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TCGF: How Much? Oh, and by the Way, How Many?

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Pillars of Immunology

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1916 100 years of The JI 2016

A Century of Excellence

Selections from the 100 most highly cited articles in The JI

The *Pillars of Immunology* article (1) selected for comment by the editors in this issue is a bit unusual. It is second on the list of the 100 most cited publications in *The Journal of Immunology* (*The JI*) with 4201 citations, and it was published in 1978, 38 years ago. Only 12 papers on this list were published at an earlier date. It not only had a significant influence but played that role for a long time.

At its founding in 1916, *The JI* was intended to provide "correct knowledge of vaccine therapy, serology and immunology from an authoritative and nonpartisan source" (2). Through its first four decades, *The JI* published articles concerned mainly with development of more effective active and passive immunization in the treatment of human and animal diseases and with characterizing immunological disease processes, using the tools that were available at the time. By the 1960s, things were beginning to change. Technological and conceptual advancements developed through basic research in the biological and physical sciences during World War II were being applied profitably in immunology research as well. Although past efforts were devoted mainly to studies of the outcome of the immune process (the final result of Ag stimulation of immunity), new technologies facilitated the study of the events involved in the initiation of the immune processes (the interactions of Ag with cells to induce immune responses).

Large numbers of lymphocytes were required to study the molecular processes by which Ag stimulation activates an immune response, and the improvements in tissue culture technology facilitated this approach. Successful long-term culture of normal human T lymphocytes was found to be strictly dependent on the addition of a growth factor. Culture supernatants from PHA-stimulated human blood cells were a convenient source of such a growth factor (3).

In their studies, Gillis et al. (1) devised a sensitive microassay for T cell growth factor (TCGF) based on the incorporation of tritiated thymidine ([^{3}H]TdR) by long-term cytotoxic T cell lines. They cultured growth factor–dependent long-term murine tumor-specific cytotoxic T lymphocytes for 24 h with serial dilutions of the experimental sample. [^{3}H]TdR was added for an additional 4 h, cultures were harvested onto glass fiber filter strips, and [^{3}H]TdR incorporation was determined. A quantitative measure of TCGF activity was obtained by comparison of the activity of the unknown sample with that of a standard growth factor sample by plotting the data from a $\log_2$ dilution series of samples as a percentage of maximum cpm on probability (probit) paper.

To compare the levels of TCGF production induced by different stimuli, the investigators cultured the stimulated cells for 48 h, the time at which maximum TCGF levels were found in stimulated rat spleen cell cultures. TCGF, produced by T cell mitogen- or Ag-stimulated mouse and rat spleen cells, caused growth of human, murine, and rat lymphocytes. This suggested that either a single type of TCGF molecule had activity on lymphocytes from three widely different species of animals or several different TCGF molecules were produced by one species. TCGF activity was first detected at 12 h after stimulation with Con A; maximum activity was detected at 48 h after stimulation, and no activity remained after 96 h of culture. The absence of TCGF activity after culture for 96 h was not due to the presence of an inhibitor of the growth factor; rather, active removal seemed to occur. Growth factor production was depressed if spleen cells were pretreated with anti–Thy-1 serum and rabbit complement, suggesting the essential involvement of T lymphocytes in TCGF production. Spleen cells from several mouse strains and from rats produced TCGF activity when stimulated with several mitogens (Con A, PHA, and PWM), as did human peripheral blood cells stimulated with PHA and by mouse spleen cells in mixed leukocyte cultures. LPS stimulation did not induce TCGF production. Conditioned medium from mouse BALB/c fibroblasts had no TCGF activity.

These results and information gathered using newly developed techniques in genetics and molecular biology documented with certainty the amazing complexity of immune responses. Intriguing discoveries suddenly made immunology a most exciting area for investigation, and multiple components of the immune response rapidly came to be investigated in increasing detail. Many distinct factors having inhibitory or stimulatory activity on the various cell types involved in immunity were discovered and characterized; soon numbers, rather than letters, were used as names (among these, TCGF was renamed as IL-2). Inbred mice had already proved their value in studying the genetic involvement in immune responses. The application of molecular approaches proved more valuable in characterizing the control mechanisms involved in regulation of immunity. The number of distinct cell types known to be involved in stimulating or inhibiting

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Abbreviations used in this article: [^{3}H]TdR, tritiated thymidine; TCGF, T cell growth factor; *The JI*, *The Journal of Immunology*.

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immunity also grew. Fluorescence microscopy, flow cytometry, mAbs, radioisotopes, and improved tissue culture methods became essential tools to readily obtain more relevant and reliable information. Space limitation prevents documenting the clinical relevance of these advances in the understanding of immunological processes. Suffice it to say that the immunological revolution had a great impact there, as well.

This article merits its selection as a *Pillars of Immunology* publication for at least two reasons. First, it describes a relatively simple, reliable, and quantitative, but inexpensive, assay to measure TCGF (IL-2) concentration. This technique was an improvement on past methods for quantifying growth factor activity by modifying the same rules and standards that were generally practiced. Thus, it can be categorized as "Normal Science," as defined by T.S. Kuhn (4). However, the Gillis and Smith studies (1) of the properties of TCGF made possible by their assay for TCGF activity posed several additional questions. The same TCGF sample stimulated proliferation of cells from several species: mouse, rat, and human. Was this a property of a single entity or multiple distinct TCGF molecules? Were the same or different cell types stimulated in the several animal species? Were there other unrecognized factors present in the TCGF-containing conditioned medium? Did the absence of growth factor activity in some conditioned medium indicate the absence of TCGF molecules or the presence of an inhibitor for TCGF? Of more basic importance, what were the detailed molecular mechanisms through which TCGF influenced the outcome of an Ag-induced immune response?

These questions were sufficiently unprecedented and open-ended to stimulate a distinct shift in thinking. They contributed to the adjustment in the standards of what constitutes an admissible problem or a solution to that problem. This paradigm shift really defines the structure of the scientific revolution in immunology (4). This is the second and most important reason why this article clearly merits its choice as a *Pillars of Immunology* publication. Today, scientific revolutions are continuing to take place in immunology, as well as in all segments of biomedical investigation. Vive la Révolution!

Disclosures

The author has no financial conflicts of interest.

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