

Sex difference in innate inflammatory response and macrophage polarization in *Streptococcus agalactiae*-induced pneumonia and potential role of microRNA-223-3p

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Supplementary data

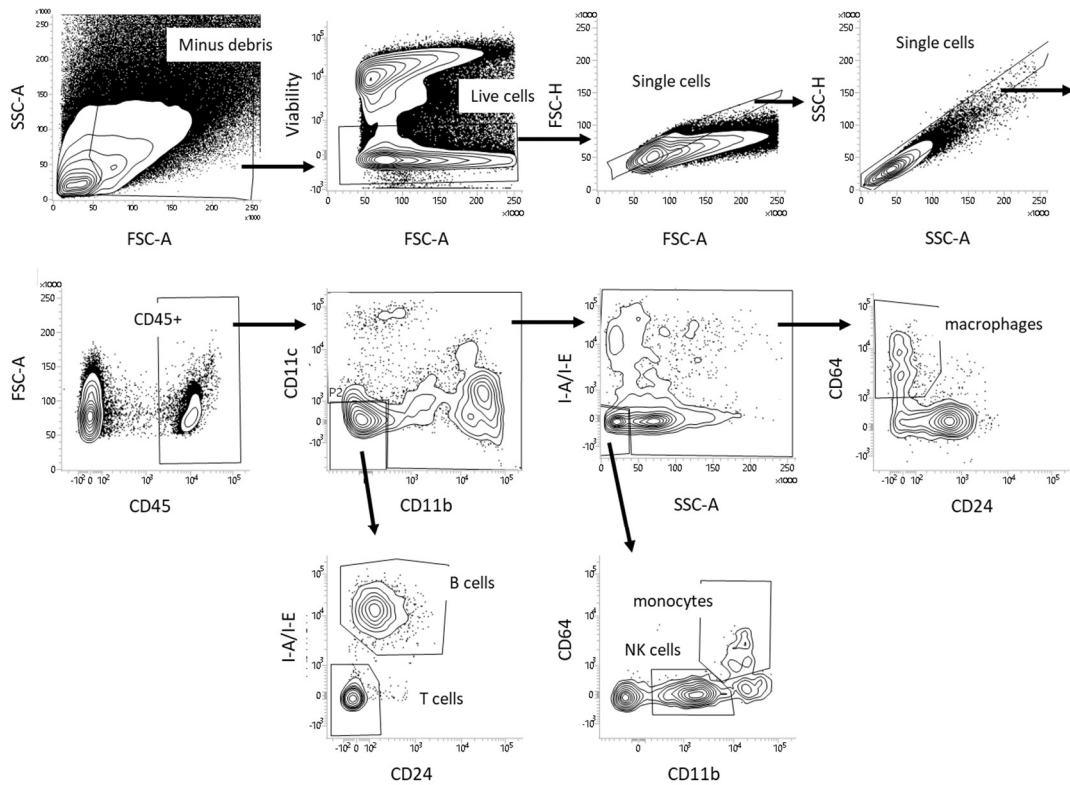


Figure S1. Gating strategy followed in flow cytometry analysis of monocytes, macrophages, B cells, T cells and NK cells in the lungs of naive and GBS-infected mice.

