followed by an additional 10 hours with a 120-s switch interval at 2°C.
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20. Cells were grown overnight in rich medium at 23°C to early log phase. Cells were diluted to an absorbance at 600 (A_{600}) of 0.1 and incubated at 37°C. Samples were tested every hour for 9 hours. Cell viability was calculated as the number of viable colonies formed after plating at 23°C at each time point divided by the number of colonies that formed after plating at 23°C at the 0 hour time point.
21. Supplementary material is available at www.sciencemag.org/feature/data/974048.shl.
22. A. Dillin and J. Rine, data not shown.
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24. Cdc28p was isolated and assayed essentially as described (*33*). The *orc5-1* cultures were grown to A_{600} = 0.1 and either arrested with α-factor, hydroxyurea, or nocodazole for 3 hours at 23°C or shifted to 37°C. Cells (1×10^8) were harvested and lysed for each hourly time point.
25. The *orc5* ts alleles were created by in vitro mutagenesis of the cloned *ORC5* gene by the polymerase chain reaction (PCR). PCR-mutagenized DNA pools were cotransformed into yeast cells with a gapped plasmid missing most of the *ORC5* reading frame such that in vivo recombination would restore plasmid-borne copies of *ORC5*. In one screen, an *orc5-1* strain (JRY4253) was transformed and the transformants were screened for their inability to complement the ts defect of *orc5-1* (*34*). Putative new ts alleles were then recovered and transformed into an *orc5* Δ strain. *orc5-2* through *orc5-12* were identified in this manner. The remaining alleles were identified by directly transforming the *orc5*Δ strain (JRY4154) with the mutagenic PCR reaction and the gapped plasmid in a plasmid-shuffle protocol. Transformants were screened for their ability to complement *orc5*Δ at 23°C, but not at 37°C.
26. Cells were prepared and analyzed by flow cytometry as described (*12*), with the exception that 10 ml of cells were harvested at A_{600} = 0.05 for each time point. All strains were made ρ°, hence lacking mitochondrial DNA, to avoid interference of mitochondrial DNA.
27. WT or *orc5-1* cells were grown and diluted to A_{600} = 0.05 in rich medium. Diluted cultures were arrested by the addition of nocodazole (10 μg/ml) for 3 hours at 23°C. Cells were harvested and resuspended in either 23° or 37°C rich medium containing nocodazole (10 μg/ml) and incubated for an additional 2 hours at either 23° or 37°C. For DNA content analysis, the cultures were washed two times with prewarmed medium and resuspended at A_{600} = 0.05 at either 37° or 23°C. For PFGE, cells were similarly released from the M phase block and cells were harvested after 200 min at either 37° or 23°C.
28. WT and mutant cells were arrested in M phase (*27*). Cells (3×10^6) were released from the M phase block by filtration onto a 0.45-μm filter and washed two times with 37°C YPD. WT cells (3×10^6) of the opposite mating type were filtered onto the same filter and placed onto a YPD plate and incubated at 37°C for 4 hours to allow mating. The filter was washed with minimal medium and the cells were plated onto solid selective medium and incubated at 37°C for up to 4 days. To select for cytoductants during the *kar1-1* mutant matings, cells were plated onto YP plates containing 2% glycerol and cyclohexamide (3 mg/liter) to select for rho$^+$ cytoductants that retained the chromosomes of the *kar1-1* (*cyh*^{R}) parent but not the chromosomes of the Kar$^+$ parent (*cyh*^{S}).
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35. Strains used were as follows: JRY2334 (*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*) (R. Rothstein), JRY3009 (JRY2334 *MAT*α), JRY4012 (JRY2334 *ADE2 lys2*Δ), JRY4249 (JRY3009 *orc5-1*), JRY4253 (JRY3009 *HMR-ss ΔI orc5-1*), JRY4503 (JRY3009 *hmr*Δ*::URA3 orc2-1*), JRY4885 (JRY3009 *HMR-ss*Δ*I orc5-1 ρ°*), JRY5117 (JRY3009, *orc5-1 rad9*Δ*::URA3*), JRY5136 (JRY3009 *orc5-1 mec1-1*), JRY5142 (JRY4012 *orc5-1 mec2-1*), JRY5144 (JRY3009 *ADE2 orc5-1 rad17*Δ*::LEU2*), JRY5449 (*MAT***a** *ade2-1 lys2-801 trp1-1 cyh*^{r} *ρ° kar1-1*) (M. Rose), JRY5487 (JRY3009 *orc5*Δ*ρ°* pRS316-*ORC5*), JRY5493 (JRY3009 *ADE2 lys2*Δ *hmr*Δ*::URA3 orc5-1*).
36. We thank members of the Rine lab, especially M. Neff, for insightful discussions; C. Beh for help with the karyogamy experiments; M. Botchan, S. Martin, and S. Okamura for comments on the manuscript; M. Rose and T. Weinert for strains; and B. Hyun (University of California, San Francisco) for help with FACScan analysis. Supported by an NIH predoctoral fellowship and a Genentech Distinguished Predoctoral Research Fellowship to A.D., a grant from the National Institutes of Health (GM-31105), and by a Mutagenesis Center grant from the National Institute of Environmental Health Sciences for core support (P30ESO1896-12).

