Two Human ULBP/RAET1 Molecules with Transmembrane Regions Are Ligands for NKG2D

Louise Bacon, Robert A. Eagle, Martina Meyer, Nicholas Easom, Neil T. Young and John Trowsdale

*J Immunol* 2004; 173:1078-1084; doi: 10.4049/jimmunol.173.2.1078

http://www.jimmunol.org/content/173/2/1078

References

This article cites 45 articles, 16 of which you can access for free at:

http://www.jimmunol.org/content/173/2/1078.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
Two Human ULBP/RAET1 Molecules with Transmembrane Regions Are Ligands for NKG2D

Louise Bacon,2,3 Robert A. Eagle,2 Martina Meyer,4 Nicholas Easom, Neil T. Young, and John Trowsdale

We characterized two novel members of the RAET1/ULBP gene cluster, RAET1E and RAET1G. The encoded proteins were similar to the ULBP in their class I-like α1 and α2 domains, but differed in that, instead of being GPI-anchored, their sequences were type I membrane-spanning molecules. Both proteins were capable of being expressed at the cell surface. Both proteins bound the activating receptor NKG2D, and RAET1G bound the human CMV protein UL16. The expression of diverse NKG2D-binding molecules in different tissues and with different properties is consistent with multiple modes of infection- or stress-induced activation. The Journal of Immunology, 2004, 173: 1078–1084.

Inhibitory receptors of the C-type lectin-like (CTLL),5 killer cell inhibitory receptor (KIR), and leukocyte Ig-like receptors (LILR; also known as ILT or LIR) families that recognize MHC class I molecules have provided a molecular basis for the “missing self” hypothesis (1) and have led to a renaissance in the theory of tumor immunosurveillance (2). Malignant or virus-infected cells that lose MHC class I, as a means of evading the CD8+ immune response, are vulnerable to attack by KIR- and LILR-bearing cells that have had inhibitory signals removed (3).

More enigmatic are the activating members of the NK cell receptor families (4). Ligands for activating KIR/LILR are largely unexplored, but it has been proposed that the balance of inhibitory and activating signals transmitted by receptors expressed on the NK cell surface may determine the outcome of target recognition. The C-type lectin-like receptor NKG2D is unique among activating receptors in that it has a number of defined, MHC class I-related ligands in both mice and humans. The murine ligands include the retinoic acid early transcript 1 (Rae-1) family, the minor histocompatibility Ag H60, and the recently identified MULT1 molecule (5–7). Human ligands consist of the MHC class I chain-related genes MICA and MICB (8) and the unique long 16 (UL16)-binding protein (ULBP) family (9). MIC proteins have three α domains structurally similar to those of classical MHC class I molecules, but they do not bind peptides or associate with β2-microglobulin. H60, ULBP1–3, and the Rae-1 family only possess MHC-like α1α2 domains. The human ULBP and murine Rae-1 proteins are distinct from the other NKG2D ligands, as they are GPI-anchored to the membrane, rather than possessing a transmembrane (TM) region.

NKG2D exists as a homodimer at the cell surface. It was thought to achieve promiscuous recognition of its diverse ligands by induced fit (10–12). However, with the recent solution of the structure of human NKG2D, there is little direct evidence for induced fit recognition (13). Diverse ligands are thought to bind to only five conserved hot spots within the NKG2D binding site. NKG2D is not limited to NK cells and is also expressed on activated CD8+ T cells, γδ T cells, and activated macrophages (14). A remarkable property of NKG2D is that it can be either directly stimulatory or costimulatory (15, 16) based on its association with either the DAP10 or DAP12 signaling adapter molecules, respectively (17, 18).

Requirements for the expression of NKG2D ligands are poorly understood. Some are induced upon cellular stress. As MIC was frequently expressed on tumors of epithelial origin (19), it was proposed that up-regulation of NKG2D ligands on tumors could be a mechanism for immune recognition and elimination of malignant cells. In mouse models it has been shown that implanted tumor cells transfected with NKG2D ligands invoked potent antitumor immunity and rejection of tumor cells in vivo (6, 20–22). NKG2D ligands may also have a role in the immune response to pathogens, including CMV (15), Mycobacterium tuberculosis (23), and Escherichia coli (24).

