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NK Cell Killer Ig-like Receptor Repertoire Acquisition and Maturation Are Strongly Modulated by HLA Class I Molecules

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The interaction between clonally distributed inhibitory receptors and their activating counterparts on NK cells and HLA class I molecules defines NK cell functions, but the role of HLA class I ligands in the acquisition of their receptors during NK development is still unclear. Although some studies demonstrated that HLA-C affects the expression of killer Ig-like receptors (KIR), other studies showed that NK cells acquire their KIR repertoire in a stochastic manner. Only when infected with human CMV is an expansion of self-specific KIR$^+$ NKG2C$^+$ NK cells detected. To gain more insight into this question, we compared the coexpression of different KIR molecules, NKG2A, CD8, and CD57, on NK cells in healthy donors and seven patients with deficient HLA class I expression due to mutations in one of the TAP genes. Our results show a correlation between the presence/absence of HLA class I molecules and the coexpression of their receptors. In an HLA class I low-expression context, an increase in KIR molecules' coexpression is detected on the NKG2A$^+$ CD8$^+$ subset. In functional assays, hyporesponsiveness was observed for TAP-deficient NK cells derived from four patients. In contrast, NK cells from patient five were functional, whereas CD107a$^+$ and IFN-γ^+ CD56^{dim} NK cells presented a different pattern of HLA class I receptors compared with healthy donors. Taken together, our results provide strong evidence for the role of HLA class I molecules in NK cell maturation and KIR repertoire acquisition. ***The Journal of Immunology*****, 2014, 192: 2602–2610.**

Natural killer cell functions are governed by a balance between activating and inhibitory signals transmitted through a large panel of activating receptors and inhibitory receptors (IR), respectively. Killer Ig-like receptors (KIR) bind selectively to some of the HLA-A, HLA-B, and HLA-C alleles. Furthermore, CD94/NKG2A heterodimers are specific for HLA-E, which presents peptides derived from the leader sequences of classical HLA class I molecules (1–3). These IR deliver inhibitory messages when mature NK cells meet normal autologous targets, but they also promote normal NK cell education. Only NK cells expressing at least one IR specific for self-HLA class I molecules become fully functional in this NK cell–licensing process. In contrast, in the absence of a self-HLA class I–specific IR, NK cells remain in a state of hyporesponsiveness. This mechanism allows the generation of a NK cell repertoire tuned to the optimal detection of "missing-self" (downregulation or absence of autologous HLA class I molecules) while avoiding autoreactivity (4–6).

A particular scenario for NK cell education exists in patients with genetic defects in the subunit 1 or 2 of the TAP gene. In these patients, the surface expression of HLA class I molecules is significantly reduced (1–10% of normal levels) (7, 8). NK cells from these patients were shown to be hyporesponsive ex vivo (low or absent cytotoxicity against K562 cells) (9–11). Such data support the above-mentioned models of NK cell education, in which NK cells remain "unlicensed" as a result of the strongly reduced interactions between the NK cells expressing IR and a severely HLA class I–defective environment.

An unanswered question is how NK cells acquire their clonally distributed repertoire of HLA class I binding molecules and, in particular, their KIR repertoire, because this is a distinctive phenomenon from NK cell education. An effect of HLA-C on the KIR repertoire was demonstrated by some investigators (12, 13), whereas others found the acquisition of KIR molecules to be stochastic (14, 15) except after human CMV infection, which leads to the expansion of self-specific KIR$^+$ NKG2C$^+$ NK cells (16).

In this study, we dissected the NK cell repertoire in seven TAP-deficient patients (17–20) and a panel of healthy donors (HD) by carrying out a detailed analysis of HLA class I receptors (CD8, NKG2A, KIR2DL1/DS1, KIR2DL2/3/DS2, KIR3DL1) and CD57 expression and coexpression using 14-color flow cytometry. Our results show an inverse correlation between the presence of HLA

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Abbreviations used in this article: HD, healthy donor; IR, inhibitory receptor; KIR, killer Ig-like receptor.

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class I molecules and the coexpression of its receptors, providing new evidence in favor of a strong role for HLA class I molecules in NK cell KIR repertoire acquisition.

Materials and Methods

Human subjects and blood samples

Peripheral venous blood was obtained from seven patients with a confirmed TAP deficiency and from 18 HD (8 males, 10 females; age 25–60 y; mean age [± SD]; 36 ± 8 y). PBMC were isolated by Ficoll-Paque density gradient centrifugation. All individuals included in the project gave written informed consent according to the Declaration of Helsinki. The study was approved by the National Research Ethics Committee of Luxembourg (201109/05).

KIR and HLA class I genotyping

Genomic DNA was isolated either from fresh blood using a QIAamp DNA Blood Mini Kit (QIAGEN) or from EBV-transformed B-lymphoblastoid cells. Both genotypings were performed using PCR sequence–specific primers. For HLA-A, HLA-B, and HLA-C, commercial Micro SSPTM plates and kits (One Lambda, Canoga Park, CA) were used, according to the manufacturer's instructions.

Abs and flow cytometry

For phenotype studies, PBMC were stained for 9- or 14-color flow cytometry with fluorochrome-conjugated mAb (Table I). Stained cells were measured on a FACSAria SORP (BD Biosciences). At least 3×10^5 live cells were acquired per sample. Analyses were done with Kaluza Analysis Software v1.2 (Beckman Coulter). Dead cells and doublets were excluded from the analyses. NK cells were subsequently identified as $CD14^-$ $CD19^-$ $CD3^-$ $CD56^{dim}$, and the percentage of positive cells for each studied marker was determined. Coexpression was calculated using the "Boolean" and the "Tree" functions (Fig. 1).

Cell sorting

PBMC were stained for five-color flow cytometry sorting with the corresponding mAb (Table I). During all steps, cells were kept in cell culture medium (RPMI 1640 supplemented with 10% heat-inactivated FBS, 10 mM HEPES, 1 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin). Hoechst 33342 (Invitrogen; 100 ng/ml) was added just before measurement to stain DNA of the permeable cells (dead or late apoptotic). NK cells were identified and selected as described above.

Cytotoxicity assays

For cytotoxicity assays, both NK cells and K562 cells were sorted prior to coincubation. K562 cells were stained using a CellTrace CFSE (Molecular Probes); 10^6 K562 cells were incubated in 1 ml PBS/0.1% BSA with CFSE (5 μM) for 15 min at 37°C, 5% CO_2. The excess CFSE was quenched by addition of five volumes of ice-cold culture medium to the cells and incubated for 5 min on ice and then cells were washed and resuspended in fresh medium. Hoechst 33342 (Invitrogen; 100 ng/ml) was added just before measurement. Live/$CFSE^+$ K562 cells were sorted and added to previously sorted $CD56^{dim}$ NK cells (see *Cell sorting* section above) at different NK/K562 ratios (1:1, 5:1, and 10:1) and then centrifuged. The pellet was resuspended in 100 μl fresh medium in 96-well V-bottom plates, which were briefly centrifuged before a 5-h incubation at 37°C, 5% CO_2. In parallel, live/$CFSE^+$ K562 cells were incubated alone to calculate their spontaneous death. After the incubation, TO-PRO-3 (Molecular Probes) was added at a final concentration of 1 μM to identify the percentage of dead K562 cells. After flow cytometry measurement, the percentage of specific death was calculated as specific death = [(death − spontaneous death)/(100 − spontaneous death)] × 100.

Functional assays (IFN-γ production and degranulation assay)

A total of 10^6 PBMC was cocultured with K562 cells at a ratio of 7:1 in 100 μl cell culture medium with 1000 U/ml rIL-2 (R&D Systems, Abingdon, U.K.). Negative controls were performed with NK cells alone. For degranulation measurement, the anti-CD107a mAb was added. After 1 h of incubation, monensin and brefeldin A (GolgiStop and GolgiPlug, respectively; both from BD Biosciences) were added. After another 5 h of incubation, cells were stained with the corresponding mAb (Table I). For IFN-γ assay, cells were fixed and permeabilized using Cytofix/Cytoperm solution (BD Biosciences) and then stained with anti–IFN-γ mAb.

