

TITLE PAGE: *Pseudomonas aeruginosa* infection impairs NKG2D-dependent NK cell cytotoxicity through regulatory T-cell activation

Mickael Vourc'h, MD, PhD (1,2); Gaelle David (3,4); Benjamin Gaborit, MD, PhD (1); Alexis Broquet, PhD (1); Cedric Jacqueline, PhD (1); Tanguy Chaumette, MD, PhD (1), Jocelyne Caillon, PharmD, PhD (1); Antoine Roquilly MD, PhD (1,2); Christelle Retiere PhD (3); Karim Asehnoune, MD, PhD (1,2)#.

Affiliations

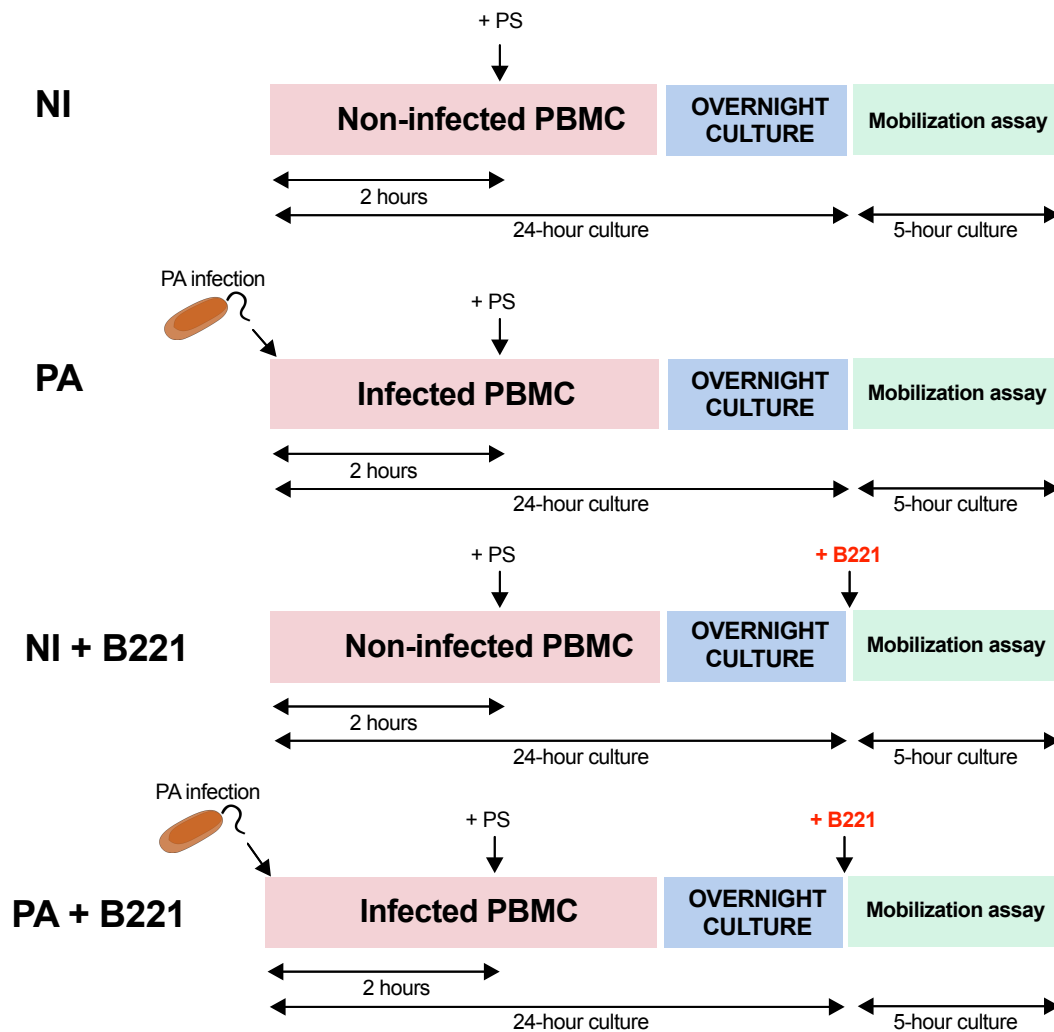
1. School of Medicine, UPRES EA 3826, Thérapeutiques Cliniques et Expérimentales des Infections, IRS2 Nantes Biotech, France.
2. Intensive Care Unit, Anesthesia and Critical Care Department, Hôtel Dieu, University Hospital of Nantes, Nantes, France.
3. EA4271 – “Immunovirologie et Polymorphisme Génétique”, Etablissement Français du Sang/Pays de la Loire, Nantes, France.
4. CRCINA, INSERM U1232, CNRS, Université d'Angers, Université de Nantes, France.

