipitated with purified mAb 56 and protein A/G beads and immunocomplexes washed. Aliquots were treated with N-glycanase (PNGase F, New England Biolabs) as recommended by the manufacturer. Dissociated and dithiothreitol-reduced immunocomplexes were separated by SDS/PAGE and electroblotted onto nitrocellulose using a Trans-Blot Semi-Dry Transfer Cell (Bio-Rad). After overnight incubation of membranes in PBS containing 10% nonfat dry milk, 0.05% Tween 20, and 0.02% sodium azide, they were repeatedly washed in TST (0.15 M NaCl/10 mM Tris-OH, pH 7.4/0.3% Tween 20) and reacted with avidin-horseradish peroxidase (Vector Laboratories) in TST (2.5 μ g/ml) for 1 hr at 4°C. Membranes washed with TST were treated with enhanced chemiluminescent reagent (Amersham) and exposed to x-ray film. For pulse-labeling and chase, 5×10^6 cells per time point were labeled with 0.5 mCi (1 Ci = 37 GBq) [35 S]methionine for 5 min as described (20). For pulse-chase, cells were spun through PBS with 10 mM methionine and resuspended in growth media for the indicated time periods. Cells were lysed and MICA protein was precipitated using mAb 2C10 as described above. Isolated and denatured MICA was treated with endoglycosidase H (Endo H, New England Biolabs), as recommended by the manufacturer and analyzed by SDS/PAGE. Fixed gels were treated with Amplify (Amersham) and dried for autoradiography.

Tissues and Immunohistology. Tissue samples from autopsies, biopsies, or surgical specimens were embedded in Tissue-Tec II OCT compound (Baxter Scientific Products, McGaw Park, IL) and frozen in liquid N₂ precooled methylbutane. Cryostat (4- μ m sections mounted on poly L-lysine-coated slides) were airdried, fixed in cold acetone, and overlaid with purified primary mAb appropriately diluted in staining buffer (PBS with 2% goat and 2% human serum/0.1% Tween 20). After overnight incubation at 4°C, slides were washed and overlaid with fluorescein-conjugated goat F(ab')₂ anti-mouse IgG (Tago) diluted 1:150 in staining buffer. Coverslipped sections were examined by confocal fluorescence microscopy. In the double stainings shown in Fig. 4B and C, nuclei were visualized with propidium iodide. In Fig. 4F, the epithelial desmosomal cadherin desmoglein-I was detected with biotinylated mAb DG3.10 (28) (Progen, Heidelberg) and Texas Red-conjugated streptavidin as the second layer. Autopsy tissue specimens included brain, heart, lung, thyroid, liver, kidney, skin, adrenal gland, placenta, tonsil, and spleen and were obtained from Swedish Hospital (Seattle). Gastric and small and large intestinal biopsy specimen were kindly provided by D. Levine (University of Washington Medical Center, Seattle). Thymus specimens from corrective infant cardiac surgery were kindly provided by D. Hall and F. Lupinetti (Children's Hospital, Seattle). For antibody staining and flow cytometry analysis, thymocyte suspensions were prepared by

passing minced tissue through wire mesh. Peripheral blood mononuclear cells from randomly selected donors were isolated by density gradient centrifugation through Ficoll-Hypaque (Pharmacia). The use of the human tissues from these sources in this research was conducted under human subject protocols approved by the institutional review board at the Fred Hutchinson Cancer Research Center.

Promoter Mapping and Sequencing, RNA Blot Hybridization, and Heat Shock Induction. *MICA* and *MICB* exon 1 and promoter sequences were mapped to 5.9-kb *XhoI/KpnI* and 0.6-kb *BamHI/BglII* fragments of the cosmid M32A and R5A, respectively, by blot hybridization with a [32 P]dCTP-labeled exon 1-derived oligonucleotide (15). Sequences were obtained by dideoxynucleotide chain termination from the double-stranded templates using the exon 1 oligonucleotide and upstream primers, [35 S]dATP and Sequenase (United States Biochemical). RNA extractions, electrophoresis (20 μ g per lane), blot transfers, and hybridizations with [32 P]dCTP-labeled DNA probes were according to standard protocols (29). The HLA-B-specific probe was the 885-bp *XbaI* fragment from the genomic clone B7-14 (30). The human HSP70 probe was a 2.3-kb *BamHI/HindIII* genomic DNA fragment (31). For heat shock treatment, HeLa cells grown in Petri dishes were floated on a 42°C water bath for various periods of time.

