SUPPLEMENTARY DATA

# Expression and Function of Granzymes A and B in *Escherichia coli* Peritonitis and Sepsis

M. Isabel García-Laorden[1], Ingrid Stroo[1,2], Sanne Terpstra[1], Sandrine Florquin[3], Jan Paul Medema[1,4], Cornelis van´t Veer[1], Alex F. de Vos[1], Tom van der Poll[1,5].

[1]Center for Experimental and Molecular Medicine (CEMM), Academic Medical Center/ University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands.

[2]Department of Immunopathology, Sanquin Research, Plesmanlaan 125, 1066 CX, Amsterdam, The Netherlands.

[3]Department of Pathology, Academic Medical Center/ University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands.

[4]Laboratory for Experimental Oncology and Radiobiology (LEXOR), Academic Medical Center, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands.

[5]Division of Infectious Diseases. Academic Medical Center/ University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands.

Correspondence should be addressed to: M. Isabel García-Laorden, Academic Medical Center, Meibergdreef 9, Room G2-130, 1105 AZ Amsterdam, The Netherlands. Phone: +31-20-5665910; Fax: + 31-206977192; E-mail: ihalemgl@yahoo.es

**Supplementary Table 1:** Percentage and median fluorescence intensity (MFI) of gzmA and gzmB in diverse lymphocyte populations from WT mice during *E. coli* peritonitis.

| **A** | | Granzyme A | | | | Granzyme B | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | CD8+T | CD4+T | γδ T | NK1.1+T | CD8+T | CD4+T | γδ T | NK1.1+T |
| 0h | % | 1.43 | 0.18 | 1.43 | 3.97 | 1.07 | 0.12 | 0.44 | 3.03 |
| | MFI | 384.00 | 408.00 | 375.00 | 522.50 | 549.00 | 429.00 | 404.50 | 595.00 |
| 6h | % | 5.38 | 0.55 | 2.62 | 7.67 | 3.88 | 1.27 | 1.45 | 6.76 |
| | MFI | 420.50 | 380.00 | 421.50 | 1125.00 | 653.00 | 545.00 | 496.00 | 815.00 |
| 14h | % | 9.42 | 2.55 | 2.47 | 25.00 | 13.55 | 3.44 | 8.64 | 34.25 |
| | MFI | 353.50 | 344.00 | 524.00 | 997.00 | 497.50 | 485.00 | 614.00 | 3173.00 |
| 20h | % | 28.70 | 9.60 | 39.96 | 45.15 | 6.38 | 7.09 | 14.20 | 18.30 |
| | MFI | 436.00 | 470.00 | 1080.50 | 780.00 | 631.00 | 404.00 | 580.50 | 667.50 |

| | % | MFI | % | MFI | % | MFI | % | MFI | % | MFI | % | MFI | % | MFI | % | MFI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P 0 vs 6 | * | ns | * | ns | ns | ns | ** | * | ** | ns | ** | ns | * | ns | * | ns |
| P 0 vs 14 | ** | ns | ** | ns | ns | ns | ** | ** | ** | ns | ** | ns | ns | ns | ** | ** |
| P 0 vs 20 | ** | ns | ** | ns | ** | * | ** | ns | ** | ns | ** | ns | ** | ns | ns | ns |
| P 6 vs 14 | ns | ns | ** | ns | ns | ns | ** | ns | * | ns | * | ns | ns | ns | ** | ** |
| P 6 vs 20 | ** | ns | ** | ns | * | ns | * | ns | ns | ns | ns | ns | ns | * | ns | ns |
| P14 vs 20 | ** | ns | * | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ** |