Statistical analysis

A two-tailed *t* test was used to assess the difference in the expression of markers by NK cells from HD and TAP-deficient patients. ANOVA and a post hoc Tukey test were used to evaluate whether KIR molecules are expressed differently in HD and TAP-deficient patients for each combination of two or all three of the following markers: CD8, CD57, and NKG2A. Graphs were constructed and statistical tests were performed using R version 2.15.2.

Results

Clinically, TAP-deficient individuals, including patients 1–4, 6, and 7 of our cohort (17–19), present a characteristic association of chronic inflammation, infection of the airways, and debilitating

Table I. Ab conjugates used for the different NK cell studies (phenotyping, sorting, or functional tests)

Usage							
Sorting	Functional	Phenotyping	Supplier	Clone	Ag	Conjugated Fluorochrome	Laser
X			Invitrogen		Dead cell marker	Hoechst 33342	UV
	X	X	Invitrogen		Dead cell marker	Blue fluorescent dye	UV
	X	X	eBioscience	61D3	CD14	Qdot 605	UV
	X		BioLegend	H4A3	CD107a	BV-421	Violet
		X	BioLegend	HCD56	CD56	BV-421	Violet
	X	X	BioLegend	HCD56	CD56	BV-510	Violet
X		X	BioLegend	OKT-3	CD3	BV-510	Violet
	X	X	BioLegend	UCHT1	CD3	BV-570	Violet
	X	X	BioLegend	RPA-T8	CD8	BV-605	Violet
	X	X	BioLegend	HCD57	CD57	FITC	Blue
X		X	BioLegend	H1B19	CD19	Alexa Fluor 488	Blue
	X	X	eBioscience	HP-MA4	KIR2DL1/DS1	PerCP-Cy5.5	Blue
		X	BioLegend	W6/32	HLA-A/B/C	PerCP-Cy5.5	Blue
		X	BioLegend	dG9	Perforin	PerCP-Cy5.5	Blue
	X		BioLegend	4S.B3	IFN-γ	PE	Yellow-green
		X	BioLegend	DH2	Granulysin	PE	Yellow-green
		X	BioLegend	3D12	HLA-E	PE	Yellow-green
	X	X	ImmunoTools	LT19	CD19	PE-DY647	Yellow-green
	X	X	Beckman Coulter	Z199	NKG2A	PE-Cy7	Yellow-green
X			BioLegend	HCD56	CD56	PE-Cy7	Yellow-green
	X	X	Beckman Coulter	GL183	KIR2DL2/3/DS2	Allophycocyanin	Red
		X	Invitrogen	FGB12	Granzyme B	Allophycocyanin	Red
X			ImmunoTools	MEM-15	CD14	Allophycocyanin	Red
	X	X	BioLegend	DX9	KIR3DL1	Alexa Fluor 700	Red
	X	X	BioLegend	3G8	CD16	Allophycocyanin-Cy7	Red

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skin ulcers. Patient 5 (20) presented the same clinical features but, more recently, an increase in the severity of the lung infections and skin ulcers was observed. This was accompanied by facial lesions, eventually leading to complete destruction of the nasal cartilage. Because of the patient's severe illness, the planned bone marrow transplantation could not be performed. She then developed a generalized vasculitis with cerebral involvement and seizures. After a few months in an intensive care unit, unfortunately the patient died.

With regard to NK cell investigations, we previously described two TAP-deficient patients who expressed KIR and NKG2A at frequencies comparable to HD (9). However, the expression level of NKG2A on individual NK cells was much higher in the patients (9). In the current study, we aimed at examining the HLA class I receptor repertoires in seven TAP-deficient patients and a panel of HD using 14-color flow cytometry according to the Ab panel in Table I.

NK cells were selected among single/live cells, followed by gating on $CD14^-$ $CD19^-$ $CD3^-$ cells and then on the $CD56^{dim}$ population (Fig. 1A). These NK cells were examined for expression of different KIR (KIR2DL1/DS1, KIR2DL2/3/DS2, KIR3DL1), NKG2A, CD8, and CD57 (Fig. 1B–E), which are present on donor-dependent subgroups of NK cells (Fig. 2A). Because only very limited numbers of $CD56^{bright}$ NK cells express KIR (1), this subset was not considered further in the present work. In the phenotypic studies, patient 7 was considered an outlier compared with the others, because his NK cell subsets were similar to the HD ones.

TAP-deficient $CD56^{dim}$ NK cells exhibit an increase in their KIR molecules' coexpression, especially on the $NKG2A^+$ subset

The percentages of NK cells expressing the studied markers varied between TAP-deficient patients and HD (Fig. 2A), with the exception of NK cells expressing 0, 1, or 3 KIR, which were not different between the two groups (only two patients had a higher frequency of NK cells expressing three KIR). In this patient cohort, the most striking difference compared with HD was the very high percentage of $NKG2A^+$ cells. Conversely, we found a slightly lower frequency of KIR^- and $CD57^+$ NK cells in the patients. The percentage of $CD8^+$ NK cells also was significantly increased in the TAP-deficient individuals.

When analyzing $NKG2A^-$ and $NKG2A^+$ NK cell subsets, we observed marked differences in KIR expression between TAP-deficient patients and HD (Fig. 2B). TAP-deficient $NKG2A^-$ and $NKG2A^+$ NK cells exhibited strong variations in their KIR coexpression distribution pattern, with a much higher percentage of KIR coexpression on $NKG2A^+$ NK cells compared with $NKG2A^-$ NK cells. In contrast, the distribution pattern of KIR coexpression was very similar in $NKG2A^{+/-}$ subsets in HD, with a higher percentage of KIR^+ NK cells on the $NKG2A^-$ subset.

The comparison of KIR-expressing cells among $CD8^-$ and $CD8^+$ NK cells showed a nonrandom distribution in the patients (Fig. 2C). Indeed, KIR^+ NK cells were more frequent in the