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Recognition of Stress-Induced MHC Molecules by Intestinal Epithelial γδ T Cells

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T cells with variable region $V_\delta1$ γδ T cell receptors (TCRs) are distributed throughout the human intestinal epithelium and may function as sentinels that respond to self antigens. The expression of a major histocompatibility complex (MHC) class I–related molecule, MICA, matches this localization. MICA and the closely related MICB were recognized by intestinal epithelial T cells expressing diverse $V_\delta1$ γδ TCRs. These interactions involved the α1α2 domains of MICA and MICB but were independent of antigen processing. With intestinal epithelial cell lines, the expression and recognition of MICA and MICB could be stress-induced. Thus, these molecules may broadly regulate protective responses by the $V_\delta1$ γδ T cells in the epithelium of the intestinal tract.

T cells expressing γδ TCRs recognize antigens without restriction by polymorphic MHC class I or class II molecules and their associated peptide ligands (*1–3*). Of two main subsets in humans, $V_\gamma2/V_\delta2$ T cells predominate in the circulation and respond to bacterial infections by recognizing soluble nonpeptide antigens (*4*). The other subset defined by expression of $V_\delta1$, however, is of unknown function and no antigens have been identified. These T cells represent 70 to 90% of the γδ T cells in the intestinal epithelium (*5*). Because they are oligoclonal and uniformly distributed, they are believed to recognize self antigens that may be stress-induced (*6, 7*).

The localization of the intestinal intraepithelial $V_\delta1$ γδ T cells is matched by the restricted expression of MICA, a divergent MHC class I–related molecule of unknown function (*8*). Its characteristics include the lack of association with β_2-microglobulin (β_2M), stable expression without conventional class I peptide ligands, and the absence of a CD8 binding site (*8, 9*). Notably, the 5′-end flanking regions of the genes for MICA and a closely related molecule, MICB, include putative heat shock elements similar to those of *hsp70* genes, and the encoded mRNAs are increased in heat shock–stressed epithelial cells (*8*).

To explore a functional relation, we established T cell lines from lymphocytes extracted from intestinal epithelial tumors (*10*). Other adequate sources of human intestinal epithelium are generally unavailable. Freshly isolated tumor cells gave positive stainings with monoclonal antibodies (mAbs) 2C10 and 6D4, which are specific for MICA and for MICA and MICB, respectively (*8, 11, 12*). $V_\delta1$ γδ T cells isolated by cell sorting were grown as two lines, δ1A and δ1B, which were cultured in the presence of cytokines and irradiated C1R cells transfected with cDNA for MICA or MICB, respectively (*10, 13*). After expansion, the T cell lines were tested for phenotype and function. They were homogeneously positive for $V_\delta1$ γδ TCRs, $CD4^-$, and $CD8^-$ (Fig. 1A). As is characteristic of intestinal intraepithelial T cells, they expressed the $\alpha_E\ \beta_7$ integrin (*12, 14*). In chromium release assays with C1R- MICA or C1R-MICB cells as targets, the δ1A and δ1B T cells were cytotoxic against both of these transfectants, but not against untransfected C1R cells (Fig. 1B) (*15*). Two $CD8^+$ αβ T cell lines grown and tested under identical

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conditions gave negative results. The same observations were made with T cell lines from a second intestinal epithelial tumor and when T cells were expanded in the absence of MICA and MICB (*12*).