| **B** | | Granzyme A | | | | Granzyme B | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | CD8+T | CD4+T | γδ T | NK1.1+T | CD8+T | CD4+T | γδ T | NK1.1+T |
| 0h | % | 0.34 | 0.26 | 1.14 | 18.80 | 0.05 | 0.04 | 0.24 | 6.57 |
| | MFI | 760.00 | 879.00 | 684.00 | 1078.00 | 786.00 | 764.00 | 1445.00 | 653.00 |
| 6h | % | 1.16 | 0.39 | 2.75 | 19.60 | 1.08 | 0.43 | 3.16 | 21.10 |
| | MFI | 470.00 | 528.50 | 398.50 | 1049.50 | 569.50 | 544.50 | 472.50 | 755.00 |
| 14h | % | 1.68 | 0.42 | 6.85 | 44.00 | 2.34 | 0.80 | 6.64 | 51.55 |
| | MFI | 470.00 | 566.00 | 870.50 | 1438.00 | 549.00 | 592.00 | 696.00 | 1786.50 |
| 20h | % | 1.76 | 1.15 | 3.36 | 36.30 | 2.13 | 1.04 | 4.03 | 36.75 |
| | MFI | 510.00 | 475.00 | 407.00 | 1133.00 | 513.50 | 458.00 | 434.00 | 1090.50 |

| | % | MFI | % | MFI | % | MFI | % | MFI | % | MFI | % | MFI | % | MFI | % | MFI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P 0 vs 6 | * | * | ns | ** | ns | * | ns | ns | * | ns | ** | * | ns | * | ns | ns |
| P 0 vs 14 | ** | * | ns | * | ** | ns | ** | ns | ** | ns | ** | * | ** | ns | ** | * |
| P 0 vs 20 | ns | * | ** | * | * | * | ns | ns | * | ns | * | ** | ns | * | ns | ns |
| P 6 vs 14 | ns | ns | ns | ns | * | * | ** | * | * | ns | ns | ns | ns | ns | ** | ** |
| P 6 vs 20 | ns | ns | * | ns | ns | ns | ns | ns | ns | ns | ns | * | ns | ns | ns | ns |
| P14 vs 20 | ns | ns | ns | ns | ns | * | ns | ns | ns | ns | ** | ns | ** | ns | ns | ns |

Values are medians from 5-6 mice of the percentage of each population of lymphocytes expressing gzmA or gzmB and the MFI in these cells, in uninfected mice and 6, 14 and 20h after infection with $1.3*10^4$ CFU *E. coli*. A. Values in lymphocyte populations from PLF. B. Values in lymphocyte populations from blood. P values of the comparisons between time points in each lymphocyte population were determined by Mann-Whitney U test. ns: non-significant , * P<0.05, ** P<0.01.

**Supplementary Table 2:** Cell counts of leukocytes, NK cells and granzyme-positive NK cells in peritoneal lavage fluid from wild-type mice during *E. coli* peritonitis.

