and C8-C1P-treated monocytes showed lower expression levels of CD80 and CD44, and CD80 and HLA-DR after 24 and 48h, respectively. Interestingly, hMø differentiated with a higher (20μM) or a lower (1μM) concentration of C8-C1P upregulated genes related to tissue-repair and resolution of inflammation like VEGFA, MER and PPARG and downregulated IRF1, a pro-inflammatory signature. Moreover, C8-C1P-differentiated hMø supernatants increased ECFC *in vitro* tubule formation only with 20μM, probably due to increased levels of proangiogenic secreted factors. In conclusion, C8-C1P not only augmented monocytes survival and reduced their activation, but also affected hMø differentiation by conferring them pro-resolving and tissue-repair functions. Our results highlight the therapeutic potential of C1P to improve wound healing.

456. (240) **CONSTRUCTION OF FLUORESCENT-TAGGED ADENOVIRAL VACCINE CANDIDATE AS A TOOL FOR STUDYING IMMUNE RESPONSES UPON VACCINATION**

Delfino MA[1,2,3], Trinitario SN[1,2,3], Cardoso A[1,2,3], Russo M[1,2], Cerny N[1,2], Bivona A[1,2,3], Malchiodi EL[1,3], Sánchez Alberti A[1,2,3]

1. Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Instituto de Estudios de la Inmunidad Humoral (IDEHU). Facultad de Farmacia y Bioquímica. Buenos Aires, Argentina.

2. Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPAM). Facultad de Medicina. Buenos Aires, Argentina.

3. Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Cátedra de Inmunología. Buenos Aires, Argentina.

DNA vaccines are efficient Th1 and CD8 inducers and have shown efficacy to control intracellular pathogens such as *Trypanosoma cruzi*. Live attenuated vectors, like rare serotype Adenovirus, used as vaccine DNA-delivery system, improve immunogenicity and guarantee a strong and long-lasting response.

Considering these facts, we generated a vaccine based on rare serotype human adenovirus (Ad48) carrying Traspain gene, a novel *T. cruzi* chimeric antigen developed in our laboratory. With the aim of studying immune activation by this Ad serotype and the spatiotemporal tracking of the antigen we developed an Ad48 carrying Traspain gene fused with the monomeric red fluorescent protein mScarlet and analyzed its performance.

mScarlet tagged Traspain was constructed by traditional cloning. Ad48-Traspain-mScarlet virus was obtained by homologous recombination in HEK-293 cells, 15 days post-transfection.

Seven clones were isolated by agarose plaque assay and further analyzed. Traspain-mScarlet gene was detected by PCR, *in vitro* expression demonstrated by Western-Blot and Fluorescent Microscopy in infected cells showed full cytopathic effect.

Three brighter clones were compared employing a high-throughput imaging system (*IN-Cell Analyzer 2200*, GE). Clone 2 was selected because it showed a signal/noise ratio of 100 and 2-fold mScarlet MFI compared to other ones. Purification of this clone by sucrose density gradient ultracentrifugation, resulted in titers higher than 2.10^8 TCID50/ml. Low rate of impurities were found by SDS-PAGE and A_{280}/A_{260} ratio = 1.40-1.60.

Traspain specific immune response was assessed by flow cytometry after immunization of C57BL6 mice with two subcutaneous doses of the virus. A strong antigen-specific CTL response was detected by tetramer staining of whole blood from immunized mice.

In conclusion, the recombinant viral vector Ad48 carrying Traspain-mScarlet was generated and its *in vitro* and *in vivo* performance confirmed the feasibility of the vaccine approach.

457. (247) **INNATE CD8⁺ T CELLS: FROM THE THYMUS TO THE SECONDARY LYMPHOID ORGANS (SLO) IN STEADY STATE VERSUS *TRYPANOSOMA CRUZI* INFECTION**

Viano ME[1], Savid-Frontera C[1], Baez NS[1], Matellón M[1], Cerbán F[1], Rodriguez-Galán MC[1]

[1.] Centro de Investigaciones en Bioquímica Clínica e Inmunología – CIBICI-CONICET. Facultad de Ciencias Químicas - Universidad Nacional de Córdoba

