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T Cell Antigen Receptor Engagement and Specificity in the Recognition of Stress-Inducible MHC Class I-Related Chains by Human Epithelial γδ T Cells[1] FREE

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T Cell Antigen Receptor Engagement and Specificity in the Recognition of Stress-Inducible MHC Class I-Related Chains by Human Epithelial γδ T Cells[1]

Jennifer Wu, Veronika Groh, and Thomas Spies[2]

Human γδ T cells with the TCR variable region $V_\delta1$ occur mainly in epithelia and respond to stress-induced expression of the MHC class I-related chains A and B, which have no function in Ag presentation. MIC function as ligands for NKG2D-DAP10, an activating receptor complex that triggers NK cells, costimulates CD8 αβ and $V_\gamma9V_\delta2$ γδ T cells, and is required for stimulation of $V_\delta1$ γδ T cells. It is unresolved, however, whether triggering of $V_\delta1$ γδ TCRs is also mediated by MIC or by unidentified cell surface components. Soluble MICA tetramers were used as a binding reagent to demonstrate specific interactions with various $V_\delta1$ γδ TCRs expressed on transfectants of a T cell line selected for lack of NKG2D. Tetramer binding was restricted to TCRs derived from responder T cell clones classified as reactive against a broad range of MIC-expressing target cells and was abrogated when TCRs were composed of mismatched γ- and δ-chains. These results and the inability of $V_\delta1$ γδ T cells to respond to target cells expressing the ULBP/N2DL ligands of NKG2D, which are highly divergent from MIC, indicate that MIC delivers both the TCR-dependent signal 1 and the NKG2D-dependent costimulatory signal 2. This dual function may serve to prevent erroneous γδ T cell activation by cross-reactive cell surface determinants. ***The Journal of Immunology,*** **2002, 169: 1236–1240.**

Unlike T cells with αβ TCRs, which recognize Ag-derived peptides bound to MHC molecules, most T cells with γδ TCRs recognize Ags directly, without known requirements for Ag processing and presentation (1–4). In humans, $V_\gamma9/V_\delta2$ T cells recognize soluble mycobacterial prenyl pyrophosphate and alkamine compounds (5–8). They are cytotoxic, secrete Th1-type cytokines, and proliferate during bacterial infections. As with all human γδ T cells, their TCRs display substantial sequence diversity in the rearranged γ- (V-N-J) and δ- (V-NDN-J) chains, including variability in nontemplated (N) sequences and numbers of D segments (2). Considerations of δ-chain complementarity-determining region 3 length distributions and the crystal structure of a $V_\gamma9/V_\delta2$ TCR have indicated that Ag recognition is more akin to Igs than to αβ TCRs (9, 10). This is supported by an in vivo experimental model showing conservation of similar conformations among natural and different replacement γδ TCRs expressed on dendritic epidermal T cells from normal and TCR γ-chain mutant mice, respectively (11).

