Mechanisms DAP12-Dependent and DAP12-Independent Modulate T Cell Activation through Stimulatory Killer Ig-Like Receptors

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Stimulatory Killer Ig-Like Receptors Modulate T Cell Activation through DAP12-Dependent and DAP12-Independent Mechanisms

Melissa R. Snyder, Takako Nakajima, Paul J. Leibson, Cornelia M. Weyand, and Jörg J. Goronzy

Stimulatory killer Ig-like receptors (KIRs) are expressed by various lymphocytes, including NK cells and subsets of T cells. In NK cells, KIRs associate with the adapter molecule KARAP/DAP12, which confers the ability to function as an independent activation unit. The function of KIRs and killer cell activating receptor-associated protein (KARAP)/DAP12 in T cells is unclear. By flow cytometry, we demonstrated that CD4+CD8null T cells heterogeneously express KIRs and/or KARAP/DAP12. In clones that lacked expression of KARAP/DAP12, the stimulatory KIR KIR2DS2 signaled through the JNK pathway, but did not activate the ERK pathway. However, in the presence of KARAP/DAP12, stimulation through KIR2DS2 led to phosphorylation of both JNK and ERK. Transfection experiments confirmed that KIR2DS2-mediated ERK phosphorylation was dependent on KARAP/DAP12. The differential signaling of KIR2DS2 through association with alternative adapter molecules resulted in differential regulation of cellular activity. In clones that lacked expression of KARAP/DAP12, stimulation of KIR2DS2 did not induce cytotoxicity. However, KIR2DS2 did augment suboptimal TCR stimulation, leading to enhanced IFN-γ production. In clones that expressed KARAP/DAP12, KIR2DS2 directly activated both cytotoxicity and IFN-γ production without the need for TCR-derived signals. The function of stimulatory KIRs in T cells is determined by the expression of the appropriate adapter molecule. Expression of KARAP/DAP12 is sufficient to convert a costimulatory KIR into a stimulatory molecule. These differing functions mediated by alternative signaling pathways have implications for the pathogenesis of diseases such as rheumatoid arthritis and acute coronary syndromes, in which aberrant expression of KIRs on T cells is frequently observed.


Lymphocyte activation is initiated by the ligation of stimulatory receptors. The mechanism by which this occurs in NK cells and T cells differs significantly. In NK cells, multiple stimulatory receptors, including NKp44, NKp46, NKG2D, and stimulatory members of the C-type lectin receptor and killer Ig-like receptor (KIR) families, function as direct activators of cellular activity (1). In contrast, T cells require two synergistic signals. One signal is initiated by the interaction between the TCR and the Ag/MHC complex, while the second signal comes from a costimulatory molecule such as CD28 or inducible costimulator protein (ICOS) (2, 3). The different stimulation requirements of NK and T cells reflect the functional differences between the innate and adaptive immune systems. Rapid NK cell activation is required in the early stages of viral or microbial infection and during cellular transformation. In contrast, adaptive immune responses are associated with sustained remodeling of the immune repertoire. The initiation of adaptive responses is strictly controlled by professional APCs in part through the expression of ligands for costimulatory receptors.

Receptors that have stimulatory function in NK cells are also expressed on subsets of T cells. Various members of the KIR family are expressed on subsets of CD8+ and CD4+ T cells (4–7). Most individuals possess KIR+CD8+ T cells. Expression of KIRs on CD4+ T cells is more restricted and frequently found in individuals with chronic inflammatory diseases, such as rheumatoid arthritis (RA) and acute coronary syndromes (ACS) (8–10). KIR+ T cells are oligoclonally expanded and are characterized by the loss of CD28 (11–13). CD4+CD28null T cells are autoreactive, resistant to apoptosis, and have acquired cytotoxic capabilities (14–16). One particular KIR, KIR2DS2 (encoded by KIR2DS2), is expressed on CD4+CD28null T cells more frequently than other stimulatory KIRs and is a genetic risk factor for extra-articular complications of RA (17) and for psoriatic arthritis (18).

