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Gillis S, Ferm M M, Ou W & Smith K A. T cell growth factor: parameters of production and a quantitative microassay for activity.
J. Immunology 120:2027-32, 1978.
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In vitro culture of mitogen-stimulated spleen cell conditioned medium together with cytokine dependent cytolytic T cells resulted in dose-dependent T-cell proliferation that could be monitored by tritiated thymidine incorporation. Culture of such cells in the absence of conditioned medium resulted in cell death within 48 hours. These results led to the hypothesis that a discrete molecular entity initially penned as "T-cell growth factor," or TCGF, present in such conditioned media was responsible for indefinite *in vitro* proliferation of antigen reactive T cells. [The *SCI*® indicates that this paper has been cited in over 1,905 publications.]

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Ten years ago the field of biological response modifiers was in its infancy. Hormones of the immune response, collectively classified as cytokines or lymphokines, were in the main mythical components of tissue culture media that had previously been used to support *in vitro* immune responses such as mitogen-induced T-cell proliferation or mixed leukocyte cultures from individuals or animals of disparate histocompatibility backgrounds. In 1976 D.A. Morgan, F.W. Ruscetti, and R.C. Gallo first published the observation that culture supernates harvested from mitogen-stimulated peripheral blood leukocytes could be used to support the continuous *in vitro* culture of homogeneous populations of bone marrow-derived T lymphocytes.[1]

At the same time at Dartmouth, Kendall A. Smith and I developed methods allowing for the *in vitro* generation of tumor-specific cytolytic T cells and questioned whether adaptation of Morgan's protocol might allow us to develop a means for culturing (for the first time) clonal populations of effector T lymphocytes. Results of our successful experiments, published in *Nature* in 1977, formed the backdrop of the studies summarized above.[2]

We hypothesized that some entity present in mitogen-conditioned medium was responsible for the *in vitro* proliferation of such effector T cells. In an effort to develop an assay for this activity, we cultured such conditioned medium-dependent cloned T cells in the presence of a variety of concentrations of conditioned medium and found that the cells proliferated in response to the conditioned medium in a dose-dependent manner as manifested by tritiated thymidine incorporation. Comparison of the amount of proliferation inducing factor activity present in a given supernate to that present in a standard supernate could be afforded by probit analysis of regression lines drawn through the linear portions of a sample's dose-response curve. With publication of these results, we termed the entity responsible for this activity "T-cell growth factor" (TCGF) and established methods useful in quantifying other cytokines whose activities would be demonstrated in years to come.

Since the time of its first publication, the TCGF assay has been used by hundreds of laboratories to quantify the levels of TCGF (later called interleukin-2 [IL-2]) present in given culture fluids. The use of the assay was instrumental in purification of IL-2 to homogeneity[3] and in the molecular cloning of IL-2 genes.[4] Perhaps the most important contribution as a result of publication of this assay technology was the lesson it taught immunologists for years to come—namely, the necessity for developing unambiguous *in vitro* assays for cytokine function as an aid in the biochemical and molecular analysis of immunoregulatory biological response modifiers.

1. **Morgan D A, Ruscetti F W & Gallo R C.** Selective *in vitro* growth of T lymphocytes from normal human bone marrows. *Science* 193:1007-8, 1976. (Cited 1,065 times.)
2. **Gillis S & Smith K A.** Long-term culture of tumor-specific cytotoxic T cells. *Nature* 268:154-6, 1977. (Cited 945 times.)
3. **Stern A S, Pan Y-C E, Urdal D L, Mochizuki D Y, DeChiara S, Blacher R, Wideman J & Gillis S.** Purification to homogeneity and partial characterization of interleukin-2 from a human T-cell leukemia. *Proc. Nat. Acad. Sci. USA* 81:871-5, 1984. (Cited 45 times.)
4. **Taniguchi T, Matsui H, Fujita T, Takaoka C, Kashima N, Yoshimoto R & Hamuro J.** Structure and expression of a cloned cDNA for human interleukin-2. *Nature* 302:305-10, 1983. (Cited 455 times.)

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