While $V_\gamma9/V_\delta2$ T cells predominate in the circulation, a smaller subset of γδ T cells defined by the expression of $V_\delta1$ is enriched in intestinal epithelium and other epithelial sites (12, 13). Some of these T cells recognize CD1c, a member of the CD1 family of lipid Ag-presenting molecules that is expressed on professional APC (14, 15). In addition, numerous $V_\delta1$ γδ T cell lines and clones have been shown to recognize the stress-inducible MHC class I-related chains A and B (MICA and MICB)[3] and MICB, which have no function in Ag presentation (16–20). MIC have a restricted tissue distribution in intestinal epithelium, are frequently expressed in epithelial tumors, and are induced by viral and mycobacterial infections (17, 19, 21, 22). This expression pattern coincides with the main occurrences of $V_\delta1$ γδ T cells, which have previously been postulated to recognize self Ags that might be stress-induced, a model originally proposed for mouse intraepithelial γδ T cells with invariant TCRs (23–26). $V_\delta1$ γδ T cells reactive with MIC are cytotoxic and produce Th1-type cytokines (19). Inhibition of cytotoxicity by Abs specific for Vδ1 and increased frequencies of $V_\delta1$ γδ T cells among lymphocyte infiltrates from tumors with induced MIC expression provide tentative evidence for TCR-mediated MIC recognition (18, 19). However, this has become controversial because of the subsequently demonstrated interaction of MIC with the activating NKG2D receptor, which is required for triggering of $V_\delta1$ γδ T cell functions (27). NKG2D forms homodimers that are associated with DAP10, an adaptor protein that signals similar to CD28 by recruitment of phosphatidylinositol 3-kinase (28). Engagement of NKG2D amplifies Ag-specific CD8 αβ T cell responses and costimulates cytokine production and proliferation of CD8 αβ and $V_\gamma9/V_\delta2$ γδ T cells (21, 22). As with most T cells, it is almost certain that TCR triggering provides signal 1 in the activation of MIC-reactive $V_\delta1$ γδ T cells, although the ligand interactions remain to be conclusively resolved. Because these T cells respond to diverse untransfected or transfected target cell types expressing MIC, including a mouse B cell line, their TCRs may recognize either MIC or, alternatively, some unidentified ubiquitous cell surface moieties (18, 19).

The present study demonstrates specific binding of MICA tetramers to TCRs derived from responder, but not nonresponder, $V_\delta1$ γδ T cell clones, as defined by their reactivity against MIC-expressing target cells and explores the mode of these interactions

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[3] Abbreviations used in this paper: MICA, MHC class I-related chain A; MICB, MHC class I-related chain B.

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using T cell transfectants expressing TCRs composed of mismatched γ- and δ-chains. Together with functional experiments showing the insufficiency of the ULBP/N2DL ligands of NKG2D, which are highly divergent from MIC, to activate $V_\delta1$ γδ T cells, the results, in aggregate, provide firm evidence for a dual function of MIC as ligands for a subset of $V_\delta1$ γδ TCRs and NKG2D.

Materials and Methods

Cell lines, Abs, and transfections

The human J.RT3-T3.5 TCR β-chain mutant and HPB-ALL T cell lines, which are negative for NKG2D by surface staining with specific mAb, were obtained from American Type Culture Collection (Manassas, VA). The origin, specificity, and culture conditions of the δ1B $V_\delta1$ γδ T cell line have been described previously (18). The NKL NK cell line was provided by Dr. M. J. Robertson (29). C1R cell transfectants expressing MICA (alleles *1 or *4) or N2DL1, -2, or -3 have been described previously (30). TCR-γ/δ-1 mAb (anti-Cγ) and secondary Ab reagents were purchased from BD Biosciences (San Jose, CA); mAb δTCS1 (anti-Vδ1) was obtained from Endogen (Cambridge, MA); biotinylated anti-FLAG tag mAb M2 (bio-M2) was purchased from Sigma (St. Louis, MO); mAb 1D11 (anti-NKG2D) has been previously described (27); mAb 3F1 (anti-N2DL1, mouse IgM) was recently generated in our laboratory (unpublished observations). Previously cloned and sequenced TCR γ- and δ-chain cDNAs (18, 19) in pcDNA3.1 vectors (Invitrogen, Carlsbad, CA) with linked neomycin or hygromycin B drug resistance genes were transfected into J.RT3-T3.5 cells by electroporation with a Gene Pulser (Bio-Rad, Hercules, CA) or using Lipofectamine reagent (Life Technologies, Gaithersburg, MD) for subsequent selection of stable transfectants or transient TCR expression, respectively. For transfection of HPB-ALL T cells, the TCR γ- and δ-chain cDNAs were subcloned into the episomal pREP4 and pREP9 expression vectors (Invitrogen), which contain hygromycin B and neomycin resistance genes, respectively. HPB-ALL cells (5×10^6) were cotransfected with pREP4-γ and pREP9-δ chain constructs (10 μg each) by electroporation using the Gene Pulser with 960 μFD and 250 V settings. Stable transfectants were selected with G418 (1.0 mg/ml) and hygromycin B (0.3 mg/ml), screened for binding of mAbs TCR-γ/δ-1 and δTCS1, and purified by flow sorting.