The ULBP molecules were first identified through their ability to interact with the UL16 glycoprotein of human CMV (HCMV). The expression of UL16 is proposed as a mechanism by which HCMV may evade immune recognition by interfering with NKG2D binding to its ligands (9, 25). Not all human MIC and ULBP proteins are targeted. MICB, ULBP1, and ULBP2 are bound by UL16, whereas MICA and ULBP3 are not, suggesting that different NKG2D ligands are not all functionally equivalent. Similarly, different murine ligands have variable affinities for NKG2D (7, 26, 27). MIC and ULBP proteins can be expressed independently of each other on cells of different lineages, which is also consistent with nonredundant functions (28).

We previously characterized ULBP-related genes in a cluster on chromosome 6p24.2-q25.3 (29), officially the RAET1-like transcripts (RAET1). Several new genes distinct from ULBP1–3 were identified. In this study we set out to investigate the ULBP-related...
genes RAET1E and RAET1G, which encode MHC class I-like α1α2 domains, but differ from ULBP1–3 because they possess TM domains and cytoplasmic tails (CYT).

During the preparation of this study, RAET1E/ULBP4 was described as capable of binding NKG2D (30). We expand on these data, showing cloning of the full-length transcript of RAET1G, which encodes a protein with a TM domain and a long cytoplasmic tail with many potential functionalities. We show the expression of RAET1G in normal tissues, ligand binding, and that RAET1E and RAET1G both induce NK cell cytotoxicity.

Materials and Methods
Molecular cloning of the ULBP family

The 5′ end of RAET1G was predicted by alignment of the established sequence tag (EST) sequences AW510737, BE711112, and BFS13861 and the genomic DNA (contig NT_023451.10). The predicted sequence matched with two IMAGE clones, 307030 and 2911855. IMAGE clone 307030 had a truncated 3′ end and missed the stop codon. The correct 3′ end was predicted from EST AS583860 and confirmed by PCR. The signal peptide was predicted with Signal PV1.1 (http://cbs.dv.tdk.), and the predicted TM region was deleted with Tmpred (http://ch.embnet.org). The exon structures were analyzed with the GCG program (Wisconsin package, version 10.3; Accelrys, Cambridge, U.K.).

The alignment was based on the global amino acid sequences or on local domains of RAET1G (NM_130900.1), RAET1E (AY176317), RAET1G (AY172579), ULBP1 (NM_022521.1), ULBP2 (NM_025217.2), ULBP3 (NM_024518.1), MICA (BC016929), MICB (NM_005922.1), and MULTI (AK020784) and conducted using ClustalW (http://ebi.ac.uk/clustalw/) and PileUp. The UPGMA (unweighted pair group method with arithmetic mean) tree was constructed by the program MEGA version 2.1 (31). The consistency of the branches was assessed by bootstrap based on 1000 samples/runs.

The following clones were obtained from the I.M.A.G.E. clone collection (HGMP, Hinxton, U.K.): RAET1G, IMAGE 307030 and 2911855; RAET1E, IMAGE 3464637; and ULBP2, 474726 (GenBank accession no. BFS13861, AW510737, BE511112, and BFS5401, and BG675590).

DNA sequencing was performed using BigDye and ABI 377 sequencer analysis (Applied Biosystems, Warrington, U.K.) using Sequence Navigator software. Full-length receptor constructs were cloned as FLAG epitope fusions in vector p3XFLAG-CMV-9 (Sigma-Aldrich, Peck, U.K.). Topo cloning of PCR fragments was performed using the Topo cloning kit (Invitrogen, Paisley, U.K.) and the manufacturer’s instructions.