We used the δ1B line to analyze the apparent recognition of MICA and MICB. MICA transfectants of Daudi cells, which lack β_2M and surface MHC class I (*16*), were as effectively lysed as Daudi-β_2M-MICA double transfectants with normal expression of class I (Fig. 2A) (*13*). Transfectants of the lymphoblastoid cell line mutant 5.2.4, which lacks expression of most MHC class II molecules (*17*), were also recognized (Fig. 2B) (*13*), as were transfectants of mouse T cell lymphoma EL4 cells (*12*). No lytic activity was observed against B cell lines with diverse MHC haplotypes. Thus, the δ1B T cell responses were independent of MHC class I and class II and were not secondary to cross-reactivity with some alleles of these molecules. Cytotoxicity against the transfected target cells was inhibited when these cells were preincubated with mAbs 2C10 or 6D4 (Fig. 2, C and D). The epitopes recognized by these mAbs are within the α1α2 domains of MICA and MICB, as determined by stainings of C1R transfectants expressing mouse class I H-2D^b or K^b hybrid molecules in which the α1α2 or α3 domains have been substituted with the corresponding sequences of MICA (*8*, *12*). The δ1B T cells lysed C1R-MICAα1α2-D^b cells, but not C1R-MICAα3-K^b cells (*12*). Thus, $V_\delta1$ γδ T cells from intestinal epithelium were restricted by MICA and MICB and recognized an epitope or epitopes associated with the α1α2 domains of these molecules.

We examined whether the recognition of MICA involved antigen processing and presentation of peptide ligands. With conventional MHC class I, the peptides are generated by proteasomes in the cytosol and are translocated into the endoplasmic reticulum by the transporters associated with antigen processing (TAP) (*18*). Treatment of C1R-MICA cells with lactacystin, which blocks proteasome functions (*19*), had no effect on the recognition of MICA by the δ1B T cells, but this did not preclude the presence of long-lived MICA-peptide complexes. However, the transfected mutant 5.2.4 cells, which lack TAP (*17*), were also proficient targets (Fig. 2B); this result implies that MICA has no function in the MHC class I pathway.

We sought physical evidence for MICA-peptide complexes by gel filtration chromatography of acid-dissociated immunoprecipitates that were isolated with mAb 2C10 from lysate of C1R-MICA cells after metabolic labeling with tritiated amino acids. The eluted fractions contained a single peak of radiolabeled polypeptide that was of high molecular weight and corresponded to MICA (Fig. 2E) (*20*). Analysis of MHC class I complexes isolated with mAb W6/32 (anti–HLA-A, -B, and -C) yielded fractions of high and low molecular weights (Fig. 2F) (*21*). Thus, under these experimental conditions, there was no evidence for an association of MICA with peptides. This was consistent with its recognition by the δ1B T cells independent of conventional class I antigen processing.

These results were in agreement with previous models of antigen recognition by γδ T cells (*2*, *3*) and supported a role of MICA and MICB as self antigens. We used intestinal epithelial cell lines to investigate the expression, regulation, and T cell recognition of these molecules. Semiconfluent DLD-1, Lovo, HCT116, and HUTU-80

