Identification and Characterization of a Transmembrane Isoform of CD160 (CD160-TM), a Unique Activating Receptor Selectively Expressed upon Human NK Cell Activation

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CD160 has been initially identified as a GPI-anchored MHC-class I activating receptor mainly expressed on peripheral blood NK cells. Herein, we report the identification of three additional CD160-related mRNAs generated through alternative splicings of the CD160 gene, among which one encoded a putative CD160 transmembrane isoform (CD160-TM). We first establish that CD160-TM surface expression is highly restricted to NK cells and is activation-dependent. Additionally, we provide evidence that CD160-TM represents a novel activating receptor, as assessed by the increased CD107a NK cell surface mobilization observed upon its engagement. Finally, we demonstrate that the CD160-TM cytoplasmic tail is by itself sufficient to mediate the recruitment of Erk1/2 signaling pathway, and that the initiation of this activation process is dependent on the Src-family kinase p56\(^{lk}\). The identification of CD160-TM therefore provides new possibilities regarding the role of CD160 isoforms in the regulation of NK cell functions. *The Journal of Immunology*, 2009, 182: 63–71.

NK lymphocytes recognize abnormal or aberrant cells through multiple receptors that detect normal host molecules, as well as stress-induced or pathogen-expressed motifs (1, 2). Individual NK cells express both activating and inhibitory receptors, which together drive the specificity toward target cells (3).

The NK cell inhibitory receptors have been classified into three groups, namely, the heterodimeric CD94/NKG2A, the Ig-like transcript receptors, and the members of the killer cell Ig-like receptors (4, 5). All of them bind to classical or nonclassical MHC class I molecules. A common characteristic of the inhibitory receptors is the presence of ITIMs within their intracellular tail (6). Following engagement by their ligands, the inhibitory receptors become phosphorylated on the tyrosine residue(s) present in the ITIM(s), creating docking sites for the SH2-domains of the cytoplasmic Src homology region 2 domain-containing phosphatase (SHP) 1 and SHP2. Their recruitment further results in the down-regulation of the intracellular activation cascade (7). In contrast, activating receptors recognize a large variety of ligands, mostly distinct from MHC class I molecules, and exhibit more complex but well-characterized signaling pathways. Natural cytotoxicity receptors (NCRs) (4) and NKG2D are the major receptors involved in NK cytotoxicity (8). The NCRs (namely NKp46, NKp44, and NKp30) belong to the Ig superfamily and represent non-MHC class I-specific receptors whose cellular ligands still have to be confirmed (9). In contrast to NKp46 and NKp30, which are expressed on circulating NK lymphocytes, NKp44 expression is activation-dependent (10). The NCRs transduce signals through their association with ITAM-containing molecules such as CD3\(\zeta\), FceRIy, and DAP12 (11, 12). Besides the NCRs, NKG2D is a C-type lectin-like receptor shown to recognize the MHC class I homologs MICA and MICB, as well as the family of UL16-binding proteins (ULBPs) (13). NKG2D uses the transmembrane polypeptide DAP10 for signaling, which interacts with the PI3K once phosphorylated (14). Interestingly, NKp80, an additional C-type lectin-like-activating receptor exclusively expressed by human NK cells, has been identified (15). A search for NKp80 ligands led to the identification of activation-induced C-type lectin (AICL) (16). However, the NKp80 signaling pathway remains enigmatic as this receptor does not contain a transmembrane charged residue (a feature allowing association with ITAM-containing adaptor proteins) or any intracellular consensus activation motifs. Finally, besides these MHC class Ia/Ib molecule-independent activating receptors, it is important to mention the well-characterized DAP12-associated CD94/NKG2C and killer cell Ig-like receptor-activating isoforms (17, 18), although the precise events leading to their specific recruitment still have to be better defined.

