1051

Increased susceptibility to *Streptococcus pneumoniae* pulmonary infection in the Eμ-TCL1 adoptive transfer mouse model of CLL

Ana Colado[1], Juliana Bernatowiez[1], Chiara Cassarino[1], Valeria Sarapura Martinez[1], Martin Bertini[2], Fernando Bezares, Mónica Vermeulen[1], Romina Gamberale[1], Mirta Giordano[1], Mercedes Borge[1]. [1]*Laboratorio de Inmunología Oncológica, Instituto de Medicina Experimental-CONICET-Academia Nacional de Medicina, Bs. As., Argentina.* [2]*Hospital General de Agudos Dr. Teodoro Álvarez, Bs. As., Argentina.* Contact: **coladoana@gmail.com**

BACKGROUND AND AIM Infectious complications continue to be a major cause of morbidity and mortality in patients with CLL. The reason of this increased risk is multifactorial and is associated with immunosuppression generated by treatment, but also by the disease itself. In fact, treatment-naïve patients are at higher risk of infections than healthy population, and infections during the treatment-naïve period are associated with worse treatment-free survival and overall survival (Andersen, M. A. et al. Haematologica 2018). Bacterial pneumonia, involving *Streptococcus pneumoniae* (*Spn*), is a frequent serious infection among untreated patients (Clare Sun, et al. Hematol Oncol Clin N Am. 2021). Early immune response to Spn is critical to control bacterial burden and disease outcome. Although innate immune alterations were described in CLL, if these mechanisms are defective in the Spn in vivo response is unknown. By using a murine adoptive transfer model of CLL (AT-TCL1) **we aimed to study the susceptibility to Spn pulmonary infection in the context of untreated CLL and to characterize innate immune parameters involved in the early response to the bacteria.**

METHODS Female C57BL/6 mice (8-10 weeks old) were intraperitoneally injected with 20 x 10^6 splenocytes from Eμ-TCL1 mice (C57BL/6 background). Adoptively transferred (AT)-TCL1 mice were considered leukemic when they have > 60% of $CD5^+$ $CD19^+$cells in peripheral blood (PB) assessed by flow cytometry (FC). AT-TCL1 leukemic mice and control mice (age-matched C57BL/6) were intranasally (i.n.) infected with 2 x 10^6 colony forming units (CFU) of *Spn* serotype 3 (clinical isolate). For survival experiments, mice were daily controlled for 11 days. In other set of experiments, mice were euthanized 24 hs after infection and bronchoalveolar lavage fluid (BALF) or lungs were obtained. BALF was used to determine: CFU by serial dilution in blood agar plates; cell number and phenotype by FC; TNF-α, IL-1β and CXCL1 by ELISA and; total protein, Lactate Dehydrogenase (LDH) and myeloperoxidase (MPO) by colorimetric assays. For cryopreservation, lungs were filled with a mixture of OCT:PFA 8% (1:1), embedded in OCT and then sectioned in a cryostat in 20-μm thickness. Sections were then stained and analysed by confocal microscopy (CM). Non-infected control and leukemic mice were used to evaluate natural a*Spn*-IgM by ELISA in plasma, peripheral blood neutrophils phenotype with CD62L and CD11b staining and also to obtain bone marrow (BM) for *ex vivo* phagocytosis experiments. Phagocytosis was confirmed by CM. *Spn* was labelled with CFSE. Statistical analysis was performed with Prism v7 (GraphPad). Values of p<0.05 was considered significant.

Figure 1. *Spn*-Infected AT-TCL1 mice have a higher mortality rate and a higher bacterial burden in BALF than control mice.

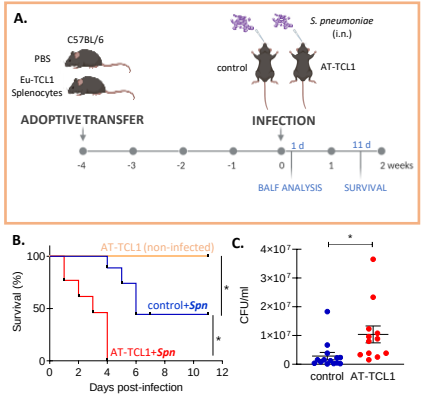

Fig 1. A. Study design. **B.** Survival of control + *Spn* (infected), AT-TCL1+ *Spn* (infected) and AT-TCL1 (non-infected) mice. n=4/group, 3 independent exp. Log-rank (Mantel-Cox) test, *statistically significant, p values corrected by the Bonferroni method for multiple comparisons . **C.** Control and AT-TCL1 were euthanized 24 h after infection, BALF was obtained, and CFU/ml were counted by plating serial dilutions in blood agar plates. 4 independent exp. Mean ± SEM *p<0.05, Mann-Whitney test.

Figure 2. *Spn*-Infected AT-TCL1 mice have higher levels of inflammatory parameters in BALF than control mice.

