Brief Definitive Report

Fibroblasts Mediate T Cell Survival: A Proposed Mechanism for Retention of Primed T Cells

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Summary

This report describes a salvage pathway whereby activated T lymphocytes revert to nonproliferating cells in the absence of antigen or mitogenic signals. After the removal of mitogenic cytokines, cultured T lymphocytes cease dividing and rapidly begin to undergo cell death. However, the addition of fibroblasts to interleukin 2 (IL-2)-propagated T cells results in prolonged survival of the previously activated T lymphocytes in the absence of proliferation. The prevention of cell death is also achieved by conditioned medium from the fibroblasts. T lymphocytes cultured with fibroblasts or the conditioned medium retain the ability to be restimulated if mitogenic stimuli are added to the culture. The activity is not accounted for by IL-1–7. The studies suggest a stromal cell–mediated, nonspecific mechanism for survival of primed T lymphocytes in a nonproliferating state.

Lymphocyte responses to antigens can be divided into three main phases: (*a*) antigen-driven activation; (*b*) clonal expansion and differentiation of effector cells; and (*c*) after removal of antigen, the retention of "primed" cells, which allows an accelerated response upon antigenic rechallenge. Although many details on the first two phases are understood, little is known about how primed cells are preserved. In fact, when activated cells are removed from the presence of antigenic stimuli or proliferation-inducing cytokines, they rapidly cease dividing and begin to deteriorate (1). We have observed that the presence of the tissue enhanced the survival and activation of T lymphocytes (2). Stimulatory antigens could account for this enhanced activation (3), but we have now shown that fibroblasts alone, or even supernatants derived from fibroblast cultures, are also able to support the survival of T lymphocytes.

Materials and Methods

Culture of Fibroblasts and Preparation of Fibroblast-conditioned Medium (FCM). Fragments of synovial tissues were obtained from patients undergoing surgery for destructive joint disease from chronic rheumatoid arthritis (RA) in order to obtain parallel cultures of adherent synovial fibroblasts and IL-2-dependant T lymphocytes, as previously described (4). A cell-free supernatant from the fibroblasts, or fibroblast conditioned medium (FCM), was prepared by collecting the spent 3-4-d conditioned medium from the cultures of confluent fibroblasts.

Cultures of T Lymphocytes. Human T lymphocytes were propagated in long-term culture using rIL-2 at 10 U/ml (2). T cells were obtained from peripheral blood stimulated with 1 μg/ml PHA, and from inflamed tissues, including allografts and rheumatoid synovia, from which the infiltrating lymphocytes were propagated in IL-2, as reported (2, 4). Such cultured lymphocytes generally will divide in response to IL-2 for a period of up to 4 wk, at which time log phase growth requires restimulation of IL-2Rs, with PHA and irradiated mononuclear cells (5). If IL-2 is withdrawn, T cells rapidly cease dividing and begin to die unless substitute stimuli are provided.

Lymphocyte-Fibroblast Coculture and FCM Assay. To test the effects on lymphocyte survival, T cells were cocultured with the synovial fibroblasts (isolated as above) in the absence of any added cytokines. Viability was assessed microscopically. For proliferation studies, the fibroblasts were irradiated with ^{137}Cs (3,000 rad). Periodically, lymphocyte aliquots were placed into individual wells of 96-well plates to monitor cell proliferation in 4-h [^{3}H]thymidine incorporation assays. At various intervals, IL-2 (100 U/ml) was added back to the T cell cultures, and both visual observations and determinations of [^{3}H]thymidine incorporation were made at 2–5 d after re-addition of IL-2.

PHA and irradiated feeder cells were added to test both viability and proliferative capacity of the cultures. The T cell lines tested included both clones and bulk culture lines obtained from RA patients (including the source of the synovial fibroblasts and unrelated individuals), T cell lines, and clones of both CD4 and CD8 phenotypes from renal allografts.

Comparison between FCM and Some Known Cytokines. Comparisons were made between FCM and a group of well-characterized cytokines (IL-1–7). FCM was tested for IL-1 activity by the ability of this medium to support the proliferation of the D10.G4.1 cell line (6), and IL-1α and -β (kindly provided by DuPont de Nemours, Inc., Wilmington, DE) were tested for their ability to prolong survival of the lymphocyte cultures in the FCM assay. The presence of IL-2 in the FCM was assayed by the ability to induce proliferation of IL-2-dependent human cell lines and murine CTLL (7). rIL-3, -4, -5, and -6 were each tested for the ability to enhance T lympho-

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cyte survival in comparison with FCM. Five different preparations of FCM were tested for IL-7 activity by a bioassay on the IL-7-dependent IxN/2b cell line (8).