RESULTS AND DISCUSSION

The substantial divergence of the MICA amino acid sequence translated from cloned genomic and corresponding cDNA from all class I sequences indicated that none of the available

FIG. 1. Construction and surface expression of MICA-K^b and -D^b hybrid molecules on C1R transfectant cells (see *Materials and Methods*). (A) Schematic representation of the cDNA hybrid constructs. Open and solid bars correspond to MICA and K^b (hybrid 1) or D^b (hybrids 2–4) domains, respectively. Domain boundaries are indicated at the top. L, leader peptide; $\alpha 1\alpha 2\alpha 3$, extracellular domains; TM, transmembrane segment; CY, cytoplasmic tail. (B) Expression of the hybrid cDNAs in stably transfected C1R cells. By flow cytometry, all of the encoded hybrid molecules were present at intermediate or high levels on the cell surfaces (solid profiles). Hybrid 1 was detected with mAb Y3 (anti-K^b $\alpha 1\alpha 2$) and hybrids 2–4 were detected with mAb 28-14-8 (anti-D^b $\alpha 3$) (24, 25). Open profiles are stainings of untransfected C1R cells, and shaded profiles in histograms 1 and 2 are stainings of C1R-K^b and -D^b transfectants, respectively.

anti-class I antibody reagents could be expected to recognize this putative gene product (15). We initially chose an indirect approach to monitor expression of MICA, by transfecting C1R cells with hybrid cDNA constructs in which all domain sequences of MICA were variously substituted with corresponding mouse H2-K^b or -D^b sequences encoding epitopes recognized by defined mAbs (Fig. 1A). Indirect immunofluorescence stainings with the mAbs Y3 (anti-K^b $\alpha 1\alpha 2$) (24) or 28-14-8 (anti-D^b $\alpha 3$) (25) and flow cytometry revealed that all of the epitope-tagged hybrid molecules were present at substantial levels on the surfaces of the stably transfected cells (Fig. 1B). This indicated that the MICA cDNA encoded a class I-like membrane-anchored cell surface protein, thereby providing the rationale for the further investigation of the expression of MICA. To obtain reagents suitable for diverse experimental applications, we generated specific mAbs after immunizing mice with C1R cells transfected with the MICA cDNA and differentially screening hybridoma supernatants by indirect immunofluorescence and flow cytometry. The isolated mAbs 56, 83, and 2C10 bound to C1R-MICA transfectant cells but not to untransfected C1R cells and were not cross-reactive with putative MICB on multiple independent C1R-MICB cDNA transfectants (Fig. 2A; data not shown).

To analyze MICA molecules, we performed immunoprecipitations with mAb 56 from lysates of surface-biotinylated C1R-MICA and C1R control cells. Samples were treated with *N*-glycanase or left untreated. After SDS/PAGE and transfer onto nitrocellulose, biotin-labeled protein was detected with avidin-peroxidase and chemiluminescent reagent. With *N*-glycanase digestion, a single compact band of 43 kDa was evident in the C1R-MICA lane, which was missing in the C1R negative control lane and matched the predicted molecular mass of the MICA polypeptide (Fig. 2B, lanes 2 and 3) (15). It showed the same electrophoretic mobility as glycosylated