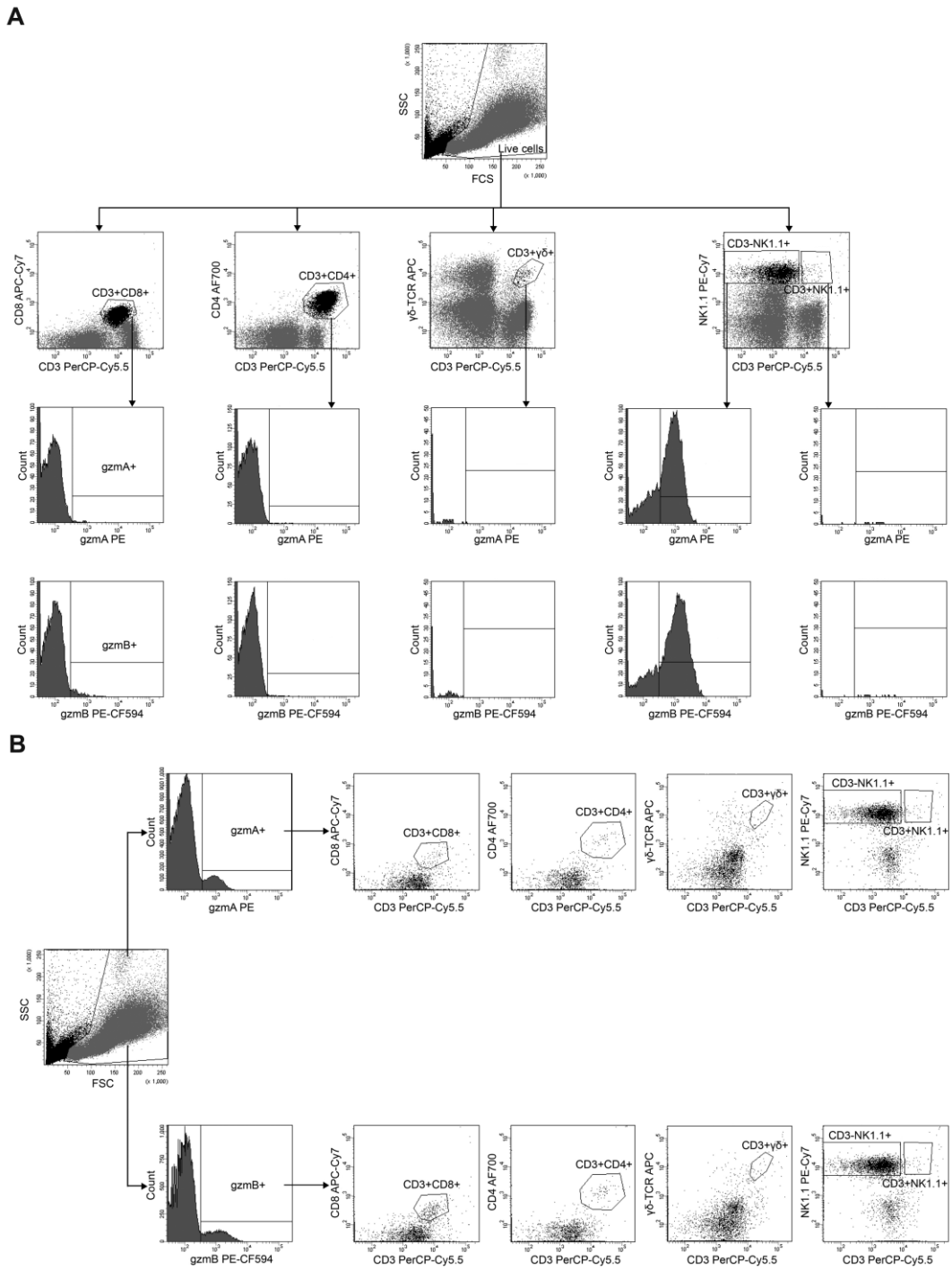
| | 0h | 6h | 14h | 20h |
|---|---|---|---|---|
| Leukocytes (cells/ml) | $5.5*10^5$ [$5.1*10^5$-$6.72*10^5$] | $2.1*10^5$ * [$1.7*10^5$-$3.8*10^5$] | $1.1*10^6$ * ** [$1.0*10^6$-$1.2*10^6$] | $2.8*10^6$ * ** [$1.3*10^6$-$4.2*10^6$] |
| NK (%) | 2.18 [2.1-2.3] | 4.1 * [3.8-4.3] | 5.1 [4.4-5.8] | 14.7 * ** [6.1-26.6] |
| NK (cells/ml) | $1.3*10^4$ [$1.1*10^4$-$1.4*10^4$] | $1.0*10^4$ * [$7.7*10^3$-$1.3*10^4$] | $4.8*10^4$ ** [$4.7*10^4$-$6.8*10^4$] | $2.7*10^5$ * ** # [$1.8*10^5$-$3.4*10^5$] |
| $gzmA^+$ NK cells (cells/ml) | $2.7*10^3$ [$2.6*10^3$-$3.5*10^3$] | $1.4*10^3$ * [$1.1*10^3$-$2.0*10^3$] | $3.1*10^3$ [$2.1*10^3$-$3.8*10^3$] | $3.7*10^3$ ** [$3.2*10^3$-$5.1*10^3$] |
| $gzmB^+$ NK cells (cells/ml) | $5.5*10^2$ [$4.0*10^2$-$7.2*10^2$] | $1.2*10^3$ [$5.2*10^2$-$2.0*10^3$] | $3.6*10^3$ * ## [$2.6*10^3$-$5.7*10^3$] | $2.8*10^3$ § [$1.2*10^3$-$3.7*10^3$] |

Values are medians (interquartile range) from 6 mice per group in uninfected mice and 6, 14 and 20h after infection with $1.3*10^4$ CFU *E. coli*. P values of the comparisons between time points were determined by Mann-Whitney U test. *P<0.01 *vs* 0h, **P<0.01 *vs* 6h, #P<0.01 *vs* 14h, ##P<0.05 *vs* 6h, §P<0.05 *vs* 0h.

