Analysis of T Suppressor Cell-Mediated Tumor Escape Mechanisms Is Facilitated by the Selective In Vitro Activation of Tumor-specific Ts Cells

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We have shown previously that tumor-specific T suppressor (Ts) cells were induced in vivo in BALB/c mice by the syngeneic plasmacytoma (PC) ADJ-PC-5 at very early stages of tumorigenesis [1, 2]. These Ts cells, which suppress a strong primary cytotoxic T cell response, have been characterized in detail [1–3].

There is evidence that Ts cell-inducing antigens (Ts-Ag) on ADJ-PC-5 plasmacytoma cells are expressed to some extent on normal BALB/c spleen cells and are therefore "self" antigens rather than tumor-specific neoantigens [4]. These data were subsequently confirmed by independent comparable studies using the EL4 thymoma of C57Bl/6 mice [5]. Thus, the induction of Ts cells by tumor-associated self antigens seems to be a more general rule and might be an important tumor escape mechanism.

To characterize Ts-Ag in more detail we have developed an in vitro system for the selective induction of tumor-specific Ts cells. Ts cell function would be masked in the in vitro Ts assay in the presence of activated cytotoxic T cells, which, like specific cytotoxic T cell clones, are not susceptible to suppression [2]. Activation of cytotoxic T cells is prevented by pretreatment of the ADJ-PC-5 stimulator cells with glutardialdehyd (GA) (Fig. 1). In contrast, specific Ts cells were activated by this approach which suppress the activation of specific cytotoxic T cells in the course of a primary mixed-lymphocyte tumor cell culture (MLTC) of BALB/c spleen cells against ADJ-PC-5

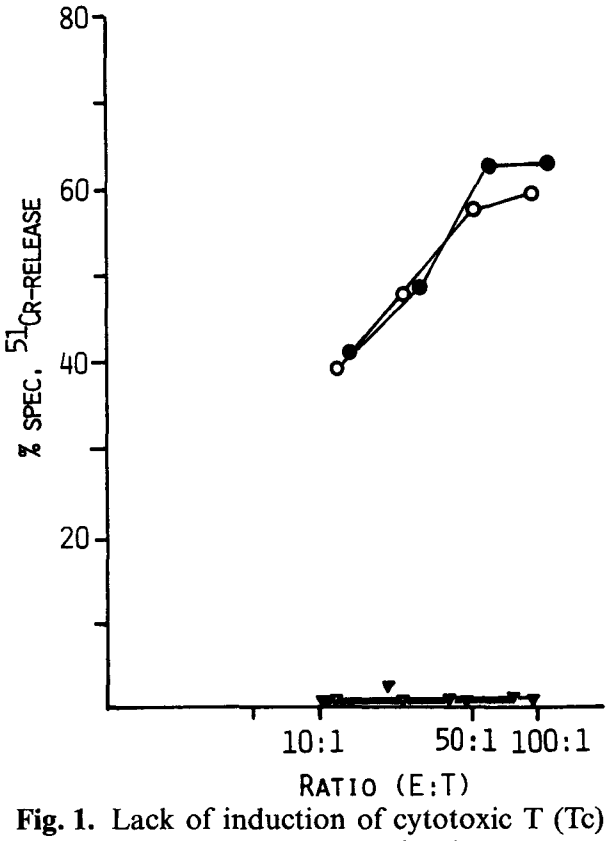

Fig. 1. Lack of induction of cytotoxic T (Tc) cells by glutaraldehyde (GA)-fixed ADJ-PC-5 plasmacytoma cells: 2×10^7 normal BALB/c spleen cells (SC) were incubated with 1×10^6 ADJ-PC-5 tumor cells in 10 ml MLTC medium containing 10% FCS in tissue culture flasks for 6 days. Thereafter, cells were harvested and tested for cytolytic activity against ADJ-PC-5 in a 6-h ^{51}Cr release assay. Several types of stimulator cells were used: (**a**) ADJ-PC-5 mitomycin-C-treated (o—o); (**b**) ADJ-PC-5 mitomycin-C-treated and subsequently fixed by GA (▼—▼); (**c**) a 1 : 1 mixture of ADJ-PC-5 mitomycin-C-treated and ADJ-PC-5 mitomycin-C-treated and GA-fixed (●—●). A control culture without stimulator cells is also shown (▽—▽)

