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Clones of Murine Functional T Cells

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A. Introduction

The serologic and biochemical analysis of different classes of T cells, such as cytotoxic, helper, and suppressor T cells, has been impeded by the lack of pure populations of functional T cell lines. None of the established well characterized AKR thymoma and lymphoma T cell lines exhibit any T cell functions besides the expression of serologic T cell surface markers. To date all attempts to transform functional T cells using oncogenic viruses have failed. Although several groups reported T cell hybridomas which secrete specific and nonspecific helper or suppressor factors (Kontiainen et al. 1978; Taniguchi et al. 1979; Taussig et al. 1979), most of the described hybridomas are not very stable and have to be subcloned and selected for activity over short periods. During the past 3 years, however, several groups have succeeded in establishing and cloning functional human and mouse T cells (reviewed in Schreier et al. 1980, Immunological Reviews, Vol. 51, Ed. Möller, G., Munksgaard, Kopenhagen).

Functional T cells were expanded in vitro after in vivo immunization and kept under optimal tissue culture conditions for the appropriate T cell type. Alloreactive proliferating T cells can be kept functional by periodical restimulation using irradiated spleen cells (Fathman and Nabholz 1977), whereas the growth of cytotoxic or helper T cells after initial antigen specific stimulation, in some cases in vivo and then in vitro, is strictly dependent on the presence of T cell growth factors in the culture medium (Gillis and Smith 1977).

All attempts to establish long-term cultures of cloned or uncloned T cells appears to depend upon the following principles:

1. Expansion of the antigen-specific T cells by in vivo immunization;
2. Secondary immunization in vitro followed by the biologic assay for the appropriate T cell specificity; and
3. Maintaining the functional T cells either by periodical restimulation or by supplementing the cultures with T cell growth factor.

To prevent overgrowth by T cells which are not antigen specific, it is important to clone the cells at this point and select those clones which exhibit the biologic activity desired.

In our laboratory we have been dealing over the past 4 years with the cloning of T cells of the following types: alloreactively proliferating T cells (Fathman and Hengartner 1978, 1979; Hengartner and Fathman 1980), H-Y antigen (14) and hapten (9) specific H-2 restricted cytotoxic T cells in the mouse.

B. Alloreactive T Cells

Alloreactive T cells can be generated in an ordinary mixed lymphocyte culture where lymph node cells are stimulated by X-ray-irradiated spleen cells. Every 10 to 14 days the viable responder T cells are diluted into fresh medium and restimulated by X-ray-irradiated stimulator spleen cells. Fathman et al. (1977) described the following two long term culture systems: A/J anti-C57BL/6 (A[B6]) and A/J anti-(C57BL/6 × A/J)F_1 (A[B6A]). The bulk cultures consisted mainly of haplotype-specific proliferating T cells lacking any cytotoxic activity. After three to four restimulations, the specificity of the two systems may be analysed by stimulating 10^4 responder T cells with 10^6 X-ray-irradiated spleen cells of different haplotypes. The stimulatory effect is measured

by ^{3}H-thymidine uptake during[a] 16-h period 48 or 72 h after the initiation of the cultures (Fathman et al. 1977).

The initial bulk culture A(B6) exhibited identical stimulation indexes against C57BL/6 and (C57BL/6 × A/J)F_1 stimulator cells, and the stimulation indexes against third party stimulator cells stayed constant even after the 20th restimulation.

The bulk culture A(B6A) showed a stimulation index against (C57BL/6 × A/J)F_1 stimulator cells which was twice as high as that against the C57BL/6 stimulator cells, suggesting a unique F_1 MLR determinant on the semiallogeneic stimulator cells. These proliferating T cells could be cloned in soft agar. The responder T cells were stimulated in suspension for 24 h and then seeded on soft agar in a petri dish. After 5 days colonies were picked from the agar and subsequently expanded in 0.2-ml, 2-ml, 15-ml and 45-ml cultures by restimulation with X-ray-irradiated stimulator spleen cells every 10 to 14 days. Between two restimulations the cloned alloreactive T cells undergo an activation, a proliferative, and a resting phase. The continous stimulation by allogeneic stimulator cells in vitro leads to this expansion of the alloreactive T cells.

The analysis of such cultures clearly demonstrated the existence of an F_1 MLR determinant expressed on semiallogeneic stimulator cells which is absent on C57BL/6 cells. The analysis of such F_1 alloreactive proliferating T cell clones using different recombinant F_1 stimulator spleen cells further demonstrated the existence of at least four different clone types with different fine specificities (Fathman and Hengartner 1979).

In the A(B6) bulk cultures we have demonstrated through cloning and subcloning the existence of a T cell clone type which can be stimulated only by C57BL/6 stimulator cells but not by the semiallogeneic (C57BL/6 × A/J)F_1 stimulator cells. This suggests the expression of a unique MLR determinant on the homozygous C57BL/6 stimulator cells (Hengartner and Fathman 1980).

The alloreactive T cells can be thawed and restimulated after freezing and storage in liquid nitrogen. The chromosome number of the clones is 40 and the chromosomes do not show any obvious abnormalities.

Fathman and Weissman (1980) injected 10^7 A/J cells of one of the F_1 specific clones (clone A [B6A] 1-1] intraperitoneally into A/J, C57BL/6, and (C57BL/6 × A/J)F_1 animals. After 4 weeks the F_1 animals exhibited enlarged lymph nodes, and the H-2 typing demonstrated the A/J origin of a large number of the lymph node cells. This clearly demonstrated the generation of a T cell lymphoma by exposing proliferating T cells to a constant stimulatory environment in the (C57BL/6 × A/J)F_1 mouse.

C. Cytotoxic T Cells

The MHC-restricted cytotoxic T cells against the H-Y antigen and the hapten sp were generated in secondary mixed lymphocyte cultures 14 days after the primary immunization in vivo. Analogous to the alloreactive T cells, the cytotoxic T cells were cloned in soft agar or under conditions of limited dilution (Nabholz et al. 1980; Von Böhmer et al. 1979). In contrast to alloreactive proliferating T cells, cytotoxic T cells have to be kept in medium containing T cell growth factor. We routinely use 10% supernatant of ConA-activated (48 h) rat spleen cells in the culture medium as a source for T cell growth factor.

The growth of cloned cytotoxic T cells is strictly dependent on T cell growth factors and cells usually die within hours in regular medium. Cytotoxic T cells can not be stimulated by the specific antigens, which are X-ray-irradiated syngeneic male spleen cells or haptenated syngeneic spleen cells (Nabholz et al. 1980; Von Böhmer et al. 1979). The chromosome number of such cells is higher than 40 and occasionally even metacentric chromosomes can be demonstrated.

D. Summary

Using techniques of cloning, it became possible to generate alloreactively proliferating (Hengartner and Fathman (1980), cytotoxic (Von Böhmer et al. 1979), and helper (Schreier and Tees 1980; Von Böhmer et al. 1979; Watson 1979) T cells. The homogeneity of functional T cells makes it possible to start to tackle problems such as the chemical nature of the T cell receptor and the organization of genes which lead to the T cell differentiation and specificity. The specific proliferation or killing observed in a complex mixture of cells after activation in a mixed lymphocyte culture

can be dissected by cloning. Utilizing such clones, it might also be possible to study the phenomenon of specific activation and the still obscure phenomenon of cytolytic activity.

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