In NK cells, KIR2DS2 functions as a stimulatory receptor, inducing cytotoxicity and cytokine production (19). This activity is mediated through the adapter molecule killer cell activating receptor-associated protein (KARAP)/DAP12 (20–22). Ligation of KIR2DS2 results in KARAP/DAP12 phosphorylation, leading to Syk/ZAP-70 phosphorylation, phospholipase C-γ1 phosphorylation, and calcium mobilization. In contrast to NK cells, CD4+CD28null T cells heterogeneously express KARAP/DAP12 at the clonal level. In this study, we demonstrate that KIR2DS2 associates with alternative adapter molecules, leading to differential signaling mechanisms and cellular activation. In the absence of KARAP/DAP12, KIR2DS2 augments suboptimal TCR-mediated stimulation, leading to enhanced proliferation and cytokine production. This costimulatory function is mediated through an unidentified adapter molecule, resulting in the activation of the JNK.
signaling pathway. However, in the presence of KARAP/DAP12, KIR2DS2 is a direct stimulatory receptor. In this case, KIR2DS2 triggering leads to cytotoxicity and cytokine production without any requirement for TCR stimulation.

Materials and Methods

Cloning of CD4<sup>+</sup> CD28<sup>+</sup> null T cells

The protocol was approved by the Mayo Clinic Institutional Review Board, and all individuals gave written, informed consent. CD4<sup>+</sup> CD28<sup>-</sup> null T cell clones were generated, as previously described (23). CD4<sup>+</sup> CD28<sup>-</sup> null T cells were sorted by FACS from PBMCs using anti-CD3<sup>+</sup> and anti-CD28<sup>-</sup> mAbs (BD Biosciences, San Jose, CA). Lines were established by anti-CD3 (Orthoclone OKT3, CRL 8001; Ortho Diagnostics, Raritan, NJ) stimulation of the total population of sorted cells, and clones were generated through limiting dilution. Clones and lines were maintained on 1 × 10<sup>5</sup> irradiated heterologous PBMCs/ml, 2 × 10<sup>5</sup> irradiated EBV-transformed lymphoblastoid cells/ml, 30 ng/ml anti-CD3 mAb, and 50 U/ml human rIL-2 (Proleukin; Chiron, Emeryville, CA). Clones and lines were phenotyped by flow cytometry using anti-CD28<sup>+</sup>, anti-KIR2DS2/2DL2/2DL3<sup>PE</sup> (GL183; Beckham Coulter, Miami, FL), and anti-CD4<sup>HPC</sup> (BD Biosciences) mAbs.

RT-PCR

Total RNA was extracted using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA), and cDNA was synthesized using an oligo(dT) primer and avian myeloblastosis virus reverse transcriptase (Roche Molecular Biochemicals, Indianapolis, IN). CD4<sup>+</sup> CD28<sup>-</sup> null KIR<sup>-</sup> T cell clones were analyzed for the expression of KIR2DS2, KIR2DL2, and KIR2DL3 transcripts, which encode for KIR2DS2, KIR2DL2, and KIR2DL3, respectively, using receptor-specific PCR primers described by Uhrberg et al. (24). The nomenclature for the cell surface molecules and for the corresponding genes was used, as suggested by World Health Organization Nomenclature Committee (25). CD4<sup>+</sup> CD28<sup>-</sup> null CD28<sup>-</sup> T cell clones were characterized for the expression of KARAP/DAP12 transcript using the primers 5'-CATGTGGTCATCCGGTGAGC-3' and 5'-TGTGTTGTGAG GTCGCTGT-3'. For each RT-PCR, β-actin transcript, amplified by the primers 5'-ATGCCACGGTGTTCGAC-3' and 5'-CAGGAGGAC CAGTGATCTTGAT-3', was used as a control.

KARAP/DAP12 intracellular flow cytometry

CD4<sup>+</sup> CD28<sup>-</sup> null T cell lines were phenotyped for the expression of KARAP/DAP12 using intracellular flow cytometry. Cells were stained on the cell surface with anti-CD28<sup>+</sup> and anti-CD4<sup>+</sup> PE mAbs, followed by fixation and permeabilization using the Cytofix/Cytoperm kit (BD Biosciences), according to the manufacturer's instructions. After permeabilization, rabbit anti-human KARAP/DAP12 antisera were added, followed by donkey anti-rabbit IgG<sup>PE</sup> (Research Diagnostics, Flanders, NJ).