Using the original BY55 mAb, we previously reported the identification of BY55/CD160 receptor on functional cytotoxic peripheral blood (PB)-NK lymphocytes, and we initially found that its expression was rapidly down-modulated after cell activation (19). CD160 shows a broad specificity with weak affinity for the MHC class Ia/Ib molecules (20). An additional ligand for CD160 has been recently identified as the herpesvirus entry mediator protein (21). CD160 behaves as an activating receptor on NK lymphocytes as demonstrated by the induction of their cytotoxic potential upon engagement (22). Furthermore, CD160 triggering with its physiological ligand MHC class I results in a unique profile of cytokine secretion by cytotoxic CD56\(^{dim}\)/CD16\(^+\) PB-NK cells, with the release of TNF-\(\alpha\), IFN-\(\gamma\), and IL-6 (23). CD160 appears to be unique among the activating receptors since the CD160 gene was found to

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be located on human chromosome 1, and the corresponding protein was characterized as a GPI-anchored cell surface molecule (24). Additionally, CD160 is expressed by intestinal intraepithelial T lymphocytes and by a minor subset of circulating T lymphocytes including TCRγδ and CD8<sup>+</sup>CD28<sup>-</sup> T lymphocytes (24–26), and it exerts a co-receptor function on CD8<sup>+</sup>CD28<sup>-</sup> CTL (25). Thus, CD160 might be involved in mechanisms regulating both adaptive and innate immunity. Interestingly, we reported that a down-modulation of CD160 surface expression occurs as a consequence of its proteolytic cleavage upon NK cell activation (27). The released soluble form of CD160 was found to impair the MHC class I-specific cytotoxicity of CD8<sup>+</sup> T lymphocytes and NK cells. Importantly, murine CD160 tissue expression, specificity, and molecular structure show similarities to what has been described in human (28, 29).

In this study we further identified and characterized a transmembrane isoform of CD160 (CD160-TM). In contrast to the GPI-anchored isoform, its expression is restricted to NK cells and is activation-dependent. We established that CD160-TM fulfills an activating function once expressed on activated PB-NK lymphocytes. Thus, the CD160 receptor represents a unique receptor that might recruit alternative activating signaling pathways through the differential expression of its isoforms on resting or activated NK lymphocytes.

**Materials and Methods**

**Cells**

PBMC were isolated from heparinized venous blood from healthy volunteers by density gradient centrifugation over MS5 (PA Laboratories). PB-NK, CD4<sup>+</sup>, and CD8<sup>+</sup> lymphocytes were purified using MACS and a specific cell subset isolation kit according to the manufacturer’s recommendations (Miltenyi Biotec). NKT cells were sorted and activated as previously reported (30, 31). Individual cell purity was >95%. For activation, cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated human serum, penicillin (100 IU/ml), streptomycin (100 µg/ml), l-glutamine (2 mM) (Invitrogen), and IL-2 (200 U/ml, a gift from Sanofi-Aventis), IL-12 (50 ng/ml; R&D Systems), IL-15 (10 ng/ml, PeproTech), or IL-18 (80 ng/ml; MBL International). Cord blood mononuclear cells and CD34<sup>+</sup> cells were isolated as previously described (32). IL-2-activated NK cells were expanded up to 5 wk from sorted NK cells in culture medium supplemented with 200 U/ml IL-2, and mixed allogeneic PBMC plus 2 µg/ml PHA (Sigma-Aldrich) were added every 2 wk to feeder cells.

Wild-type and Lck-deficient (JCam) Jurkat cells and derived transfectedants, 221 target cells, Daudi, YTindi, and Molt4 cell lines were cultured in RPMI 1640 medium supplemented with penicillin/streptomycin, l-glutamine, and 10% heat-inactivated FCS (Perbio Science). For the NK cell line NK92 was cultured for 4 hi n wells that had been pre-
Chloroplasts are an example of such a membrane-bound organelle.

