Sars-Cov-2 Spike 1 protein control Natural killer cells activation via HLA-E/NKG2A pathway.

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Method Article

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Abstract

Natural killer (NK) cells are important in the control of viral infections. However, the role of NK cells during Sars-Cov-2 infection has previously not been identified. Peripheral blood NK cells from Sars-Cov and Sars-Cov-2 naïve subjects were evaluated for their activation, degranulation, interferon-gamma expression in the presence of Sars-Cov and Sars-Cov-2 spike proteins. K562 and lung epithelial cells were transfected with spike proteins and co-cultured with NK cells. The analysis was performed by flow cytometry and immune-fluorescence. Sars-Cov and Sars-Cov-2 spike proteins did not alter NK cell activation in K562 in vitro model. On the contrary, Sars-Cov-2 spike 1 protein (SP1) intracellular expression by lung epithelial cells resulted in NK cell reduced degranulation. Further experiments revealed a concomitant induction of HLA-E expression on the surface of lung epithelial cells and the recognition of a SP1-derived HLA-E-binding peptide. Simultaneously, there was the up-modulation of the inhibitory receptor NKG2A/CD94 on NK cells when SP1 is expressed in lung epithelial cells. We ruled out GATA3 transcription factor as responsible for HLA-E increased levels and HLA-E/NKG2A interaction as implicate in NK cells exhaustion. We show for the first time that NK cells are affected by SP1 expression in lung epithelial cells via HLA-E/NKG2A interaction. The resulting NK cells exhaustion might contribute to immunopathogenesis in Sars-Cov-2 infection.

Introduction

Natural killer (NK) cells are important in the control of viral infections. However, the role of NK cells during Sars-Cov-2 infection has previously not been identified. Peripheral blood NK cells from Sars-Cov and Sars-Cov-2 naïve subjects were evaluated for their activation, degranulation, interferon-gamma expression in the presence of Sars-Cov and Sars-Cov-2 spike proteins. K562 and lung epithelial cells were transfected with spike proteins and co-cultured with NK cells. The analysis was performed by flow cytometry and immune-fluorescence. Sars-Cov and Sars-Cov-2 spike proteins did not alter NK cell activation in K562 in vitro model. On the contrary, Sars-Cov-2 spike 1 protein (SP1) intracellular expression by lung epithelial cells resulted in NK cell reduced degranulation. Further experiments revealed a concomitant induction of HLA-E expression on the surface of lung epithelial cells and the recognition of a SP1-derived HLA-E-binding peptide. Simultaneously, there was the up-modulation of the inhibitory receptor NKG2A/CD94 on NK cells when SP1 is expressed in lung epithelial cells. We ruled out GATA3 transcription factor as responsible for HLA-E increased levels and HLA-E/NKG2A interaction as implicate in NK cells exhaustion. We show for the first time that NK cells are affected by SP1 expression in lung epithelial cells via HLA-E/NKG2A interaction. The resulting NK cells exhaustion might contribute to immunopathogenesis in Sars-Cov-2 infection.

Reagents

Flow cytometry

CD3-PerCp-Cy5.5, CD56-FITC moAbs (e-Bioscience, Frankfurt, DE); CD3-PE-Cy7 (clone SP34-2), CD16-PE (clone B73.1), CD56-APC (clone B159), NKG2A-PE (clone 131411) (BD, Italy); HLA-I (HLA-A,-B,-C)-PE (BD Biosciences, Italy); anti-IFN- γ MoAb (27726; R&D Systems); anti-GATA3 (BV421; BD, Italy); anti-spike 1 (clone 2C1), anti-spike 2 and anti Sars-Cov spike antibodies (myBiosource, USA); anti-CD107a-PE; clone H4A3; BD Biosciences.

IntraPrep reagent (Beckman Coulter). 7AAD/CFSE Cell-mediated cytotoxicity assay kit (1180 E. Ellsworth Rd Ann Arbor, MI, USA).

Viral RNA Detection

MagMAX Viral/Pathigen Nuclei Acid Isolation kit (ThermoFisher, Italy); TaqMan 2019nCoV assay kit v1 (ThermoFisher, Italy).

Cell culture

Beas-2B (ATCC CRL-9609); BEGM culture medium (BEGM Kit Catalog No. CC-3170 Lonza/Clonetics Corporation, USA); K562 (ATC CCL-243); RPMI (Gibco, Italy); FCS (fetal calf serum, Euroclone, Pero, MI, Italy); penicillin, streptomycin.