Vaccinia virus infection

Jurkat T cells were infected for 5 h at a ratio of 10:1 (viral PFU:T cell ratio) with recombinant vaccinia virus containing KIR2DS2 cDNA alone or in combination with recombinant vaccinia virus containing KARAP/DAP12 cDNA. CD4<sup>+</sup> CD28<sup>-</sup> KIR2DS2<sup>-</sup> KARAP/DAP12<sup>-</sup> T cell clones were infected for 5 h at a ratio of 10:1 with recombinant vaccinia virus containing KARAP/DAP12 cDNA. Cell surface expression of KIR2DS2 was confirmed by flow cytometry, and the expression of KARAP/DAP12 was confirmed by RT-PCR.

Western blots

Resting CD4<sup>+</sup> CD28<sup>-</sup> null KIR2DS2<sup>-</sup> T cell clones, transfected T cell clones, or transfected Jurkat T cells were treated with mouse IgG (Valeant Pharmaceuticals, Costa Mesa, CA), anti-CD3 mAb, anti-KIR2DS2 mAb (GL183; Beckham Coulter), or anti-CD3 and anti-KIR2DS2 mAbs, and then cross-linked with rabbit anti-mouse IgG Ab (Valeant Pharmaceuticals). The cells were stimulated for 20 min and then harvested. The samples were incubated on ice in 50 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1.0% Triton X-100, 0.5 mM PMSF, 5.0 μg/ml aprotinin, 10.0 μg/ml leupeptin, and 1.0 mM orthovanadate. Clarified cell lysates were collected after centrifugation at 10,000 × g for 10 min. The amount of total protein in each sample was quantified with the Protein Assay Kit II (Bio-Rad, Richmond, CA). Lysates were analyzed by SDS-PAGE and transferred onto a nitrocellulose membrane. The membranes were blotted in series with specific Abs against phospho-JNK and JNK or phospho-ERK 1/2 (EKR1/2) and EKR1/2 (all Cell Signaling Technology, Beverly, MA). Blots were developed using HRP-conjugated goat anti-rabbit IgG Ab (Cell Signaling Technology) and the SuperSignal West Pico Chemiluminescent Detection System (Pierce, Rockford, IL).

Cytotoxicity assay

HUVEC (CRL1730; American Type Culture Collection, Manassas, VA) were labeled with calcine acetoxyethyl ester (Molecular Probes, Eugene, OR), then surface biotinylated, according to the manufacturer’s instructions (Pierce), and coated with avidin-conjugated mouse IgG, anti-CD3, or anti-KIR2DS2 mAbs. Resting CD4<sup>+</sup> CD28<sup>-</sup> null T cell clones were incubated with the coated HUVEC at various E:T ratios. Supernatants were harvested after 4 h, and calcine release was determined on a fluorescence plate reader (CytotoFluor; Applied Biosystems, Framingham, MA). Results are expressed as a percentage of maximal lysis after correction for background release.

IFN-γ analysis

Resting CD4<sup>+</sup> CD28<sup>-</sup> null KIR2DS2<sup>-</sup> T cell clones were stimulated with plate-immobilized mouse IgG mAb, anti-CD3 mAb, anti-KIR2DS2 mAb, anti-CD3, and anti-KIR2DS2 mAbs, or anti-CD3 and mouse IgG mAbs. Alternatively, mAbs used for stimulation were immobilized using P815 mastocyteoma cells. Cell supernatants were harvested after 20 h, and IFN-γ was determined by ELISA (BioSource International, Camarillo, CA, or BD Pharmingen, San Diego, CA), according to the manufacturer's instructions.