Results and Discussion

Fibroblasts Preserve T Lymphocyte Viability. The removal of IL-2-propagated T lymphocytes from medium containing IL-2 leads to the cessation of cell division and subsequent cell death. When such IL-2-dependent cell lines are cocultured with tissue or fibroblasts derived from inflamed synovium, they also cease dividing, but, after clustering around the adherent fibroblasts, many of the T cells remain viable for several months. Although this could be the result of antigenic stimulation, the observation that synovial fibroblasts had a T cell viability preserving activity (TVPA) on several IL-2-dependent T cell lines derived from different inflamed tissues suggested a nonspecific fibroblast-mediated activity possibly due to the production of one (or more) nonspecific cytokine(s). As shown in Fig. 1, viable stable T cell cultures can be maintained for extended periods (> 8 mo at the time of manuscript submission) in the presence of allogeneic fibroblasts. Renewed division by these cells can be stimulated with PHA and accessory cells. A similar phenomenon can be observed when FCM is added to the T cells. In FCM, culture viability is less dramatically extended, but live T cells can be maintained for as long as 6–8 wk only by periodic replenishment of FCM. In addition to synovial fibroblasts, FCM from dermal fibroblasts can also extend T lymphocyte viability, although such cells generally produce less TVPA than recently established synovial fibroblasts. In the absence of IL-2 or FCM, cell viability deteriorates much more rapidly, rarely being retained for >10 d (Fig. 2).

Although the synovial fibroblasts did not stimulate significant proliferation among IL-2-dependent T cells, they did enhance IL-2 responsiveness of the primed lymphocytes. At an optimal concentration of 5×10^3 fibroblasts/ml, lymphocytes (2×10^5/ml) showed approximately threefold enhanced IL-2-driven proliferation (7,613 ± 2,333) vs. lymphocytes plus IL-2 (100 U/ml) without added fibroblasts (2,778 ± 1,039). As shown in Table 1, the fibroblast-enhanced IL-2 responsiveness can also be demonstrated by the supernatant fluid from the fibroblasts.