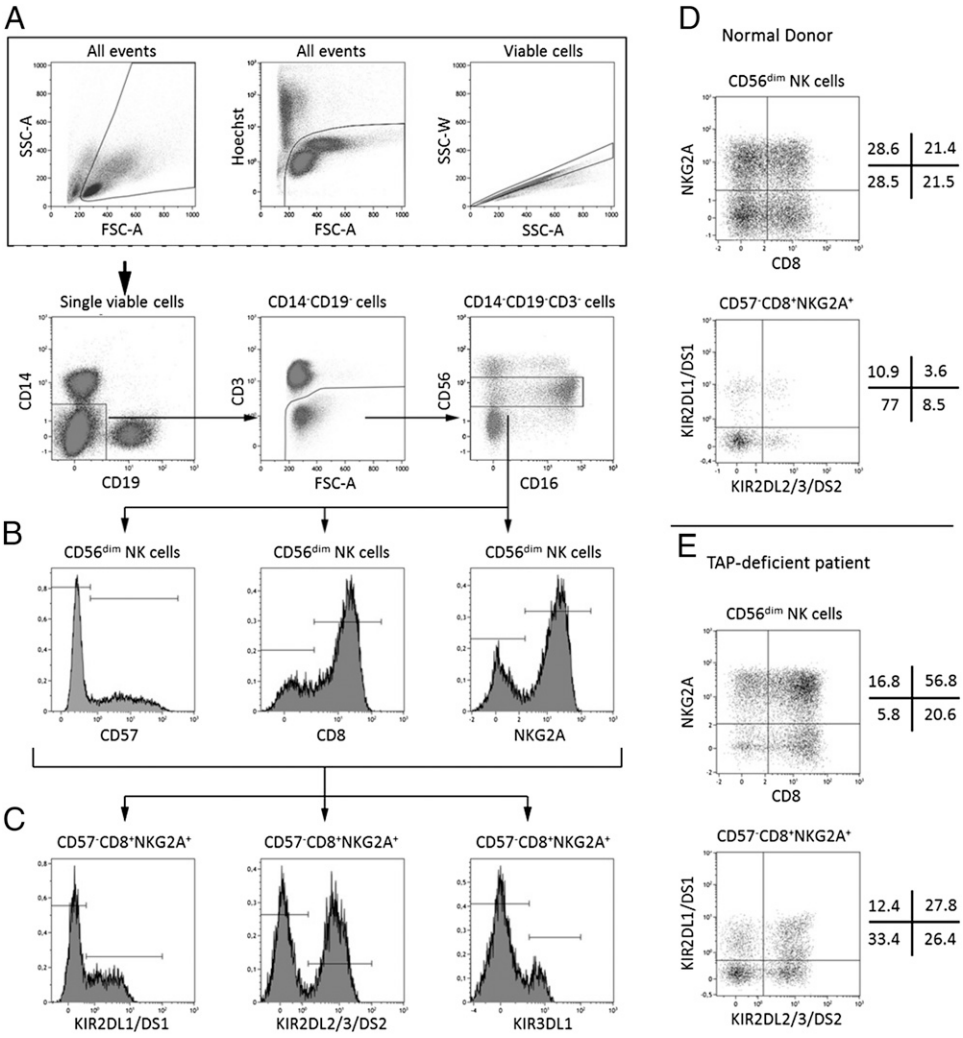

FIGURE 1. Gating strategy used to study CD57, CD8, NKG2A, and KIR coexpression on $CD56^{dim}$ NK cells. (**A**–**C**) Results of the phenotyping of a representative TAP-deficient patient. The same gating strategy was used for phenotyping the six other patients, as well as the HD. For coexpression of the different marker, the "Boolean" and "Tree" functions were applied to the gating of (B) and (C). (C) The different KIR expressions in one of the "Boolean" combinations of (B): $CD57^-$ $CD8^+$ $NKG2A^+$. Four representative dot plots showing the coexpression of NKG2A and CD8 on $CD56^{dim}$ NK cells, as well as KIR2DL2/3/DS2 and KIR2DL1/DS1 on $CD57^-$ $CD8^+$ $NKG2A^+$ NK cells derived from one HD (**D**) and one TAP-deficient patient (**E**).

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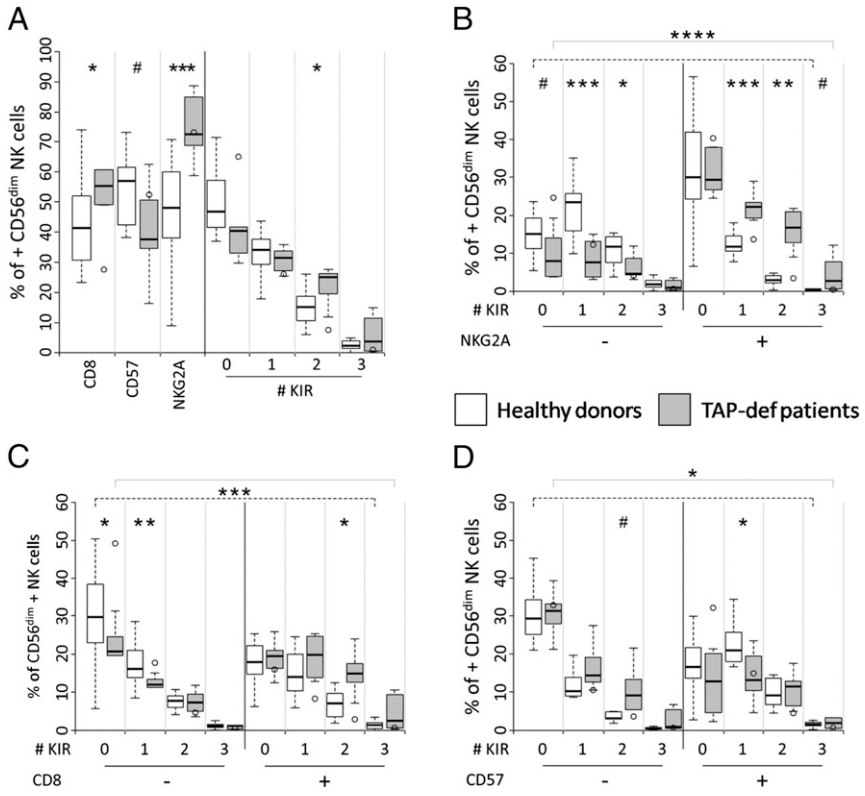

FIGURE 2. The expression of KIR molecules is influenced by the presence of NKG2A when comparing $CD56^{dim}$ NK cells from HD and TAP-deficient patients. Box plots represent the frequency of $CD56^{dim}$ NK cells expressing (**A**) or coexpressing (**B**–**D**) the studied markers in HD ($n = 9$ when CD57 is implicated; $n = 18$ when CD57 is not implicated) and six TAP-deficient patients. Circles represent TAP-deficient patient 7, who was considered an outlier. (A) By comparing NK cells from HD and TAP-deficient patients, significant differences are observed in the expression of CD8, NKG2A, and two KIR. When dissecting NK cells considering NKG2A (B), CD8 (C), or CD57 (D) expression, it appears that the presence of NKG2A and CD57 (individually) on HD NK cells has no effect on KIR distribution (horizontal dashed line). In TAP-deficient patients, KIR distribution is strongly affected by NKG2A [(B) horizontal gray line] but much less by CD57 [(D) horizontal gray line]. $^{****}p < 1 \times 10^{-6}$, $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $^{\#}p < 0.1$.

$CD8^+$ subset than in the $CD8^-$ subset, which was not the case for the HD.

Next, we studied the influence of the maturation marker CD57 on the distribution of KIR molecules (Fig. 2D). As expected, and as previously described (21, 22), the majority of HD KIR^- cells was contained in the $CD57^-$ population, whereas the KIR^+ NK cells were better represented among the $CD57^+$ subset. Regarding the TAP-deficient NK cells, they followed the pattern of HD cells in the $CD57^-$ population. However, among the $CD57^+$ NK subset, the frequency of cells expressing one KIR was significantly lower than in HD.

HLA class I molecules strongly influence their receptors' acquisition and NK cell maturation

We compared the different combinations of these markers to explore the coinfluence of CD8 and NKG2A on KIR repertoire shaping in a HLA class I low-expression environment (Fig. 3A). The greatest difference in the percentages of KIR^+ NK cells be-