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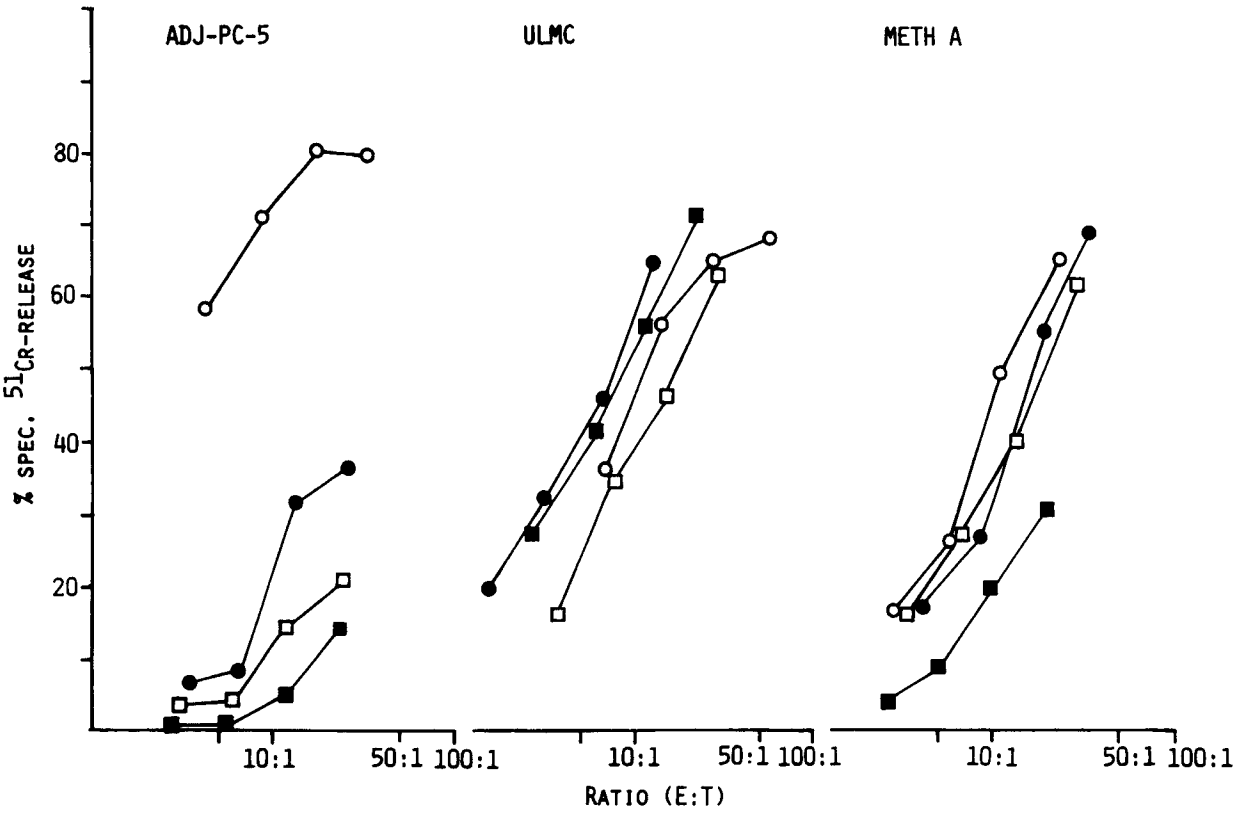

Fig. 2. Induction of specific Ts cells by stimulation of BALB/c SC with GA-fixed ADJ-PC-5 plasmacytoma cells: 4×10^6 normal BALB/c SC were incubated with 2×10^5 GA-fixed ADJ-PC-5 stimulator cells in MLTC medium containing 0.5% syngeneic normal mouse serum in Costar plates in a volume of 2 ml for 6 days. Subsequently, cells were harvested, 800 R x-irradiated, and preincubated with rIL2 (5 U/ml) for 1 h. They were then washed and used as a source for Ts cells. Graded numbers of Ts cells were added to primary mixed-lymphocyte tumor cultures (MLTC) of normal BALB/c SC against syngeneic tumor targets. After 6 days cells were harvested and tested for cytolytic activity. MLTC without Ts cells (o—o), with 2×10^5 (●—●), 6×10^5 (□—□), and 2×10^6 Ts cells (■—■). Tumor targets are ADJ-PC-5 (plasmacytoma), ULMC (lymphoma) and MethA (fibrosarcoma)

plasmacytoma cells, but not against the syngeneic control tumors ULMC (lymphoma) and MethA (fibrosarcoma) (Fig. 2). These Ts cells have been further characterized. Even in lectin-kill assays they have no cytolytic or NK-like activity, excluding a veto effect. In addition, suppression is not due to nonspecific effects like IL2 consumption, toxic effects by glutaraldehyde or PGE_2 release (data not shown).

The phenotype of these Ts cells was Thy 1.2^+, Lyt 2.2^+, L3T4$^+$, I-A^{d-}, I-E^{d+} as evidenced by treatment with cytotoxic monoclonal antibodies and complement.

This in vitro system will be helpful for the isolation and characterization of Ts-Ag, but it also allows us to study in more detail the requirements for the induction of Ts cells and Ts-cell effector mechanisms.

References

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