Characterisation of Trojan: a novel leukocyte protein

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Aiming to identify molecules involved in T cell development, we cloned and characterised a novel avian cell surface protein, called “Trojan”, from embryonic thymocyte cDNA library.

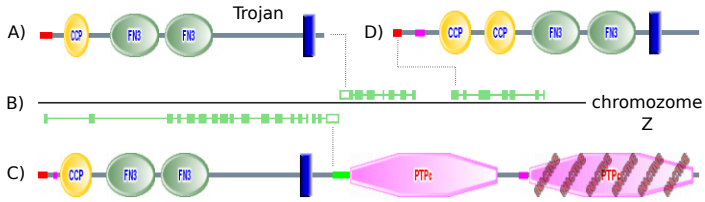

Figure 1. Trojan and family members. Trojan (A) is a type I transmembrane protein, predicted to have a Complement control protein (CCP) domain, two Fibronectin type III (FN3) domains and a short cytoplasmic tail with two possible serine phosporylation sites. Its gene is matched to chromosome Z (B) of the publicly available databases. The adjacent upstream and downstream genes encode for hypothetical Trojan-like proteins: receptor type protein tyrosine phosphatase (C) and a membrane protein having pairs of CCP and FN3 domains (D).

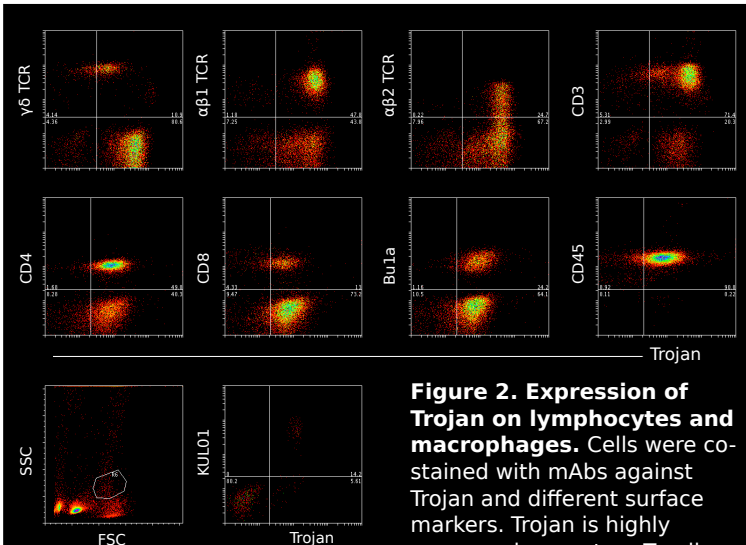

Figure 2. Expression of Trojan on lymphocytes and macrophages. Cells were co-stained with mAbs against Trojan and different surface markers. Trojan is highly expressed on mature T cell populations (defined by CD3, the three types of TCR, CD4 and CD8), B cells (defined by Bu1a), macrophages (defined by KUL01) and leukocytes in general (defined by CD45).

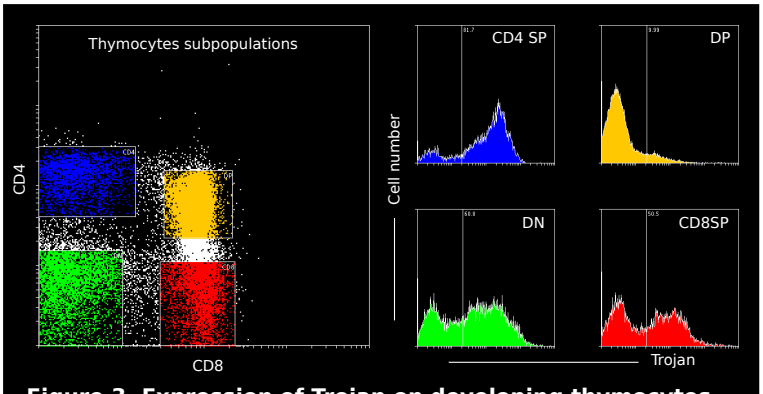

Figure 3. Expression of Trojan on developing thymocytes. Cells were analysed for their expression of CD4 and CD8 (left), then sub-populations were gated and further analysed for their expression of Trojan (right). Trojan has a moderate expression on DN cells, diminishes from the surface of DP cells and re-appears on the surface of SP cells. This expression pattern, being similar to that of anti-apoptotic proteins like IL-7R and Bcl-2, makes Trojan an attractive candidate for an anti-apoptotic or proliferative role.