FCM Preserves T Lymphocyte Viability. Table 1 shows two

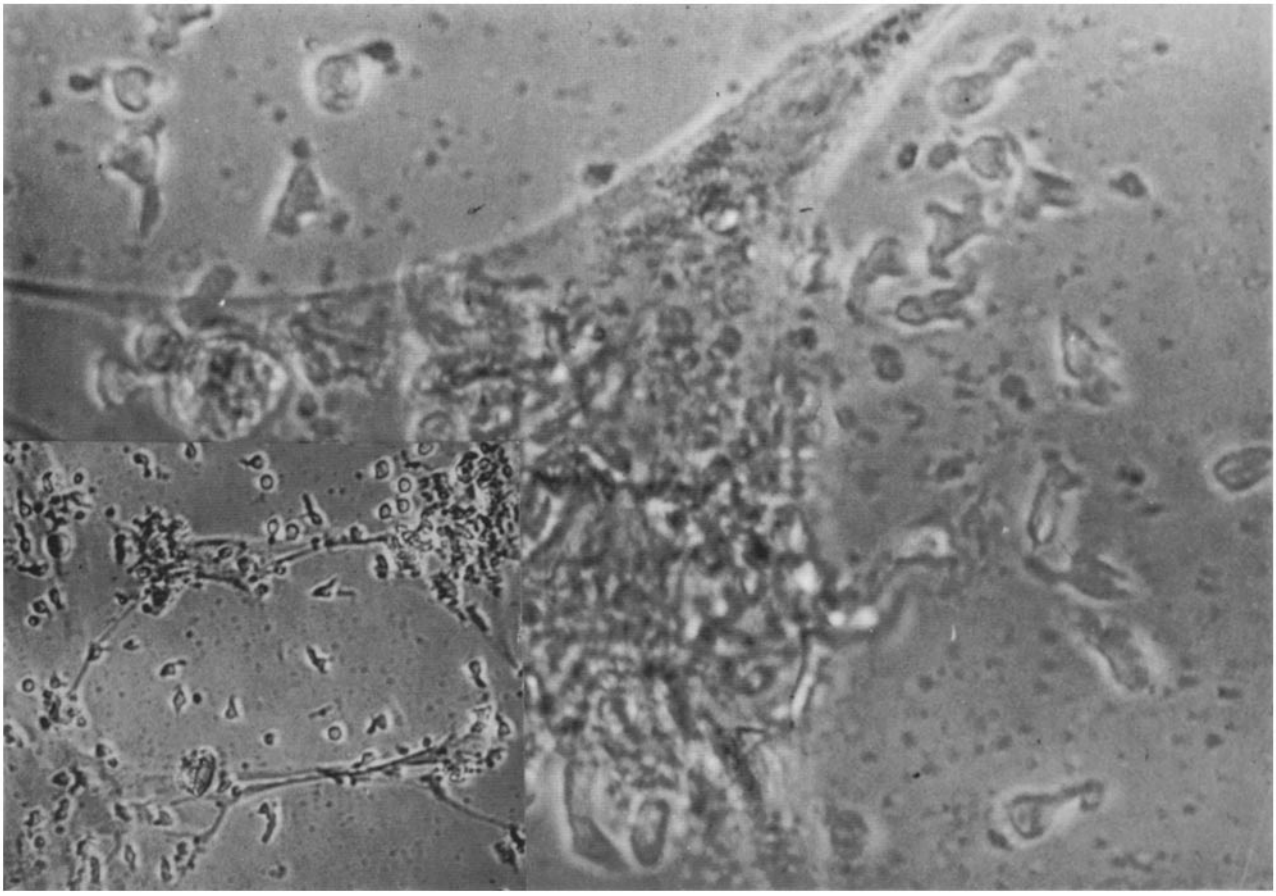

Figure 1. Survival of lymphocytes in presence of fibroblasts and FCM. Shown is the appearance of an IL-2-dependent T cell line (cultured from a renal allograft [2]) after 6 mo (185 d) in the presence of allogeneic synovial fibroblasts, which did not induce proliferation. The appearance was virtually identical after 8 mo of culture (× ~1,000, main figure; × ~300, inset).

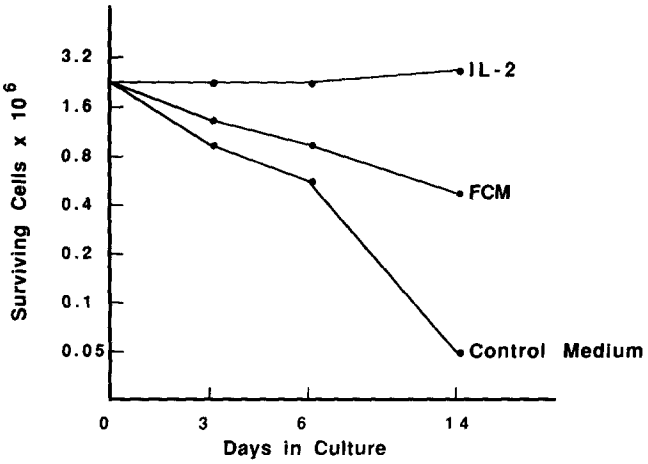

Figure 2. The number of surviving primed T lymphocytes in IL-2 vs. FCM vs. control medium over a 14-d period. Data shown are means of triplicate experiments of different cultures derived from tissue-derived lymphocytes.

types of assays to demonstrate the retained viability and activation of the lymphocytes. To determine retained IL-2 responsiveness, IL-2 was re-added at various times after culture in FCM. While IL-2-depleted cells in control medium lose the ability to respond to IL-2, together with the deterioration of their viability after 3 d, the FCM-cultured cells retain IL-2 responsiveness for the first 5 d, then diminish by 8 d, and are nonproliferative in response to IL-2 by 14 d. However, the lack of proliferation in response to IL-2 at this time is not due to lost viability. In fact, the cells can be stimulated with PHA in the presence of accessory cells, which allows the resumption of log phase growth and IL-2 responsiveness.

Table 1. *Preservation of Proliferative Potential by FCM*

Culture conditions	Days without IL-2	[^{3}H]Thymidine incorporation
		cpm
DMEM	3	476 ± 206
FCM	3	29,238 ± 2,565
DMEM	5	181 ± 57
FCM	5	47,608 ± 7,209
DMEM	8	63 ± 55
FCM	8	6,135 ± 3,603
DMEM then PHA + APC	14	5,601 ± 256
FCM then PHA + APC	14	37,407 ± 3,021

FCM was assayed for TVPA both in the presence and absence of added IL-2. Parallel cultures of IL-2-dependent human T cells in DMEM with 5% FCS (with and without FCM) were established in tissue culture plates at 2 × 10^5/ml. After varying periods of time, IL-2 (100 U/ml) was added back to the cultures, and [^{3}H]thymidine incorporation was assayed at 2 d after readdition of IL-2. The ability of the T cell cultures to respond to mitogenic stimuli was further tested by the addition of PHA and irradiated APC as an indication of both viability and proliferative capacity of the cultures.