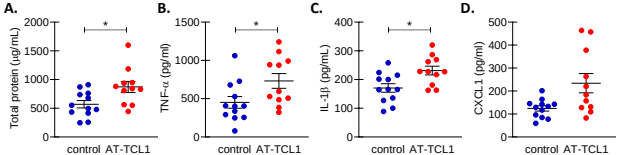

Fig 2. Control and AT-TCL1 mice were euthanized 24 h after infection and the BALF was obtained to determine: total protein **(A)** with the BCA Protein Assay Kit and TNF-α **(B)**, IL-1β **(C)** and CXCL1 **(D)** by ELISA. Mean ± SEM *p<0.05, Mann-Whitney test.

Figure 3. *Spn*-Infected control and AT-TCL1 mice have similar lung damage.

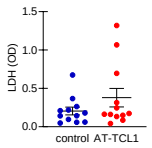

Fig 3. Control and AT-TCL1 were euthanized 24 h after infection and the BALF was obtained to determine Lactate Dehydrogenase (LDH) with a colorimetric commercial kit. Mean ± SEM.

Figure 4. Despite similar neutrophil number, *Spn*-Infected AT-TCL1 mice have lower levels of MPO in BALF than control mice.

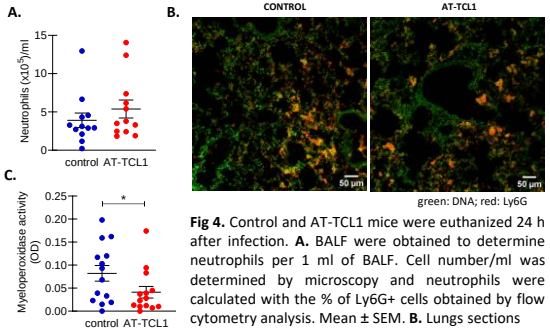

Fig 4. Control and AT-TCL1 mice were euthanized 24 h after infection. **A.** BALF were obtained to determine neutrophils per 1 ml of BALF. Cell number/ml was determined by microscopy and neutrophils were calculated with the % of Ly6G+ cells obtained by flow cytometry analysis. Mean ± SEM. **B.** Lungs sections were stained with Ly6G and DAPI, mounted and analysed by CM. **C.** Myeloperoxidase (MPO) was determinate with a colorimetric commercial kit in the BALF. Mean ± SEM *p<0.05, Mann-Whitney test.

NON-INFECTED MICE

Figure 5. Neutrophils from AT-TCL1 mice show an altered phenotype and similar ex vivo phagocytic capacity, compared to control mice.

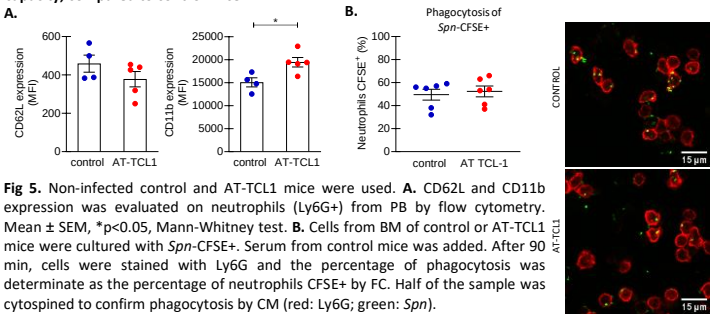

Fig 5. Non-infected control and AT-TCL1 mice were used. **A.** CD62L and CD11b expression was evaluated on neutrophils (Ly6G+) from PB by flow cytometry. Mean ± SEM, *p<0.05, Mann-Whitney test. **B.** Cells from BM of control or AT-TCL1 mice were cultured with *Spn*-CFSE+. Serum from control mice was added. After 90 min, cells were stained with Ly6G and the percentage of phagocytosis was determinate as the percentage of neutrophils CFSE+ by FC. Half of the sample was cytospined to confirm phagocytosis by CM (red: Ly6G; green: *Spn*).

Figure 6. Lower levels of anti-*Spn* IgM in AT-TCL mice is accompanied with a lower capacity to induce *Spn* phagocytosis by control neutrophils.

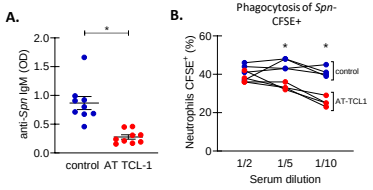

Fig 6. Non-infected control and AT-TCL1 mice were used. **A.** Natural anti-*Spn* IgM was evaluated by ELISA in plasma from control and AT-TCL1 mice. Mean ± SEM. **B.** Cells from control mice-BM were cultured with *Spn*-CFSE+. Different serum dilutions from control or AT-TCL1 mice were added to cultures. After 90 min, cells were stained with Ly6G and the percentage of neutrophils CFSE+ were evaluated by FC. *p<0.05, Mann-Whitney test.

CONCLUSIONS

Our results show that CLL predisposes to an impaired control of bacterial growth and to a higher mortality rate in a mouse model of *Spn* pulmonary infection. In addition, we found that leukemic mice have defects in early immune mechanisms that might contribute to the increased susceptibility to Spn such as a decrease on natural IgM anti-Spn, an altered neutrophil phenotype and lower levels of MPO released by neutrophils during Spn infection.