ROLE OF TRANSFORMING GROWTH FACTOR β 1 IN LYMPHOCYTE DEVELOPMENT AND DEATH

Ramireddy Bommireddy*, Ilona Ormsby, Moying Yin, and Thomas C. Doetschman

Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati
College of Medicine, Cincinnati, OH 45267-0524, USA

* bommirr@email.uc.edu

INTRODUCTION. Transforming growth factor β 1 (TGF β 1) is a polypeptide growth factor known to exert multiple functions during development and in the adult stage as well (1-2). TGF β 1 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 Further studies revealed that T lymphocytes are the primary effectors in this phenotype (Doetschman, unpublished observation).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 TGF β 1 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 1x10⁶/ml. 5 μ l of PE labeled annexin-V (binds to phosphatidyl serine on apoptotic cells) and 5 μ l of 7-AAD (viability marker) added to 100 μ l of cell suspension, mixed gently and incubated at room temperature for 20 minutes in the dark. After the incubation 400 μ l 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 TGF β 1 in vivo (Fig. 1). Extent of apoptosis is varied from 10-20% in thymocytes and 20-30% in splenocytes irrespective of age, and TGF β 1 genotype. Analysis of proliferation of thymocytes revealed that T cells from TGF β 1^{-/-} 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 TGFβ1^{+/+} and TGFβ1^{-/-} lymphocytes might be the result of hyperresponsiveness of TGFβ1^{-/-} 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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