Production of IL-20 cytokines limits bacterial clearance and lung inflammation during infection by *Streptococcus pneumoniae*.

Fahima Madouri^{1, 2, 3, 4*}, Olivia Barada^{1, 2, 3, 4*}, Gwenola Kervoaze^{1, 2, 3, 4}, François Trottein^{1, 2, 3, 4}, Muriel Pichavant^{1, 2, 3, 4}, Philippe Gosset^{1, 2, 3, 4}

Short title: IL-20 cytokines in pneumonia

Supplementary Tables and Figures

¹Institut Pasteur de Lille, Centre d'Infection et d'Immunité de Lille, 59019 Lille, France

²Université Lille Nord de France, 59000 Lille, France

³Centre National de la Recherche Scientifique, UMR 8204, 59021 Lille, France

⁴Institut National de la Santé et de la Recherche Médicale, U1019, 59019 Lille, France CHRU de Lille, 59000 Lille, France

^{*} Equal contribution for both authors

Supplementary Table 1: Lung injury scoring.

The cumulative score ranged from 0 to 30 was evaluated in a blind fashion.

Lung injury	Score				
Scale	0	1	2	3	4
Extent of lung injury	Absence	<25 %	26 to 50 %	51 to 75 %	>75 %
Alveolar wall thickness	≤1 rbc	> 1 ≤ 2 rbc	3 to 5 rbc	6 to 10 rbc	> 10 rbc
Hyaline mem- branes	Absence	Presence	NA	NA	NA
Neutrophilic alve- olitis	Absence	<10 neutrophils/HPF	10 to 20 / HPF	21 to 50 / HPF	> 50 / HPF
Suppuration	Absence	Presence	NA	NA	NA
Bronchial epi- thelial degener- ation	Absence	Presence	NA	NA	NA
Neutrophilic peribronchitis	Absence	<10 neutrophils/HPF	10 to 20 / HPF	21 to 50 / HPF	> 50 / HPF
Lympho- hiosti- ocytic peribron- chitis	Absence	<10 mononuclear cells/HPF	10 to 20 / HPF	21 to 50 / HPF	> 50 / HPF
Vasculitis	Absence	Presence	NA	NA	NA

Abbreviations: rbc: red blood cell; HPF: high power field; NA: not applicable

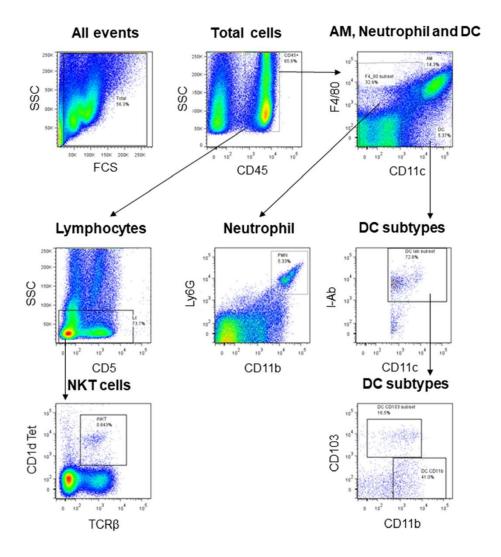
Supplementary Table 2: Sequence of primer used for RT-qPCR

Forward and reverse sequences of primer used during RT-qPCR experiment are represented in this table.

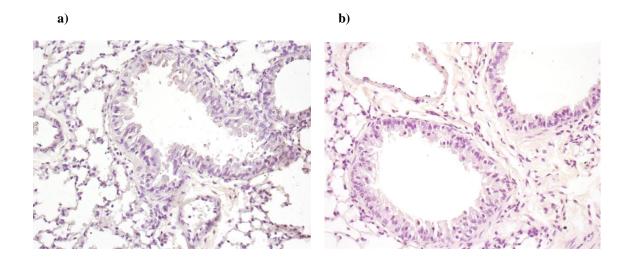
Target	Primer	Sequence
Mouse GAPDH	Forward	5'-TGTTTCCTCGTCCCGTAGACAA
	Reverse	5'-GGCAACAATCTCCACTTTGCC
Mouse IL-19	Forward	5'-TGTGTGCTGCATGACCAACAA
	Reverse	5'-GGCAATGCTGCTGATTCTCCT
Mouse IL-20	Forward	5'-TCTTGCCTTTGGACTGTTCTCC
	Reverse	5'-GTTTGCAGTAATCACACAGCTTC
Mouse IL-22	Forward	5'-AGCCGGACGTCTGTGTTGTTA
	Reverse	5'-TTGAGGTGTCCAACTTCCAGCA
Mouse IL-24	Forward	5'-CCACTCTGGCCAACAACTTCAT
	Reverse	5'-TCTGCGGAACAGCAAAAACC
Mouse IL-20Ra	Forward	5'-CTGGCCGCTTCGGGACGC
	Reverse	5'-AACCACAGACACAAGGAACTG
Mouse IL-20Rb	Forward	5'-CAGGTGCTTCCAGTCCGTCT
	Reverse	5'-CTCTCCTGGAATCCCCAAAGT
Mouse IL-22Ra	Forward	5'-ATATTGTCCAAGGAAAGTGCCC
	Reverse	5'-AGCCGACGAGGAAACCCAT