Corresponding author: Karim Asehnoune, MD, PhD. Intensive Care Unit, Anesthesia and Critical Care Department, Hôtel Dieu, University Hospital of Nantes, Nantes, France, F-44093, France, Karim.asehnoune@chu-nantes.fr +33 (0)2 40 08 33 33.

Figure A1

Title Figure A1: NK cell CD107a mobilization assay.

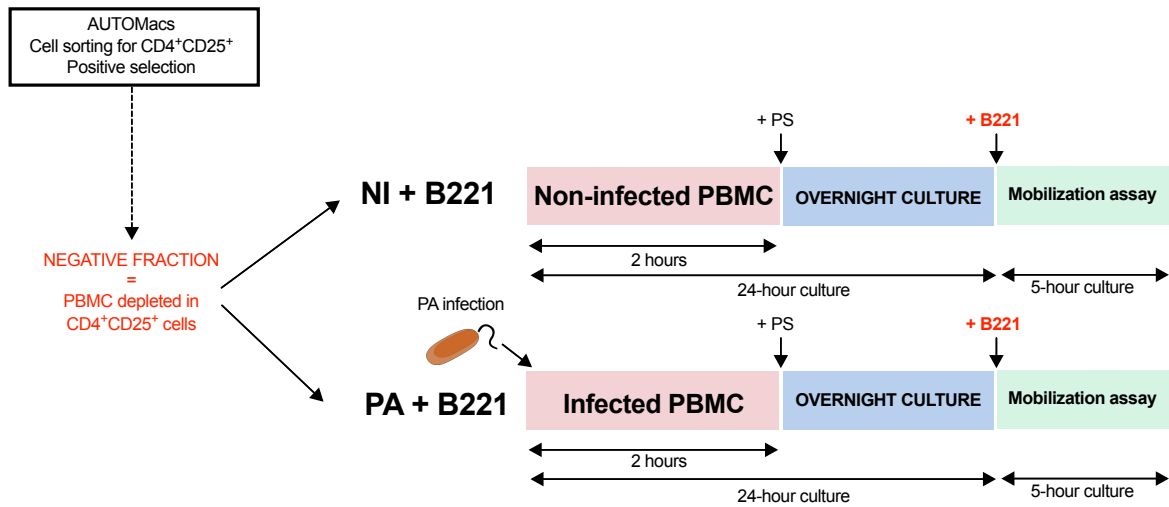
INFECTION AND CYTOTOXICITY ASSAY



Legend Figure A1: Experimental protocol to study the effect of *Pseudomonas aeruginosa* infection on NK cells CD107a activity. The mobilization assay was performed in non-infected (NI) or 24-hour *Pseudomonas aeruginosa* (PA) infected PBMC. **B221:** 5-hour exposition to B221 cells with a B221:PBMC ratio of 1:1, **PS:** Penicillin/Streptomycin, **PBMC:** Peripheral Blood Mononuclear Cells.