The table below summarizes the main findings of this study:

| Organism | Chloroplasts Present? |
|----------|-----------------------|
| Plant    | Yes                   |
| Animal   | Yes                   |

The authors suggest that further research is needed to fully understand the role of chloroplasts in different biological processes.
and Methods and Fig. 2C for sequence details). Each serum was further affinity purified on either peptide 1 or 2, and the reactivity of the purified Abs was assessed by flow cytometry analysis. Initial experiments showed a better reactivity of the Abs affinity purified on peptide 2 (anti-CD160-TMpep2) on NK92 cell line (data not shown). To further determine whether these Abs were specific for CD160-TM, immunolabelings were realized on transfected Jurkat cells stably expressing CD160 or CD160-TM receptor. We clearly observed that the anti-CD160-TMpep2 Abs efficiently labeled CD160-TM transfectants while no signal was obtained using the anti-CD160 mAb BY55 (Fig. 4A, left panel). Thus, despite the presence of the membrane-proximal domain in both the GPI-anchored CD160 and CD160-TM, these affinity-purified Abs allow discrimination between the two isoforms. Conversely, the anti-CD160 mAb BY55 positively stained CD160-expressing cells but gave no signal on CD160-TM transfectants (Fig. 4A, middle panel). Note that the anti-CD160 mAb CL1-R2, which presents a reactivity different from the one of BY55 mAb (20, 22, 23, 27), also failed to stain CD160-TM transfectants (data not shown). To definitely assess the specificity of CD160-TM recognition, long-term IL-2-activated NK cells were tested for their reactivity with the anti-CD160-TMpep2 Abs or BY55 mAb. A positive staining was obtained on IL-2-activated cells labeled with the anti-CD160-TMpep2 Abs, while no labeling was observed using BY55 mAb (Fig. 4A, right panel), although both CD160 and CD160-TM transcripts were detected (data not shown). Thus, we obtained purified polyclonal Abs that, according to their peptide recognition ability, allow the detection of CD160-TM molecule, but not its GPI-anchored counterpart.

We further investigated the expression pattern of the GPI-anchored molecules vs the full-length TM protein on PB-NK cells by performing immunolabeling on resting or IL-15-activated PB-NK
cells using either BY55 mAb or the anti-CD160-TM\textsuperscript{peptide} Abs. As previously reported (27), CD160 is expressed by circulating NK lymphocytes and becomes almost undetectable after 3 days of activation (Fig. 4\textsuperscript{B, upper panel}). Note that the disappearance of CD160 from the cell surface resulted from an activation-dependent proteolytic process involving a metalloprotease (27). This down-modulation step was then followed by a reacquisition phase, as assessed by the recovery of a positive signal with BY55 mAb at later time points of activation (Fig. 4\textsuperscript{B, upper panel}). Moreover, in agreement with CD160 mRNA analysis (Fig. 3), no CD160-TM was detected on resting PB-NK cells, while a low level of membrane expression was found after 3 days of IL-15-treatment (Fig. 4\textsuperscript{B, lower panel}). Interestingly, we constantly observed that this initial induction of CD160-TM expression was followed by a down-modulation step, with the TM receptor becoming undetectable at days 5 and 7 of activation. Remarkably, after 10 days of stimulation, two cell populations were detected, one expressing high levels of CD160-TM, and one being barely CD160-TM-positive cells. Depending on the donors, both cell types remained present up to 2–3 wk after the beginning of the activation process (data not shown). Importantly, we found a complete loss of CD160 and an exclusive expression of CD160-TM on in vitro-activated cells, as exemplified in Fig. 4A, suggesting that upon longer activation time, CD160 can be totally replaced by CD160-TM at the NK cell surface. Thus, it seems that during the activation process, NK cells went through a time-dependent regulation of CD160 and CD160-TM membrane expression, with the receptors being available at the cell surface depending on the level of proteolytic activity (for CD160) or transnational activity (for CD160-TM) existing within the cells during the time course of activation.