Generation of MICA tetramers

Tetrameric recombinant MICA was generated following the approach described by Altman et al. (31) and Gütgemann et al. (32), except that the polypeptide was expressed as a secreted protein in High-Five insect cells (Invitrogen) using the BAC-TO-BAC baculovirus expression system (Life Technologies). Using appropriate oligonucleotide primers and PCR, the extracellular sequences of MICA corresponding to the signal peptide and the α1α2α3 domains were amplified from template cDNA and purified. In subsequent rounds of PCR and amplicon purification, the truncated MICA sequence was fused to the 15-aa biotinylation recognition sequence (LHHILDAQKMVWNHR) (33) using the primers 5′-TCTGGATCCATGG GGCTGGGCCCGGTC-3′ (MICA 5′-end) and 5′-ACGATGAATCCAC ACCATTTTCTGTGCATCCAGAATATGATGCAGGCTTCCTTTCCCA GAGGGGACAGGGGTGAG-3′ (biotinylation recognition sequence-glycine-serine linker-MICA 3′-end overlap) and to sequences for a hexa-histidine tract and the 8-aa FLAG tag (DYKDDDDK) M2 Ab epitope (34). The amplicon was flanked by restriction sites for insertion into pFAST-BAC1 (Life Technologies). The construct was sequenced and transfected into Sf9 cells for production of high titer recombinant Baculovirus, which was used to infect High-Five cells. Medium was harvested from cell cultures grown in Spinner flasks 4–5 days postinfection, cleared, filtered, and applied to a Ni^{2+}-charged chelating Sepharose Fast Flow resin column (Pharmacia, Piscataway, NJ). Recombinant protein was eluted with imidazol in PIPES/NaCl/NaN_3 buffer and was further purified by Superdex S75 gel filtration chromatography (Pharmacia). Soluble MICA (1.8 mg) was biotinylated by overnight incubation at 27°C in the presence of 15 μg BirA enzyme (AVIDITY, Boulder, CO), 80 μM biotin, 10 mM ATP, 10 mM MgOAc, 20 mM bicine, and 10 mM Tris (pH 8.3). Free biotin was removed by Sephadex G-25 (Pharmacia) chromatography, and MICA was tetramerized with streptavidin (Molecular Probes, Eugene, OR) at a ratio of 5:1. Tetramer was analyzed by Superdex S200 filtration chromatography and concentrated using a BIOMAX-100K (Millipore, Bedford, MA) ultrafiltration device.

Tetramer stainings of γδ TCR transfectants

The HPB-ALL TCR transfectants were incubated with MICA tetramer (50–200 ng/ml) and bio-M2 mAb, washed, and stained with PE-streptavidin (each step was 30 min on ice). Washed cells were analyzed using a FACScan cytometer and CellQuest software (BD Biosciences). For testing of Ab blocking, cells were preincubated with mAb δTCS1 or negative control mouse IgG1 for 30 min on ice before washing and incubation with tetramer.

Cytotoxicity assays

The origin, specificity, and culture conditions of the δ1B $V_\delta1$ γδ T cell and NKL NK cell lines have been described previously (18, 27, 29). Cytotoxicity assays with [^{51}Cr]sodium chromate-labeled target C1R-MICA or -N2DL transfectants, Ab inhibition experiments, and the standard calculation of specific lysis (as a percentage) were carried out as previously described (18). The redirected lysis assays with FcR^+ P815 mouse mastocytoma target cells in the presence of anti-NKG2D (mAb 1D11) or anti-CD3 (mAb OKT3; BD Biosciences) were performed as previously described (27).