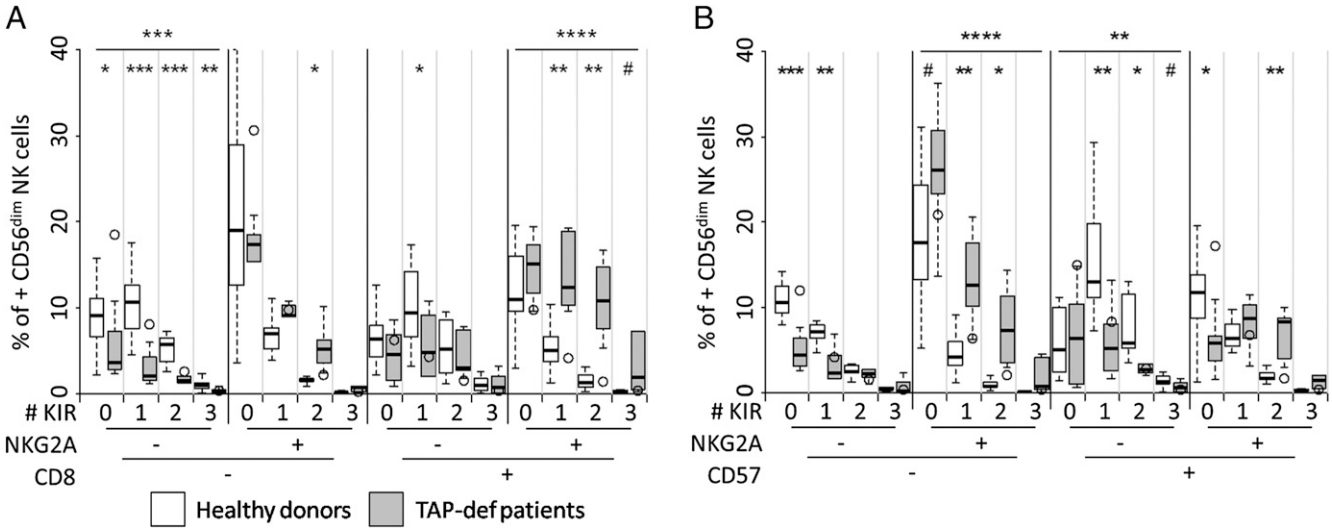

FIGURE 3. HLA class I molecules strongly influence their receptors' acquisition and NK cell maturation. Box plots represent the frequency of $CD56^{dim}$ NK cells coexpressing the studied markers in HD ($n = 9$ when CD57 is implicated; $n = 18$ when CD57 is not implicated) and six TAP-deficient patients. Circles represent TAP-deficient patient 7, who was considered an outlier. (**A**) With regard to the effect of the coexpression of CD8 and NKG2A, no significant differences were observed between HD and TAP-deficient patients for the single-positive cells ($CD8^-$ $NKG2A^+$ or $CD8^+$ $NKG2A^-$). The presence or absence of both markers together seems to affect the expression of KIR molecules on NK cells in an HLA class I low expression context. (**B**) With regard to the effect of CD57 and NKG2A on KIR distribution on $CD56^{dim}$ NK cells, significant differences were only observed for the single-positive cells for these two markers ($CD57^-$ $NKG2A^+$ and $CD57^+$ $NKG2A^-$). $^{****}p < 1 \times 10^{-6}$, $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $^{\#}p < 0.1$.

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tween the two donor groups was found in the $CD8^+$ $NKG2A^+$ and $CD8^-$ $NKG2A^-$ fractions compared with the single-positive subsets ($CD8^-$ $NKG2A^+$ and $CD8^+$ $NKG2A^-$). The major difference between KIR-expressing $NKG2A^+$ NK cells in the patients compared with HD was confirmed only in the $CD8^+$ $NKG2A^+$ subset and not among the $CD8^-$ $NKG2A^+$ cells, which is consistent with a synergistic influence of both CD8 and NKG2A on the KIR repertoire of the patients.

When combining the two markers CD57 and NKG2A (Fig. 3B), it appeared that more KIR^+ NK cells are present in the $CD57^-$ $NKG2A^+$ subset from the patients than the HD. The $CD57^-$ $NKG2A^-$ population showed the lowest amount of KIR^+ $CD56^{dim}$ NK cells. Likewise, KIR^- NK cells represented the majority in the $CD57^-$ $NKG2A^+$ subtype. When focusing on $CD57^+$ NK cells, we observed that there were more KIR^+ cells within the $NKG2A^-$ population of the HD, whereas this was not true for the patients' NK cells. KIR^+ cells were relatively rare in the $CD57^+$ $NKG2A^+$ subset in both HD and patients. Nevertheless, the HD had more KIR^- cells than the patients, with the inverse observed for $NKG2A^+$ cells expressing two KIR.

To complete the phenotypic studies, we looked at the triple combination of CD57, CD8, and NKG2A (Supplemental Fig. 1). NK cells from TAP-deficient patients and HD showed some differences, in particular among the $NKG2A^+$ cells, as well as in the $CD57^-$ $CD8^-$ $NKG2A^-$ population. A higher percentage of $CD57^-$ $CD8^+$ $NKG2A^+$ KIR^+ NK cells was observed in patients compared with HD, indicating a block of CD57 acquisition, but not for KIR molecules, in the patients.

TAP-deficient $CD56^{dim}$ NK cells exhibit hyporesponsiveness against K562 cells but express cytotoxic molecules and HLA-E

In our first study of TAP-deficient NK cells, we demonstrated that they have, in the absence of IL-2–mediated activation, no natural cytotoxic activity against the target cell line K562 or against HLA class I–deficient EBV-transformed B cell lines (no missing self recognition) (9). This result was confirmed in cytotoxicity assays using nonactivated, but purified, NK cells from four of the seven patients as effectors and K562 cells as targets (Fig. 4A). Indeed, NK cells from three patients (patients 1, 2, and 3) were not cytotoxic. Interestingly however, NK cells from one patient (patient 5) killed K562 cells to a significant degree, although not to the same extent as HD NK cells, suggesting that NK cells from this patient were able to overcome the missing education due to the lack of HLA class I molecules.

The lack of cytotoxic activity in NK cells from patients 1–3 was paralleled by the absence of a significant degranulation in the presence of K562, measured in terms of the percentages of $CD107a^+$ NK cells, which were extremely low in patients 1–4. As expected, NK cells from patient 5 degranulated upon stimulation, albeit less strongly than did NK cells from most HD. A similar hyporesponsiveness was shown for IFN-γ production by NK cells from the patients, again with the exception of patient 5 (Fig. 4B).

KIR and HLA genotyping were performed on PBMC from the seven patients (Supplemental Table I), hoping to find a unique or different genotype for the functional outlier, patient 5. KIR genotypes were identified according to Pyo et al. (23). The three possible KIR haplotype compositions were found among the seven patients (homozygous haplotype A for patient 2; heterozygous haplotypes AB for patients 1, 6, and 7; and homozygous haplotype B for patients 3, 4; and 5, Supplemental Table II). Among the three patients exhibiting haplotype B, characterized by the expression of activating KIR, only patient 5 had functional $CD56^{dim}$ NK cells. Unfortunately, such results do not explain the functional differences in NK cells from patient 5.

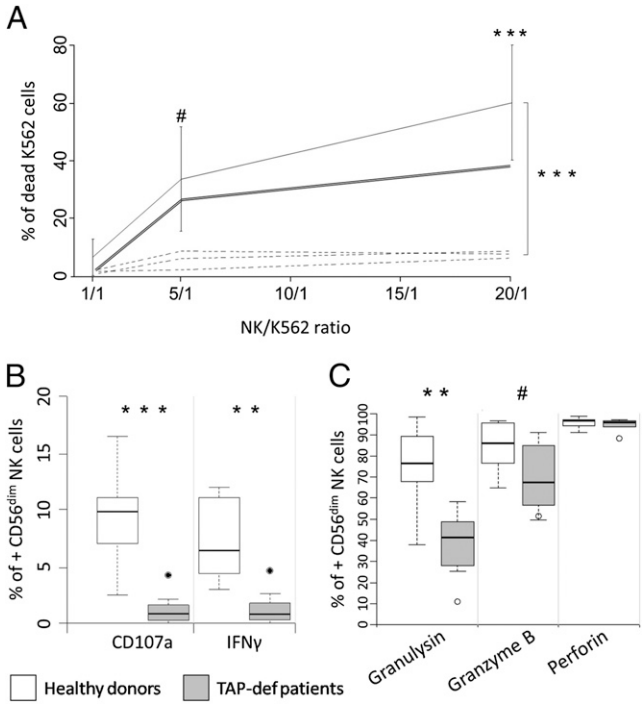

FIGURE 4. TAP-deficient $CD56^{dim}$ NK cells exhibit hyporesponsiveness toward K562 cells, although they express cytotoxic molecules. (**A**) The gray line represents HD, the dashed lines represent TAP-deficient patients 1, 2, and 3; and the bold line represents TAP-deficient patient 5. (**B** and **C**) Box plots showing the frequency of $CD56^{dim}$ NK cells derived from seven HD and six TAP-deficient patients. Outliers do not represent the same patient in (B) and (C): patient 5 is the outlier in (B) (solid dots), and patient 7 is the outlier in (C) (open dots). (A) Cytotoxicity assays were performed with purified $CD56^{dim}$ NK cells and K562 target cells incubated together for 5 h. NK cells show hyporesponsiveness for patients 1, 2, and 3 in contrast to patient 5, who presents cytotoxic $CD56^{dim}$ NK cells. (B) To confirm these results, PBMC from seven HD and five TAP-deficient patients (patients 1, 2, 3, 4, and 5) were incubated with K562 cells (ratio 7:1) and 1000 U/ml of IL-2 for 6 h. The percentage of $CD56^{dim}$ NK cells expressing CD107a or IFN-γ was determined, showing hyporesponsiveness for the studied patients with the exception of patient 5, represented as an outlier. (C) The percentage of $CD56^{dim}$ NK cells expressing granulysin, granzyme B, or perforin was determined. TAP-deficient NK cells showed no major difference compared with normal NK cells except for granulysin (circles represent patient 7, considered an outlier). $^{***}p < 0.001$, $^{**}p < 0.01$, $^{\#}p < 0.1$.