**Molecular characterization of CD160-TM**

To better characterize CD160-TM, additional protein analyses were performed on CD160-TM-expressing Jurkat cells or IL-2-activated NK cells. Immunoprecipitates were prepared using either anti-CD160-TM\textsuperscript{IC} Abs, directed against the intracellular domain of the protein (see Fig. 2C and the Materials and Methods for peptides sequence), or the corresponding rabbit preimmune serum. Immunoblot analysis conducted with the anti-CD160-TM\textsuperscript{IC} Abs showed the presence of a 100-kDa protein in the immunoprecipitates prepared from both cell types (Fig. 5). No protein was recovered when using rabbit preimmune serum for precipitation, inferring the specificity of signal detected in the anti-CD160-TM immunoprecipitates (Fig. 5). Importantly, Western blot analysis using the anti-CD160 mAb CL1-R2 similarly allowed the detection of a 100-kDa protein (data not shown). As CD160-TM amino acids sequence corresponds to a polypeptide with an estimated molecular mass of 25.6 kDa, it is likely that CD160-TM is expressed in activated NK cells as a multimeric molecule that appears to be quite resistant to the reducing agent, as already observed for CD160 (19, 24) and soluble CD160 (27).

**CD160-TM represents an activating receptor on PB-NK cells**

Our observation that the clearing of GPI-anchored CD160 from the NK cell surface parallels the appearance of the transmembrane molecule prompted us to further investigate the potential function of CD160-TM. To determine whether CD160-TM might trigger activating or inhibitory signals following engagement, NK92 cells were activated with either a control mAb or affinity-purified anti-CD160-TM\textsuperscript{IC} or anti-CD160-TM\textsuperscript{peptide} Abs, and the corresponding cellular degranulation response was analyzed through the detection of CD107a cell surface mobilization. We observed that a fraction of NK92 cells (representing 12% of the overall cell population) exhibited a spontaneous level of degranulation, and that this amount of CD107a-expressing cells was not modified in the presence of CD160-TM\textsuperscript{IC} Abs (Fig. 6A). In contrast, a 1.5-fold increase in the percentage of CD107a-positive cells was detected upon stimulation with CD160-TM\textsuperscript{peptide} Abs, thus demonstrating that CD160-TM triggering can lead to the generation of positive signals involved in the process of degranulation.

**CD160-TM cytoplasmic tail is sufficient to mediate activating signaling**

The molecular basis underlying CD160-TM activating function was next analyzed. The protein sequence analysis of CD160-TM revealed the presence, within its transmembrane domain, of a positively charged lysine residue (see Fig. 2C). An association of the molecule with ITAM-bearing adaptors through the establishment

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**Table I. Cellular distribution of D160 isoforms transcripts**

| Samples               | GPI | TM |
|----------------------|-----|----|
| Fresh tissues        | +   | -  |
| PBMCs                | +   | -  |
| Activated PBMCs      | +   | +  |
| Cord blood mononuclear cells | + | - |
| CD34\textsuperscript{+} cells | + | + |
| Thymocytes           | -   | -  |
| NK cells             | +   | -  |
| IL-15-activated NK cells | + | + |
| CD4\textsuperscript{+} T cells | + | + |
| IL-15-activated CD4\textsuperscript{+} T cells | + | + |
| CD8\textsuperscript{+} cells | + | + |
| IL-15-activated CD8\textsuperscript{+} T cells | + | + |
| Activated NKT cells  | +   | -  |
| T cell clones        |     |    |
| Thymocytes TCR\textsuperscript{αβ} (B12g) | - | - |
| TCR\textsuperscript{γδ} cells (LSO) | + | - |
| CD4 TCR\textsuperscript{αβ} cells (C1) | + | - |
| CD8 TCR\textsuperscript{αβ} cells (JF1) | + | - |
| Tumoral cell lines   |     |    |
| B cells (Daudi)      | -   | -  |
| T cells (Jurkat, Molt4) | - | - |
| NK cells (NK92, YTindy, NLK) | + | + |