Table 2. *Comparison of FCM with Known Cytokines.*

Culture conditions	Days without IL-2	[^{3}H]Thymidine incorporation
		cpm
DMEM	3	131 ± 49
FCM	3	244 ± 50
IL-2	3	7,188 ± 1,477
IL-2 + FCM	3	19,082 ± 1,616
DMEM	3, then IL-2	3,923 ± 538
FCM	3, then IL-2	79,973 ± 7,403
IL-6	3, then IL-2	5,183 ± 292
FCM + anti-IL-6	3, then IL-2	65,590 ± 1,757
DMEM + anti-IL-6	3, then IL-2	1,429 ± 278
IL-6 + anti-IL-6	3, then IL-2	1,605 ± 68
DMEM	4, then IL-2	3,159 ± 367
FCM	4, then IL-2	12,268 ± 1,111
IL-4	4, then IL-2	3,137 ± 913
DMEM	2, then IL-2	3,477 ± 452
FCM	2, then IL-2	12,550 ± 2,331
IL-3	2, then IL-2	3,302 ± 664
IL-5	2, then IL-2	2,821 ± 789

IL-2-propogated lymphocytes were washed and cultured 3 d in FCM vs. IL-6 (20 U/ml, also tested at between 100 and 0.1 U/ml, not shown), then IL-2 was re-added for 2 d before pulsing with [^{3}H]thymidine. Anti-IL-6 was used at a concentration sufficient to neutralize 20 U of IL-6. IL-4 (shown at 25 U/ml., also tested at 5 and 2.5 U/ml); IL-3 (shown at 10 U/ml, tested at 0.1–100 U/ml), and IL-5 (not stimulatory at any dose tested >300-fold range) were compared with FCM.

Surface staining indicated that both $CD4^+$ and $CD8^+$ subsets of $CD3^+$ T cells could be maintained in the presence of FCM. Although some cell death does occur in cultures maintained in FCM without IL-2 for 14 d, there is not a significant alteration in the proportions of $CD4^+$ or $CD8^+$ T cells.

Comparison of FCM with Known Cytokines. Several known cytokines were tested to determine if the TVPA was due to a previously characterized factor. No IL-2 proliferation-inducing activity was demonstrated in the FCM when tested on IL-2-dependent human cell lines. IL-1, IL-3, IL-4, and IL-5 all failed to prolong T cell line viability. IL-6, which is known to enhance IL-2 responsiveness (9), had minimal TVPA (Table 2). Anti-IL-6 antibody had minimal effect on the activity of the FCM, indicating that this is not likely to be an important component of the TVPA. No IL-1 was detected in FCM as determined in the D10-G4 proliferation assay. FCM also did not contain detectable IL-7. FCM contained PGE_2, but no TVPA was observed using this prostaglandin.

TVPA is produced constitutively for at least several months in cultured synovial fibroblasts and is not enhanced by stimulation of the fibroblasts with IL-1 or IFN-γ. Lower amounts of TVPA could also be recovered from cultures of fibroblasts obtained from normal skin. Although it is possible that the activity seen in FCM may be due to a novel cytokine(s) or to a newly recognized activity of already described factors, the identification of the responsible molecule(s) remains undetermined. Preliminary attempts to characterize the factor(s) present in FCM indicated that the TVPA was stable at 4°C, survived 1 h at 56°C, but was lost after treatment for 1 h at pH >10 or <4. The activity was retained in a dialysis membrane with a 5,000-dalton pore size.

A proposed Mechanism for Retention of Primed T Cells. The ability of fibroblasts and FCM to retain lymphocyte viability could provide some clues as to the fate of activated T cells after removal of antigen. As with the nonspecific cytokines responsible for regulation of the induction phase of the cellular immune response, the cytokine(s) produced by the stromal cells described in this report suggest a nonspecific mechanism for retention of activated T cells. These factors can play a role not only in the normal immune response, but could be relevant also in the pathogenesis of chronic inflammatory states, such as RA, where activated cells are maintained at the site of inflammation. Our findings suggest a possible general mechanism for the retention of "primed" cells (10), as antigen rechallenge and/or IL-2 can lead to the rapid reactivation and deployment of cells specific for previously encountered antigens. Although this study was confined to activated T cells, the stromal cell activity described could also play a role in intrathymic development and selective retention of naive as well as primed lymphocytes. The hypothesis is offered that T lymphocytes undergo cell death as a physiological result of maturation and proliferation after antigenic or TCR triggering (11, 12). A salvage pathway mediated by stromal cells is then responsible for maintaining viability in certain lymphoid tissues and inflammatory sites. This hypothesis, in which T cell preservation occurs in the absence of proliferation, represents an alternative to the proposition that continued antigenic stimulation, or recognition of cross-reactive antigens, is responsible for T cell memory (13). Further elucidation of the responsible factor(s) and the characteristics of retained cells will provide insights into a crucial and as yet poorly understood aspect of the immune response.

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