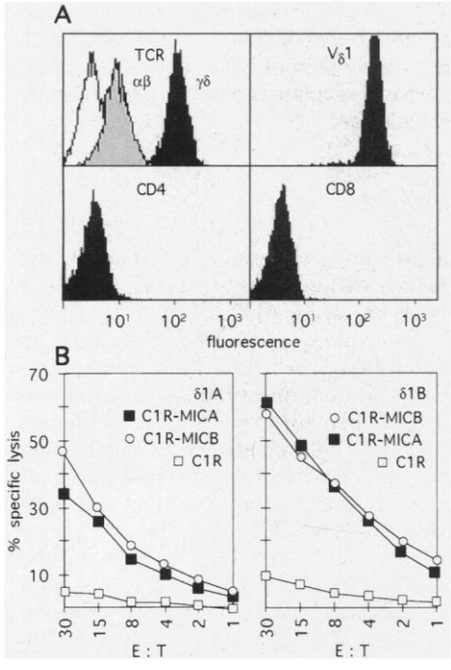

Fig. 1. $V_\delta1$ γδ T cell lines from intestinal epithelium recognize C1R transfectants expressing MICA or MICB. (**A**) Surface phenotype of the δ1B T cells by immunofluorescence stainings and flow cytometry with the following mAbs: αβ TCR (anti-TCR- α/β-1) (shaded profile), γδ TCR (anti-TCR-γ/δ-1), $V_\delta1$ (mAb δTCS1), CD4 (mAb Leu-3a), and CD8 (mAb Leu-2a) (*10*). Note that mAb anti-TCR- α/β-1 weakly stains γδ TCR$^+$ T cells. The open profile is an isotype-matched control staining. Similar profiles were obtained with the δ1A T cells. (**B**) In chromium release assays, the δ1A and δ1B T cells lysed C1R-MICA and C1R-MICB transfectants but not untransfected C1R cells. Data are means of triplicate experiments with less than ±5% deviation. E:T, effector-to-target cell ratio.

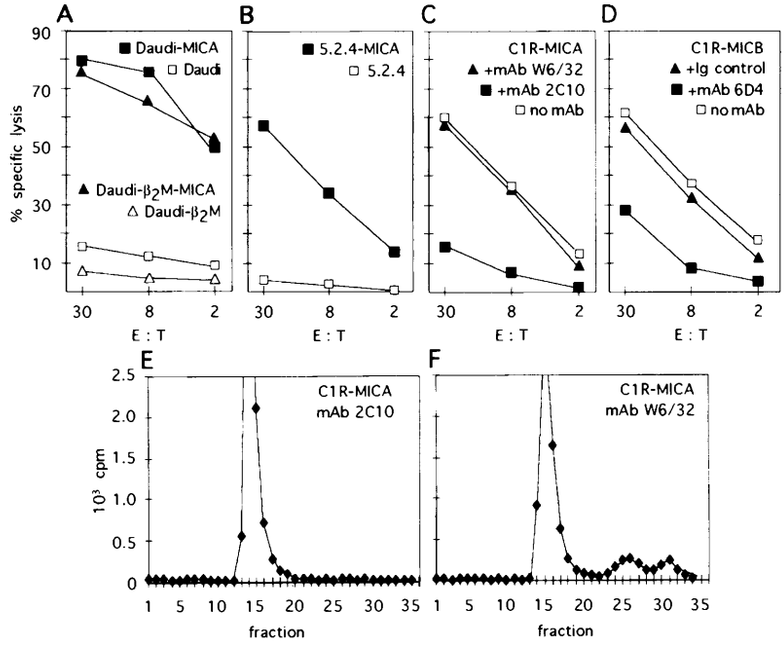

Fig. 2. $V_\delta1$ γδ T cell responses are restricted by MICA and MICB and are independent of β_2M and conventional class I antigen processing. (**A** and **B**) The δ1B T cells lysed Daudi-MICA (β_2M^-, class I$^-$), Daudi-β_2M-MICA (class I$^+$), and 5.2.4-MICA (DR$^-$, DQ$^-$, TAP$^-$) transfectants but showed no or minimal lytic activity against the untransfected cells. (**C** and **D**) Binding of mAbs 2C10 (anti-MICA) and 6D4 (anti-MICA and -MICB) inhibited lysis of C1R-MICA and C1R-MICB targets, respectively. Treatment with the anti–HLA-A, -B, and -C mAb W6/32 (*21*) or isotype control IgG had no effect. (**E** and **F**) Gel filtration analysis of acid-dissociated immunoprecipitates isolated with mAbs 2C10 or W6/32 from C1R-MICA cells after metabolic labeling with [^{3}H]amino acids (*20*).

cells that were rapidly proliferating expressed large amounts of MICA and MICB. They were lysed by the δ1B T cells in a specific interaction that was inhibited by mAb 6D4 (*12*, *22*).