Results

KARAP/DAP12 is present in CD4<sup>+</sup> CD28<sup>-</sup> null T cells

CD4<sup>+</sup> CD28<sup>-</sup> null T cells found in patients with RA and ACS frequently express KIR2DS2 on their cell surface (8–10). In NK cells, this receptor associates with the adapter molecule KARAP/DAP12. However, it is generally accepted that most T cells do not express KARAP/DAP12 (20, 22). To assess the expression of KARAP/DAP12<sup>+</sup> T cell clones, lines were generated from multiple individuals, which were then analyzed by RT-PCR and intracellular flow cytometry. By RT-PCR, we found that CD4<sup>+</sup> CD28<sup>-</sup> null T cells from some, but not all, individuals possessed transcript for KARAP/DAP12 (Fig. 1A). In intracellular flow cytometry confirmed that the T cell line that did not express transcript did not contain any KARAP/DAP12<sup>+</sup> T cells. However, in the T cell line that did possess transcript, ~10% of the total CD4<sup>+</sup> CD28<sup>-</sup> null population expressed KARAP/DAP12. Therefore, KARAP/DAP12 can be heterogeneously expressed within a population of CD4<sup>+</sup> CD28<sup>-</sup> null T cells.

KIR2DS2 induces JNK phosphorylation independent of KARAP/DAP12

In NK cells, KIR2DS2 primarily signals through KARAP/DAP12 to induce effector function. However, on CD4<sup>+</sup> CD28<sup>-</sup> null T cells, KIR2DS2 is expressed in the presence and absence of KARAP/DAP12. This led to the hypothesis that KIR2DS2 uses different signaling pathways depending upon the identity of the adapter molecule with which it associates. CD4<sup>+</sup> CD28<sup>-</sup> KIR2DS2<sup>-</sup> KARAP/DAP12<sup>-</sup> and CD4<sup>+</sup> CD28<sup>-</sup> KIR2DS2<sup>+</sup> KARAP/DAP12<sup>+</sup> T cell clones were stimulated and analyzed for JNK phosphorylation (Fig. 2A). Optimal stimulation of either clonotype through the TCR induced JNK phosphorylation, which was dramatically reduced with suboptimal TCR stimulation. Additionally, in both KARAP/DAP12<sup>+</sup> and KARAP/DAP12<sup>-</sup> null clones, stimulation through either KIR2DS2 alone or in combination with suboptimal stimulation through the TCR led to JNK phosphorylation. These results were confirmed in Jurkat T cells transfected with KIR2DS2 in the absence or presence of KARAP/DAP12 (Fig. 2B). In Jurkat T cells, stimulation through the TCR did not activate the JNK cascade. However, stimulation through KIR2DS2 either in the presence or absence of KARAP/DAP12 resulted in the phosphorylation of JNK.
KIR2DS2 induces ERK phosphorylation only in the presence of KARAP/DAP12

In NK cells, the KIR2DS2-KARAP/DAP12 complex induces cytotoxicity and cytokine production partially through activation of the ERK pathway. Although KIR2DS2 can induce NKG2D phosphorylation in the presence or absence of KARAP/DAP12, we hypothesized that KIR2DS2 would be capable of activating the ERK pathway in T cells only in the presence of KARAP/DAP12. CD4+CD28null KIR2DS2+ KARAP/DAP12null and CD4+CD28null KIR2DS2+ KARAP/DAP12+ T cell clones were stimulated and analyzed for ERK phosphorylation (Fig. 3A). In each clone analyzed, stimulation of the TCR at either optimal or suboptimal levels resulted in activation of the ERK pathway. However, only in the T cell clones that expressed KARAP/DAP12 did stimulation of KIR2DS2 lead to ERK phosphorylation. These results are paralleled by experiments using transfected Jurkat T cells (Fig. 3B). Jurkat T cells transfected only with KIR2DS2 did not induce ERK phosphorylation after stimulation, although TCR-mediated activation of this pathway was intact. In contrast, after cotransfection of KARAP/DAP12, stimulation through KIR2DS2 led to phosphorylation of ERK. Despite the observation that KIR2DS2 can activate the NKG2D pathway in the absence of KARAP/DAP12, this adapter molecule is required for activation of the ERK pathway.