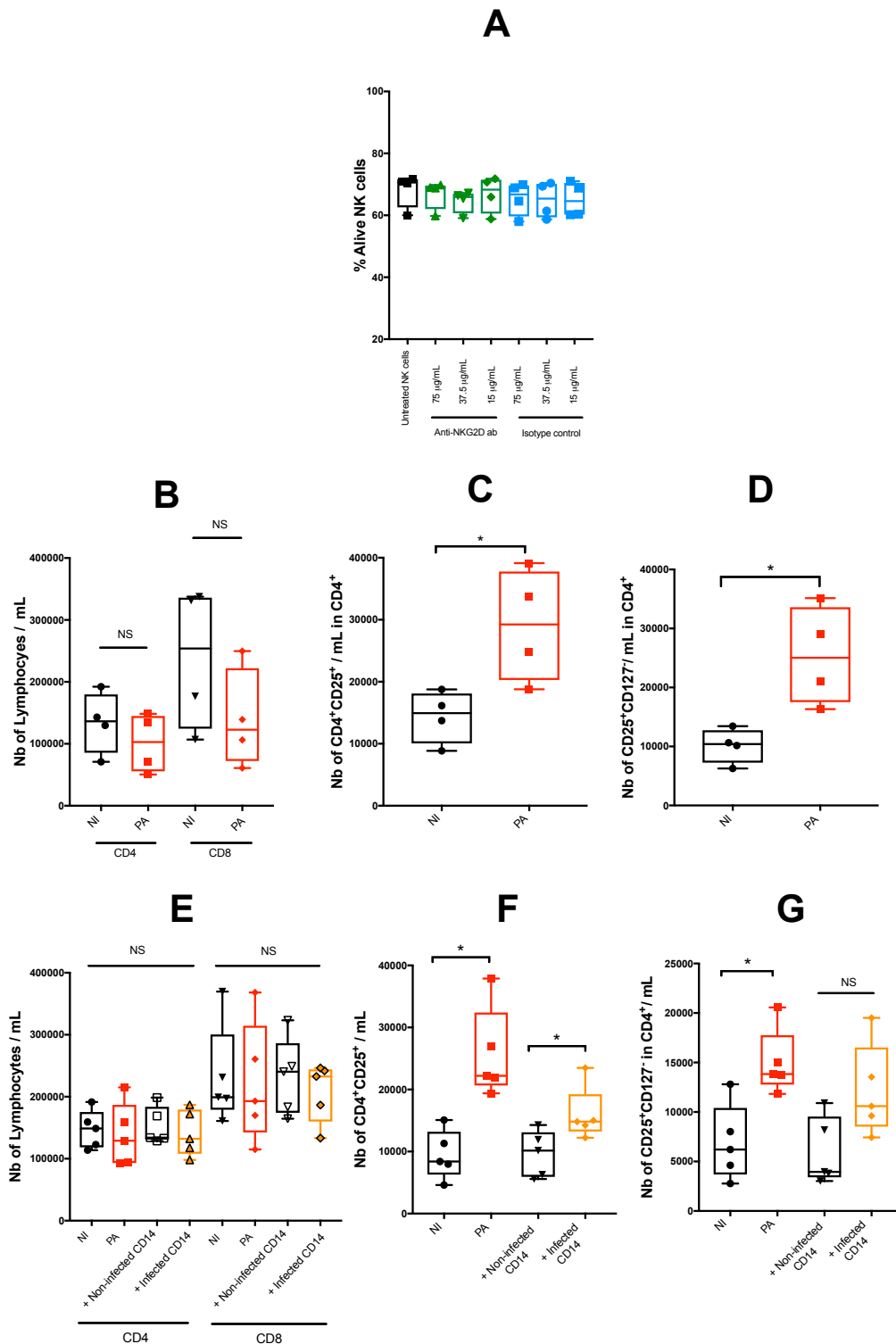
Figure A2

Title Figure A2: CD4⁺CD25⁺ cell depletion followed by mobilization assay



Legend Figure A2: Experimental protocol to study the effect of CD4⁺CD25⁺ depletion before infection on NK cell CD107a activity. After CD4⁺CD25⁺ depletion, 24-hour PA infection was performed when appropriate. Then, NK cell mobilization assay was performed in non-infected (NI) or 24-hour *Pseudomonas aeruginosa* (PA) infected PBMC. **B221:** 5-hour exposition to B221 cells with a B221:PBMC ratio of 1:1, **PS:** Penicillin/Streptomycin, **PBMC:** Peripheral Blood Mononuclear Cells.

Figure A3
Title Figure A3: Supplementary results



Legend Figure A3: (A) Representing histograms of the viability of sorted NK cells after 1-hour incubation with or without anti-NKG2D blocking antibody or its isotype followed by 5-hour exposition to B221 cells for CD107a mobilization assay (**experiments corresponding to Figure 2E**). Histograms

illustrating number of CD4⁺ and CD8⁺ (**B**), CD4⁺CD25⁺ (**C**) and CD4⁺CD25⁺CD127⁻ (**D**) cells in non-infected (NI) or *Pseudomonas aeruginosa* (PA) infected conditions. Histograms illustrating the effect of adoptive transfer of non-infected or PA-infected CD14⁺ on the number of CD4⁺ and CD8⁺ (**E**), CD4⁺CD25⁺ (**F**) and CD4⁺CD25⁺CD127⁻ (**G**) cells.

*: $P < 0.05$, NS: Non-significant difference