The expression of MICA in intestinal epithelium may be stress-induced rather than constitutive (*8*). In proliferating cell lines, however, transcription of the *hsp70* gene is activated in the absence of cellular stress (*23*). We used Lovo, HCT116, and HUTU-80 cells grown as nonproliferating confluent monolayers to investigate the expression of MICA and MICB before and after heat shock induction. Uninduced cells had very low steady-state levels of MICA and MICB mRNA and expressed small amounts of the encoded surface molecules. However, heat shock induction resulted in large increases in mRNA and protein expression (Fig. 3, A and B) (*24*). Concurrently, hsp70 mRNA was potently induced, whereas class I HLA-B mRNA and surface class I HLA-A, -B, and -C detected with mAb W6/32 on HCT116 and HUTU-80 cells (Lovo lacks β_2M and thus class I surface expression) were unchanged (Fig. 3A) (*12*). The heat shock–treated cells were sensitized to lysis by the δ1B T cells, whereas minimal lytic activity was observed with the uninduced target cells. As with the proliferating cell lines, cytotoxicity was inhibited by mAb 6D4 (Fig. 3C). Thus, the expression of MICA and MICB and their recognition by the δ1B T cells were regulated by cell stress. Because these results were obtained with cell lines derived from intestinal epithelium, which is the only peripheral site where expression of MICA has been observed (*8*), MICA and presumably MICB were functionally associated with $V_\delta1$ γδ T cells in this compartment.

We investigated whether MICA and MICB were recognized by T cells expressing diverse γδ TCRs and sought evidence for TCR engagement. A total of 16 T cell clones derived from the δ1A and δ1B lines showed functional activity against C1R-MICA and C1R-MICB targets. Analysis of cDNA sequences identified five distinct γ and δ chain pairs (Fig. 4, A and B) (*25*). The γ chains included V_γ1.3, 1.4, 1.5, or 1.8, and J_γ2.1 or 2.3. All of the δ chains expressed $V_\delta1$ and $J_\delta1$ with diverse junctions encoded by one or two D segments and nontemplated N region nucleotides (Fig. 4A) (*1*, *26*). Because prolonged culture frequently resulted in loss of functional activity of T cell clones, these sequences were a minimal representation of different $V_\delta1$ γδ T cells capable of recognizing MICA and MICB. We tested the ability of T cell clones 1, 3, and 5 to recognize C1R-MICA targets in the presence of the $V_\delta1$ mAb δTCS1 (*10*, *15*). With all three clones, inhibitory effects were observed (Fig. 4C). These data showed that MICA and MICB were recognized by $V_\delta1$ γδ T cells expressing diverse TCRs and supported an engagement of these molecules by these TCRs. The diversity of TCRs implied that most, if not all, intestinal epithelial $V_\delta1$ γδ T cells may be capable of interacting with MICA and MICB.

Our results define a T cell subset–MHC ligand interaction. Intestinal epithelial $V_\delta1$ γδ T cells recognize epithelial cell lines without restriction by polymorphic MHC class I or class II molecules (*27*). We have now shown that stress-induced MHC class I–related molecules, MICA and MICB, function as target antigens recognized by these T cells. A number of allelic variants of MICA of uncertain significance have been identified (*28*). We observed no differences in the recognition by the δ1B T cells of C1R transfectants expressing three alleles representing most of the sequence variation in the α1α2 domains of MICA (*29*). Thus, although MICA and MICB are encoded in the MHC, their recognition was "MHC-unre-