Results and Discussion

Failure to redirect specificity by expression of γδ TCRs in a Jurkat mutant T cell line

To obtain direct evidence for TCR-mediated γδ T cell recognition of MIC, initial efforts were aimed at demonstrating transfer of specificity by transfection of the Jurkat mutant J.RT3-T3.5 T cell line, which lacks endogenous TCR-β and surface NKG2D and is most commonly used for transfer of human αβ or γδ TCRs. Upon TCR triggering, J.RT3-T3.5 cells respond by secretion of IL-2. In several repeat experiments matched TCR γ- and δ-chain cDNAs (corresponding to sequences 1–3 in Fig. 1*A*) derived from previously studied $V_\delta1$ γδ T cell clones were cotransfected in pcDNA3.1 vectors with different drug marker genes, and stable transfectants were selected. All transfectants in several hundred cell culture wells screened by flow cytometry were negative for binding of Abs specific for Vδ1 (mAb δTCS1) or Cγ (TCR-γ/δ-1), although control transfections of HLA-A2 and -B5 cDNA constructs yielded numerous isolates expressing both the encoded surface molecules. However, ~8–12% of J.RT3-T3.5 cells were positive for γδ TCR by staining with both mAbs 72 h after transient transfections (data not shown). These discrepancies implied that during the long term selection of stable transfectants, cell viability may have been negatively affected by the expression of γδ TCRs. This was supported by the unexpected finding that J.RT3-T3.5 cells are positive for surface MIC, suggesting that continuous stimulation of TCR transfectants might have induced cellular death. Efforts using transient transfectants for detection of IL-2 production gave no reproducible results, presumably because of their small numbers in addition to heterogeneity in signaling pathways among J.RT3-T3.5 T cells.

Binding of MICA tetramers to γδ TCRs expressed on HPB-ALL T cell transfectants

As an alternative to the failed functional approach to demonstrate γδ TCR specificity for MIC, we investigated whether binding of MICA tetramers could provide evidence for specific interactions with γδ TCRs. Tetramers of polymorphic MHC class I- or class II-peptide complexes have been widely used as high avidity binding reagents to detect Ag-specific T cells (31, 32, 35, 36). Moreover, tetramers of the mouse nonclassical MHC class I T22 molecule have been shown to specifically interact with the TCRs of a small population of splenic γδ T cells (37). Soluble recombinant MICA, including its extracellular domains, a biotinylation site, a hexa-histidine tract for affinity purification, and the FLAG tag M2 Ab epitope, was produced in insect cells and isolated from culture supernatant. Protein chains were biotinylated using BirA enzyme, tetramerized with streptavidin, and purified (see *Materials and Methods*). By staining with biotinylated mAb M2 (bio-M2) and PE-conjugated streptavidin and analysis by flow cytometry, MICA

