Products of Lymphoid Cells in Continuous Culture

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LYMPHOID CELL LINES derived from human peripheral blood have been established in apparently permanent suspension culture from large numbers of patients with varied benign [1] and malignant [2, 3] lymphoproliferative disorders and from many normal individuals.[4, 5] Considerable numbers of these cell lines have been maintained for 3 or more years of continuous passage without loss of vitality. They grow as free-floating pleomorphic forms, singly and in clumps, with a fundamental lymphoid character on light and electron microscopic analysis. The cell population in general resembles the immature "blast-like" transformed cells seen after the stimulation *in vitro* of peripheral small lymphocytes by phytomitogens and antigens.[6] Occasional cells with morphologic features reminiscent of plasma cells and macrophages have been observed. The remarkable proliferative ability of these cell lines, doubling their numbers every 24–48 hr and reaching a stable population of 1–2 $\times$ 10^6 cells/ml of culture fluid, suggested early in their study that they would be a useful tool for controlled investigations of lymphoid functions and products.

Intensive study of these long-term lymphoid cell lines in many laboratories has confirmed the remarkable versatility of these cell systems and is contributing much to our rapidly expanding concepts of the multiple functions of lymphoid cells in the immune response. These studies have shown, among other properties, the ability of these cells to synthesize a variety of products, some of which are secreted into the medium.

Immunoglobulins

Synthesis of immunoglobulins of IgG (γ), IgA (α), and IgM (μ) heavy chain classes and K (κ) and L (λ) light chain types has been described.[7–10] Many lines simultaneously produce immunoglobulin molecules of several distinct heavy chain classes and K (κ) and L (λ)

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light chain specificities. Further characterization by combined polyacrylamide gel filtration, immunodiffusion and autoradiography indicates the synthesis of intact immunoglobulin molecules with both heavy and light polypeptide chains in close association, as well as free light and perhaps free heavy polypeptide chain synthesis.[8] Synthesis of immunoglobulins in synchronized cell cultures involves 90% or more of the cell population, suggesting that all cells may retain potential for this activity and that transcription of genes for immunoglobulin production occurs during a limited portion of the mitotic cycle.[11] Cloning studies [12] have suggested that the offspring of single cells can produce at least two heavy chain classes of immunoglobulins but only one light chain type. In view of the fact that these cells tend to grow in clumps and that serial dilution technics were used in the cloning studies, these results should be confirmed by technics, recently developed in our laboratory (S. W. Broder, work in progress), of picking single cells by micromanipulation. Such experiments, however, do support work done with peripheral lymphocytes [13] showing that cell differentiation for light chain synthesis occurs prior to differentiation for heavy chain production. Of interest is the possibility that these lymphoid cell lines may produce immunoglobulins with antibody specificity.[14] Sequential study of several cell lines further indicates qualitative differences in individual biosynthetic profiles with time.[8] Loss of previous biosynthetic capacities, as well as synthesis of additional classes of heavy and light polypeptide chains, has been observed. These findings suggest that lymphoid cell lines are capable of undergoing differentiation and selection *in vitro*.

Mediators of Cellular Immunity

The relationship of long-term lymphoid cell lines to cell-mediated immune responses (delayed-type hypersensitivity) had until recently received little attention. These cells in permanent culture, however, share striking morphologic and biochemical similarities with "blast-like" transformed cells seen after phytomitogen and antigen stimulation of peripheral small lymphocytes in short-term culture [15, 16] and the large pyroninophilic "activated" lymphoid cells observed in regional lymph nodes during the acquisition *in vivo* of delayed cutaneous hypersensitivity.[17] A close relationship has been ascribed to the responses of lymphocytes to mitogenic stimuli *in vitro* and their capacities to mainfest cell-mediated immune responses *in vivo*.[18] This activation of small lymphocytes is attended by the release of a variety of potent soluble factors, including a macrophage migration inhib-

itory factor,[19] a cytotoxicity factor,[20] blastogenic factors,[21, 22] a leukotactic factor [23] and a transfer factor.[24] These substances appear to be the effector molecules of cellular immunity with the capacity to recruit nonsensitized cells and to cause cell damage in ways that rather closely mimic the various responses associated with delayed hypersensitivity *in vivo*. The similarity of lymphoid cells in long-term suspension culture to stimulated lymphocytes argues for a significant relationship of these versatile cell systems to cell-mediated immune responses.

Initial studies in our laboratories and in collaboration with Dr. G. A. Granger [25] have demonstrated a rapid destruction of syngeneic, allogeneic and xenogeneic fibroblast monolayers by continuously cultured lymphoid cells and by their supernatant culture fluids. These samples contain a soluble substance with many of the physicochemical and biologic features of lymphotoxin (See Granger, this Symposium).