HLA-C isolated with mAb W6/32 from C1R-MICA (HLA-A⁻, -B⁻) cell lysate (Fig. 2B, lane 4) (19, 32). Without *N*-glycanase digestion, we detected a broad band in the 65–75 kDa molecular mass range (Fig. 2B, lane 1). Thus, bona fide MICA molecules were expressed on the surfaces of C1R-MICA cells and were highly glycosylated, consistent with the eight potential N-glycosylation acceptor sites in the $\alpha 1\alpha 2\alpha 3$ domain sequences of MICA (15). This distinguished MICA from all conventional and nonclassical MHC class I molecules, which have a single glycosylation site at position 86 in the $\alpha 1$ domain that is not conserved in MICA (1, 15). Similar results were obtained using mAbs 83 and 2C10, of which the former reacted less efficiently in these experiments, and with mouse LTK cells transfected with a cosmid encoding the MICA gene (Fig. 2A and B, lanes 8–10).

The subunit composition and transport of MICA molecules was investigated using C1R-MICA cells and mutant lymphoblastoid cell line Daudi (β_2m^-) and 5.2.4 (TAP⁻) cells transfected with the MICA cDNA (21, 22). Immunoprecipitation with the anti-human β_2m mAb BBM1 coprecipitated HLA-C but not MICA from lysates of surface-labeled C1R-MICA cells (Fig. 2B, lanes 6 and 7) (33). Moreover, C1R-MICA and untransfected C1R cells showed the same fluorescence intensity after staining with mAb BBM1 (data not shown) and Daudi-MICA cells displayed large amounts of surface MICA, as observed by staining with mAb 56 and by immunoprecipitation of surface-labeled MICA molecules (Fig. 2A and B, lanes 14–16). In further support of these results, no β_2m was coprecipitated with MICA from lysates of metabolically labeled C1R-MICA cells using mAb 2C10, and newly synthesized MICA molecules were transported through the Golgi and matured at similar rates in C1R-MICA and Daudi-MICA cells, as determined by metabolic pulse-labeling and chase and by treatment of immunoprecipitated protein with endoglycosi-

FIG. 2. Detection and analysis of MICA molecules on transfected and untransfected cell lines using the anti-MICA mAbs. (A) By indirect immunofluorescence staining with mAb 56 and flow cytometry, large amounts of MICA were present on the surfaces of C1R-MICA, LTK-MICA, mutant 5.2.4-MICA (TAP⁻), and Daudi-MICA (β_2m^-) cells, as well as on HT29 colon carcinoma and U373 astrocytoma cells. Open profiles show negative stainings of untransfected cells and IgG2a antibody isotype-control stainings of HT29 and U373 cells. (B) Immunoprecipitation of MICA molecules with mAb 56 from lysates of surface-labeled C1R-MICA, LTK-MICA, 5.2.4-MICA, Daudi-MICA, HT29, and U373 cells, and SDS/PAGE of the immunocomplexes treated (+) or nontreated (-) with *N*-glycanase. Control lanes (C) show negative results obtained with untransfected cells. In lanes 4–7, HLA-C but not MICA was immunoprecipitated from C1R-MICA lysate with mAb W6/32 and with the anti-human- β_2m mAb BBM1, respectively (32, 33). Numbers on the right indicate molecular masses in kDa.

dase H before SDS/PAGE (Fig. 3 *A* and *B*). Thus, unlike all other MHC class I and related molecules, MICA molecules were not associated with β_2m . Moreover, normal surface levels of MICA were also observed with 5.2.4-MICA cells, which lack the TAP peptide transporter, which supplies the peptides mainly bound by class I molecules from the cytosol into the lumen of the endoplasmic reticulum (Fig. 2 *A* and *B*, lanes 11–13) (22, 34, 35). Thus, distinct from most conventional MHC class I molecules, the transport and apparently stable surface expression of MICA was independent of cytosolic peptide ligands—a characteristic shared with the mouse nonclassical MHC class I T10^b, T22^b, and TL molecules (8–10, 36).