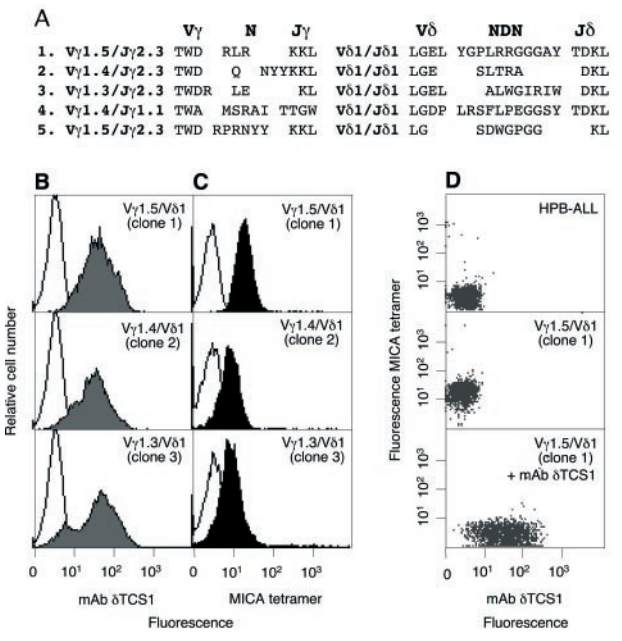

FIGURE 1. Specific binding of MICA tetramers to γδ TCRs expressed on transfectant HPB-ALL T cells. *A*, The variable region amino acid sequences (single-letter code) in *lanes 1–3* correspond to γ- and δ-chain cDNAs obtained from responder $V_\delta1$ γδ T cell clones that are cytotoxic against diverse MIC-expressing target cells (18). Sequences in *lanes 4* and *5* were derived from unresponsive T cell clones (19). *B* and *C*, Flow cytometric histograms of representative isolates of HPB-ALL cells stably transfected with γ- and δ-chain expression constructs corresponding to the sequences 1–3 shown in *A*. The transfectants bound the Vδ1 specific mAb δTCS1 (▨) and MICA tetramers detected by staining with biotinylated anti-FLAG tag mAb M2 (bio-M2; ■). The tetramer staining of clone 3 apparently does not replicate the bimodal Ab staining. However, Abs and tetramers have different binding modalities, as can be seen with clones 1 and 2. Moreover, as with clone 2, the left flank of the clone 3 tetramer staining profile overlaps the mAb M2-negative staining control and thus includes the cell population with very low levels of TCR. □, Control stainings of untransfected HPB-ALL cells. *D*, Two-color flow cytometric analysis of transfected HPB-ALL cells stained with MICA tetramers/bio-M2 (*y*-axis) after preincubation with mAb δTCS1 (*bottom dot plot; x*-axis) showed specific inhibition of tetramer binding. The *upper* and *middle dot plots* are tetramer stainings of untransfected and transfected HPB-ALL cells, respectively. Similar results were obtained with transfectants expressing the other $V_\delta1$ γδ TCRs.

tetramers bound strongly to the NKL NK cell line and to peripheral blood $CD8^+$ T cells and $CD56^+$ NK cells, but not to $CD4^+$ T cells and $CD20^+$ B cells (data not shown). This binding pattern replicated the distribution of NKG2D, which stably interacts with MICA (27, 30). Binding was inhibited by anti-NKG2D mAb (data not shown). Thus, these results confirmed the utility and specificity of the tetramer reagent.

To generate γδ TCR transfectants suitable for testing of MICA tetramer binding, HPB-ALL was selected from a number of TCR-$\alpha\beta^+$ T cell lines screened for lack of surface MIC and NKG2D. Cells were cotransfected with either of three pairs of γ- and δ-chain cDNAs (corresponding to sequences 1–3 in Fig. 1*A*), which were derived from responder T cell clones that are cytotoxic against target cells expressing MIC, in episomal pREP4 and pREP9 vectors. After drug selection, TCR-$\gamma\delta^+$ HPB-ALL transfectants positive for staining with mAbs δTCS1 and TCR-γ/δ-1 were readily obtained (Fig. 1*B* and data not shown). All these transfectants, but not untransfected HPB-ALL cells, bound MICA tetramers, as revealed by staining with mAb bio-M2 and PE-streptavidin (Fig. 1*C*). Tetramer binding was inhibited by prior incubation of transfectants with mAb δTCS1, thus providing definitive evidence that the tetramers interacted with the expressed γδ TCRs (Fig. 1*D*). Because HPB-ALL T cells were refractory to stimulation with PMA, ionomycin, and TCR-CD3 complex ligation, the transfectants could not be used to obtain functional results.