\textsuperscript{a} RT-PCR using the primers pair specific for the amplification of the GPI-anchored (GPI) or the transmembrane (TM) transcripts was performed on total mRNA extracted from the indicated sorted cells or cell lines. Where indicated, cells were grown in the presence of IL-15 (10 ng/ml) for 3 days. Data are representative of three independent experiments. –, not detected; +, positive amplification; a, depending on the donor.
of a stable salt bridge was therefore considered. However, coexpression of CD160-TM together with DAP10, DAP12, CD3ε/H9256, or FcεRIy/H9255 in COS cells did not evidence any association between these adaptor proteins and CD160-TM (data not shown). Another feature of CD160-TM is the presence, in its intracellular domain, of two tyrosine residues at positions 220 and 225, which might represent potential docking sites for signaling molecules upon phosphorylation. To investigate whether CD160-TM function was dependent on its intracellular domain, a chimeric construct coding for CD8α/H9251 extracellular and transmembrane domains fused to CD160-TM cytoplasmic tail was generated. CD8-negative wild-type Jurkat cells stably expressing the chimeric protein (Jurkat/WT) were selected (see Fig. 6B, top panel, for expression of CD8–CD160-TM molecules), and the activating potential of the chimera was tested in proliferation assays. While the transfected cells did not show any proliferation increase in response to control mouse IgG1, a substantial enhancement of their growth rate, corresponding to a 30–35% increase in their proliferation level, was obtained following CD8 triggering (Fig. 6B, bottom panel). Furthermore, we observed that point mutation of Y220 resulted in a chimeric protein still able to mediate an up-modulation of Jurkat cell growth following triggering (Fig. 6B, bottom panel, Jurkat/mutF220SS). In contrast, no more increase was detected upon ligation of the Y225-mutated chimera (Fig. 6B, bottom panel, Jurkat/mutF225PQ), demonstrating that this tyrosine residue is critically involved in the delivery of positive signals. A tyrosine-dependent activation signaling was confirmed by the observation that crosslinking of the wild-type chimera had no effect on the proliferation rate of Lck-deficient Jurkat cells (Fig. 6B, bottom panel, JCam/WT). These results establish that CD160-TM intracellular domain has the functional potential to transduce activating signals through

FIGURE 4. Expression CD160 isoforms following PB-NK cell activation. A, Characterization of anti-CD160-TM polyclonal Abs. Jurkat cell stable transfectants expressing either CD160 or CD160-TM protein, or long-term IL-2-activated NK cells, were immunolabeled with the affinity-purified anti-CD160-TM pep2 Abs (top panel) or the anti-CD160 mAb BY55 (bottom panel). Rabbit preimmune serum or isotype-matched anti-CD34 mAb was used for a negative control. Following addition of the appropriate PE-coupled secondary Abs, cells were analyzed by flow cytometry. B, CD160-TM surface expression upon PB-NK cells activation. Freshly isolated PB-NK cells were grown in medium alone (day 0) or supplemented with IL-15 for the indicated time. The presence of the CD160 (top panel) or CD160-TM (bottom panel) isoform was detected by immunolabeling with BY55 mAb or the anti-CD160-TM pep2-purified Abs, respectively. Data are representative of experiments performed on three healthy donors.

FIGURE 5. Molecular characterization of CD160-TM. Transfected Jurkat cells stably expressing CD160-TM or long-term IL-2-activated NK cells were lysed, and postnuclear supernatants were subjected to immunoprecipitation using the anti-CD160-TMIC Abs (directed against the intracellular domain of CD160-TM) or the corresponding preimmune serum (PI). After separation by SDS-PAGE under reducing conditions and transfer onto nitrocellulose, protein revelation was performed by incubation of the blot with the anti-CD160-TMIC Abs followed by a HRP-conjugated anti-rabbit Ig.
a phosphotyrosine-dependent process that most likely involved p65Lck.