RT-PCR

The following PCR primers were used for determining tissue distribution: 1G forward, 5′-AGCCTGGGCTTCCCTTCA; reverse, 5′-TGT ATACAGGCAAGGAGGGC; 1E forward, 5′-TATCCCTAGTCTCTGCGCT; reverse, 5′-GCCACCTACATTTGGC; and GAPDH forward, 5′-ACCACAGTCTCCATGCA; reverse, 5′-TCCACACCCCTGTTGTA.

Cell line RNAs were made as previously described (32). Tissue cDNA was a gift from Dr. D. Simmons (Celltech Group, Berkshire, U.K.). The expected sizes of the products were 935 bp for RAET1G, 835 bp for RAET1G2, and 810 bp for RAET1E. GAPDH was used as a control reaction for each cDNA.

Transfections and flow cytometry

Transient transfections were performed into CV-1 cells using Lipofectamine 2000 (Invitrogen) and the manufacturer’s standard protocol. Flow cytometry was performed on a FACSCalibur machine (BD Biosciences, Oxford, U.K.). Detection of the full-length receptors was performed via a FITC-conjugated mAb to the FLAG epitope (Sigma-Aldrich). Detection of NKG2D binding was via the human Fc fusion, using an anti-human IgG FITC-conjugated polyclonal Ab (DakoCytomation, Ely, U.K.). Detection of NKG2D binding was via the human Fc fusion, using an anti-human Fc HRP-conjugated Ab (DakoCytomation).

Results

Two RAET1 proteins with TM regions

Initial analysis of the ULBP/RAET cluster called for six expressed genes encoding GPI-linked molecules (Fig. 1) (29). More detailed analysis of the sequences identified potential TM regions in RAET1E and RAET1G. An analysis of the genes encoding RAET1E, RAET1G, and ULBP2 (RAET1E, RAET1G, and RAET1H, respectively) revealed a conserved exon structure, where exon 1 encoded the signal peptide and the start of the protein, exons 2 and 3 encompassed the α1 and α2 domains, and exon 4 encoded a hydrophobic sequence. In the ULBP2s, this exon encoded the GPI anchor region and 3′ UTR, but in both RAET1E and RAET1G, the sequence was compatible with a TM as well as a short CYT. Exon 5 in RAET1G encoded the remainder of the putative cytoplasmic domain. The equivalent exon in RAET1H was silent. Although the RAET1H locus also potentially encoded an intact protein, no RAET1L EST sequences have been identified, and it is not considered further in this study.

To clarify the sequences of the expressed gene products, we fully sequenced clones corresponding to RAET1E and RAET1G (29). We confirmed that RAET1G was very similar to ULBP2 over the first four exons. A comparison of the novel protein sequences with those of existing murine and human NKG2D ligands showed that RAET1G was most closely related to ULBP2 (85% overall similarity). The highest level of amino acid identity was in the α1α2 domains. The remaining translated sequence encoded a TM and a 100-aa CYT (Fig. 2).

Similarly, the RAET1E sequence encoded two α domains, then a hydrophobic TM, followed by a cytoplasmic domain of 20 aa. RAET1E was the most divergent member of the cluster, sharing <43% identity with the other ligands, whereas ULBP1-3 shared ~55–60% identity with each
RAET1G, which has version 2.1 (31). Bootstrap values were used to determine the consistency of the tree and are based on 1000 sample runs.

FIGURE 1. Upper panel. Arrangement of expressed genes in the RAET1 cluster on chromosome 6q24.2-q25.3. All genes have four coding exons, except RAET1G, which has five. Exon 5 of ULBP2 is not coding. RAET1 pseudogenes are not shown in this study (29). Lower panel. Phylogenetic tree of murine and human NKG2D ligands. The alignment was based on the global amino acid sequences using ClustalW (http://ebi.ac.uk/clustalw/). The tree was computed from the matrix of distances values using the UPGMA (unweighted pair group method with arithmetic mean) method of the program MEGA2 version 2.1 (31). Bootstrap values were used to determine the consistency of the tree and are based on 1000 sample runs.