Simple positive CD8⁺ thymocytes (SP8) that develop in the thymus could give rise either to conventional SP8 or to Innate CD8⁺ T cells (T_{IM}). T_{IM} acquire a memory phenotype during their thymic maturation and are exported to SLO as a conventional T cell. T_{IM} play a protective role during the early phase of infectious processes as reported for certain bacteria, viral and parasite infections. Our previous results demonstrated that during *T. cruzi* infection, a large number of T_{IM} mature in the thymus due to local production of IL-4 and IL-15, 2 cytokines responsible for their maturation/maintenance process. T_{IM} functionally act in a TCR-independent way; instead they are activated through cytokines as IL-12 and IL-18. By using OT-I mice (not RAG2 KO, that carry an OVA specific TCR in most of SP8 cells) we could compare the expression of a large number of markers between OVA tetramer⁺ (OVAt⁺) SP8 cells (not specific for the parasite) and conventional polyclonal SP8 cells simultaneously present in the thymus of control and *T. cruzi* infected mice. Data demonstrate that OVAt⁺ SP8 cells expressed higher levels of CD44, CD122, CD5, CD69, QA2 and decreased levels of CD24 compared to conventional SP8 cells while other markers like CD62L, PD-1 and CD5 seem not to be differentially expressed ($p<0,05$). Moreover, this pattern is even more pronounced after *T. cruzi* infection ($p<0,05$) demonstrating that OVAt⁺ SP8 cells adopt a T_{IM} phenotype in and Ag-independent way after infection. Expression of S1PR1 and S1PR4, that allow mature SP8 thymocyte to be exported to SLO, is downregulated in the bulk thymocyte population from *T. cruzi*-infected compared to control mice. In correlation with these data, exportation experiments performed by labeling thymocytes with CFSE (using intrathymic injection) demonstrated a significant lower number of CD8-CFSE⁺ cells in SLO of *T. cruzi* infected mice ($p<0,05$). Our data contribute to understand the maturation and exportation process of T_{IM} that is still poorly described in the scientific literature.

458. (249) **THYMIC STROMAL LYMPHOPOIETIN (TSLP) IS A NEUTROPHIL CELLS MODULATOR: RELEVANCE IN THE PATHOGENESIS OF BRAIN TUMORS**

Coronel JV [1], Iturrizaga J [1;2], Infante A [1], Jancic C [1;3], Vermeulen M [1;3], Candolfi M [5], Villaverde M [6],Rabadán A [2], Lenicov FR [4], Salamone G [1;3].

[1] Instituto de Medicina Experimental (IMEX) CONICET – Academia Nacional de Medicina, Buenos Aires, Argentina.

[2] División Neurocirugía del Instituto de Investigaciones Médicas A Lanari. Universidad de Buenos Aires. Universidad de Buenos Aires.

[3] Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, Universidad de Buenos Aires. Buenos Aires, Argentina.

[4] Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), Universidad de Buenos Aires – CONICET, Argentina.

[5] Instituto de Investigaciones Biomédicas (INBIOMED UBA-CONICET), Facultad de Medicina, UBA.

[6] Unidad de Transferencia Genética, Área Investigación, Inst. de Oncología Ángel H. Roffo, Facultad de Medicina, UBA.

Background: Glioblastoma (GBM) is the most devastating brain tumor, with an associated poor prognosis. Despite the advances in surgery and chemoradiation, the survival of GBM patients has not improved significantly in the past three decades. Thymic stromal lymphopoietin (TSLP) is a cytokine produced primarily by activated epithelial cells and it has been shown to be a key factor in maintaining immune homeostasis and regulating inflammatory responses at mucosal barriers. However, recent studies have found an expanding role for TSLP in inflammatory diseases and cancer.

This work aimed to elucidate the relevance of TSLP in the interaction between neutrophils and GBM cells. First, we evaluated the production of TSLP by tumor cells through RT-PCR and then, we determined whether the TSLP treatment affects the cross-talk between neutrophils and tumor GBM cells. For those purposes, human U251 cell line or tumoral cells obtained from a GBM patient were co-cultured with human neutrophils. We observed that the U251 cell line or the primary cell culture incubated with Epidermal Growth Factor (EGF) produced TSLP ($p<0.05$). Sequencing of the PCR product confirmed it was TSLP. Additionally, the neutrophils obtained from