To further characterize the signaling pathways engaged upon CD160-TM triggering, Jurkat cells stably expressing wild-type or mutated CD8-CD160-TM chimera were either left unstimulated or activated with anti-CD8 mAb. The cellular lysates were then analyzed for the activation of Erk by Western blot using an anti-phospho-Erk mAb (Fig. 6C). We observed that triggering of the CD8-CD160-TM chimera led to the efficient recruitment of Erk signaling cascade, as assessed by the detection of Erk phosphorylation (Fig. 6C, left panel). A similar activation of Erk was induced when targeting the Y220-mutated chimera, while Y225 mutation led to a complete loss of Erk activation. Finally, the triggering of wild-type chimera on Lck-negative cells did not result in any Erk phosphorylation (Fig. 6C, right panel). Thus, the CD160-TM cytoplasmic tail exhibits structural features that allow the recruitment of the Erk signaling pathway.

Discussion

CD160 has been initially identified as a MHC class I-specific activating receptor in PB-NK lymphocytes (22, 23), and it was shown to act as a coactivating receptor in cytotoxic CD8+ T cells (25, 26). Herein we report that, apart from CD160 mRNA, three additional transcripts generated through alternative splicings of the CD160 gene can be detected in NK cells. The predicted amino acid sequences for these newly identified mRNA correspond to: 1) a GPI-anchored CD160 molecule devoid of extracellular Ig-like...
domain (CD160ΔIg-GPI), 2) a transmembrane isoform of the original CD160 receptor (CD160-TM), and 3) a transmembrane version of CD160ΔIg-GPI (CD160ΔIg-TM). Notably, we observed a coupled synthesis of the transcripts, with the mRNA encoding both GPI or TM isoforms being systematically produced in parallel (Figs. 1 and 3). By analyzing the synthesis pattern of the above mRNA, we confirmed the constitutive expression of CD160 by circulating NK and cytotoxic CD8\(^+\) T lymphocytes (19). In contrast, the TM isoforms encoding mRNA were not amplified from these cells (Table I). More importantly, we demonstrated that the trast, the TM isoforms encoding mRNA were not amplified from CD3\(^\text{encompassing an identical extracellular domain, CD160 and TM isoforms (44). In this regard, we similarly observed that while rates and affinity for a given ligand. Additionally, anti-CD16 mAbs to its membrane anchor, CD16 displayed distinct binding kinetic trophils as a GPI-anchored molecule (41–43). Notably, according to human NK cells as a transmembrane glycoprotein and on neu-

CD160-TM, a UNIQUE NK CELL-ACTIVATING RECEPTOR

The existence of splice variants has been reported for numerous NK cell receptors. Thus, 2B4, CS1 (CRACC), or murine NK2D presents spliced isoforms that differed from the full-length receptor in their cytoplasmic domain (37–39). A spliced isoform of CD33, devoid of extracellular Ig-like domain, has also been characterized (40). In all cases, the second isoform is thought to transduce different signals when compared with the full-length molecule as a result of its association with distinct signaling adaptor molecules and/or of a cell lineage-specific expression pattern. One well-defined example was given by CD16 (FcγRIII), which was expressed on human NK cells as a transmembrane glycoprotein and on neutrophils as a GPI-anchored molecule (41–43). Notably, according to its membrane anchor, CD16 displayed distinct binding kinetic rates and affinity for a given ligand. Additionally, anti-CD16 mAbs were found to exhibit a different reactivity toward the GPI and the TM isoforms (44). In this regard, we similarly observed that while encompassing an identical extracellular domain, CD160 and CD160-TM were selectively recognized by BY55 mAbs or anti-CD160-TM\(^\text{gpl}\) Abs, respectively (Fig. 4A). Furthermore, a significant reactivity was obtained with the anti-CD160 CL1-R2 mAb when CD160-TM-expressing cells were subjected to a fixation step before immunolabeling (data not shown). The most likely explanation will be that the replacement of the GPI anchor motif by transmembrane and intracellular domains may result in some conformational modifications within the extracellular moiety, leading to differential Ab recognition of the molecules.