These results and the previously observed restricted transcription of the *MICA* gene in epithelial cell lines raised the question of the tissue distribution of MICA (15). Indirect immunofluorescence stainings using mAbs 56 and 83 confirmed the absence of MICA on the surfaces of B-, T-, and monocyte cell lines and on freshly isolated peripheral blood mononuclear cells and thymocytes (data not shown). Variable amounts of MICA were detected on epithelial tumor cell lines such as HT29 colon carcinoma and U373 astrocytoma cells, as indicated by antibody binding and flow cytometry and by immunoprecipitation of the surface-labeled protein (Fig. 2 *A* and *B*, lanes 17–20). On some epithelial cell lines, surface MICA was low or undetectable despite the presence of similar

amounts of MICA mRNA in all of the cell lines tested (15). This discrepancy may suggest that the post-translational processing and transport of MICA molecules was dependent on factors that were limited or missing in these cell lines and that were different from those required for the normal processing of conventional class I molecules (35). The tissue distribution of MICA was examined by immunofluorescence microscopy of mAb 83 and fluorescein isothiocyanate-goat F(ab')₂ anti-mouse IgG-stained cryosections. Most of the tissues were negative for binding of mAb 83. These included brain, heart, lung, thyroid, liver, kidney, skin, adrenal gland, placenta, tonsil, and spleen. Strongly positive staining was seen in gastric and in small and large intestinal mucosa biopsies from several individuals and was confined to surface and glandular epithelial cells (Fig. 4*A–C*). In intestinal epithelium, fluorescent cells lined the villi but were not apparent in the undifferentiated crypts (Fig. 4*B*). Notably, the observed staining was often discontinuous, with variably sized interspersed areas of non-fluorescent epithelium, which occasionally included single fluorescent cells (Fig. 4*D*). IgG1-matched isotype controls and stainings of dog intestinal mucosa sections gave negative results. In specimens of infant thymus, mAb 83 stained a population of stellate cells in the subcapsular cortex, which were epithelial cells because they were positive for the desmoglein-I epithelial cell marker (Fig. 4 *E* and *F*) (28).

The remarkably restricted and varied expression of MICA in intestinal epithelium suggested an unusual transcriptional regulation of the *MICA* gene. 5'-end-flanking region sequences of *MICA* and *MICB* upstream of their translation initiation codons were derived from mapped cosmid fragments. Both sequences were unrelated to the transcriptional control regions of conventional class I genes. Surprisingly, they contained heat shock elements defined in *HSP70* genes and shared further nucleotide sequence homologies with the human *HSP70* promoter (Fig. 5*A*) (37). Notably, *MICA* and *MICB* are closely linked to *HSP70* genes in the MHC (38, 39). Exposure of HeLa cells to heat shock resulted in moderate and substantial increases of the steady-state levels of MICA and MICB mRNA, respectively, with induction kinetics that were similar to data obtained in parallel for *HSP70* mRNA (Fig. 5 *B* and *C*). Because these results were obtained with rapidly proliferating tissue culture cells in which *MICA* but not *MICB* is constitutively transcribed at a significant rate (15), they may quantitatively underestimate the induction of MICA mRNA in quiescent gastrointestinal epithelial cells in which the *MICA* gene appeared not to be constitutively expressed (Fig. 4*D*). This deregulated transcription of the *MICA* gene in epithelial cell lines with resultant intermediate or high surface expression of MICA and the only moderate mRNA induction may at least partially explain why our repeated attempts with various cell lines to detect increased surface levels of MICA after heat shock treatment were unsuccessful. This demonstration will require a suitable experimental system but should be readily achievable for MICB once specific mAbs become available. Nevertheless, because heat shock is one condition that triggers the general cell stress response, our results argue that *MICA* and more obviously *MICB* are cell stress response genes (40, 41).