One explanation for the hyporesponsiveness of TAP-deficient NK cells could be the absence of the cytotoxic molecules contained in the granules normally released upon sensitive target cell encounter, although this would not explain the absence of IFN-γ production. Therefore, we analyzed the presence of granulysin, granzyme B, and perforin in normal and TAP-deficient NK cells (Fig. 4C). Although perforin was present in all NK cells from both cohorts, we found a slightly reduced percentage of TAP-deficient NK cells expressing granzyme B compared with HD. A more striking difference was observed for granulysin, which was present in only ~30–50% of patients' NK cells compared with 70–90% in most HD. Nevertheless, the three molecules are clearly present in TAP-deficient NK cells, so that the missing cytotoxic activity of these cells is probably not due to a lack of such factors.

The fact that most of the NK cells from the patients are $NKG2A^+$ prompted us to check for expression of HLA-E (Fig. 5A). We hypothesized that patient 5 might exhibit stronger expression of HLA-E, which would interact with NKG2A and lead to a potential education of her NK cells. The phenotyping showed that all seven patients expressed HLA-E on their NK cells, which was reduced

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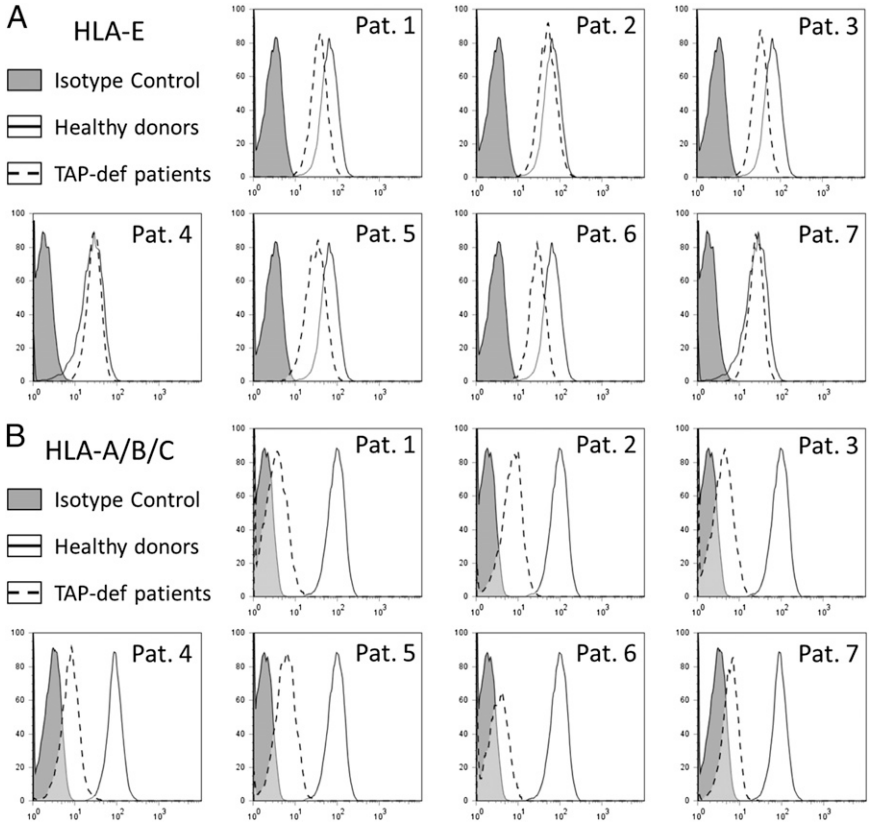

FIGURE 5. HLA-E and HLA-A, HLA-B, and HLA-C expression on HD and TAP-deficient CD56^{dim} NK cells. HLA-E (**A**) and HLA-A, HLA-B, and HLA-C (**B**) expression on CD56^{dim} NK cells for three HD and seven TAP-deficient patients. The same expression was observed for CD3$^+$, CD19$^+$, and CD14$^+$ cells.

30–50% compared with HD (50% for patient 5). In contrast, as expected and as previously shown (7–11), expression of the classical HLA class I molecules (HLA-A, HLA-B, and HLA-C) was decreased significantly in all patients (Fig. 5B), between 95 and 99% reduction compared with HD (96% for patient 5).

Different patterns for functional CD56^{dim} NK cells with regard to their HLA class I receptors in HD and TAP-deficient patient 5

We then investigated which subpopulations of NK cells from HD preferentially degranulated and/or produced IFN-γ. With regard to the patients, this analysis only made sense for patient 5 (represented as an outlier in the box plots in Fig. 6). First, we observed that most of the degranulating TAP-deficient NK cells were NKG2A$^+$ and CD8$^+$, whereas only ∼20% of the degranulating cells were CD57$^+$ (Fig. 6A). With regard to IFN-γ–producing NK cells (Fig. 5B), patient 5 presented the same pattern as HD, with the exception of CD8: 70% of IFN-γ$^+$ CD56^{dim} NK cells were positive for this marker.

Next, we analyzed the functionality, with respect to KIR expression, of NKG2A$^+$ NK cells compared with NKG2A$^-$ NK cells (Fig. 6C, 6D). A significant percentage of HD KIR$^+$ NK cells were reactive to stimulation, regardless of the presence or absence of NKG2A, but KIR$^-$ NK cells from the NKG2A$^+$ subpopulation were best represented among the functional cells. Only very few NKG2A$^-$ KIR$^+$ NK cells from patient 5 could respond to K562, whereas most of the functional cells were NKG2A$^+$. Interestingly, 20% of the IFN-γ$^+$ NK cells from the patient were NKG2A$^-$ KIR$^-$, and this percentage was twice as high as in the HD.

With respect to CD8 (Fig. 6E, 6F), the overall pattern was that, in CD8$^-$ cells, a higher number of KIR$^-$ NK cells were functional compared with KIR$^+$ cells, and this phenomenon was even more pronounced in the patient. When CD8 was expressed, NK cells without a KIR or with only one KIR were the most responsive to stimulation, with a slightly higher fraction of TAP-deficient cells compared with the cohort of HD. With regard to KIR$^-$ NK cells from the patient, the percentage of functional cells was in the same range in the presence or absence of CD8.

Cells expressing one KIR were the most functional among the CD57$^+$ population, whereas the KIR$^-$ NK cells were predominantly reactive among the less mature CD57$^-$ subset (Fig. 6G, 6H). There were more CD57$^-$ KIR$^-$ cells among the functional NK cell subset from patient 5 than all other populations, including CD57$^+$ cells.

The investigation of CD8 and NKG2A together (Fig. 7A, 7B) added little to the overall picture, but it confirmed that NKG2A$^-$ cells from patient 5 were not functional and that, in the CD8$^+$ NKG2A$^+$ population, a higher percentage of patient 5's NK cells were reactive compared with normal NK cells, with KIR absent or present.

When analyzing CD57 and NKG2A together, we found the same pattern among the CD57$^-$ NKG2A$^+$ NK cells but not the CD57$^+$ NKG2A$^+$ NK cells (Fig. 7C, 7D). No major differences between normal and TAP-deficient NK cells were noticed when three markers (CD57, CD8, NKG2A) were compared, with the exception that, again, a higher percentage of functional cells among the CD57$^-$ CD8$^+$ NKG2A$^+$ subpopulation was found in patient 5 compared with HD (Supplemental Fig. 2).

An overall conclusion is that, for TAP-deficient patient 5, functional NK cells are more represented among the CD57$^-$ CD8$^+$ NKG2A$^+$ KIR$^-$ CD56^{dim} subpopulation.