**Supplementary Table 3:** Cytokines and chemokine plasma levels of wild-type, *gzmA*$^{-/-}$, *gzmB*$^{-/-}$ and *gzmAxB*$^{-/-}$ mice during *E. coli* peritonitis.

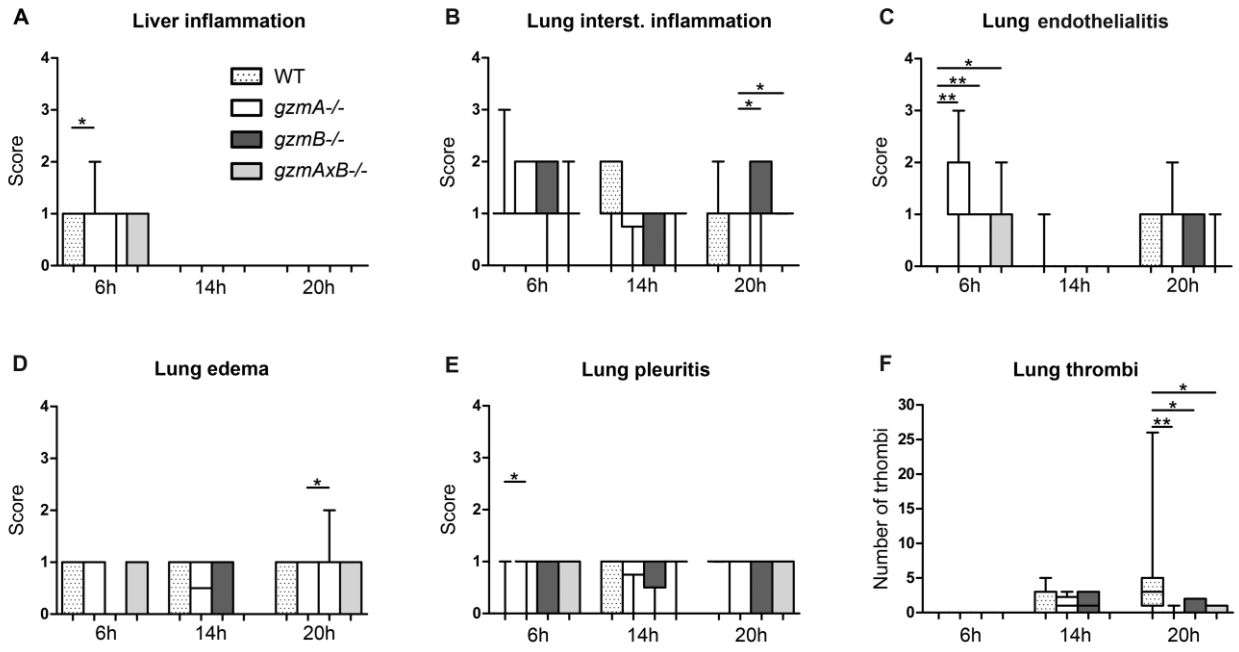
| | TNF-α (pg/ml) | IFN-γ (pg/ml) | IL-10 (pg/ml) | MCP-1 (pg/ml) |
|---|---|---|---|---|
| 6h | | | | |
| WT | 333.1 [65.0-449.8] | 2.1 [2.0-2.8] | 5.0 [0.0-5.0] | 461.4 [211.2-2478.1] |
| *gzmA*$^{-/-}$ | 380.4 [246.8-1154.5] | 1.9 [1.8-2.4] | 5.0 [5.0-10.2] | 2402.4 [1784.3-8856.3] |
| *gzmB*$^{-/-}$ | 509.1 [277.9-567.6] | 1.6 [1.3-3.7] | 11.1 [5.0-15.0] | 4944.6$^{*}$ [2913.9-10000.0] |
| *gzmAxB*$^{-/-}$ | 352.6 [160.7-722.3] | 2.1 [1.7-3.3] | 5.0 [0.0-20.6] | 3109.9 [1673.3-7177.0] |
| 14h | | | | |
| WT | 242.9 [191.1-658.5] | 13.9 [8.7-18.7] | 26.0 [10.3-39.3] | 3226.9 [2048.5-9119.0] |
| *gzmA*$^{-/-}$ | 504.3 [345.1-905.8] | 4.9 [3.7-8.2] | 42.8 [35.4-55.3] | 4794.1 [3412.8-9057.4] |
| *gzmB*$^{-/-}$ | 340.0 [211.8-403.5] | 3.4 [3.0-5.5] | 39.2 [27.2-87.2] | 4194.4 [2537.9-5862.2] |
| *gzmAxB*$^{-/-}$ | 500.5 [343.9-594.6] | 3.7$^{A}$ [3.0-4.2] | 55.0$^{A}$ [48.2-60.3] | 6612.6 [3768.6-8089.8] |
| 20h | | | | |
| WT | 359.8 [306.0-379.1] | 2.9 [2.2-3.6] | 30.1 [21.8-38.7] | 5106.1 [3644.4-5946.9] |
| *gzmA*$^{-/-}$ | 471.0 [206.6-595.7] | 2.1 [0.0-5.0] | 38.7 [13.3-94.1] | 5759.5 [2819.2-7402.9] |
| *gzmB*$^{-/-}$ | 460.7 [423.1-683.0] | 11.7 [8.9-30.6] | 47.2 [36.6-49.8] | 6246.2 [5911.7-6921.4] |
| *gzmAxB*$^{-/-}$ | 305.0 [263.6-737.4] | 0.0$^{\#}$ [0.0-2.2] | 34.5 [24.3-74.9] | 4724.6 [3326.6-5834.8] |