Lack of MICA tetramer binding to nonresponder γδ TCRs

The γ and δ cDNAs used for transfection were originally derived from T cell clones that were established from lymphocyte infiltrates extracted from intestinal epithelial tumors and displayed strong cytolytic responses against targets that were positive for MIC (18). Hence, MICA tetramer binding correlated with expression of these responder γδ TCRs. To further explore the Ag specificity of $V_\delta1$ γδ T cells for MIC, two pairs of γ- and δ-chain cDNA constructs (sequences 4 and 5 in Fig. 1*A*) corresponding to nonresponder TCRs were expressed on transfectant HPB-ALL cells (Fig. 2*A*). These sequences were derived from T cell clones that were established from lymphocytes isolated from an ovarian and a lung carcinoma, respectively, and showed no significant cytotoxicity against MIC-expressing targets (19) (V. Groh and T. Spies, unpublished observations). However, cytolytic responses by these T cells could be triggered by anti-CD3 or anti-NKG2D mAbs

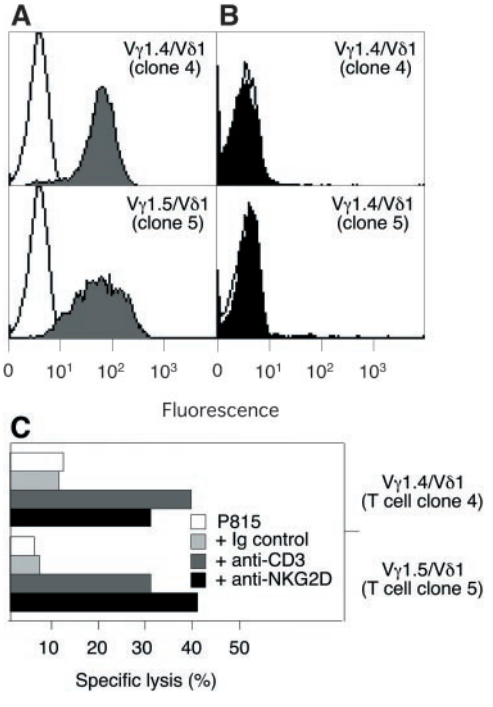

FIGURE 2. Lack of binding of MICA tetramers to γδ TCRs derived from T cell clones that are unresponsive to MICA-positive target cells. *A* and *B*, The γ- and δ-chain heterodimers expressed on stably transfected HPB-ALL cells correspond to the sequences shown in *lanes 4* and *5* in Fig. 1*A*. Histograms represent stainings with the Vδ1-specific mAb δTCS1 (▨) and with MICA tetramers/bio-M2 (■). □, Control stainings of untransfected HPB-ALL cells. Profiles are representative of several independent transfectant isolates. *C*, In redirected lysis assays with FcR^+ P815 target cells, both anti-CD3 (mAb OKT3) and anti-NKG2D (mAb 1D11) triggered lysis by the two $V_\delta1$ γδ T cell clones from which the γ- and δ-chain sequences used for transfection were derived. This confirmed the intact cytotoxic potential of these T cells, thus corroborating their apparent lack of specificity for MIC. The data shown represent averages of three independent experiments with deviations of not >5% and were obtained at an E:T cell ratio of 10:1.

against FcR-bearing P815 mouse mastocytoma cells in redirected lysis assays (Fig. 2*C*). This indicated that the TCR- and NKG2D-dependent cytolytic capacity of these T cells was fully intact, thus suggesting that their unresponsiveness was due to lack of Ag specificity for MIC. Accordingly, the nonresponder TCRs expressed on the HPB-ALL transfectants failed to bind MICA tetramers, although they were expressed at surface levels similar to those of the responder TCRs (Fig. 2, *A* and *B*). Moreover, Jurkat J.RT3-T3.5 T cell transfectants stably expressing these TCRs could be readily obtained (data not shown). Thus, altogether these results demonstrated that MICA tetramer binding accurately replicated γδ TCR recognition of MIC.