Culture fluids from lymphoid cell lines markedly impede the migration of guinea pig peritoneal macrophages. Initial characterization confirms that some of the inhibitory products are nondialyzable and stable at 56 C for 30 min, similar to the migration inhibitory factor (MIF) of Bloom and Bennett.[19] A striking finding has been the demonstration that lymphoid cells from lines producing such materials will migrate in ways closely mimicking guinea pig peritoneal macrophages (Fig 1). Furthermore, they respond to inhibitory substances produced by lymphocytes stimulated in short-term culture by phytomitogens and antigens as well as to supernatant culture fluids from syngeneic and allogeneic lymphoid cell lines. Of particular interest is the observation that the migration of these lymphoid cells appears to be more sensitive than guinea pig macrophages to human migration inhibitory products (Table 1). These findings suggest that there may be species specificity for the extracellular mediators of cell immune reaction. The ability of permanently established lymphoid cell lines to produce other factors associated with delayed hypersensitivity is under careful scrutiny (*eg*, transfer factor, blastogenic factor). The remarkable proliferative and synthetic potential of these cell systems should facilitate the isolation and precise characterization of these biologically active immunologic factors. Such studies should determine whether these extracellular mediators are multiple and distinct or one or two molecular species with multiple biologic effects.

Interferon

Additional and perhaps unexpected lymphoid functions with pos-

sible consequences for host defense mechanisms have been uncovered by the analysis of lymphoid cells in long-term suspension culture. Large quantities of a viral inhibiting factor with the biologic characteristics of interferon are produced by these cell lines.[26-28] Viral inhibitory activity is detectable at about the time that initial samples of peripheral blood destined to become established in long-term suspension culture demonstrate increased cellular proliferation.[26] Although a latent viral infection with the herpes-like virus (HLV, Epstein-Barr) appears to exist in most lymphoid cell lines, the mechanisms involved in the production of interferon by lymphoid cells in culture remain obscure. By nature, interferon lacks specificity for any particular viral species. Accumulating evidence further suggests that the association between HLV and interferon production by lymphoid cell lines may be fortuitous.[29] Small lymphocytes stimulated by phytomitogens and antigens are known to produce interferon-like substances.[30] Interferon may be another of the nonspecific effector molecules released by lymphoid cells activated in cell-mediated immunologic reactions. This response could be of distinct advantage to the immunocompetent host in the localized recognition and restriction of intracellular microbial parasitism and would function in situations known to be resistant to high titers of circulating antibody.

Complement

Synthesis of C'3 (B_{1A}/B_{1C}-globulin) has similarly been demonstrated for these cells in long-term suspension culture.[31] These cells also show active phagocytosis of particulate matter,[32] perhaps with bactericidal activity (work in progress with Steven Douglas, MD).

Table 1. Response of Guinea Pig Peritoneal Macrophages and Cultured Human Lymphoid Cells to Human Migration Inhibitory Products

Sample	Average radius (mm)	% inhibition*
Guinea pig cells plus control medium	12.5	
Guinea pig cells plus supernatant culture fluid	7.5	64
Cultured lymphoid cells plus control medium	19.75	
Cultured lymphoid cells plus supernatant culture fluid	4.25	96

* % migration inhibition $= 1 - \frac{\text{area (unknown)}}{\text{area (control)}} \times 100.$

This process is not associated, however, with the release of endogenous pyrogens. These rather diverse capacities are not ordinarily ascribed to lymphoid cells and traditionally have been considered the proper function of macrophages. Several of the lines appear to produce leukocyte alkaline phosphatase as well.

This stem cell nature of lymphoid cells in long-term culture supports the thesis that peripheral lymphocytes activated during immune responses *in vivo* are derepressed cells with enormous biologic potentials.[38] The ability of such cells to ingest and process foreign material, with the synthesis and release of a variety of specific and nonspecific immune factors may be of considerable biologic importance in the defenses of the immunocompetent host. It provides the organism with a uniquely simple, mobile system for the localized, vigorous restriction and destruction of aberrant tissues and foreign antigens. Long-term lymphoid cell lines continue to offer an uncommonly versatile model for the analysis of these lymphoid functions of immunologic significance.

Genetic Studies

The relationship of permanently established lymphoid cell lines to autochthonous donor cells is of considerable interest. Studies in our laboratories have demonstrated the general stability of the diploid human chromosomal pattern of these cells. Unusual morphologic features observed in the donor are retained by cells in long-term culture as seen in lines derived from patients with the Chediak-Higashi syndrome.[34] Further investigations demonstrate the similarity of donor cells and lymphoid cell lines in the synthesis of a variety of enzymes with wide genetic polymorphism, particularly phosphoglucomutase (PGM) and glucose-6-phosphate dehydrogenase (G-6-PD).[35] Collaborative studies with Ceppellini confirm the identity of HL-A surface antigens of host cells and lymphoid cells in long-term culture (Table 2). Similar findings are being reported by other laboratories.[36]

The blastogenic response of fresh lymphoid cells to autochthonous cells in long-term culture by the one-way mixed lymphocyte test is currently under investigation in our laboratories. Preliminary findings [37] demonstrate that lymphoid cells propagated in long-term suspension culture are blastogenic for fresh foreign lymphocytes. Occasionally, stimulation of autochthonous fresh lymphocytes by the mitomycin-treated long-term cells has been noted despite their identical HL-A surface antigens. Although the reason for this stimulation is unknown, the possibilities include minor alterations in surface antigenicity (neo-