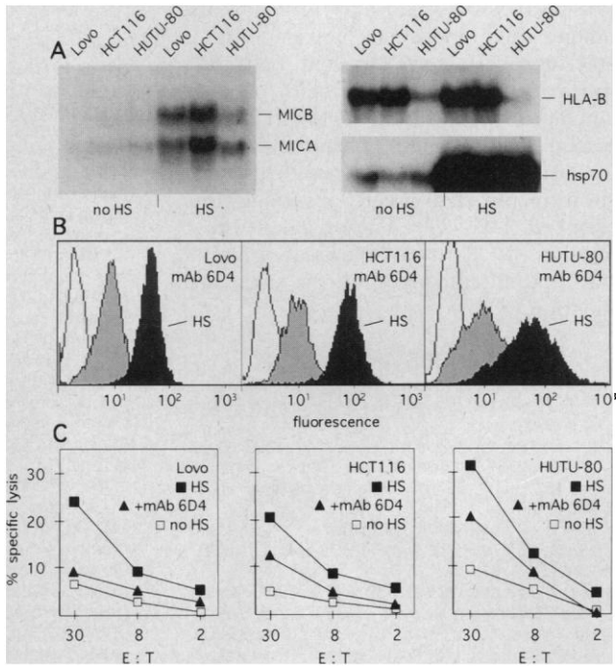

Fig. 3. Stress-induced expression and T cell recognition of MICA and MICB on quiescent intestinal epithelial cell lines. Lovo, HCT116, and HUTU-80 cells cultured for 8 days as confluent monolayers had very low steady-state levels of MICA and MICB mRNAs by blot hybridization of total cellular RNAs (**A**) (*24*). They expressed small amounts of the encoded cell surface proteins by indirect immunofluorescence staining with mAb 6D4 and flow cytometry (shaded profiles in **B**) and were poorly lysed by the δ1B T cells (**C**). Heat shock treatment strongly increased MICA and MICB mRNA (A) and protein expression (filled profiles in B), and also sensitized target cells to lysis, which was inhibited by mAb 6D4 (C); hsp70 mRNA was potently induced and control HLA-B mRNA was unaltered (A). The hsp70 blot was exposed to film for a much shorter time. Open profiles in (B) are isotype IgG1 control stainings. HS, heat shock.

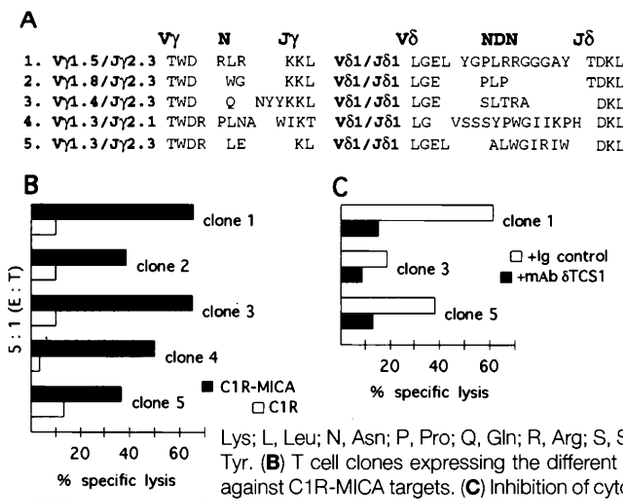

Fig. 4. MICA is recognized by T cell clones expressing diverse $V_\delta1$ γδ TCRs. (**A**) Five different γ and δ chain sequence pairs were identified by reverse transcription–PCR and direct sequencing among the 16 T cell clones derived from the δ1A and δ1B lines. Of the V and J region sequences flanking the variable N or N(D)N regions, only a few amino acids are shown (*25*). Abbreviations for the amino acid residues are as follows: A, Ala; D, Asp; E, Glu; G, Gly; H, His; I, Ile; K, Lys; L, Leu; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. (**B**) T cell clones expressing the different γδ heterodimers were cytotoxic against C1R-MICA targets. (**C**) Inhibition of cytotoxicity of T cell clones expressing TCR sequences 1, 3, and 5 by $V_\delta1$ mAb δTCS1 (*10*). Control IgG1 antibody was used under the same conditions. Data shown are representative of several independent assays and were obtained at a constant effector-to-target ratio of 5 to 1.

stricted." This was in accord with the recognition of all of the intestinal epithelial cell lines tested. Because MICA and MICB were recognized on diverse target cells without an apparent requirement for antigen processing, and there was no evidence for associated peptide ligands, it seems probable that these molecules alone conferred specificity in the recognition by the $V_\delta1$ γδ T cells. This inference would be consistent with current models of γδ T cell recognition of antigen but remains tentative until the absence of peptide or nonpeptide ligands is conclusively demonstrated.

The stress-induced expression of MICA and MICB and their recognition by diverse $V_\delta1$ γδ T cells may serve as an immune surveillance mechanism for the detection of damaged, infected, or transformed intestinal epithelial cells, or may stimulate T cell secretion of growth factors for the maintenance of epithelial homeostasis, as originally proposed for murine intraepithelial T cells expressing invariant γδ TCRs (*7*). The irregular distribution of MICA in variable areas of intestinal epithelium may reflect such an induction (*8*).