Alternative splicing of RAET1G

The sequence of IMAGE clone 2911855 was colinear with that of RAET1G except for a 100-bp deletion at the start of exon 4. This arrangement is compatible with alternative splicing at this boundary, with a second potential splice start shifted 3’ by 100 bp. Translation of this deleted form of RAET1G showed that the alternative splicing caused a frameshift and premature termination of the protein sequence. This truncated protein is predicted to be soluble, as the frameshift causes termination before the TM region. This splice form is termed RAET1G2, and its alternative sequence ending is shown below that of RAET1G in Fig. 2. Exon structures for RAET1G and -1G2 are shown also in Fig. 2. Truncated NKG2D ligands such as this may have a role in blocking receptor interactions, and their expression could be part of an immune evasion strategy used by tumors or infected cells (2, 6, 25).

Expression patterns of RAET1G/1G2 and RAET1E

Specific PCR primers were designed to establish the expression profiles of RAET1G/1G2 and RAET1E. Several tumor cell lines contained mRNA for RAET1E or RAET1G (Fig. 3), and the genes were expressed independently of each other, in cells of different lineages. This is in contrast to MICA and MICB where expression appears to be restricted to cells of epithelial origin, and it is unclear whether they are expressed independently of each other (37, 38).

The T cell leukemia-derived line HSB-2 expressed a truncated RAET1G transcript. This cDNA product was cloned using Topo cloning and, when sequenced, was identical with the splice form RAET1G2 in IMAGE clone 2911855. The expression of a splice variant encoding a soluble protein is potentially important given the proposed role of soluble NKG2D ligands in impairment of NK and T cell recognition of tumors (39). A limited range of normal human tissues tested showed no expression of RAET1E or the splice form RAET1G2. RAET1G was strongly expressed in colon, but not in the other tissues screened (Fig. 3). An EST matching RAET1G has also been identified from a larynx cDNA library (data not shown).

RAET1E and RAET1G expressed on the cell surface bind NKG2D and UL16

We next asked whether RAET1E and RAET1G were capable of binding to their proposed ligand, NKG2D. Full-length cDNAs for RAET1E, RAET1G, and ULBP2 were cloned as N-terminal, FLAG-tagged fusion proteins. These reached the cell surface in transient transfections of CV-1 cells and detection with anti-FLAG Abs in flow cytometry (Fig. 4). NKG2D, expressed as a recombinant soluble Fc fusion protein, bound to CV-1 cells transiently transfected with ULBP2, RAET1G, and RAET1E by flow cytometry (Fig. 4). Similarly, recombinant, soluble, His-tagged UL16 bound FLAG-tagged RAET1G expressed on the surface of transfected cells, but not to untransfected cells, by flow cytometry (Fig. 4). No other transfected cell lines were analyzed for UL16 binding.
RAET1E and RAET1G are capable of inducing NK cell cytotoxicity via NKG2D

We next wanted to determine whether RAET1E and RAET1G were biologically functional in triggering cytotoxicity. To show this, stable transfectants of the two molecules in CV-1 cells introduced to cytotoxicity assays using NK effectors (Fig. 5). CV-1 cells expressing either molecule were targets for NK killing under conditions in which untransfected CV-1 cells did not trigger a response. NK cell killing of the transfected cells was 100% blocked by the addition of saturating amounts of an anti-NKG2D antibody, showing that the killing was specifically due to the NKG2D-ligand interaction. Relative killing data for the two ligands and untransfected cells are shown in Fig. 5. These data demonstrate that both TM-linked RAET molecules are potentially efficient targets for NK cells after engagement by NKG2D.