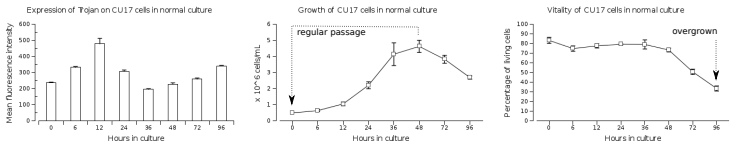

Figure 4. Expression of Trojan on a T cell line (CU17) in normal culture. Expression level was analysed by flow cytometry on living, non-apoptotic cells, as defined by Annexin V staining and FSC/SSC properties. Trojan showed highest expression at the *lag/log* phase transition and in the death phase, in consent with the hypothesised proliferative and/or anti-apoptotic role.

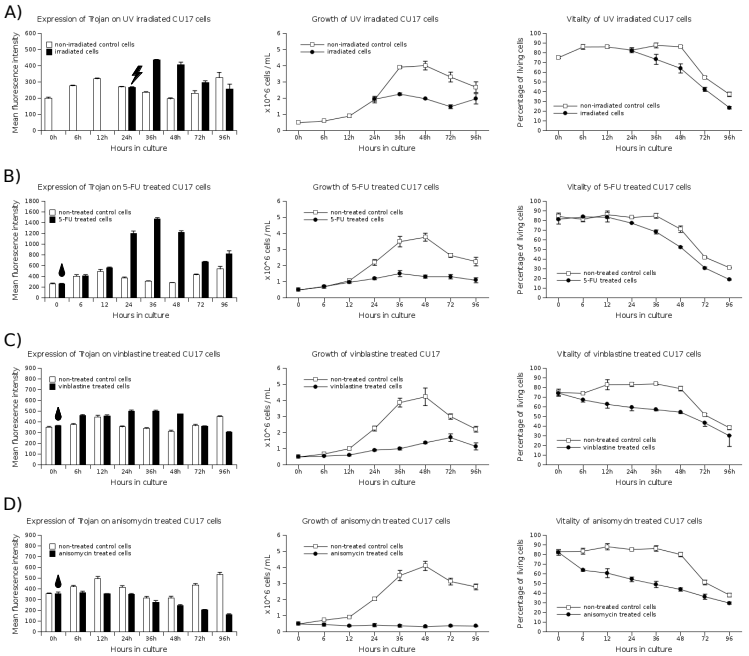

Figure 5. Expression of Trojan on CU17 cells after apoptosis induction. Following the experimental strategy of Fig. 4, Trojan expression was analysed on cells treated with: A) Ultraviolet light (DNA damage); B) 5-fluorouracil (dTTP depletion); C) Vinblastine (mitotic spindle block); D) Anisomycin (protein synthesis inhibition). Upon apoptosis induction, Trojan expression generally rises on the surface of non-apoptotic cells, in support of the proposed pro-survival/anti-apoptotic role. Treatment with anisomycin is an exception, suggesting that *de novo* protein synthesis is required for the increase of Trojan expression.

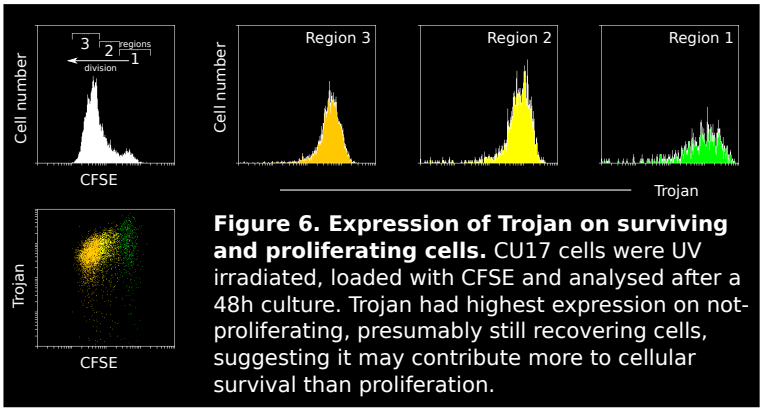

Figure 6. Expression of Trojan on surviving and proliferating cells. CU17 cells were UV irradiated, loaded with CFSE and analysed after a 48h culture. Trojan had highest expression on not-proliferating, presumably still recovering cells, suggesting it may contribute more to cellular survival than proliferation.

Trojan belongs to a novel protein family and is highly expressed on the surface of leukocytes. During T cell development, Trojan shows an expression pattern similar to that of anti-apoptotic proteins. In addition, its expression rises upon cell proliferation, nutrients depletion and in responce to apoptotic inducers *in vitro*. This data suggests that Trojan has a proliferative and/or anti-apoptotic role.