Values are medians (interquartile range) from 7-8 mice per group 6, 14 and 20h after infection with 1.3*10$^{4}$ CFU *E. coli*. $^{*}$P<0.05 *vs* WT, $^{\#}$P<0.05 *vs gzmB*$^{-/-}$ by Mann-Whitney U test.




**Supplementary Figure 1: Gating strategy for the analysis of the expression of granzymes A and B by lymphocyte populations in wild-type mice.** Intracellular expression of gzmA and B by lymphocyte populations was analysed in peritoneal lavage fluid (PLF) and blood from wild-type mice by flow cytometry. Leukocytes region was gated on the basis of forward (FSC) and side scattering (SSC) characteristics. A. $CD8^+$ T ($CD3^+CD8^+$), $CD4^+$ T ($CD3^+CD4^+$), γδ T ($CD3^+$γδ $TCR^+$), $NK1.1^+$ T ($CD3^+NK1.1^+$) and NK ($CD3^-NK1.1^+$) cells were identified by dot-plots, and the percentage of $gzm^+$ cells in each lymphocyte population as well as the median fluorescence intensity (MFI) of the positive expression were determined in histogram plots. B. $GzmA^+$ and

gzmB$^+$ cells were identified by histogram plots, and the percentage of cells corresponding to each lymphocyte population within the gzm$^+$ cells were determined in dot-plots. Data shown are of blood from a representative individual (gating of PLF samples was done similarly as for blood).




**Supplementary Figure 2: Histopathology of liver and lung from wild-type, *gzmA*$^{-/-}$, *gzmB*$^{-/-}$ and *gzmAxB*$^{-/-}$ mice during *E. coli* peritonitis.** Mice were infected intraperitoneally with 1.3*10$^4$ CFU *E. coli* and sacrificed at 6, 14 and 20h after infection. Data are box-and-whisker diagrams depicting the smallest observation, lower quartile, median, upper quartile and largest observation. N = 7-8 per group at each time point. * P<0.05, ** P<0.01 determined by Mann-Whitney U test.