Antibodies

Anti-HLA-E (clone MEM-E/08, Exbio, Praha, CZ); anti-CD94/NKG2A (clone 131411, BD, Italy)

Inhibitors

Pyrrothiogatain (Santa Cruz; cat#sc-352288A).

Cell migration assay

Sars-Cov-2 proteins (spike protein S1 subunit, spike protein S2 subunit) (RayBiotech); control Sars-Cov spike protein (MyBiosource); CXCL12 (BioLegend).

Protein Transfection

Pierce Protein Transfection Kit (ThermoFisher).

Lactate dehydrogenase (LDH) assay

A colorimetric-based lactate dehydrogenase (LDH) assay (Cytotoxicity Detection KitPLUS; Switzerland).

Equipment

Flow cytometry

The flow cytometry analysis were performed with FACS Cantoll flow cytometer (BD, Milan, Italy) and FlowJo LLC analysis software (Ashland, Oregon, USA).

Immunofluorescence assay

The immunofluorescence images were acquired with Nikon Eclipse TE2000S, equipped with a digital camer.

HLA-E binding prediction

The HLA-E binding prediction was made using the IEDB analysis resource ANN aka NetMHC (ver. 4.0) tool at the <http://tools.iedb.org/mhci/help/>, using the viral spike 1 protein from Sars-Cov-2 sequence (QH062112.1).

Procedure

NK cells purification

Primary NK cells were separated from peripheral blood samples using the negative magnetic cell separation (MACS) system (Miltenyi Biotech, Gladbach, Germany). The analysis of purified cell fraction by flow cytometry with CD3-PerCp-Cy5.5, CD56-FITC moAbs (e-Bioscience, Frankfurt, DE), demonstrated that the NK cell content was >90% (data not shown).

Viral RNA Detection

RNA extraction was performed by using MagMAX Viral/Pathigen Nuclei Acid Isolation kit (ThermoFisher, Italy) according to the manufacturer's instructions. The RT-PCR was performed with the TaqMan 2019nCoV assay kit v1 (ThermoFisher, Italy).

Cell culture

Beas-2B (ATCC CRL-9609) bronchial epithelial cell line was grown in BEGM culture medium (BEGM Kit Catalog No. CC-3170; Lonza/Clonetics Corporation, USA) K562 (ATC CCL-243) lymphoblastoid cell line was cultured in RPMI (Gibco, Italy) supplemented with 10% of FCS (fetal calf serum, Euroclone, Pero, MI, Italy) and 100 U/mL penicillin, 100 µg/mL streptomycin. Cell cultures were maintained at 37° C in humidified atmosphere of 5% CO₂ in air.

The role of HLA-E and NKG2A was evaluated incubating the cells with anti-HLA-E (clone MEM-E/08, Exbio, Praha, CZ) or anti-CD94/NKG2A (clone 131411, BD, Italy) antibodies (7.5 ng/ml).

GATA3 DNA-binding activity was inhibited adding pyrrothiogatain (Santa Cruz; cat#sc-352288A) to cell cultures (10uM) ¹⁷.

Flow cytometry

1x10⁵ eNK cells were labeled with fluorophore-conjugated antibodies: CD3-PE-Cy7 (clone SP34-2), CD16-PE (clone B73.1), CD56-APC (clone B159), NKG2A-PE (clone 131411) (BD, Italy) and matched isotype controls.

5x10⁵ bronchial epithelial cells epithelial cells were stained specific Ab HLA-I (HLA-A,-B,-C)-PE (BD Biosciences, Italy), HLA-E (clone MEM-E/08, Exbio, Praha, CZ) and matched isotype controls.

Interferon-gamma (IFN-gamma) and GATA3 expression were evaluated in fixed and permeabilized cells with IntraPrep reagent (Beckman Coulter) and stained with anti-IFN-γ MoAb (27726; R&D Systems) or anti-GATA3 (BV421; BD, Italy) MoAb.

SP1, SP2 and S proteins expression in K562 and Beas-2B transfected cells were evaluated with anti-spike 1 (clone 2C1), anti-spike 2 and anti Sars-Cov spike antibodies (myBiosource, USA).

Data were analyzed using FACS Cantoll flow cytometer (BD, Milan, Italy) and FlowJo LLC analysis software (Ashland, Oregon, USA). Ten thousand events were acquired.