In summary, these results demonstrate the surface expression of a previously unrecognized human MHC class I molecule with unusual structural and biological characteristics. The expression of MICA without associated β_2m , independent of TAP, and almost exclusively in gastrointestinal epithelium is unique among all class I-like molecules although partial similarities exist among the mouse nonclassical MHC class I T10^b, T22^b, and TL molecules and human CD1b and CD1d (8–10, 42–44). Unlike certain class I-related molecules that are encoded outside the MHC and have conserved functions not associated with T-cell recognition (45, 46), the characteristics of the MHC class I MICA and putative MICB molecules imply

FIG. 3. MICA molecules were not detectably associated with β_2m and matured with similar kinetics in C1R-MICA and Daudi-MICA cells. Immunoprecipitation of MICA molecules with mAb 2C10 from lysates of C1R-MICA and Daudi-MICA cells after pulse-labeling and chase is shown for increasing periods of time (indicated in minutes at top) by SDS/PAGE. For each time point, immunoprecipitated protein was left untreated (– Endo H) or was treated (+ Endo H) with endoglycosidase H to measure the acquisition of cleavage-resistant complex-type carbohydrates during transport in Golgi vesicles. Control lanes in both panels are immunoprecipitations with mAb 2C10 from lysates of untransfected labeled C1R and Daudi cells. Numbers on the right indicate molecular masses in kDa.

FIG. 4. Immunofluorescence micrographs of gastrointestinal epithelium and thymus sections stained with mAb 83. (A) Gastric antrum mucosa. (B and C) Duodenal villi with red counterstaining of nuclei. (D) Section of terminal ileum showing highly variable binding of mAb 83. (E and F) Thymic cortex with mAb 83-stained cells and double-labeling with the anti-desmoglein-I mAb DG3.10 (F, red punctuate staining) (28).

that these are recognized by a subset of T cells in gastrointestinal epithelium in an interaction that is probably independent of conventional MHC class I antigen processing. This is supported by the expression of MICA in thymic cortical epithelial cells, suggesting a possible role of MICA in T-cell selection, and by the existence of a minimum of 16 allelic variants with clustered amino acid substitutions in the $\alpha 1\alpha 2\alpha 3$ domains of MICA (15, 52). Because the expression of MICA and putative MICB is to some degree coupled to cell stress, these molecules could be recognized alone or possibly complexed with ligands derived from heat shock proteins. In human intestinal epithelium, the oligoclonal expansion of T cells with $\gamma\delta$ (V δ 1) or $\alpha\beta$ T-cell receptors are unexplained phenomena (47–50). However, stimulation of these T cells by

autologous antigens that may be stress induced has been postulated (48, 50, 51). Thus, MICA and possibly MICB may represent a “missing link” in the gut mucosal immune system.

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FIG. 5. Transcriptional regulation of *MICA* and *MICB* by heat shock promoters. (A) Alignment of human HSP70, MICA, and MICB promoter sequences. The HSP70 sequence contains a consensus heat shock element (boxed) that is almost perfectly matched by MICA and MICB promoter sequences (37, 41). Dashes in the MICA and MICB sequences indicate identities with the HSP70 promoter sequence. Vertical lines in the MICB sequence represent nucleotides shared with the MICA sequence. Numbering on the right side indicates distances from the translation initiation codon (ATG). (B and C) Increased steady-state levels of MICA and MICB mRNA in HeLa cells after heat shock, shown by RNA blot hybridizations with a labeled MICA cDNA probe. MICB mRNA of 2.4 kb is larger than MICA mRNA due to a longer 3' untranslated region (16). (B) Heat shock induction at 42°C for increasing periods of time. (C) Kinetics of induction after a 1-hr exposure to 42°C. In both experiments the kinetics of HSP70 mRNA induction were very similar. Blots hybridized with the HSP70 probe were exposed to film for much shorter time. Control HLA-B mRNA remained unaltered in these experiments.

