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ROLE OF TRANSFORMING GROWTH FACTOR β1 IN LYMPHOCYTE DEVELOPMENT AND DEATH

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INTRODUCTION. Transforming growth factor β1 (TGFβ1) is a polypeptide growth factor known to exert multiple functions during development and in the adult stage as well (1-2). TGFβ1 knockout mice are normal at the time of birth and do not exhibit any developmental defect. After one week of birth these mice start developing multifocal inflammatory lesions and eventually die around three weeks of age (3). Further studies revealed that T lymphocytes are the primary effectors in this phenotype (Doetschman, unpublished observation). During T cell development in the thymus, T cell progenitors undergo massive proliferation and around 95% of them undergo apoptosis. Positively selected $CD4^+CD8^+$ double positive T cells undergo thymic selection where cells that recognize self-antigens with high affinity are induced to undergo apoptosis (negative selection) (4-5). Any perturbations in the thymic education process might lead to export of self-reactive T cells to the periphery. In order to find the role of TGFβ1 in preventing the inflammation, we have studied the lymphocyte apoptosis and proliferation.

METHODS. Annexin-V apoptosis kit from BD-Pharmingen was used to detect apoptosis. Single cell preparations from thymus and spleen were prepared, RBCs were depleted using ammonium chloride lysis buffer, washed twice with chilled PBS and suspended in 1x binding buffer at $1x10^6$/ml. 5µl of PE labeled annexin-V (binds to phosphatidyl serine on apoptotic cells) and 5µl of 7-AAD (viability marker) added to 100µl of cell suspension, mixed gently and incubated at room temperature for 20 minutes in the dark. After the incubation 400µl of 1x binding buffer was added to the cell suspension and analyzed in a Beckman Elite flow cytometer.

Proliferation was measured using BrdU flow kit from BD-Pharmingen.

RESULTS. Analysis of apoptosis levels revealed no significant role for TGFβ1 in vivo (Fig. 1). Extent of apoptosis is varied from 10-20% in thymocytes and 20-30% in splenocytes irrespective of age, and TGFβ1 genotype. Analysis of proliferation of thymocytes revealed that T cells from $TGF\beta1^{-/-}$ mice are hyper responsive as assessed by tritiated thymidine and BrdU incorporation (results will be presented and discussed during poster presentation).

Apoptosis of Thymocytes and Splenocytes (Day 9)

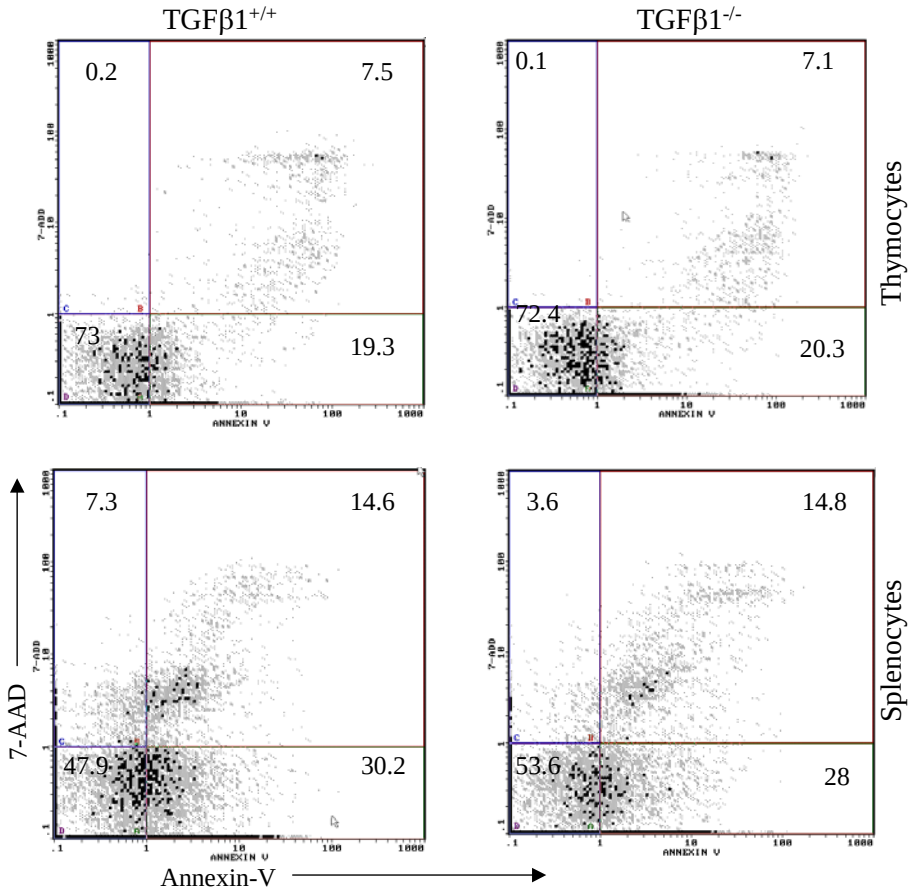

Fig. 1

DISCUSSION. Based on our observations, we propose that TGFβ1 doesn't prevent apoptosis. The minor differences observed between $TGF\beta1^{+/+}$ and $TGF\beta1^{-/-}$ lymphocytes are might be the result of hyperresponsiveness of $TGF\beta1^{-/-}$ T cells. TGFβ1 negatively regulates lymphocyte proliferative responses. Altered activation signal threshold level of lymphocytes might lead to activation and accumulation of self-reactive T cells in the periphery.

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REFERENCES.

1. Massague, J., Blain, S.W, and Lo, R.S. (2000) Cell 103, 295-309
2. Massague, J. (2000) Nat. Rev. 1, 169-178
3. Shull, M.M., Ormsby, I., Kier, A.B., Pawlowski, S., Diebold, R.J., Yin, M., Allen, R., Sidman, C., Proetzel, G., Calvin, D., et al. (1992) Nature 359, 693-699

4. Vasquez, N.J., Kaye, J., and Hedrick, S.M. (1992) J. Exp. Med. 175, 1307-1316
5. van Meerwijk, J.P., Marguerat, S., Lees, R.K., Germain, R.N., Fowlkes, B.J., and MacDonald, H.R. (1997) J. Exp. Med. 185, 377-383
6. Gong, Q., Cheng, A.M., Akk, A.M., Alberola-Ila, J., Gong, G., Pawson, T., and Chan, A.C. (2001) Nat. Immunol. 2, 29-36