Immunofluorescence assay

HLA-E expression was analyzed by immunofluorescence with anti-HLA-E antibody (clone MEM-E/08, Exbio, Praha, CZ).

Cell migration assay

The assay was performed using the Corning Transwell System, with inserts with 5µm pore polycarbonate membrane. Briefly, 1×10^6 cells were seeded in the upper chamber in 150µl of RPMI or BEGM with the 0,5% of FBS. The inserts containing cells were positioned into a 24well plate, which provides the lower reservoirs for migration system. Each reservoir was filled with 650µl of medium (RPMI) containing Sars-Cov-2 proteins (spike protein S1 subunit, spike protein S2 subunit) (RayBiotech) or control Sars-Cov spike protein (MyBiosource) at the final concentration of 100ng/ml. Medium with CXCL12 (100ng/ml; BioLegend) at the concentration of was used as positive. Migration was performed for 3h at 37°C and then the plate was briefly centrifuged at 300g for 5min in order to collect migrated cells in the lower reservoir for cell count. Every condition was tested in triplicate and results were reported as fold of cell migration compared to untreated NK cells.

Protein Transfection

K562 or Beas-2B cell lines were transfected using the Pierce Protein Transfection Kit (ThermoFisher) following product instructions. A total of 4×10^5 cells were transfected with 1µg of protein (spike protein S1 subunit, spike protein S2 subunit) of Sars-Cov-2 or Sars-Cov spike protein. Transfection was performed for 3-4h at 37°C in 1ml medium without FBS. After transfection, a volume of complete medium with 20% FBS was added to each well. K562 or Beas-2B cells treated with transfection reagent alone or transfected with 0,5µg of control fluorescent antibody (provided in the kit) were used as negative and efficiency control, respectively.

Lactate dehydrogenase (LDH) assay

LDH assay was performed to evaluate the effect of the transfection with Sars-Cov2 and Sars-Cov proteins in K562 or Beas-2B cells on cell viability. Transfected K562 or Beas-2B cells were suspended at 5×10^4 cells/mL and cultured for 4 h on a 96-well microplate at 37 °C with 5% CO₂. A colorimetric-based lactate dehydrogenase (LDH) assay (Cytotoxicity Detection KitPLUS; Switzerland) was used, according to the manufacturer's instructions.

Degranulation analysis

In vitro cytotoxicity experiments were performed using K562 or Beas-2B cells as target and NK cells as effector cells. NK cells were added to K562 or Beas-2B cells with a 5:1 effector:target ratio. NK cell degranulation was evaluated by CD107a staining (anti-CD107a-PE; clone H4A3; BD Biosciences) after 3 hours of treatment with Golgi Stop solution (BD). Labeled cells were analyzed with FACSCantoll flow cytometer (Becton Dickinson, Milano, Italy) and FlowJo software (Tree Star Inc, Ashland, OR, USA).

Carboxyfluorescein diacetate succinimidyl ester (CFSE) analysis

K562 or Beas-2B cells were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) to assess cell-mediated cytotoxicity, using 7AAD/CFSE Cell-mediated cytotoxicity assay kit (1180 E. Ellsworth Rd Ann Arbor, MI, USA). 10^7 cells/ml were resuspended in CFSE staining solution and incubated for 15 minutes at 37°C. Control target cells were resuspended in 0.1% BSA. Then, cells were washed two times with culture medium and incubated for 30 minutes at 37°C. NK cells were put in co-culture with CFSE-labeled infected cells at a 1:5 ratio. The cell mixture was incubated for 4h, centrifuged and resuspended in 7-AAD staining solution. Control target cells were resuspended in Assay Buffer. Cells were incubated for 15 minutes in the dark at 4°C. Then cells were centrifuged and resuspended in Assay buffer. Cells were analyzed with FACSCantoll flow cytometer and FlowJo software.

HLA-E binding prediction

The HLA-E binding prediction was made using the IEDB analysis resource ANN aka NetMHC (ver. 4.0) tool at the <http://tools.iedb.org/mhci/help/>, using the viral spike 1 protein from Sars-Cov-2 sequence (QH062112.1).

Troubleshooting

The procedures are well defined and previously published.