In the absence of KARAP/DAP12, KIR2DS2 does not induce cytotoxicity, but does costimulate IFN-γ production

Because KIR2DS2 activates different signaling pathways in the presence or absence of KARAP/DAP12, its activation should have different functional outcomes for the cell. Like NK cells, CD4+CD28null T cells express perforin and granzyme B and mediate cytotoxic activity toward a variety of target cells. CD4+CD28null T cell clones that expressed KIR2DS2, but lacked expression of KARAP/DAP12, were analyzed for their ability to lyse target cells in a redirected cytotoxicity assay (Fig. 4A). TCR stimulation resulted in lysis of the HUVEC target cells; however, these clones were unable to lyse the targets after stimulation of KIR2DS2. These results suggest that activation of the NKG2D pathway by KIR2DS2 in the absence of KARAP/DAP12 is insufficient to initiate cell-mediated cytotoxicity.

In CD4+CD28null KARAP/DAP12null T cells, KIR2DS2 signals through NKG2D, a primary pathway through which a variety of other costimulatory signals are mediated (26–28). Therefore, we hypothesized that KIR2DS2 functions as a costimulatory molecule in the place of CD28. CD4+CD28null KIR2DS2+ KARAP/DAP12null T cell clones were stimulated with immobilized Abs, and the cell supernatants were analyzed for secreted IFN-γ (Fig. 4B). After optimal TCR stimulation, CD4+CD28null T cells produced high levels of IFN-γ; decreased cytokine levels were detected after stimulation with suboptimal doses of anti-CD3 mAb. When the CD4+CD28null T cell clones were stimulated with anti-KIR2DS2 mAb, only slightly elevated levels of IFN-γ were detected. However, when the clones were costimulated with a suboptimal dose of anti-CD3 mAb and an optimal dose of anti-KIR2DS2 mAb, there was a dramatic increase in the amount of IFN-γ produced that was equal to or greater than the levels observed after optimal TCR stimulation. This increase was not observed after costimulation with anti-CD3 mAb and a control IgG mAb. The cytotoxicity and cytokine production results indicate that KIR2DS2 functions as a

FIGURE 1. KARAP/DAP12 is expressed by a subset of CD4+CD28null T cells. CD4+CD28null T cell lines were established by anti-CD3 stimulation in the presence of IL-2. Representative results are shown for individuals A and B. A, cDNA from PBMCs (lane 1), CD4+CD28null T cell line A (lane 2), and CD4+CD28null T cell line B (lane 3) was analyzed by RT-PCR for KARAP/DAP12 and β-actin expression. cDNA was omitted from the negative control (lane 4). B, CD4+CD28null T cell line A (left panel) and CD4+CD28null T cell line B (right panel) were analyzed for KARAP/DAP12 expression by intracellular flow cytometry. KARAP/DAP12 expression in CD4+CD28− T cells was essentially negative in all donors (data not shown), but was variably positive in CD4+CD28null T cells.

FIGURE 2. Stimulation of KIR2DS2 leads to phosphorylation of JNK. A, CD4+CD28null KIR2DS2+ KARAP/DAP12null and CD4+CD28null KIR2DS2− KARAP/DAP12+ T cell clones were stimulated with anti-CD3 and/or anti-KIR2DS2 mAbs, and then analyzed for NKG2D phosphorylation (left panel). Each blot was subsequently analyzed for total JNK (right panel). Ab concentrations (micrograms per milliliter) are shown for the mouse IgG, anti-CD3, and anti-KIR2DS2. B, Jurkat T cells were transfected with KIR2DS2 or with KIR2DS2 and KARAP/DAP12. The cells were then stimulated with anti-CD3 and/or anti-KIR2DS2 mAbs and analyzed for NKG2D phosphorylation (left panel) and for total JNK (right panel).
KIR2DS2 is capable of signaling through different pathways, depending on the adapter molecule with which it associates. In the absence of KARAP/DAP12, these signaling pathways lead to costimulatory activity without any direct activation of cytotoxicity or cytokine production. The observation that the coexpression of KARAP/DAP12 leads to activation of additional signaling cascades suggests that KIR2DS2 is capable of regulating T cell function in a manner other than that of a costimulatory receptor. CD4⁺CD28null/KIR2DS2⁺ KARAP/DAP12 null T cell clones were analyzed for the ability to lyse endothelial target cells (Fig. 5A). As with all CD4⁺CD28null T cells, stimulation through the TCR resulted in lysis of the target cells. In contrast to the KARAP/DAP12 null T cell clones, KARAP/DAP12⁺ T cell clones were also capable of KIR2DS2-mediated cytotoxicity. The activation of the cytotoxic pathway is independent of TCR stimulation. This type of stimulatory activity is also evident in cytokine production (Fig. 5B). With a KARAP/DAP12⁺ T cell clone, TCR stimulation resulted in a dramatic increase in IFN-γ production over that observed after treatment with a control Ab. This same increase was also observed after KIR2DS2 cross-linking. These data demonstrated that in KARAP/DAP12 null T cell clones, KIR2DS2 directly mediates cellular activation without any requirement for Ag-dependent TCR stimulation.