Supplementary Figure 1:

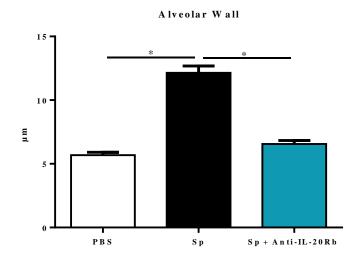
Gating strategy in order to identify NKT cells, Alveolar macrophages (AM), neutrophils and dendritic cells (DC) (Cdc1 (DC CD11 b^+) and cDC2 (DC CD10 3^+).



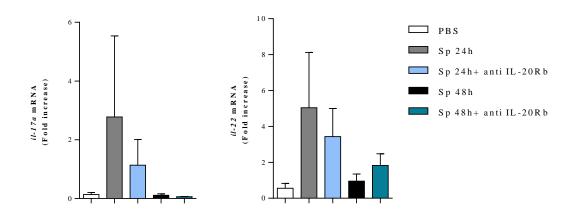
Supplementary Figure 2: Lack of staining was obtained with the isotype control. Lung sections were evaluated by immunohistochemistry a) with the isotype control for the antibodies against the cytokines and b) with the isotype control for the antibodies against the receptors (magnification x200) on lung collected at 24h after the challenge. These samples were prepared from not infected mice (PBS).



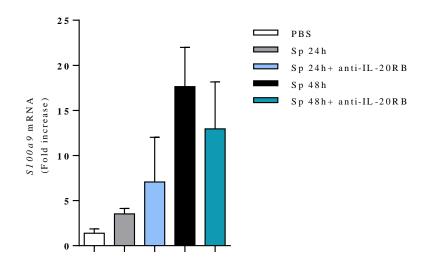
Supplementary Figure 3: Treatment with the anti-IL-20Rb antibody completely prevent the alveolar wall thickening in mice infected by Sp. We have measured the alveolar wall thickness in control mice (PBS) as well as in mice infected with Sp treated or not with the anti-IL-20Rb antibody. Alveolar wall thickening was measured in the peribronchial area by using the software Image J (NIH). We analyzed at least 10 alveolar walls in the lungs of 4 different mice for each group.



Supplementary Figure 4: Anti-IL-20Rb neutralizing antibody did not modulate the production of IL-17 and IL-22. mRNA level for the cytokines IL-17 and IL-22 were evaluated by RT-qPCR in lung tissue at 24h p.i. Results were expressed as fold increase compared to the mice exposed to PBS and using expression of GAPDH as a housekeeping gene. Results were expressed as mean \pm SEM. There was no significant difference between each group.



Supplementary Figure 5: Anti-IL-20Rb neutralizing antibody did not modulate the production of S100A9 mRNA level for the antimicrobial peptide S100A9 were evaluated by RT-qPCR in lung tissue at 24h p.i. Results were expressed as fold increase compared to the mice exposed to PBS and using expression of GAPDH as a housekeeping gene. Results were expressed as mean \pm SEM. There was no significant difference between each group.



Supplementary Figure 6: Treatment with the anti-IL-20Rb antibody did not affect the weight loss and the survival in mice infected by *Sp.* a) the weight was measured during 6 days after the infection and the results were expressed as the percentage of the initial weight. b) the percentage of the surviving mice was reported in the same experiments. The experiment was repeated 2 times with ten mice per group. There was no significant difference between each group.