Time Taken

Anticipated Results

Spike proteins induce NK cell migration

Although the role of NK cells in the immune response towards viral infections is generally accepted, there are few data on the early NK cell trafficking in response to coronavirus infections⁵. The evaluation of NK response to Sars-Cov-2 infection is important to determining the innate immune response per se and for the cross-talk with adaptive immune cells²². We explored the migration, interferon-gamma (IFN-gamma) expression and degranulation of NK cell in the presence of spike 1 (SP1) and spike 2 (SP2) Sars-Cov-2 proteins and Sars-Cov spike proteins (S). We used a cell migration system in a 5 µm transwells w/polycarbonate filter, without a cell barrier. We used peripheral blood NK cells from control subjects negative for both Sars-Cov-2 and Sars-Cov viremia (data not shown). This condition ensured that NK cells were naïve for spike proteins. We cultured NK cells in the presence of SP1, SP2 from Sars-Cov-2 or spike protein (S) from Sars-Cov. We used CXCL12 as a positive control for NK cell migration²⁰. We observed an increase in NK cell migration in the presence of both SP1 and SP2 (SP1 $p < 0.001$; SP2 $p = 0.0125$; Student T test) (Fig. 1A). Similarly, NK cell migration was induced by the Sars-Cov S protein ($p = 0.034$; Student T test) (Fig. 1A). These data suggest that both Sars-Cov-2 and Sars-Cov spike proteins are able to chemo-attract NK cells. To evaluate if spike proteins are able to induce also NK cells activation, we analyzed the expression of IFN-gamma. We observed an increase in IFN-gamma expression in the presence of SP1 protein ($p < 0.001$; Student T test), while SP2 and S proteins were not able to induce IFN-gamma (SP2 $p = 0.067$; S $p = 0.076$; Student T test) (Fig. 1B).

Spike proteins did not modify NK cell cytotoxicity

Then, we evaluated the cytotoxicity of NK cells in the presence of spike proteins, using CD107a staining, a marker of degranulation²¹. We mimicked the expression of spike proteins inside NK target cells, the K562 cell line, transfecting directly the proteins. We obtained a mean transfection efficiency of 85% for SP1, SP2 and S proteins (Fig. 1C). The K562 cells viability, evaluated by MTT assay, was not affected by protein transfection (Fig. 1D). We incubated NK cells for four hours with K562 and evaluated the expression of CD107a. We observed an increase in CD107a staining in all the culture conditions, with no difference in the presence of SP1, SP2 and S proteins in comparison with the co-culture with untreated NK cells (S1 $p = 0.078$; S2 $p = 0.087$; S $p = 0.081$; Student T test) (Fig. 1E, F). To be sure that NK cells expressing CD107a were able to kill K562 cells, CFSE (carboxyfluorescein diacetate succinimidyl ester)

staining of target cells was detected by flow cytometry ¹⁸. We observed an increase in K562 CFSE⁺/7-AAD⁺ cell percentage in all the co-culture conditions and comparable with the killing observed in the co-culture with untreated NK cells ($p < 0.001$; Student T test) (Figure 1G), confirming the result observed with CD107a staining (Figure 1E, F).

Sars-Cov-2 spike 1 protein modifies NK cell cytotoxicity when presented by lung epithelial cells

Since the target cells for Sars-Cov-2 replication are lung epithelial cells ⁴, we considered the activation status of NK cells in this context. We mimicked the expression of SP1, SP2 and S proteins inside Beas-2B lung epithelial cells, transfecting directly the proteins. We obtained a 90% efficiency of transfection with all the proteins (Fig. 2A). The transfection did not affect cell viability (Fig. 2B) and the expression of CK8 epithelial cell markers (Fig. 2A). We incubated NK cells for four hours with lung epithelial cells and evaluated the expression of CD107a degranulation marker. We observed a decrease in CD107a staining in the culture condition with SP1 transfected Beas-2B cells ($p < 0.001$; Student t test) (Fig. 2A, B). On the contrary, we observed only a slight decrease in CD107a expression in the culture condition with SP2 and S proteins transfected Beas-2B cells, that is similar to that observed with the control of transfection condition (Null-transfected) ($p = 0.043$; Student T test) (Fig. 2A, B).

