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T Cells and Suppression in Vitro

T cell–mediated suppression has been of interest for some time, yet direct demonstration of specific mechanisms has been clouded in part by the diversity of experimental systems. Although suppression is expected to be of most relevance to the prevention of autoimmune disease, for simplicity investigators have relied primarily on the demonstration of T cell tolerance in vitro. Such studies got a considerable boost with the demonstration by Lamb *et al.* (*1*) and Jenkins and Schwartz (*2*) that T cell proliferative responses in vitro might be blocked by inducing T cell receptors to trigger in the absence of co-stimulation. T cells treated in this way became unresponsive, or anergic, to restimulation. Although this mechanism appeared to be of value in explaining peripheral immunological tolerance, issues remained unsettled, including the question of whether inert T cells would be of value to a dynamic peripheral immune system.

Giovanna Lombardi *et al.* (*3*) appear to have resolved this question by demonstrating that T cell clones made anergic by an established protocol could, in mixes with nonanergic T cell clones, block proliferative responses in vitro. The blockade was specific, in that anergic cells were more potent suppressors if they matched the specificity of the nonanergic responder cells, and nonspecific, in the sense that the inert cells could absorb cytokines necessary for driving the in vitro proliferative response.

While we generally support the proposed mechanisms (*3*), we are concerned that these are not necessarily features peculiar to anergic cells in vitro, especially as Lombardi *et al.* irradiated the anergic T cells before adding them to the suppressor cultures (*3*). To test this question, we set up similar studies with the use of T cell receptor transgenic T cells in culture with spleen cells pulsed with peptide that presents antigen. As a source of putative suppressor T cells, we added T cell receptor transgenic T cells that had been stimulated (not anergized) with peptide 48 hours earlier, then irradiated before being added to the culture. These T cell receptor transgenic blast T cells were used because they would be expected to reproduce some of the features demonstrated for anergic T cells: cell enlargement because of blast transformation, retention of cell surface antigen receptors, and increased expression of surface interleukin-2 (IL-2) receptors (*2*); this protocol is similar to that of Lombardi *et al.*, except that the putative suppressor cells are not anergized and are fully functional before irradiation. In the case of T cell receptor–transgenic responder T cells (TCR-HNT) (Fig. 1, A to C), the addition of suppressor cells [irradiated TCR-HNT blasts (Fig. 1B)], at a ratio of 1:1, raised the background counts but still significantly suppressed antigen-specific proliferation. With a second T cell receptor–transgenic responder (TCR-SFE) (Fig. 1, D and E), the inhibition [by irradiated TCR-SFE blasts, (Fig. 1E)] was even stronger. Suppression was specific; so although irradiated TCR-SFE blasts suppressed TCR-SFE responders, they had no effect on TCR-HNT responders (Fig. 1C). Addition of nonlimiting amounts of both HNT and SFE peptide gave dramatically increased proliferation (Fig. 1C, right column).

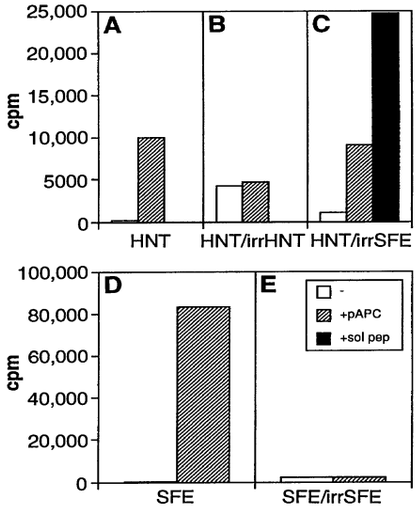

Fig. 1. T cell receptor transgenic T cells from TCR-HNT (*5*) and TCR-SFE (*6*) transgenic mice show specificity for influenza hemagglutinin peptides 126-138 (HNT) on I-A^d or 110-119 (SFE) on I-E^d, respectively. Here, 2×10^5 T cells were stimulated with either 4×10^5 irradiated (2100 R) B10.D2 spleen cells (-), peptide pulsed spleen cells [+pAPC; HNT peptide, (**A**–**C**); SFE peptide, (**D**, **E**)], or spleen cells in the presence of nonlimiting concentrations of both HNT and SFE peptides (+sol pep, 2 μg/ml each peptide). Bars indicate proliferative responses (cpm, incorporation of ^{3}H-thymidine). T cells (B, C, and E) were cultured 48 hours with peptide and spleen, then washed and irradiated (2100 R) before addition as suppressor cells at a 1:1 ratio to responder cultures (irrHNT and irrSFE).