Discussion

The existence of multiple inhibitory NK receptors able to bind MHC class I molecules has become a paradigm of innate immune recognition. NKG2D is remarkable as an activating receptor with multiple MHC class I-related ligands (40). To understand how recognition through NKG2D is controlled, it is necessary to discern the full complement of its ligands and, crucially, differences in their properties. We have shown that two novel members of the RAET1 gene cluster encode proteins that act as NKG2D ligands, and that one of them can bind the UL16 protein of HCMV. Although their extracellular domains show significant homology with the ULBPs, there are unique features. In contrast to the GPI-anchoring of the ULBPs, the RAET1E/G proteins contain TM regions and CYT domains, which may have implications for a variety of roles of NKG2D ligands in different immunological situations.

RAET1G alternative splice ending

| RAET1G | MAAASSAFLR-LPPLL-LLGWCTRGLDPSLCLYDITVIPKRRPGFPCAWQQLCVQDEK 58 |
| ULBP2 | MAAAAATTLLCPL-LLGWGAAAGRDPSLCLYDITVIPKRRPGFPCAWQQLCVQDEK 59 |
| RAET1G | MRRSLSLSSPRLLP-LLLLAIIEIMVQHLSLFCNTIKSLRPGFPCAWQQLCVQDEK 59 |
| ULBP3 | MAAASAAIPFLRFLPALLFLLDGSQGTRADAHSLWNYFIIILPREGQWQCVQDEK 60 |

RAET1G sequence alignment with ULBP2 and ULBP3.

The Journal of Immunology 1081

FIGURE 2. Upper panel, Sequence alignment of RAET1E and RAET1G with ULBP2 and ULBP3. AA sequences derived from cDNA clones were aligned to compare main features. Both splice variants of RAET1G are shown. Symbols indicate proposed α-helical (black cylinders), β-strand (gray arrow), and β strand (gray arrow). These features were derived from the crystal structure of ULBP3. The amino acids underlined in ULBP3 are those in contact with NKG2D. Lower panel, Exon structures of RAET1G and RAET1G2. Alternative splicing in RAET1G leads to a soluble protein product. The cDNA clones are consistent with contiguous sequences for the first three exons. The splice acceptor sites in the fourth exon differ by 100 bp, resulting in a truncated RAET1G2 protein.

The great diversity of RAETs: continuous evolution for disease resistance?

The diverse features of NKG2D ligands are consistent with functions in alerting the immune system to a wide variety of challenges, whether infection, stress, or malignant transformation. The marked differences in their sequences in different species indicate that some duplication of different sets of genes, namely ULBP/RAET1 and MIC in humans and Rae1, H60, and MULT1 in mice, have occurred, making it difficult to identify individual murine and human ligand orthologues. Although a number of features are shared, including a long CYT in both RAET1G and MULT1, it is...
likely that some gene duplication occurred after speciation of mouse and man. This evolutionary diversity, association to the membrane via a TM region rather than a GPI linkage, and varied expression patterns are all suggestive of selection, presumably in response to resistance to different pathogens. Consistent with this premise, human and mouse CMV have developed proteins that can prevent some, but possibly not all, ligands for NKG2D from reaching the cell surface (25, 41).

Role for soluble versions of NKG2D ligands

The presence of soluble MIC in the sera of patients with MIC+ tumors has been linked to a reduction in surface NKG2D on lymphocytes and may be a route for immune evasion by impairing the responsiveness of NKG2D-bearing NK and T cells (29). MICA is proposed to be lost from the cell surface of tumors through cleavage by metalloproteases (42), and this is likely to be the case for the TM-containing ligands, RAET1G and RAET1E. The soluble splice form of RAET1G detected in the T cell leukemia line HS2-2 could play a similar role. Genetic variation in the ULBP/RAET cluster has not been fully explored, but our preliminary data suggests that the genes are polymorphic, and this variation could have a profound effect on the interaction with NKG2D and viral products, as is proposed for variants of MICA/B (43).