To be sure that NK cells expressing CD107a were able to kill Beas2B cells, CFSE staining of target cells was detected by flow cytometry. We observed an increase in Beas-2B CFSE⁺/7-AAD⁺ cell percentage in the co-culture with NK cells in SP2 and S protein transfected Beas2B cells (Fig. 3C), while there was a decrease in Beas-2B CFSE⁺/7-AAD⁺ cell percentage in the co-culture with NK cells in SP1 transfected Beas-2B cells ($p < 0.001$; Student T test) (Fig. 3C), confirming the results observed with CD107a staining (Fig. 3A, B).

Spike 1 peptide is presented by lung epithelial cells via HLA-E molecules

Since the presence of intracellular SP1 protein in lung epithelial cells induced a decrease in the cytotoxic activity of NK cells, we analyzed the possible factors involved in the modification of NK cell status.

Since viral proteins are commonly recognized and degraded by proteasome inside the infected cells ²³, we hypothesized that SP1 peptides might be presented to NK cells. Intracellular peptide presentation is performed by Human leukocyte antigen (HLA) class I molecules, that are expressed by all nucleated cells. Firstly, we evaluated the expression of HLA class I molecules in Beas-2B cells transfected with Sp1, Sp2 and S proteins. When we stained the cells with anti-classical HLA class I molecules (HLA-A, HLA-B, HLA-C) antibody, we recognized a significant decrease in their membrane expression when lung epithelial cells were transfected with SP1 protein ($p < 0.001$; Student t test) (Fig. 3D, E). Epithelial lung cells can express also non-classical HLA-E molecule ²⁴. HLA-E binds peptides primarily derived from a specific signal

sequences²⁵ and interacts with NKG2A/CD94 NK cell inhibitory receptors¹⁴. When we performed the lung epithelial cell staining with MEM-E/06 anti-HLA-E antibody, we observed a slight expression in basal conditions that is not affected by SP2 and S transfection (Fig. 3F, G). On the contrary, there is a significant increase in HLA-E expression in the presence of SP1 protein ($p=0.011$; Student T test) (Fig. 3F, G). HLA-E expression is controlled by GATA3 transcription factor¹¹, that is known to be expressed also by lung epithelial cells^{26, 27}. We observed an increase in GATA3 expression in the presence of SP1 ($p<0.001$; Student T test) (Fig. 4A, B). To be sure that the increase in HLA-E expression in lung epithelial cells transfected with SP1 protein is controlled by GATA3 transcription factor, we treated the cells with a GATA3 inhibitor, the pyrrothiogatain¹⁷. The treatment with pyrrothiogatain of Beas-2B cells transfected with SP1 decreased the surface expression of HLA-E, reaching the basal levels of HLA-E expression in Beas-2B cells (Fig. 3F, G). The immune-fluorescence staining revealed a clear surface expression of HLA-E molecules in the presence of intracellular SP1, that is reduced with the addition of GATA3 inhibitor (Fig. 4C). To evaluate if SP1 peptides might be presented by HLA-E molecules, we performed a MHC binding predictions, made on 4/26/2020 using the IEDB analysis resource ANN aka NetMHC (ver. 4.0) tool. We observed that a 8mer peptide in the SP1 domain (270-277, LQPRTFLL) showed a high affinity mainly for the HLA-E*0101 binding groove (IC50: 0.02) and in a lower extent for HLA-E*0301 allele (IC50: 0.76) with a similar consensus motif with HLA-E binding (VMAPRTVLL)¹³. We might speculate that the induction of GATA3 and the binding of SP1 peptide might induce HLA-E membrane expression on lung epithelial cells.

HLA-E / NKG2A/CD94 interaction is responsible for NK cells exhaustion

Since the functional control of NK cells by HLA-E is possible in the presence of NKG2A/CD94 on NK cells, we evaluated the expression of this receptor on the surface of NK cells. When NK cells were co-cultured with SP1 transfected Beas-2B cells, we observed an increase in the expression of the inhibitory receptor NKG2A/CD94 ($p<0.001$; Student t test) (Fig. 5A, B). To be sure that the resulting inactivity of NK cells towards lung epithelial cells expressing SP1 was determined by HLA-E/NKG2A interaction, we treated the cell culture with anti-HLA-E or anti-NKG2A/CD94 antibodies. We incubated NK cells for four hours with SP1 transfected Beas-2B cell line and evaluated the expression of CD107a in the presence or absence of anti-HLA-E or anti-NKG2A antibodies. We observed a decrease in CD107a positive NK cells in the culture condition with SP1 transfected Beas-2B cells ($p<0.001$; Student T test) (Fig. 5C, D), that is partly recovered by the treatment with anti-HLA-E antibody ($p=0.037$; Student T test) (Fig. 5C, D), while it is totally regained with anti-NKG2A/CD94 antibody (Fig. 5C, D).

