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Grimm E A, Mazumder A, Zhang H Z & Rosenberg S A. Lymphokine-activated killer cell phenomenon: lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. *J. Exp. Med.* 155:1823-41, 1982. [Surgery Branch, National Cancer Institute, and Department of Cytopathology, Clinical Center, National Institutes of Health, Bethesda, MD]

Lymphokine-activated killing (LAK) is the term used for antigen-independent, interleukin-2 dependent activation of cytotoxic lymphocytes. This description of LAK resulted in (1) the first and unifying hypothesis to classify anomalous lymphocyte-mediated cytotoxicities and (2) a revival of cellular immunotherapy for cancer, by applying interleukin-2 and its activated lymphocyte products. [The *SCI*® indicates that this paper has been cited in over 860 publications.]

Interleukin-2 Alone Activates MHC-Unrestricted Oncolytic Lymphocytes

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This first paper defining the major characteristics of the lymphokine-activated killing (LAK) system resulted from a failure of the intended experimental negative controls. The key ingredients to realization of the LAK system were (1) my background in allo-antigen-specific cytotoxic T lymphocyte (CTL) activation signals and (2) partially purified T-cell growth factor[1] (TCGF, now called IL-2), which had been used to grow mouse and human lymphocytes; and researchers preceding me had noted the expression of tumor killing activity developing in these lymphocyte cultures. The local interpretation of this resultant tumor killing was that the cytotoxic lymphocytes must be CTL, as the effector phenotype was consistent with the available T-cell markers. One major problem with this interpretation was that the TCGF probably contained residual phytohemagglutinin (PHA), a lectin, linking specific killer lymphocytes to irrelevant targets, thereby overriding any endogenous specificity.

Therefore, in 1979, when I joined the Surgery Branch laboratory of Steve Rosenberg at the National Cancer Institute, I pursued what I thought was to be a different role for IL-2, that of the second signal in CTL induction. My experimental design was based on the hypothesis that, if immunotherapy were to have any role in cancer therapy, then tumors must be antigenic, even if they were not immunogenic. IL-2 was proposed to be the crucial accessory signal responsible for progression of antigen-primed lymphocytes through their proliferative pathway. I planned to test whether natural IL-2 preparations would provide the "second signal" for human tumor-specific CTL progression. For over a year, I struggled with the details in alloantigenic systems in which I rendered stimulator cells nonimmunogenic by ultraviolet irradiation, or heat killing. As I reported,[2] IL-2 addition to the cultures did restore specific CTL induction in the absence of any stimulator cell proliferative stimulus. Therefore, I was ready to try this with tumor stimulator cells and generate tumor-specific CTL. My *in vitro* sensitization experiments were performed with the cancer patients' lymphocytes as responders, the autologous tumor (irradiated or mytomycin-C treated) as the stimulators, and IL-2 as the source of the second signal to provide an "immunogenic" environment. For two years every intended negative control of IL-2 with responder lymphocytes alone (no tumor stimulator) yielded cytotoxicity to tumor cells, but instead of the desired CTL-like HLA restricted killing, all tumors were killed.

This time period was very frustrating for me. Steve was eager to have the "tumor-specific CTL" that we believed existed. He instructed me to "get rid" of the offending nonspecific lymphocytes, as they were masking our observations of "tumor-specific CTL." Thus began my final series of experiments that for me were going to determine whether I would stay in tumor immunology research. I tried with exhaustion to eliminate all traces of PHA. Using monoclonal antibodies to human lymphocyte subsets, I selectively depleted various populations; no matter what set I eliminated, I had IL-2 responsive lymphocytes developing into LAK.[3] I was ready to give up. I remember staying awake trying to decide how to tell Dr. Rosenberg that I was going to leave. Finally, it occurred to me that there was an alternative. Perhaps my failed negative results represented a novel activity and had no relationship to tumor-specific CTL. The more I thought about this, the more it fit. Janet K. Seeley and Sid Golub[4] (and many others I soon learned) had already reported "anomalous killing" activity occurring in certain circumstances. A review of the literature in this area found many examples of "promiscuous killers," "activated killers," and so on. Was it possible that IL-2 was the common stimulus for induction of these activities? Now we believe that this is the case. The recent application of either LAK, or of IL-2 to create LAK, endogenously in cancer patients is well known.[5]

1. **Morgan D A, Ruscetti F W & Gallo R C.** Selective in vitro growth of T lymphocytes from normal human bone marrows. *Science* 193:1007-9, 1976. (Cited 1,235 times.)
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3. **Grimm E A, Ramsey K, Mazumder A, Wilson D J, Djeu J & Rosenberg S A.** Lymphokine activated killer cell phenomenon. II. The precursor phenotype is serologically distinct from peripheral T lymphocytes, memory CTL and NK cells. *J. Exp. Med.* 157:884-97, 1983. (Cited 365 times.)
4. **Seeley J K & Golub S H.** Studies on cytotoxicity generated in human mixed lymphocyte cultures. I. Time course and target spectrum of several distinct concomitant cytotoxic activities. *J. Immunology* 120:1415-9, 1978. (Cited 185 times.)
5. **Grimm E A.** Human lymphokine-activated killer cells (LAK cells) as a potential immunotherapeutic modality. *Biochim. Biophys. Acta* 865:267-79, 1986. (Cited 30 times.)

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