Expression patterns suggest different roles in different tissues

The expression pattern of the ULBP/RAET genes presented in this study and in previous studies (9, 28) shows that multiple ligands for NKG2D can be expressed on one target cell. The ligands are also clearly capable of independent expression. The data are consistent with different NKG2D ligands expressed on different tissues. MIC products are generally expressed on epithelial cells. ULBP/RAET1 can be expressed on epithelial cells, but are also expressed in cell lines of nonepithelial origin, providing a rationale for roles distinct from MICA/B; for example, in immune responses to lymphoid malignancies and to viruses that infect lymphocytes. It may be simplistic to consider the ligands for NKG2D merely as flags of cellular stress or transformation, because a number appear to be expressed in normal tissues. MICA is known to be constitutively expressed in the intestinal epithelium and can be recognized by

FIGURE 3. Expression of RAET1E and RAET1G in cell lines and tissues. A, A panel of established cell lines was screened for expression by RT-PCR. Specific primers were used, such that RAET1G gave a product of 835 bp, and RAET1G gave a product of 935 bp, respectively. The RAET1E product is 810 bp. Upper panel, RAET1G; middle panel, RAET1E. GAPDH in the lower panel was used as a positive loading control. The cell lines used were: lane 1, Daudi (B cell); lane 2, 721.221 (B cell); lane 3, HSB-2 (T cell); lane 4, Jurkat (T cell); lane 5, THP-1 (monocyte); lane 6, HeLa (cervical carcinoma); lane 7, HT1080 (fibrosarcoma); lane 8, MelJuSo (melanoma); lane 9, K562 (erythroid); lane 10, NKL (NK cell line); lane 11, Raji (B cell); lane 12, RPMI-8402 (T cell); lane 13, HPB-ALL (T cell); lane 14, MOLT-4 (T cell); lane 15, MOLT-13 (T cell); lane 16, CCRF-CEM (T cell); lane 17, Fib (B cell); lane 18, Ind (B cell); lane 19, U937 (monocyte); lane 20, YT (NK cell line); lane 21, HUVEC (umbilical vein); and lane 22, HepG2 (liver carcinoma). Track 23 (right) contains RAET1G cDNA control in the upper panel and RAET1E control in the second panel. B confirms the expression of the RAET1G2 form in the T cell leukemia line HSB-2. PCR products from left to right: IMAGE 307030 (RAET1G), IMAGE 2911855 (RAET1G2), K562, and HSB-2 cell lines. The sequences of the products matched those shown in Fig. 2. C, Expression of RAET1G in tissues. Five normal tissues were screened for the presence of both novel genes. Upper panel, RAET1G expression. GAPDH (lower panel) was used as a positive loading control. No RAET1E was found in the panel tested. Tissue cDNA tested bone marrow (lane 1), colon (lane 2), PBL (lane 3), placenta (lane 4), and tonsil (lane 5). Lane 6, Positive control. RAET1G was detected in the colon. The expression pattern of the ULBP/RAET genes presented in this study and in previous studies (9, 28) shows that multiple ligands for NKG2D can be expressed on one target cell. The ligands are also clearly capable of independent expression. The data are consistent with different NKG2D ligands expressed on different tissues. MIC products are generally expressed on epithelial cells. ULBP/RAET1 can be expressed on epithelial cells, but are also expressed in cell lines of nonepithelial origin, providing a rationale for roles distinct from MICA/B; for example, in immune responses to lymphoid malignancies and to viruses that infect lymphocytes. It may be simplistic to consider the ligands for NKG2D merely as flags of cellular stress or transformation, because a number appear to be expressed in normal tissues. MICA is known to be constitutively expressed in the intestinal epithelium and can be recognized by