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10. Bulk lymphocytes extracted from surgical specimens of intestinal epithelial tumors were provided by K. Grabstein and H. Secrist (Corixa Corp., Seattle, WA). These were prepared by enzymatic digestion of minced tissue samples, sieving through wire mesh, and centrifugation in differential Ficoll-Hypaque (Pharmacia) gradients [T. L. Whiteside, S. Miescher, J. Hurlimann, L. Moretta, V. von Fliedner, *Int. J. Cancer* **37**, 803 (1986); E. C. Ebert, *Gastroenterology* **97**, 1372 (1989)]. Collected and washed lymphocytes were seeded in 96-well round-bottom plates (10^3 cells per well) and cultured in the presence of γ-irradiated C1R-MICA or C1R-MICB cells (*13*) (2×10^4 cells per well) in RPMI media supplemented with 8% fetal calf serum, 2% pooled human serum, recombinant human interleukin-2 (rhIL-2, 2 IU/ml; Cetus), rhIL-7 (10 ng/ml; provided by N. Vita, Sanofi Recherche, France) [M. Watanabe *et al.*, *J. Clin. Invest.* **95**, 2945 (1995)], phytohemagglutinin (0.5 μg/ml; Difco), glutamine, and antibiotics. After 1 week, pooled lymphocytes were sorted for $V_\delta1$ γδ T cells with the use of fluorescein isothiocyanate (FITC)–conjugated mAb δTCS1 (Endogen) [Y. J. Wu *et al.*, *J. Immunol.* **141**, 1476 (1988)] and a FACS VANTAGE cell sorter (Becton Dickinson), and were cultured as above with weekly restimulations. Double staining with mAb δTCS1 and biotinylated anti-TCR-γ/δ-1 (Becton Dickinson) [J. Borst *et al.*, *J. Exp. Med.* **167**, 1625 (1988)] indicated that all γδ T cells expressed $V_\delta1$. The remaining lymphocytes were αβ T cells, as shown by staining with anti-TCR-α/β-1 (Becton Dickinson) [W. J. M. Tax, H. M. Willems, P. P. M. Reekers, P. J. A. Capel, R. A. P. Koene, *Nature* **304**, 445 (1983)], and were grown under identical conditions. T cell lines were first tested 3 weeks after sorting. The δ1A and δ1B lines were $CD4^-$ and $CD8^-$, as determined with mAbs Leu-3a and Leu-2a (Becton Dickinson), respectively, and were $\alpha_E\beta_7^+$ as shown by staining with mAb CD103 (Immunotech). After another 3 weeks, T cells were grown in the additional presence of irradiated allogeneic peripheral blood mononuclear cells. T cell clones from the δ1A and δ1B lines were derived after the first week of culture by limiting dilution plating and expanded for at least 3 weeks before functional testing.
11. The mAb 6D4 was generated by immunization of RBF/DnJ mice (Jackson Laboratories) with mouse LTK-MICA transfectants as described (*8*) and identified by screenings of hybridoma supernatants by indirect immunofluoresence stainings and flow cytometry of C1R, C1R-MICA, and C1R-MICB transfectants (*13*). This mAb was subcloned twice and is of the immunoglobulin G1 (IgG1) isotype.
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13. MICA cDNA transfectants of C1R, Daudi, and 5.2.4 have been described (*8*). Transfections of EL4 and Daudi-β_2M cells [B. Sadasivan, P. J. Lehner, B. Ortmann, T. Spies, P. Cresswell, *Immunity* **5**, 103 (1996)] with MICA cDNA (*9*) in RSV.5neo or in pREP8Δ (*8*) [D. Arnold *et al.*, *Nature* **360**, 171 (1992)], and of C1R cells with MICB cDNA [S. Bahram and T. Spies, *Immunogenetics* **43**, 230 (1996)] in RSV.5neo, were done as described [A. G. Grandea III, M. J. Androlewicz, R. S. Athwal, D. E. Geraghty, T. Spies, *Science* **270**, 105 (1995)]. Stable transfectant isolates had large amounts of surface MICA or MICB, as shown by indirect immunofluorescence stainings with mAb 2C10 or 6D4 and flow cytometry (*8, 11*).
