EXTRATHYMIC GENERATION AND ANTIGEN RECOGNITION OF IL3-INDUCED CD4$^-$CD8$^-$ $\alpha\beta$T CELLS

Hiroshi KUBOTA

Department of Immunology and Cell Biology, Faculty of Medicine, Kyoto University, Kyoto 606, Japan

Analysis of T cell receptors (TCR) of a series of CD4$^-$CD8$^-$ (double negative, DN) $\alpha\beta$T cell lines induced by the combined interleukin 3 (IL3) and IL2 culture system revealed that their V gene usage was biased for Vα4 and Vβ2. This has been confirmed in the primary short-term cultures. Thus, IL3, but not IL2, induced the generation of DN $\alpha\beta$T cells with predominant Vβ2 gene expression from the CD4$^+$/CD8$^+$ T cell-depleted spleen or bone marrow (BM) cells of normal BALB/c mice within 10 days. It was strongly suggested that the DN $\alpha\beta$T cells were derived from the prethymic progenitors, because they could be similarly generated with IL3 from the spleen and BM cells of young nude mice as well as from fetal spleen cells, both of which were devoid of post thymic mature T cells. It was further indicated that the Vβ2$^+$ β chain genes contained few junctional N region in both IL3-induced primary DN $\alpha\beta$T cells and continuous lines. Interestingly, the majority of such generated DN $\alpha\beta$T cells carried homologous $\alpha\beta$TCR (Vα4JαTA28/Vβ2Dβ1.1Jβ2.6). Search for the *in vivo* counterpart of *in vitro* IL3-induced DN $\alpha\beta$T cells revealed that BM, but not spleens, of normal BALB/c and B6 mice did contain a significant proportion of DN $\alpha\beta$T cells, and that the majority of them expressed Vβ2$^+$ β chain genes with few junctional N region. The presence of Vβ2$^+$ DN $\alpha\beta$T cells was similarly observed in the BM of BALB/c nude mice, but their proportion varied markedly among various strains of mice, which was not linked to H-2 haplotypes. The results indicated that Vβ2$^+$DN $\alpha\beta$T cells in the BM represented one of the thymus-independent T cell populations, whose development was under the MHC-unlinked genetic contol. In order to gain insight into the recognition manner of the unique TCR repatoire, the identification of the antigen corresponding to the homogeneous Vα4/Vβ2 TCR was attempted, taking advantage of the fact that growth of T cell hybridomas was arrested by their TCR stimulation. Results indicated that both syngeneic and allogeneic thymocytes, particularly from the newborn mice, could specifically inhibit the proliferaton of a T cell hybridoma with the particular Vα4/Vβ2 TCR (15H1.2). It was also found that embryonal carcinoma (EC) cells without classical MHC antigens could specifically inhibit the proliferation of 15H1.2 cells. Then, a monocloal antibody, 14.37, against at EC line, OTF9, that could interfere with the ability of them to inhibit the growth of 15H1.2 was established. Pretreatment of 15H1.2 cells with anti-Vβ2 and OTF9 cells with 14.37moAb, respectively, completely

abrogated the growth inhibition of 15H1.2 by OTF9. Ontogenically 14.37 antigen (Ag) was strongly expressed in fetal and newborn mice, rather rapidly declined thereafter, and remained at very low level in adult organs. In a given stage, the expression of 14.37 Ag exhibited an apparently inverse relationship with that of class I MHC antigens. Molecular cloning of the 14.37 Ag revealed it to be a murine homologue of human 4F2 heavy chain. Taken together, these results suggested that IL3 could extrathymically induce differentiation and/or proliferation of DN $\alpha\beta$T cells with uniquely limited repertoire, which existed preferentially in BM *in vivo*, and that a subset of the DN $\alpha\beta$T cells, which expressed the dominant TCR, Vα4/Vβ2, could specifically recognize the fetal/activation Ag, 4F2, on the target cells in a MHC-independent fashion.

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