Our results indicate that, as long as antigen reactive T cells are blocked from proliferation by irradiation, they can act as suppressors of in vitro proliferation. This effect is seen regardless of whether the suppressor T cells are anergic or fully responsive. This point is relevant to the fact that (unirradiated) anergic T cells have been shown to retain the ability to proliferate in response to cytokines such as IL-2, and that the IL-2–driven proliferation will actually induce recovery from the anergic state (*2*, *4*). That is, unirradiated anergic T cells under the conditions described, might contribute to, rather than suppress, proliferative responses. Thus, suppression of T cell proliferation in vitro as described by Lombardi *et al.* is probably not a property specific to anergic T cells, but rather a redemonstration of cold target inhibition.

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Response: The comment by Scott *et al.* is pertinent and thought provoking. They raise the point that the model we propose to account for suppression, mediated by "anergic" T cells, would equally be applicable to the effects of activated T cells paralyzed by irradiation. Data are included in support of his contention.

However, we would like to draw an important distinction between the "anergic" T cells as used in our experiments (*1*) and T cell blasts as used in their system. The stimuli used to induce this anergic state (T

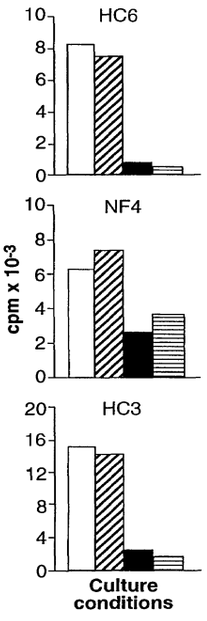

Fig. 1. Proliferation assays were conducted with three DR1-restricted, haemagglutinin-specific human T cell clones, in the absence (open bars) or the presence of irradiated responsive (sloping hatch marks), peptide-induced anergic (black bars), or anti-CD3–induced anergic (horizontal hatch marks) cells from the same T cell clone.

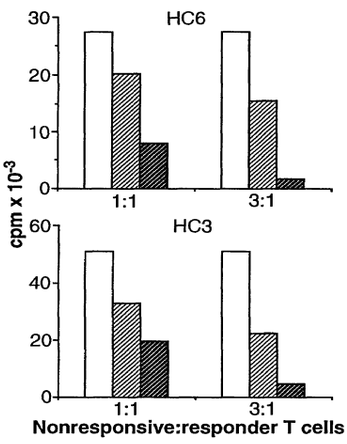

Fig. 2. Assays were conducted as in Fig. 1, except that anti-CD3–induced anergic cells were not used in this experiment.

cell antigen presentation or immobilized antibody to CD3) did not induce proliferation, were incapable of inducing the secretion of IL-2 by the responder cells, and inactivated the IL-2 gene such that the T cells were refractory to subsequent challenge. This phenomenon has been fully characterized (*2*). In contrast, exposure of T cells to competent antigen-presenting cells, as in the experiments by Scott *et al.*, induces proliferation, IL-2 secretion, and leaves the T cells in a fully activated state. The addition of irradiated responsive cells was a control that we have included in many of our experiments. Most important, addition of irradiated *responsive* members of a T cell clone caused trivial inhibition in three quarters of our experiments (Fig. 1); in a minority of instances irradiated responsive cells did cause significant suppression (Fig. 2), however, this was never as pronounced as that caused by the "anergic" cells. The reason is specifically because "cold target competition" by T cells that are capable of secreting IL-2 is insufficient to account for the suppression that we observe. Our hypothesis is that the suppression effected by anergic cells requires the simultaneous competition for the APC surface and interference with the local delivery of paracrine cytokines. In addition, it is central to the argument presented in our report that the so-called anergic state can arise in vivo and that the effects of a cohort of such cells are mirrored by the in vitro system employed in our experiments. Irradiated T cell blasts, in contrast, do not arise in vivo. The reason for irradiating the anergic T cells in our experiments was to ensure that any proliferation measured in the assays was arising from the untreated responder T cells, rather than from the anergic cells themselves.

The observations described by Scott *et al.* fulfill predictions that could be seen as arising from the model of suppression that we proposed (*1*). The only unexpected result is that the T cell blasts did not secrete IL-2 in the second culture, given that the dose of irradiation was only 2100 rads, well below the dose needed to inhibit IL-2 secretion in our hands. This result may reflect the "premature" restimulation of T cells (48 hours after their previous stimulation), which can have inhibitory effects. Although well-recognized, the mechanisms responsible for this phenomenon are complex and poorly understood, but may have relevance to the findings of Scott *et al.*

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