Discussion

We show in this study of a cohort of seven TAP-deficient patients that the NK cell phenotype regarding KIR and NKG2A expression is significantly different in six of the seven patients compared with a panel of HD. Our results clearly identify that HLA class I

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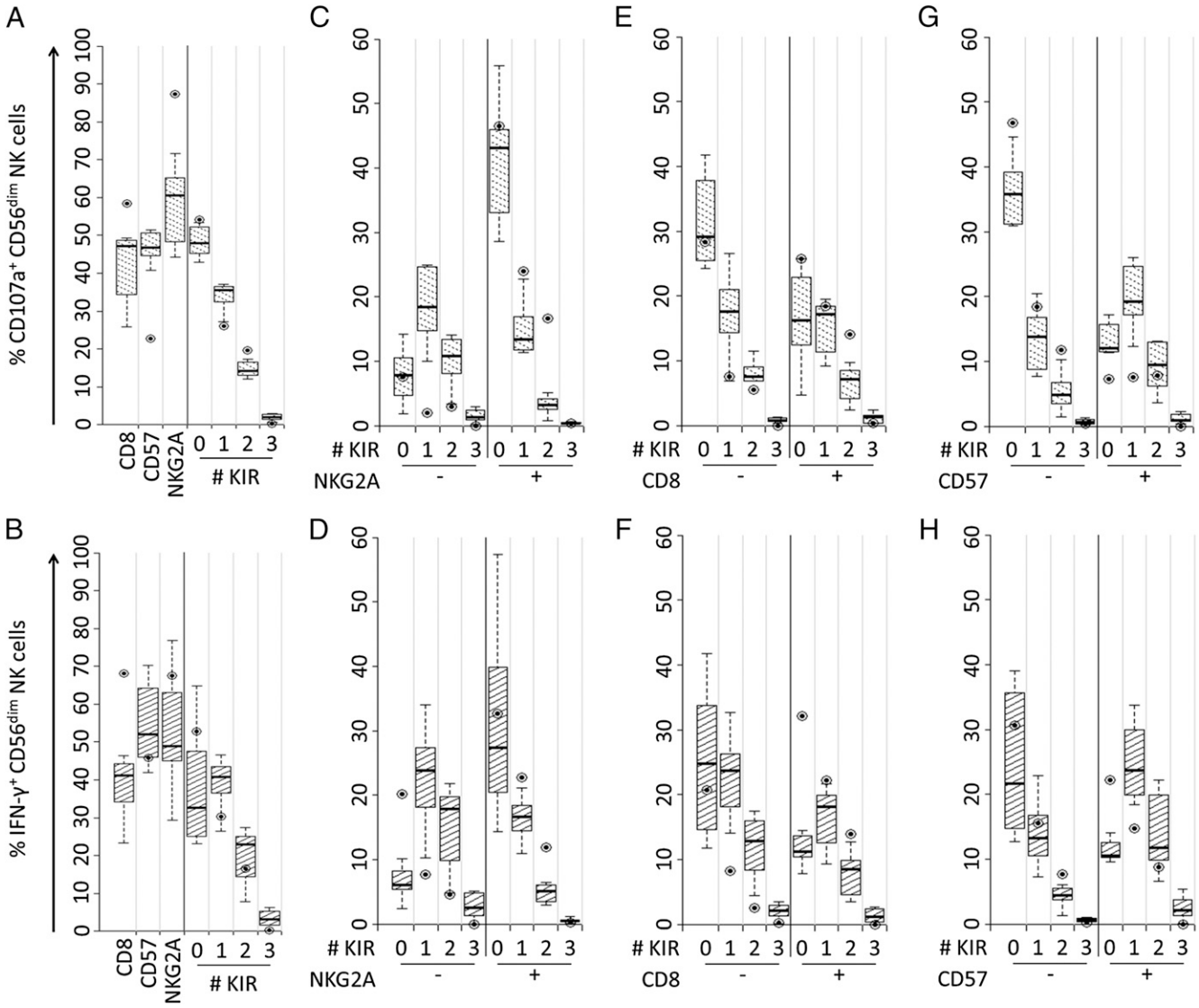

FIGURE 6. KIR molecules and NKG2A, CD8, and CD57 expression and coexpression differ in functional $CD56^{dim}$ NK cells between HD and TAP-deficient patients. Box plots represent the frequency of different markers on $CD107a^+$ and IFN-γ^+ $CD56^{dim}$ NK cells derived from seven HD. The outlier represents patient 5 (encircled black dots), who has functional $CD56^{dim}$ NK cells. When comparing NK cells from HD and TAP-deficient patient 5, differences are observed in the expression of CD8, CD57, and NKG2A on $CD107a^+$ cells (**A**) but only in the expression of CD8 on IFN-γ^+ $CD56^{dim}$ NK cells (**B**). (A and B) No differences are observed for the expression of KIR molecules. When considering the KIR distribution following the expression of NKG2A (**C**, **D**), CD8 (**E**, **F**), or CD57 (**G**, **H**) on $CD56^{dim}$ NK cells, it appears that patient 5's NK cell marker distribution on $CD107a^+$ and IFN-γ^+ $CD56^{dim}$ NK cells does not follow the same patterns as in HD. For TAP-deficient patient 5, zero KIR NK cells are always more functional than ones expressing at least one KIR, despite the presence of NKG2A or CD57.

molecules exert a strong effect on their receptors' (CD8, NKG2A, and KIR molecules) acquisition on NK cells. From a functional point of view, four of the five patients had hyporesponsive NK cells, whereas NK cells from the fifth patient were cytotoxic toward K562 and produced IFN-γ, although slightly less than the majority of HD. No major defect in TAP-deficient NK cells' cytotoxic molecules (granzyme B and perforin) was found, in contrast to a reduced expression of granulysin.

The fact that TAP-deficient NK cells are hyporesponsive was reported by us and other investigators in both human and mouse (7–11, 24). In particular, natural cytotoxic activity ex vivo was completely absent in the first two patients that we described (not included in this study) (9). According to the current models of NK cell education, only those NK cells that express at least one IR specific for self-HLA class I molecules become functional, whereas cells lacking such receptor(s) will not become licensed for cytotoxicity and cytokine production (4, 5). Following this model, NK cells from patients with TAP deficiency, in whom HLA class I expression is greatly reduced, are expected to remain hyporesponsive.

An exception to this rule is the nonclassical class I molecule HLA-E, which was reported to be subnormally expressed by TAP-deficient hematopoietic cells (25), although this was not confirmed in another patient (26). Because NK cells from one of our patients were functional, we checked for the presence of HLA-E at the cell surface and expected a higher level of this molecule on the NK cells (as representatives of lymphocytes) from patient 5. This hypothesis was based on the possibility that NK cells from this patient could have been educated through the interaction of NKG2A with its ligand HLA-E. However, HLA-E was present on the NK cells and other hematopoietic cells from the seven patients, although at reduced levels compared with HD (30–50% reduction compared with HD). Thus, if NKG2A had educated NK cells from patient 5, it would not have done so in the other

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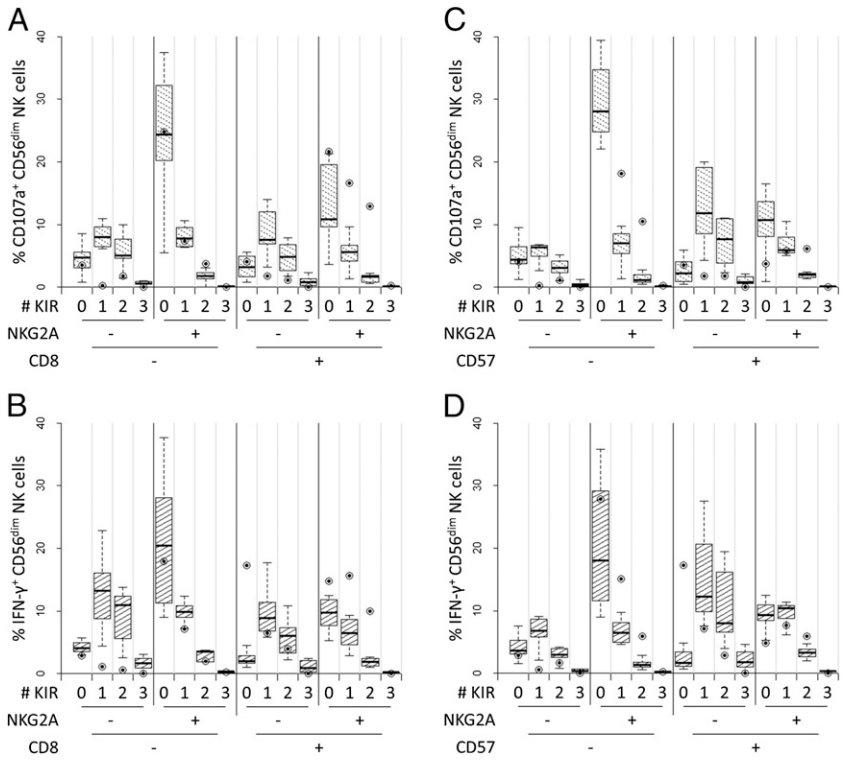