To examine whether the ability of KIR2DS2 to function as a stimulatory receptor in CD4⁺CD28null T cells is exclusively determined by the presence of KARAP/DAP12, we transfected a CD4⁺CD28null/KIR2DS2⁺ KARAP/DAP12 null T cell clone with KARAP/DAP12 and used it in a redirected cytotoxicity assay (Fig. 6A). Whether infected with a control virus or KARAP/DAP12⁺ virus, TCR stimulation resulted in cytotoxicity. However, only after expression of KARAP/DAP12 did the clone acquire the ability to lyse the target cells after cross-linking of KIR2DS2. This pattern was also observed for cytokine production (Fig. 6B). Regardless of the type of vaccinia virus infection, TCR-mediated stimulation led to increased production of IFN-γ in comparison with the control Ab. When the T cell clone was infected with the control virus, stimulation with anti-KIR2DS2 mAb did not induce detectable levels of IFN-γ. In contrast, after KARAP/DAP12 expression, cross-linking of KIR2DS2 led to substantial production of IFN-γ. The induction of both cytotoxicity and IFN-γ by KIR2DS2 in the presence of KARAP/DAP12 is not dependent on TCR stimulation. The activation of additional signaling pathways, including the ERK pathway, by the KIR2DS2/KARAP/DAP12 complex results in the conversion of a costimulatory molecule into a stimulatory receptor.

**Discussion**

KIR2DS2 is expressed by CD4⁺ T cells that are clonally expanded in individuals with chronic inflammatory conditions. Among these T cells, there is heterogeneous expression of the adapter molecule KARAP/DAP12. In the absence of KARAP/DAP12, KIR2DS2 does not initiate cytotoxicity, but it does costimulate IFN-γ production in conjunction with suboptimal TCR activation. In the presence of KARAP/DAP12, KIR2DS2 functions as a stimulatory receptor.
receptor, directly inducing cytotoxicity and IFN-γ production without any requirement for TCR stimulation. The differential functions of KIR2DS2 are controlled by different signaling cascades. In the absence of KARAP/DAP12, KIR2DS2 signals exclusively through the JNK pathway. However, when coexpressed with KARAP/DAP12, KIR2DS2 activates both the JNK and ERK signaling pathways. Based on these data, we propose a model in which the function of this receptor is determined by the cellular context in which it is expressed, including the presence or absence of various adapter molecules (Fig. 7). In general, this mechanism provides a single receptor with another level of functional flexibility via associations with different signaling molecules.

The activation of different signaling pathways by KIR2DS2 and its effect on cellular activation is solely dependent on its association with alternative adapter molecules. A KIR2DS2+/KARAP/DAP12+/− T cell clone is not capable of cytotoxicity or IFN-γ production after KIR2DS2 stimulation alone, but it does augment IFN-γ expression under conditions of suboptimal TCR triggering. After induction of KARAP/DAP12 expression, KIR2DS2 is capable of directly inducing both activities without any requirement for TCR stimulation. The introduction of KARAP/DAP12 expression, KIR2DS2 is capable of directly inducing both activities without any requirement for TCR stimulation. The introduction of KARAP/DAP12 expression, KIR2DS2 is capable of directly inducing both activities without any requirement for TCR stimulation. The introduction of KARAP/DAP12 expression, KIR2DS2 is capable of directly inducing both activities without any requirement for TCR stimulation.