Absence of $V_\delta1$ γδ T cell responses against target cells expressing the N2DL ligands of NKG2D

The previously observed inhibition of $V_\delta1$ γδ T cell lysis of MIC-positive target cells by anti-Vδ1 mAb indicated the requirement of TCR triggering, either via engagement by MIC or a ubiquitous cell surface determinant, such as a carbohydrate moiety, since these T cells were activated by MIC expressed on diverse types of cells that included mouse cell lines (18, 19). Our current results provide direct evidence for the former possibility, thus indicating that MIC delivers both the TCR-dependent signal 1 and the NKG2D-dependent costimulatory signal 2. This model was tested by comparing the cytolytic responses of a $V_\delta1$ γδ T cell line (δ1B) against C1R cell transfectants expressing MICA or the highly divergent N2DL1, -2, or -3 ligands (also termed ULBPs) of NKG2D (30, 38). The function of N2DL was confirmed with NKL effector cells, which displayed cytolytic responses against C1R-N2DL1 targets that were triggered by N2DL1 engagement of NKG2D (Fig. 3*C*). However, the δ1B T cells showed no significant responses against the C1R-N2DL transfectants, although they were triggered by MICA expressed at similar levels (Fig. 3, *A* and *B*, and data not shown). Thus, in the context of presumably identical target cell surface components, N2DL engagement of NKG2D was insufficient as a functional substitute for MICA. These results provided further evidence in support of the dual role of MIC as ligands for γδ TCR and NKG2D.

Lack of MICA tetramer binding to mismatched responder γδ TCRs

The numbers of $V_\delta1$ γδ T cells with Ag specificity for MIC are likely to be significant, as implied by the relative ease with which numerous cytotoxic T cells (38 distinct clones in one study) have been obtained (18, 19). All the analyzed γ-chains expressed $V_\gamma1.3$, 1.4, 1.5, or 1.8 and $J_\gamma2.1$ or 2.3. The δ-chains expressed $V_\delta1$ and $J_\delta1$ with highly diverse V(NDN)J junctions (18, 19). This diversity is similar to that documented for $V_\gamma9/V_\delta2$ γδ T cells, which recognize soluble mycobacterial prenyl pyrophosphate and alkamine Ags (6). This raised the question of how the many different TCRs with substantial γ- and δ-chain sequence diversity can interact with the same MIC Ags, i.e., whether specificity is embedded in clonotypic γδ TCR heterodimers or mainly conferred by γ- or δ-chains alone. The former case would be in accord with functional data suggesting that mouse epithelial γδ TCRs with the same Ag specificity share similar conformations (11). In the latter case, MIC recognition might somewhat resemble that of superantigens, possibly with limited involvement of the variable γ- or δ-chain junctional sequences representing most of the receptor diversity.

To obtain evidence favoring one or the other of these models, mismatched combinations of γ- and δ-chains from the three responder TCRs were expressed on HPB-ALL cells and tested for MICA tetramer binding. Although the surface amounts of the mismatched TCR heterodimers were similar to those of the unaltered TCRs, no significant tetramer binding was observed (Fig. 4, *A* and *B*). Hence, these results indicated that MIC recognition is determined by specific γ- and δ-chain heterodimers.

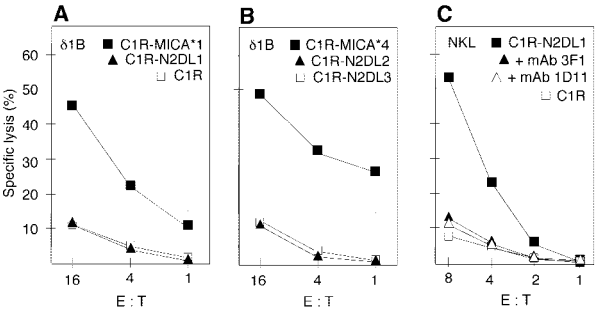

FIGURE 3. Failure of a $V_\delta1$ γδ T cell line (δ1B) (18) to respond to C1R transfectants expressing the N2DL1, -2, or -3 ligands of NKG2D (30). *A* and *B*, The δ1B T cells were cytotoxic against C1R-MICA (alleles *1 and *4), but not against the three C1R-N2DL transfectants. N2DL surface expression was confirmed using mAb 3F1 (anti-N2DL1) and a soluble recombinant NKG2D-FLAG tag-staining reagent (30). *C*, The NKL NK cell line was triggered by N2DL1 engagement of NKG2D (38). Cytotoxicity was inhibited by mAbs specific for N2DL1 (3F1) or NKG2D (1D11), thus confirming the functionality of the N2DL ligands.