FIGURE 7. Coexpression pattern of CD57, CD8, and NKG2A on CD107a$^+$ and IFN-γ$^+$ CD56^{dim} NK cells from HD and TAP-deficient patient 5. Box plots represent the frequency of the combinations of different markers on CD107a$^+$ (**A**, **C**) and IFN-γ$^+$ (**B**, **D**) CD56^{dim} NK cells derived from seven HD. Encircled black dots represent patient 5, who has functional CD56^{dim} NK cells. Differences in the frequency of the different combinations of markers are observed between the two NK cell types. For patient 5, functional NK cells are more frequent in the NKG2A$^+$CD8$^+$ population (A, B) and the CD57$^-$ subset (C, D) compared with HD.

patients, despite comparable expression levels of its ligand HLA-E. One possible reason for the responsiveness of these NK cells is that HLA-E molecules of the patient present TAP-independent peptides, which is indeed possible (27, 28), and that the combination of HLA-E with these given peptides would allow recognition by NKG2A, whereas this would not be the case for the other patients with a different set of TAP-independent peptides. Another hypothesis is that HLA-E levels are suboptimal and still too low to educate NK cells through NKG2A, but they are not higher in patient 5 than in the hyporesponsive individuals.

A more "basic" explanation may be a strong in vivo preactivation of NK cells through HLA class I–independent mechanisms and, in particular, by inflammatory cytokines. Indeed, the patient was heavily infected at the time of blood drawing and had the most severe symptoms in our cohort, as briefly described in the Introduction.

Phenotypically, at the first level of analysis, a significantly higher proportion of patient NK cells than HD NK cells expressed NKG2A and CD8. The most striking difference was the significantly higher frequency of NKG2A$^+$ KIR$^+$ NK cells in the patients compared with the panel of HD. These results suggest that, in the absence of classical HLA class I molecules, a significant fraction of NK cells continues to "desperately" express HLA class I–specific receptors to encounter sufficient "educating" amounts of matched ligands, which can actually never occur in TAP deficiency.

Our analysis of CD57 expression revealed that KIR$^+$ NK cells were more frequent among the CD57$^+$ population and that KIR$^-$ NK cells were predominant among the CD57$^-$ subset. There were no major differences between normal and TAP-deficient NK cells in this regard. An interesting result was that, in the patients, significantly more KIR$^+$ cells were found among the CD57$^-$/NKG2A$^+$ subset than in the HD. This might suggest that a portion of the TAP-deficient NK cells acquire KIR in earlier stages of differentiation or, alternatively, that their maturation is blocked in a significant fraction of the NK cells before CD57 becomes expressed.

Because it was established that the CD8α/α homodimer, the only form of CD8 expressed by NK cells, can recognize HLA class I molecules (29, 30), we decided to integrate CD8 into the list of HLA class I ligands and to investigate its potential influence on the expression of other HLA class I ligands present in NK cells. Addison et al. (31) showed that CD8$^+$ NK cells are more cytotoxic and less prone to target cell–induced apoptosis than are their CD8$^-$ counterparts. The majority of the functional TAP-deficient NK cells in our study were CD8$^+$, which would be in favor of this claim. In contrast, CD8 by itself is not sufficient to make NK cells functional, as seen in the other patients. In any event, there is no CD8 ligand expressed on K562 target cells, so that CD8 might "only" be a phenotypic marker of the strongly cytotoxic NK cells but not functionally implicated at this level. However, it seems to play a role in the development of the KIR repertoire, at least in TAP deficiency.

With regard to the acquisition of the KIR repertoire by NK cells, two models have been proposed: the "stochastic" model (14) and the "ligand-instructed" model (13). The latter claims that, although initially stochastic, the repertoire later becomes biased toward self-specific KIR. Schönberg et al. (12) demonstrated that the "instruction" process is not yet in place in the neonatal period but appears later in life. This might explain why KIR repertoires are different among individuals, because the repertoire is shaped by the personal history of infections. However, we found a very similar KIR expression among HD NKG2A$^+$ subsets, suggesting the existence of common acquisition rules, at least for some NK subpopulations. Recently, Béziat et al. (16) showed that the bias toward self-specific KIR is characteristic of patients with a history of CMV infection but not other common herpes virus infections. This might be an explanation in many cases and would validate both the "stochastic" model (initially and in the absence of CMV infection) and the "ligand-instructed" model (bias due to CMV infection). In a humanized mouse model (mice lacking Ly49 ligands but transgenic for HLA-Cw3 and a KIR B haplotype), van Bergen et al. (32) found that, in the presence of HLA-Cw3, the

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corresponding IR KIR2DL2 was reduced both in frequency and in expression level.

With regard to the expression of NKG2A and KIR in TAP deficiency, the acquisition rules are likely to be disturbed by the very low number of HLA class I molecules in the environment. Nevertheless, among NKG2A$^+$ cells, KIR molecules are present at higher levels than in HD. This could reflect that, under physiological conditions, KIR acquisition is stopped once enough "matching" IR–HLA class I interactions are attained by the individual NK cell, whereas the process continues in TAP deficiency because the limiting partner is absent. However, the reason why not all TAP-deficient NK cells express NKG2A and several KIR remains unknown. Thus, our results suggest that HLA class I molecules have a strong influence on KIR expression and point toward the existence of additional NK cell–intrinsic, HLA class I–independent factors that prevent KIR overexpression. In the context of the increasing use of NK cells in clinics, the fact that HLA class I molecules have a strong impact on the acquisition of their receptors could become important from a translational perspective.