1. Bjorkman, P. J. & Parham, P. (1990) *Annu. Rev. Biochem.* **59**, 253–288.
2. Germain, R. N. & Margulies, D. H. (1993) *Annu. Rev. Immunol.* **11**, 403–450.
3. Beckman, E. M., Porcelli, S. A., Morita, C. T., Behar, S. M., Furlong, S. T. & Brenner, M. B. (1994) *Nature (London)* **372**, 691–694.
4. Sieling, P. A., Chatterjee, D., Porcelli, S. A., Prigozy, T. I., Mazzaccaro, R. J., Soriano, T., Bloom, B. R., Brenner, M. B., Kronenberg, M., Brennan, P. J. & Modlin, R. L. (1995) *Science* **269**, 227–230.
5. Kurlander, R. J., Shawar, S. M., Brown, M. L. & Rich, R. R. (1992) *Science* **257**, 678–679.
6. Pamer, E. G., Wang, C.-R., Flaherty, L., Fischer Lindahl, K. & Bevan, M. J. (1992) *Cell* **70**, 215–223.
7. Ito, K., Van Kaer, L., Bonneville, M., Hsu, S., Murphy, D. B. & Tonegawa, S. (1990) *Cell* **62**, 549–561.
8. Schild, H., Mavaddat, N., Litzenger, C., Ehrlich, E. W., Davis, M. M., Bluestone, J. A., Matis, L., Draper, R. K. & Chien, Y.-H. (1994) *Cell* **76**, 29–37.
9. Weintraub, B. C., Jackson, M. R. & Hedrick, S. M. (1994) *J. Immunol.* **153**, 3051–3058.
10. Kaliyaperumal, A., Falchetto, R., Cox, A., Dick, R., Shabanowitz, J., Chien, Y.-H., Matis, L., Hunt, D. F. & Bluestone, J. A. (1995) *J. Immunol.* **155**, 2379–2386.
11. Hershberg, R., Eghtesady, P., Sydora, B., Brorson, K., Cheroutre, H., Modlin, R. & Kronenberg, M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 9727–9731.
12. Wu, M., Van Kaer, L., Ito, S. & Tonegawa, S. (1991) *J. Exp. Med.* **174**, 213–218.
13. Ojcius, D. M., Delarbre, C., Kourilsky, P. & Gachelin, G. (1994) *Crit. Rev. Immunol.* **14**, 193–220.
14. Cheroutre, H., Holcombe, H. R., Tangri, S., Castano, A. R., Teitell, M., Miller, J. E. W., Cardell, S., Benoist, C., Mathis, D., Huse, W. D., Peterson, P. A. & Kronenberg, M. (1995) *Immunol. Rev.* **147**, 31–52.
15. Bahram, S., Bresnahan, M., Geraghty, D. E. & Spies, T. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 6259–6263.
16. Bahram, S. & Spies, T. (1996) *Immunogenetics* **43**, 230–233.
17. Horton, R., Ho, S. N. & Pease, L. R. (1990) *Biotechniques* **8**, 528–533.
18. Waneck, G. L., Sherman, S. H., Calvin, S., Allen, H. & Flavell, R. A. (1987) *J. Exp. Med.* **165**, 1358–1370.
19. Alexander, J., Payne, J. A., Shigekawa, B., Frelinger, J. A. & Cresswell, P. (1990) *Immunogenetics* **31**, 169–178.
20. Grandea, A. G., III, Androlewicz, M. J., Athwal, R. S., Geraghty, D. E. & Spies, T. (1995) *Science* **270**, 105–108.
21. Arce-Gomez, B., Jones, E. A., Barnstable, C. J., Solomon, E. & Bodmer, W. F. (1978) *Tissue Antigens* **11**, 96–112.
22. Mellins, E., Kempin, S., Smith, L., Monji, T. & Pious, D. (1991) *J. Exp. Med.* **174**, 1607–1615.
23. Spies, T., Blanck, G., Bresnahan, M., Sands, J. & Strominger, J. L. (1989) *Science* **243**, 214–217.
24. Ozato, K. & Sachs, D. H. (1980) *J. Immunol.* **126**, 317–321.
25. Hammerling, G. J., Rusch, E., Tada, N., Kimura, S. & Hammerling, U. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4737–4741.
26. Kearney, J. F., Radbruch, A., Liesegang, B. & Rajewsky, K. (1979) *J. Immunol.* **123**, 1548–1550.