FIGURE 4. Surface expression of RAET1 proteins, and binding to NKG2D and UL16. A, Cell surface expression in CV-1 cells. The histogram denotes transfected cells stained with anti-flag Ab: cells only (solid black line); ULBP2 (solid gray line); RAET1G (dashed gray line); and RAET1E (dashed black line). The percentages of cells in the M1 populations marked are as follows: untransfected cells, 15%; ULBP2, 85%; RAET1G, 53%; and RAET1E, 71%. B, Histogram denoting transfected cells stained with NKG2D: cells only (solid black line); ULBP2 (solid gray line); RAET1G (dashed gray line); and RAET1E (dashed black line). The percentages of cells in the M1 populations marked are as follows: untransfected cells, 12%; ULBP2, 81%; RAET1G, 50%; and RAET1E, 78%. C, Histogram denoting transfected cells stained with UL16 cells only (solid black line) and RAET1G (dashed gray line).
intestinal intraepithelial lymphocytes, including γδ T cells (38). It is proposed that MICA is up-regulated on mucosal epithelial surfaces upon recognition of bacterial products (24), where it contributes to T cell alertness (44). RAET1G, a new ligand for NKG2D, is also strongly expressed in the colon, and it could have a parallel role to MICA in the gut. The identification of novel ligands for NKG2D with TM domains raises questions about the functional implications of different ligand topologies.

In the mouse, GPI-anchored proteins have modest to low affinities for NKG2D, whereas the ligands possessing CYT domains have high affinity, and the human NKG2D ligands may well conform to this pattern. In a situation where one cell bears multiple ligands with differing affinities for NKG2D, there would be competition for the binding of the receptor, and the ligand with more favorable kinetics would gain occupancy (26). However, this model is complicated by potential differences in cellular distribution between GPI-anchored and TM proteins. GPI-anchored proteins tend to be found in localized cholesterol-rich regions known as lipid rafts. Localized concentrations of specific surface proteins and signaling apparatus perform distinct roles in what has been referred to as the immune synapse (45). The ULBPs may be found preferentially in lipid rafts on cellular activation and synapse formation and would be found at high density in contact regions between cells. Their affinities may reflect the clustered nature of proteins within lipid rafts, where low to moderate affinity for NKG2D would be augmented by an increased avidity. If ligands such as RAET1G, MICA, and MULT1 have an increased affinity over GPI-anchored molecules, they would have a greater ability to bind NKG2D in the absence of the increased avidity afforded by such clustering.

Within polarized epithelial layers there is an additional difference in the subcellular localization of GPI-anchored and TM proteins. GPI-anchored proteins have a greater tendency to be found at the apical surface, whereas some TM proteins, if they possess one of several specific motifs, will be targeted to the basal or lateral sides of the cell. Basolateral targeting studies on MICA are consistent with the idea that TM ligands behave differently from those only on one side of the membrane (36). Normal wild-type MICA is targeted to the basolateral membrane of gut epithelium, in proximity to NK or T cells, where it can signal cell destruction. However, a naturally occurring allele that has a TM motif, but no cytoplasmic tail, does not target to the same part of the cell, but moves to the apical surface, where there is a greater concentration of lipid rafts and less contact with NKG2D-bearing cells. This targeting is shown to be effected by two pairs of leucine/valine residues. RAET1G could be similarly targeted to the basolateral surface, because it has a similar dihydrophobic motif. In effect, it could be a good front-line indicator of bacterial challenge. In a polarized cell layer, such as epithelial surfaces in the gut, the differences in anchorage of NKG2D ligands allow differential distribution in the same cell, different possible signaling pathways and hence, differential availability to lymphocytes. The distribution of ligands on a cell could change on bacterial challenge, transformation, or lymphocyte engagement. The relative distribution of NKG2D ligands in distinct tissues and cellular domains may be fundamental to understanding NKG2D-mediated immune recognition. The unique CYT tail carried by RAET1G provides the potential to transmit signals within the cell to modulate other molecules involved in responses to pathogens. We are currently investigating the signaling capability of the RAET1G cytoplasmic tail. This is the first NKG2D ligand described with evidence of its own signaling capability.

In conclusion, we have described two novel ligands of human NKG2D. Both ligands possess TM and CYT domains, making them functionally divergent from the ULBPs. The novel sequences contained within the CYT domain of RAET1G have implications for our understanding of the specificity of activation by NKG2D. It will be important to determine the precise conditions for induction of expression of the entire ULBP/RAET family of molecules and their distribution in normal and challenged tissues.

References