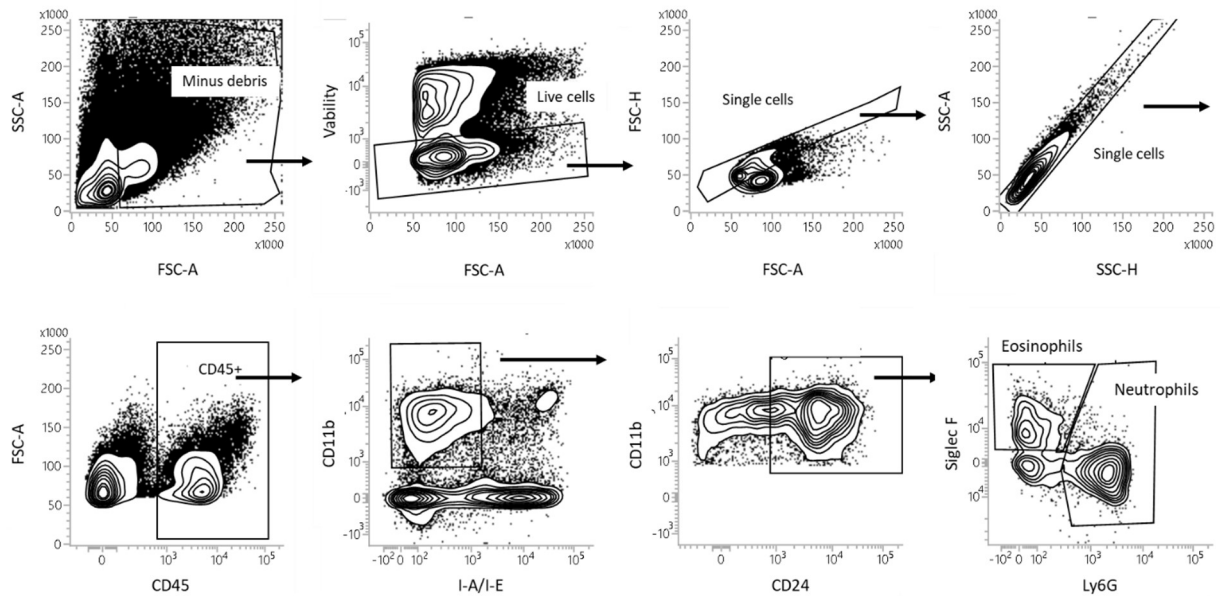


Figure S2. Gating strategy followed in flow cytometry analysis of neutrophils and eosinophils in the lungs of naive and GBS-infected mice.

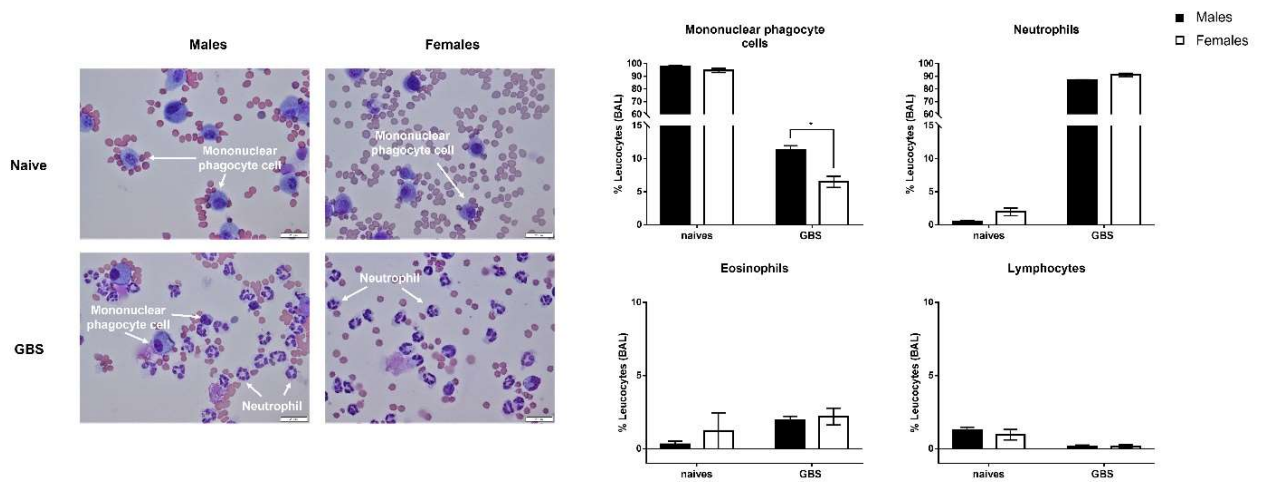


Figure S3. Sex difference in leukocyte cells in bronchoalveolar lavage (BAL) in naive and GBS-infected mice. C57BL/6 male (black) and female (white) mice were infected intranasally with $\pm 10^8$ CFUs of GBS. Age-matched naive male and female mice were used as controls (n=5 for each group). 24h pi, BALs were performed, and the recovered cells were fixed and stained with Diff-Quik staining kit. A minimum of 600 cells were counted by microscopy. Data are means \pm SD. *p<0.05 (Mann-Whitney U test).