27. Harlow, E. & Lane, D. (1988) *Antibodies: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
28. Schmidt, A., Heid, H. W., Schafer, S., Nuber, U. A., Zimbelmann, R. & Franke, W. W. (1994) *Eur. J. Cell Biol.* **65**, 229–245.
29. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
30. Barbosa, J. A., Kamarck, M. E., Biro, P. A., Weissman, S. M. & Ruddle, F. H. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6327–6331.
31. Wu, B., Hunt, C. & Morimoto, R. I. (1985) *Mol. Cell. Biol.* **5**, 330–341.
32. Parham, P., Barnstable, C. J. & Bodmer, W. F. (1979) *J. Immunol.* **123**, 342–349.
33. Parham, P., Androlewicz, M. J., Holmes, N. J. & Rothenberg, B. E. (1983) *J. Biol. Chem.* **258**, 6179–6186.
34. Spies, T., Bresnahan, M., Bahram, S., Arnold, D., Blanck, G., Pious, D. & DeMars, R. (1990) *Nature (London)* **348**, 744–747.
35. Lehner, P. J. & Cresswell, P. (1996) *Curr. Opin. Immunol.* **8**, 59–67.
36. Holcombe, H. R., Castano, A. R., Cheroutre, H., Teitell, M., Maher, J. K., Peterson, P. A. & Kronenberg, M. (1995) *J. Exp. Med.* **181**, 1433–1443.
37. Hunt, C. & Morimoto, R. I. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6455–6459.
38. Sargent, C. A., Dunham, I., Trowsdale, J. & Campbell, R. D. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1968–1972.
39. Spies, T., Bresnahan, M. & Strominger, J. L. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8955–8958.
40. Craig, E. A. & Gross, C. A. (1991) *Trends Biochem. Sci.* **16**, 135–139.
41. Morimoto, R. I. (1993) *Science* **259**, 1409–1410.
42. Porcelli, S., Morita, C. T. & Brenner, M. B. (1992) *Nature (London)* **360**, 593–597.
43. Hanau, D., Fricker, D., Bieber, T., Esposito-Farese, M. E., Bausinger, H., Cazenave, J. P., Donato, L., Tongio, M. M. & de la Salle, H. (1994) *Hum. Immunol.* **41**, 61–68.
44. Balk, S. P., Burke, S., Polischuk, J. E., Frantz, M. E., Yang, L., Porcelli, S., Colgan, S. P. & Blumberg, R. S. (1994) *Science* **265**, 259–262.
45. Araki, T., Gejyo, F., Takagaki, K., Haupt, H., Schwick, H. G., Burgi, W., Marti, T., Schaller, J., Rickli, E., Brossmer, R., Atkinson, P. H., Putnam, F. W. & Schmid, K. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 679–683.
46. Simister, N. E. & Mostov, K. E. (1989) *Nature (London)* **337**, 184–187.
47. Deusch, K., Luling, F., Reich, K., Classen, M., Wagner, H. & Pfeffer, K. (1991) *Eur. J. Immunol.* **21**, 1053–1059.
48. Van Kerckhove, C., Russell, G. J., Deusch, K., Reich, K., Bhan, A. K., DerSimonian, H. & Brenner, M. B. (1992) *J. Exp. Med.* **175**, 57–63.
49. Chowers, Y., Holtmeier, W., Harwood, J., Morzycka-Wroblewska, E. & Kagnoff, M. F. (1994) *J. Exp. Med.* **180**, 183–190.
50. Gross, G. G., Schwartz, V. L., Stevens, C., Ebert, E. C., Blumberg, R. S. & Balk, S. P. (1994) *J. Exp. Med.* **180**, 1337–1344.
51. Kagnoff, M. F. (1993) *Gastroenterology* **105**, 1275–1280.
52. Fodil, N., Laloux, L., Wanner, V., Pellet, P., Hauptmann, G., Mizuki, N., Inoko, H., Spies, T., Theodorou, I. & Bahram, S. (1996) *Immunogenetics* **44**, 351–357.