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Disclosures

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References

1. Caligiuri, M. A. 2008. Human natural killer cells. *Blood* 112: 461–469.
2. Held, W., M. Kijima, G. Angelov, and S. Bessoles. 2011. The function of natural killer cells: education, reminders and some good memories. *Curr. Opin. Immunol.* 23: 228–233.
3. Vivier, E., D. H. Raulet, A. Moretta, M. A. Caligiuri, L. Zitvogel, L. L. Lanier, W. M. Yokoyama, and S. Ugolini. 2011. Innate or adaptive immunity? The example of natural killer cells. *Science* 331: 44–49.
4. Joncker, N. T., and D. H. Raulet. 2008. Regulation of NK cell responsiveness to achieve self-tolerance and maximal responses to diseased target cells. *Immunol. Rev.* 224: 85–97.
5. Jonsson, A. H., and W. M. Yokoyama. 2009. Natural killer cell tolerance licensing and other mechanisms. *Adv. Immunol.* 101: 27–79.
6. Ljunggren, H. G., and K. Kärre. 1990. In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol. Today* 11: 237–244.
7. Gadola, S. D., H. T. Moins-Teisserenc, J. Trowsdale, W. L. Gross, and V. Cerundolo. 2000. TAP deficiency syndrome. *Clin. Exp. Immunol.* 121: 173–178.
8. Zimmer, J., E. Andrès, L. Donato, D. Hanau, F. Hentges, and H. de la Salle. 2005. Clinical and immunological aspects of HLA class I deficiency. *QJM* 98: 719–727.
9. Zimmer, J., L. Donato, D. Hanau, J. P. Cazenave, M. M. Tongio, A. Moretta, and H. de la Salle. 1998. Activity and phenotype of natural killer cells in peptide transporter (TAP)-deficient patients (type I bare lymphocyte syndrome). *J. Exp. Med.* 187: 117–122.
10. Furukawa, H., T. Yabe, K. Watanabe, R. Miyamoto, A. Miki, T. Akaza, K. Tadokoro, S. Tohma, T. Inoue, K. Yamamoto, and T. Juji. 1999. Tolerance of NK and LAK activity for HLA class I-deficient targets in a TAP1-deficient patient (bare lymphocyte syndrome type I). *Hum. Immunol.* 60: 32–40.
11. Vitale, M., J. Zimmer, R. Castriconi, D. Hanau, L. Donato, C. Bottino, L. Moretta, H. de la Salle, and A. Moretta. 2002. Analysis of natural killer cells in TAP2-deficient patients: expression of functional triggering receptors and evidence for the existence of inhibitory receptor(s) that prevent lysis of normal autologous cells. *Blood* 99: 1723–1729.
12. Schönberg, K., J. C. Fischer, G. Kögler, and M. Uhrberg. 2011. Neonatal NK-cell repertoires are functionally, but not structurally, biased toward recognition of self HLA class I. *Blood* 117: 5152–5156.
13. Schönberg, K., M. Sribar, J. Enczmann, J. C. Fischer, and M. Uhrberg. 2011. Analyses of HLA-C-specific KIR repertoires in donors with group A and B haplotypes suggest a ligand-instructed model of NK cell receptor acquisition. *Blood* 117: 98–107.
14. Andersson, S., C. Fauriat, J. A. Malmberg, H. G. Ljunggren, and K. J. Malmberg. 2009. KIR acquisition probabilities are independent of self-HLA class I ligands and increase with cellular KIR expression. *Blood* 114: 95–104.
15. Sternberg-Simon, M., P. Brodin, Y. Pickman, B. Önfelt, K. Kärre, K. J. Malmberg, P. Höglund, and R. Mehr. 2013. Natural killer cell inhibitory receptor expression in humans and mice: a closer look. *Front. Immunol.* 4: 65.
16. Béziat, V., L. L. Liu, J. A. Malmberg, M. A. Ivarsson, E. Sohlberg, A. T. Björklund, C. Retière, E. Sverremark-Ekström, J. Traherne, P. Ljungman, et al. 2013. NK cell responses to cytomegalovirus infection lead to stable imprints in the human KIR repertoire and involve activating KIRs. *Blood* 121: 2678–2688.
17. Parissiadis, A., A. Dormoy, D. Fricker, D. Hanau, H. de la Salle, J. P. Cazenave, P. Lenoble, and L. Donato. 2005. Unilateral necrotising toxoplasmic retinochoroiditis as the main clinical manifestation of a peptide transporter (TAP) deficiency. *Br. J. Ophthalmol.* 89: 1661–1662.
18. Doğu, F., A. Ikincioğullari, D. Fricker, G. Bozdoğan, C. Aytekin, M. Ileri, T. Teziç, E. Babacan, and H. De La Salle. 2006. A novel mutation for TAP deficiency and its possible association with Toxoplasmosis. *Parasitol. Int.* 55: 219–222.
19. Caversaccio, M., H. M. Bonél, R. Carter, A. P. Williams, and S. D. Gadola. 2008. TAP deficiency syndrome: chronic rhinosinusitis and conductive hearing loss. *Eur. Arch. Otorhinolaryngol.* 265: 1289–1292.
20. Villa-Forte, A., H. de la Salle, D. Fricker, F. Hentges, and J. Zimmer. 2008. HLA class I deficiency syndrome mimicking Wegener's granulomatosis. *Arthritis Rheum.* 58: 2579–2582.
21. Björkström, N. K., P. Riese, F. Heuts, S. Andersson, C. Fauriat, M. A. Ivarsson, A. T. Björklund, M. Flodström-Tullberg, J. Michaëlsson, M. E. Rottenberg, et al. 2010. Expression patterns of NKG2A, KIR, and CD57 define a process of CD56dim NK-cell differentiation uncoupled from NK-cell education. *Blood* 116: 3853–3864.
22. Lopez-Vergès, S., J. M. Milush, S. Pandey, V. A. York, J. Arakawa-Hoyt, H. Pircher, P. J. Norris, D. F. Nixon, and L. L. Lanier. 2010. CD57 defines a functionally distinct population of mature NK cells in the human CD56dimCD16+ NK-cell subset. *Blood* 116: 3865–3874.
23. Pyo, C. W., L. A. Guethlein, Q. Vu, R. Wang, L. Abi-Rached, P. J. Norman, S. G. Marsh, J. S. Miller, P. Parham, and D. E. Geraghty. 2010. Different patterns of evolution in the centromeric and telomeric regions of group A and B haplotypes of the human killer cell Ig-like receptor locus. *PLoS ONE* 5: e15115.
24. Ljunggren, H. G., L. Van Kaer, H. L. Ploegh, and S. Tonegawa. 1994. Altered natural killer cell repertoire in Tap-1 mutant mice. *Proc. Natl. Acad. Sci. USA* 91: 6520–6524.
25. Furukawa, H., T. Yabe, T. Akaza, K. Tadokoro, S. Tohma, T. Inoue, K. Tokunaga, K. Yamamoto, D. E. Geraghty, and T. Juji. 1999. Cell surface expression of HLA-E molecules on PBMC from a TAP1-deficient patient. *Tissue Antigens* 53: 292–295.
26. Matamoros, N., J. Milà, M. Llano, A. Balas, J. L. Vicario, J. Pons, C. Crespí, N. Martinez, J. Iglesias-Alzueta, and M. López-Botet. 2001. Molecular studies and NK cell function of a new case of TAP2 homozygous human deficiency. *Clin. Exp. Immunol.* 125: 274–282.
27. Prod'homme, V., P. Tomasec, C. Cunningham, M. K. Lemberg, R. J. Stanton, B. P. McSharry, E. C. Wang, S. Cuff, B. Martoglio, A. J. Davison, et al. 2012. Human cytomegalovirus UL40 signal peptide regulates cell surface expression of the NK cell ligands HLA-E and gpUL18. *J. Immunol.* 188: 2794–2804.
28. Lorente, E., S. Infantes, D. Abia, E. Barnea, I. Beer, R. García, F. Lasala, M. Jiménez, C. Mir, A. Morreale, et al. 2012. A viral, transporter associated with antigen processing (TAP)-independent, high affinity ligand with alternative interactions endogenously presented by the nonclassical human leukocyte antigen E class I molecule. *J. Biol. Chem.* 287: 34895–34903.
29. Sun, J., and P. B. Kavathas. 1997. Comparison of the roles of CD8 alpha alpha and CD8 alpha beta in interaction with MHC class I. *J. Immunol.* 159: 6077–6082.
30. Gao, G. F., B. E. Willcox, J. R. Wyer, J. M. Boulter, C. A. O'Callaghan, K. Maenaka, D. I. Stuart, E. Y. Jones, P. A. Van Der Merwe, J. I. Bell, and B. K. Jakobsen. 2000. Classical and nonclassical class I major histocompatibility complex molecules exhibit subtle conformational differences that affect binding to CD8alphaalpha. *J. Biol. Chem.* 275: 15232–15238.
31. Addison, E. G., J. North, I. Bakhsh, C. Marden, S. Haq, S. Al-Sarraj, R. Malayeri, R. G. Wickremasinghe, J. K. Davies, and M. W. Lowdell. 2005. Ligation of CD8alpha on human natural killer cells prevents activation-induced apoptosis and enhances cytolytic activity. *Immunology* 116: 354–361.
32. van Bergen, J., A. Thompson, M. van Pel, C. Retière, D. Salvatori, D. H. Raulet, J. Trowsdale, and F. Koning. 2013. HLA reduces killer cell Ig-like receptor expression level and frequency in a humanized mouse model. *J. Immunol.* 190: 2880–2885.

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