Conclusions

The present results provide definitive evidence for specific interactions of $V_\delta1$ γδ TCRs with the stress-inducible MHC class I-like

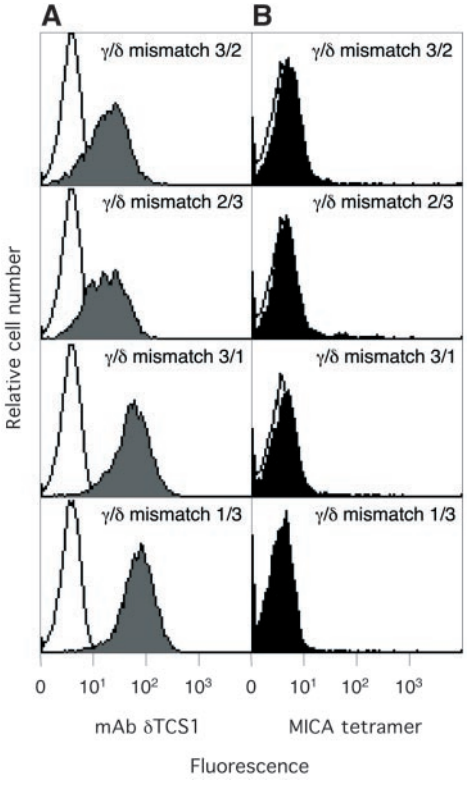

FIGURE 4. Lack of binding of MICA tetramers to TCRs composed of mismatched γ- and δ-chains derived from responder T cell clones. *A* and *B*, HPB-ALL transfectants expressing γ- and δ-chain combinations corresponding to the sequences shown in Fig. 1*A* bound the Vδ1-specific mAb δTCS1 (▨), but not MICA tetramers (■). □, control stainings of untransfected HPB-ALL cells.

MIC molecules. This resolves the previous controversy regarding the true nature of the Ags recognized by $V_\delta1$ γδ TCRs on MIC-positive target cells, which was raised by the demonstration that NKG2D engagement by MIC is required for cytolytic responses and cytokine secretion by $V_\delta1$ γδ T cells and that NKG2D triggers cytotoxicity against FcR^+ P815 cells in Ab-dependent redirected lysis assays (27). Hence, it is now clear that MIC delivers both the TCR-dependent signal 1 and the NKG2D-dependent costimulatory signal 2 for a subset of $V_\delta1$ γδ T cells. This dual function has precedent in the manifold interactions of MHC class I molecules with αβ TCR, the CD8 T cell coreceptor, isoforms of the killer cell Ig-like receptors and the leukocyte Ig-like receptor 1 (39–42). The γδ TCR-mediated recognition of MIC validates the original model derived from studies of mouse dendritic epidermal T cells, that intraepithelial γδ T cells may recognize stress-inducible self-Ags (24–26). At least in humans, this is corroborated by the colocalization of intraepithelial $V_\delta1$ γδ T cells and stress-inducible MIC in local tissue environments that include the intestinal mucosa, sites of viral infection, and epithelial tumors. The requirement of NKG2D engagement for triggering of the γδ T cell effector functions may be due to suboptimal TCR stimulation by MIC. Notably, we have never observed $V_\delta1$ γδ T cells lacking NKG2D. In addition, these dual receptor interactions of MIC might serve to preclude erroneous T cell activation by cross-reactive cell surface determinants. This does not appear to be the case with $V_\delta1$ γδ T cells specific for CD1c, which respond against target cells that, to our knowledge, lack expression of NKG2D ligands (15). Structural studies will be necessary to unravel the principles underlying the ability of numerous different $V_\delta1$ γδ TCRs to interact with MIC.

Acknowledgments

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