Table 2. Histocompatibility Antigens (HL-A) of Fresh Peripheral Lymphocytes and Autochthonous Lymphoid Lines

		Antigen Classification No.														
	HL-A	1	2	3	9	10		5	7	8	12	13				
Donor	Torino	8	9	10	12	13	14	5	20	7	11	21	2	3	4	6
M.G.																
Fresh blood	NIH*	—	—	—	—	—	—	—	—	—	(+)	+	+	+	—	+
Line (SWB-5A)	ABS/$_F$†	—	—		—	—	—	—	—	—	—	+	+			+
W.B.																
Fresh blood	NIH*	—	+	—	+	—	+	—	+	—	—	—	—	+	—	+
Line (HSGP-23)	ABS/$_F$†	—	+		+	—	+	—		—	—	—	—			+

* Cytotoxicity (NIH method).
† Fluorochromasia (Ceppellini) after absorption.
(+) indicates doubtful.

antigens? embryonal antigens?) and/or production of a nonspecific blastogenic factor by the lymphoid cell line.

Recent studies by Reisfeld [38] showed that these cell lines are ideal tools for the isolation of highly purified soluble HL-A antigens. These antigens appear in large quantities in supernatant fluids from these cell lines. The release of these cell surface antigens constitute another soluble product of these lymphoid cell lines, perhaps with equal biologic significance.

Summary

Established lymphoid cell cultures derived from human peripheral blood provide useful models for the detailed analysis of lymphoid functions. These versatile cell systems appear to retain stem cell potentials, performing multiple activities and synthesizing a variety of products which may all be of biologic significance in the defenses of the immunocompetent host (Table 3). Immunoglobulins, mediators of

Table 3. Products of Lymphoid Cells in Continuous Culture

Immunoglobulins
Mediators of Cellular Immunity
Cytotoxicity factor(s)
Migration Inhibitory factor(s)
Blastogenic factor(s) ?
Other factors ?
Interferon
C'3 (B_{1A}/B_{1C}-Globulin)
Enzymes
Histocompatibility Antigens

cellular immunity, interferon, components of complement, enzymes, and soluble histocompatibility antigens are among the known materials synthesized and released into the supernatant fluid. The remarkable proliferative and synthetic potential of these lines should facilitate greater understanding of the nature of these products and their role in the immunologic responses of the host.

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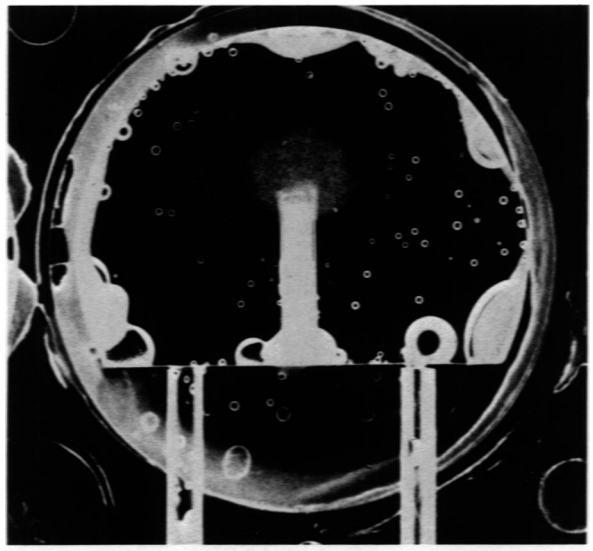
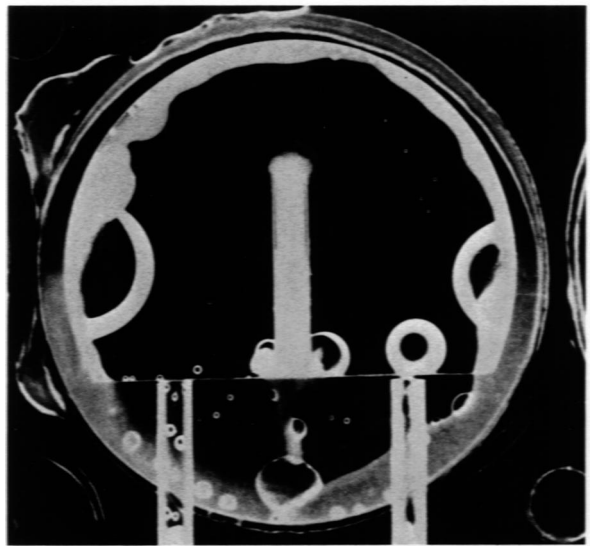
[*Illustration follows*]

Legend for Figure

Fig 1. Migration of lymphoid cells from continuous cell line. *(top)* Migration of cells in control RPMI 1640 medium. *(bottom)* Migration of cells in supernatant fluid from lymphoid cell line. Migration inhibition is approximately 60% of control at top.





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