FIGURE 5. In the presence of KARAP/DAP12, KIR2DS2 induces cytotoxicity and IFN-γ production independent of TCR stimulation. A, A CD4+CD28−/−KIR2DS2−/− KARAP/DAP12−/− T cell clone was analyzed for cytotoxic activity in a redirected cellular lysis assay using mouse IgG-, anti-CD3 mAb-, or anti-KIR2DS2 mAb-coated target cells. B, A CD4+CD28−/−KIR2DS2−/− KARAP/DAP12−/− T cell clone was stimulated with anti-CD3 or anti-KIR2DS2 mAbs. Abs concentrations (micrograms per milliliter) are shown for the mouse IgG, anti-CD3, and anti-KIR2DS2. IFN-γ production was quantified by ELISA.

FIGURE 6. Expression of KARAP/DAP12 confers KIR2DS2 with ability to directly stimulate cytotoxicity and IFN-γ production independent of TCR stimulation. A, A CD4+CD28−/−KIR2DS2−/− KARAP/DAP12null T cell clone was transfected with wild-type vaccinia virus (upper panel) or with KARAP/ DAP12−/− vaccinia virus (lower panel). The transfectants were analyzed for cytotoxic activity in a redirected cellular lysis assay using mouse IgG-, anti-CD3 mAb-, or anti-KIR2DS2 mAb-coated target cells. B, Control- or KARAP/DAP12-transfected CD4+CD28−/−KIR2DS2−/− KARAP/DAP12null T cell clones were stimulated with anti-CD3 or anti-KIR2DS2 mAbs. Abs concentrations (micrograms per milliliter) are shown for the mouse IgG, anti-CD3, and anti-KIR2DS2. IFN-γ production was quantified by ELISA. ND, not detected.

costimulatory receptors, and it seems to be a critical mechanism through which TCR-mediated signals are enhanced (26–28). Alone, the JNK pathway cannot activate IFN-γ production or cytotoxicity. However, after KIR2DS2 stimulation, signaling through the JNK pathway leads to expression and activation of the transcription factors, activating transcription factor-2 and c-Jun (23), which act in concert with TCR-induced transcription factors to activate IFN-γ production (29, 30). In contrast, when KARAP/ DAP12 is transfected into a KIR2DS2+ KARAP/DAP12null T cell clone, stimulation through KIR2DS2 leads to JNK and ERK phosphorylation. Presumably, it is the activation of the ERK, and possible other, pathways that allows KIR2DS2 to bypass the requirement for TCR activation. Clearly, the machinery required for the activation of both signaling pathways and the subsequent cellular activation is present in virtually all CD4+ T cells. Stimulation of KIR2DS2 transfected into Jurkat T cells leads only to activation of the JNK pathway. However, after cotransfection of KIR2DS2 and...
KARAP/DAP12, both the JNK and ERK pathways are activated. Therefore, once a T cell has acquired expression of an MHC class I-recognizing stimulatory receptor, the determining factor in the function of this receptor is the identity of its adapter molecule.

This mechanism, in which a receptor uses different adapter molecules, is not confined to KIR2DS2. In mice, mNKG2D can be expressed as two alternatively spliced isoforms (31). Both isoforms are expressed on activated CD8+ T cells and NK cells, and both associate with the adapter molecule DAP10. However, one of the isoforms also associates with KARAP/DAP12, which is only expressed in NK cells (31). In CD8+ T cells, the costimulatory activity of mNKG2D is mediated through DAP10 (32). In activated NK cells, both DAP10 and KARAP/DAP12 are capable of inducing the stimulatory activity of mNKG2D (31). In humans, NKG2D (hNKG2D) associates with DAP10, with no detectable association with or activation of KARAP/DAP12 (33). DAP10 mediates the costimulatory activity of hNKG2D in T cells and the stimulatory activity in NK cells. How can the hNKG2D/DAP10 complex differentially modulate cellular activity in two distinct cell types? Although hNKG2D associates with only DAP10, this adapter molecule may be capable of interacting with various proximal signaling molecules, thereby initiating different signaling cascades. This suggests a more general theme, in which differential associations between various types of molecules within the signaling complex may be responsible for the functional heterogeneity of receptors.