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15. Chromium release assays were according to standard conditions, in 96-well round-bottom plates, with target cells plated at 3×10^3 per well after labeling for 2 hours with [^{51}Cr]sodium chromate and washing. Adherent target cells were harvested with nonenzymatic cell dissociation solution (Sigma). Effectors were added at the indicated ratios and briefly centrifuged, and released radioactivity was determined after 4 hours at 37°C by means of Luma Plates and a TopCount microplate scintillation counter (Packard). Specific lysis (expressed as a percentage) was calculated using the standard formula [(experimental − spontaneous release/total − spontaneous release) × 100]. Data in Figs. 1 and 2 are the mean of triplicate experiments with usually less than ±5% deviation. All experiments were repeated at least once and in most cases several times. For blocking with mAb, labeled target cells were preincubated for 30 min with saturating amounts of 2C10, 6D4, or W6/32 ascites before exposure to T cells. TCR $V_\delta1$ mAb δTCS1 (*10*) or control IgG1 was added at 10 μg/ml to T cells 30 min before the addition of labeled targets.
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19. Target cells were exposed to 10 or 50 μM lactacystin (Calbiochem) for 2 hours before labeling and subsequent testing with the δ1B T cells [G. Fenteany *et al.*, *Science* **268**, 726 (1995)].
20. C1R-MICA cells (2×10^7) were labeled with a mixture of 15 ^{3}H-labeled amino acids (Code TRK440; Amersham) and proteins solubilized in 2 ml of NP-40 lysis buffer in the presence of protease inhibitors. After preclearing with protein A–Sepharose (Pharmacia), MICA and MHC class I (C1R cells express only HLA-C) were each precipitated from half of the lysate using ascites of mAb 2C10 and W6/32 (*21*), respectively. Washed immunoprecipitates were incubated in 0.2 ml of 1 M acetic acid for 15 min and fractionated by gel filtration using a Biogel P10 column (Bio-Rad; equilibrated with 1 M acetic acid, 1% bovine serum albumin, and 0.1% NP-40). Fractions (0.5 ml) were collected and radioactivity was counted.
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24. For heat shock treatment, the adherent cells grown in Petri dishes were immersed in a 42.5°C water bath for 90 min. One hour later, total cellular RNAs were prepared, gel-fractionated, and blot-hybridized with [^{32}P]deoxycytidine triphosphate–labeled DNA probes for MICA, HLA-B, and hsp70 as described (*8*). For surface staining with mAb and cytotoxicity assays, cells were harvested 12 to 16 hours after heat shock induction with nonenzymatic cell dissociation solution (Sigma).
25. Total RNA was isolated with STAT-60 reagent (Tel-Test) and cDNA was synthesized with oligo(dT) and AMV reverse transcriptase (Promega). TCR γ and δ sequences [B. Arden, S. P. Clark, D. Kabelitz, T. W. Mak, *Immunogenetics* **42**, 455 (1995)] were amplified by the polymerase chain reaction (PCR) using Taq polymerase (Pharmacia) and selected primers for $V_\gamma1$ segments (5′-AAGTCGACTGGTACCTACACCAGGA-3′), $V_\gamma2$ (5′-AAGTCGACTATTGGTATCGAGAGAGACC-3′), C_γ [biotin-(5′-CCGAATTCGTATGTTCCAGCCTTCTGGA-3′)], $V_\delta1$ (5′-CCGTCGACGTCAACTTCAAGAAAGCAGC-3′), and C_δ [biotin-(5′-GTAGAATTCCTTCACCAGAC-3′)]. PCR cycles included 30 s at 95°C, 20 s at 56°C, and 60 s at 72°C. Alkali-denatured DNA strands were separated with streptavidin-coupled magnetic beads (M-280; Dynal) and single-stranded DNAs were sequenced. The GenBank accession numbers of the five γ and five δ chain sequences (in order from 1 to 5) are AF025412 to AF025416 and AF025417 to AF025421, respectively.
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29. We tested C1R cDNA transfectants expressing MICA alleles 1, 4, and 5 (*13, 28*).

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