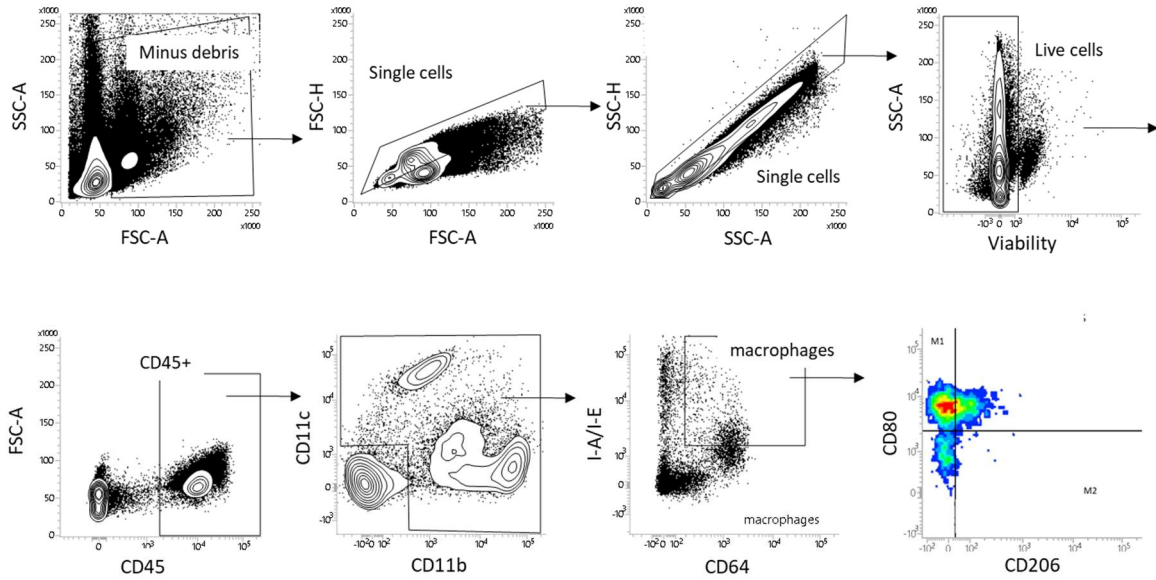


Figure S4. Gating strategy followed in flow cytometry analysis of CD80+CD206- macrophages (M1) and CD80-CD206+ macrophages (M2) in the lungs of naive and GBS-infected mice.

CFUs count before frozen at -80°C with 50% glycerol			CFUs count after thawing and resuspension with sterile PBS at the desired concentration (1*10 ⁹ /mL).		
			Test 1		
<u>Dilution factors</u>	<u>Mean CFUs count</u>	<u>number of GBS in 1 stock tube</u>	<u>Dilution factors</u>	<u>Mean CFUs count</u>	<u>number of GBS/mL</u>
6*10 ⁶	145	8.17*10 ⁸	4*10 ⁶	235	9.4*10 ⁸
1*10 ⁷	52	5.2*10 ⁸	4*10 ⁷	24	9.6*10 ⁸
mean		6.95*10⁸	mean		9.5*10⁸
			Test 2		
<u>Dilution factors</u>	<u>Mean CFUs count</u>	<u>number of GBS/mL</u>			
2*10 ⁷	75	1.5*10 ⁹			
2*10 ⁸	8	1.6*10 ⁹			
mean		1.55*10⁹			

Table S1. Representative data of CFUs (colony forming units) enumeration on Todd Hewitt Agar (THA) media plates with 15mg/mL nalidixic acid, after freezing and thawing and resuspension to the desired concentration.