Despite DAP10 mediating the costimulatory function of NKG2D in T cells, this is not the case for KIR2DS2. DAP10 was considered a potential alternate adapter molecule for KIR2DS2; however, immunoprecipitation experiments failed to detect any interaction between these two molecules (23). This correlates with previously published data of cotransfection experiments in which KIR2DS2 associated with KARAP/DAP12, but not with DAP10 (34). At this time, the identity of the adapter molecules that transduces KIR2DS2-initiated T cell costimulation remains unknown.

NKG2D and the stimulatory KIRs are a novel class of T cell costimulatory molecules that, in many aspects, are distinct from the classic T cell costimulatory receptors. Although NKG2D is expressed on all CD8+ T cells, its expression on CD4+ T cells, as well as the expression of KIRs on both CD4+ and CD8+ T cells, is restricted to cells with an effector/memory phenotype (4, 7, 35). This expression is constitutive and is not affected by TCR-mediated stimulation, unlike that of ICOS or 4-1BB (3, 23, 36). In this respect, NKG2D and the KIRs are similar to CD28. However, unlike CD28, the ligands for NKG2D and the KIRs are not restricted to professional APCs. In humans, NKG2D recognizes MHC class I chain-related genes A/B and the UL16 binding proteins (37, 38). These molecules are stress induced and, in principle, could be expressed by any cell that has been virally infected or has undergone malignant transformation. The exact ligands for the stimulatory KIRs are unknown. The stimulatory KIRs have a high degree of homology to the inhibitory KIRs, which bind to various MHC class I molecules. At least some of the stimulatory KIRs may bind the same MHC polymorphisms as their inhibitory counterparts, albeit with lower affinity (39, 40). Stimulatory KIRs may also bind to specific peptide/class I complexes, or they may bind to class I-related molecules. However, given their expression on NK cells, it seems unlikely that any ligand recognized by the stimulatory KIRs would be restricted to professional APCs. In this context, we propose that the function of stimulatory NK receptors on T cells is to augment TCR-mediated activation in the initiation and prolongation of an effector/memory immune response. This costimulatory activity would not be restricted to lymphoid tissue, but could occur in the periphery, wherever ligands for the NK receptors are expressed.

The expression of NK stimulatory receptors on effector/memory CD4+ T cells is associated with chronic inflammatory conditions. Not all effector/memory CD4+ T cells express these receptors. Only those with a phenotype indicative of replicative senescence, including oligoclonal expansion and loss of CD28 expression, are known to express these molecules (11, 12). Although CD4+CD28null T cells are infrequently found in healthy individuals, they are often expanded to significant levels in patients with RA, ACS, and multiple sclerosis (13, 41, 42). In addition, this subpopulation of T cells from individuals with chronic inflammation expresses various members of the KIR family. Both inhibitory and/or stimulatory KIRs are expressed. However, one particular receptor, KIR2DS2, is found almost exclusively on CD4+CD28null T cells from patients with RA and is a genetic risk factor for psoriatic arthritis (18) and for extra-articular manifestations in RA (17). NK cell functional activity is modulated by the balance of inhibitory and stimulatory receptors, and this may also be the case for most CD4+CD28null T cells. However, if excessive stimulatory receptors are expressed, the balance would be shifted to activation instead of inhibition. Given the association of KIR2DS2 with these disease manifestations, we propose a model in which the autoimmune inflammatory response is initiated and/or extended by the
costimulatory activity mediated by the encounter between MHC class I-recognizing receptors and their ligands. In addition, the acquisition of KARAP/DAP12 would result in a situation in which the MHC class I-recognizing receptor could initiate the immune response without any requirement for TCR activation.

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References