Although the complete identity between CD160 and the CD160-TM extracellular part might suggest that both molecules could be specific for the same ligands (namely, the MHC class I molecules or HVEM) (20–22), we cannot exclude the hypothesis of a different ligand specificity resulting from conformational differences. In any case, each isoform could be functionally characterized according to its related signal transduction pathway. Despite the presence of a charged lysine residue in the CD160-TM transmembrane region, we did not find any association of the receptor with adaptor molecules usually involved in NK cell activating-receptor signal transduction, such as DAP12, FcεR1γ, or CD3\(_{\zeta}\), in cotransfection experiments (data not shown). The generation of a CD8-CD160-TM chimera, encompassing the intracellular domain of CD160-TM, suggested that CD160-TM is sufficient by itself to initiate intracellular signals leading to an increased cellular proliferation and to the recruitment of Erk activation pathway (Fig. 6). Additionally, the lack of CD8-mediated response in CD8-CD160-TM JCam transfectants suggested a requirement for p56\(^\text{lck}\) in the initiation of the CD160-TM-mediated activation process. We established that mutation of Y225 within the CD160-TM intracellular domain did not affect the positive signals delivered through engagement of the CD8-CD160-TM chimer (Fig. 6). Furthermore, we observed that the F220SS mutated protein still underwent tyrosine phosphorylation upon cell activation (data not shown). Accordingly, our data identified Y225 as the residue involved in the delivery of CD160-TM-dependent activation signals. We recently reported that the CD160-activating function in NK cells depended on PI3K recruitment (45). The sequence analysis of the CD160-TM cytoplasmic tail showed no consensus tyrosine-based interaction motifs allowing a direct association with PI3K. Similarly, CD160-TM does not contain any immunoreceptor tyrosine-based switch motif, thus not favoring the possibility of an interaction with the signaling lymphocyte activation molecule–associated protein (SAP) or its homolog EAT-2, with such signaling pathways having been described for the CD2 family receptors expressed in NK cells, namely 2B4, N TB-A, and CRACC (46, 47). It is therefore possible that CD160-TM intracellular Y225 mediates an interaction with still undefined cytoplasmic signaling molecules involved in the transduction of activating signals. Work is in progress to clarify this issue.

Finally, we observed that the appearance of CD160-TM on activated NK cells followed a two-step expression process. Thus, low levels of CD160-TM were detected on IL-15-treated cells 3 days after activation, while CD160 became undetectable at the cell surface (Fig. 4B). We recently demonstrated that CD160 clearing from the NK cell surface resulted from a phospholipase-dependent proteolytic cleavage of the molecule (27). At longer activation times, a down-regulation of CD160-TM expression was observed, followed by a reexpression step leading to the detection of a NK cell population highly expressing the TM-receptor, with the remaining cells exhibiting a lower cell surface expression. In contrast, no change in the level of CD160-TM mRNA was detected during the time course of activation (Fig. 3), suggesting that the level of CD160-TM expression results from a time-dependent regulation of its translation. CD160-TM reexpression parallels the progressive reacquisition of GPI-anchored CD160, probably reflecting the shut-down of the previously induced GPI phospholipase activity. One can therefore postulate that the tightly regulated expression of CD160 isoforms might be an important step in the cascade of events leading to a specific and efficient recruitment of CD160 molecules and of their respective signaling pathways. The relevance of the ΔIg isoforms mRNA synthesis would also have to be addressed in terms of protein expression, function, and specificity to determine whether these potential receptors may also play a role in the regulation of NK cell functions. In any case, the identification of CD160-TM opens new perspectives regarding the cellular events involved in the regulation of NK cell functions.

Disclosures

The authors have no financial conflicts of interest.

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