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Figures

Figure 1

Peripheral blood NK cells from four control subjects negative for both Sars-Cov-2 and Sars-Cov viremia were cultured NK cells in the presence of SP1, SP2 from Sars-Cov-2 or spike protein (S) from Sars-Cov. A) NK cell migration is reported as fold of cell migration compared to untreated NK cells. We used CXCL12 as a positive control for NK cell migration. B) Expression of IFN-gamma evaluated by flow cytometry C) Representative intracellular expression of SP1, SP2 and S proteins in K562 cells after transfection. D) K563 viability, assessed by LDH assay, in basal and transfected conditions. The transfection with 0,5ug of control fluorescent antibody was used as positive control (Null-transfected). E) Representative dot plots of NK cell degranulation towards K562 cell target. NK cells were marked with CD56. The degranulation was assessed by CD107a expression. F) Percentage of CD107a positive NK cells after the co-culture with K562 target cells. G) CFSE+/7-AAD+ cell percentage in NK cell / K562 co-cultures. The values are presented as mean $\pm$ standard deviation. *, **significant p values Student t test.

Figure 2

A) Representative intracellular expression of SP1, SP2 and S proteins and surface expression of the epithelial marker CK8 in Beas-2B cells after transfection. B) Beas-2B viability, assessed by LDH assay, in basal and transfected conditions. The transfection with 0,5ug of control fluorescent antibody was used as positive control (Null-transfected). The values are presented as mean $\pm$ standard deviation. *, ** significant p values Student t test.

Figure 3

A) Representative dot plots of NK cell degranulation towards K562 cell target. NK cells were marked with CD56. The degranulation was assessed by CD107a expression. Beas-2B were transfected with SP1, SP2, S proteins or control fluorescent antibody used as positive control (Null-transfected), or treated with GATA3 inhibitor (anti-GATA3). B) Percentage of CD107a positive NK cells after the co-culture with Beas-

2B cells. C) CFSE+/7-AAD+ cell percentage in NK cell / Beas-2B co-cultures. The values are presented as mean $\pm$ standard deviation. D) Representative histograms of HLA-I expression on the surface of transfected Beas-2B. E) The histograms showed the mean $\pm$ standard deviation MFI (mean fluorescence intensity) values of HLA-I expression in three independent experiments. *significant p values Student t test; F) Representative histograms of HLA-E expression on the surface of transfected Beas-2B cells G) The histograms showed the mean $\pm$ standard deviation MFI (mean fluorescence intensity) values of HLA-E expression in three independent experiments. *significant p values Student t test.

Figure 4

A) Representative histograms of GATA3 expression on the surface of Beas-2B cells after transfection with SP1, SP2, S proteins or control fluorescent antibody was used as positive control (Null-transfected) or treated with GATA3 inhibitor (anti-GATA3). B) The histograms showed the mean $\pm$ standard deviation MFI (mean fluorescence intensity) values of HLA-E expression in three independent experiments. *significant p values Student t test. C) HLA-E expression in Beas-2B cells was characterized by immunofluorescence (Nikon Eclipse TE2000S, equipped with a digital camera). The evaluation was assessed after SP1 transfection (SP1) without or with GATA3 inhibitor (GATA3 inhibitor). Original magnification 40 $\times$.

Figure 5

A) Representative histograms of NKG2A/CD94 expression on the surface of NK cells after co-culture with Beas-2B transfected with SP1, SP2, S proteins or control fluorescent antibody was used as positive control (Null-transfected). B) The histograms showed the mean $\pm$ standard deviation MFI (mean fluorescence intensity) values of NKG2A/CD94 expression in three independent experiments.

*significant p values Student t test. C) Representative dot plots of NK cell degranulation towards Beas-2B cells transfected with SP1 protein without or with anti-HLA-E, anti-NKG2A or anti-isotype control. NK cells were marked with CD56. The degranulation was assessed by CD107a expression. D) Percentage of CD107a positive NK cells after the co-culture with Beas-2B cells transfected with SP1 protein without or with anti-HLA-E, anti-NKG2A or anti-isotype control. The values are presented as mean $\pm$ standard deviation. *significant p values Student t test.