when the subject is 21.28 years old, thus marking the end of this developmental epoch.

It should be noted that Eq. 1 predicts the average peak frequency for postural tremor from average age. Although this equation suitably describes the underlying developmental sequence, it is not appropriate for predicting the specific peak frequency for a single person. Hence, a correlation over all 287 subjects was calculated between age and peak postural frequency [r (285) = .5610, $P < .001$]. The corresponding regression equation is given as Eq. 2.

$$Y = .2901X + 4.0593 \quad (2)$$

This equation also specifies a developmental rate of approximately 0.25 Hz per year. Moreover, the Y intercept value is in rather good agreement with that found in Eq. 1. In sum, the regression equation based on all 287 individual subjects agrees rather well with the regression equation based on average age and average peak tremor frequency.

Nashold (*4*) reported that the peak frequency of postural tremor in patients with Parkinson's disease is about half (5 Hz) that of normal persons (10 Hz). Friedlander (*5*) reported the peak frequency of postural tremor in parkinsonian patients to range from 4 to 7 Hz. This same author reported the dominant frequency of postural tremor in alcoholics to be 6 Hz. He also reported that the dominant frequency of patients with unilateral damage of the central nervous system ranges from 3 to 7 Hz.

There are two striking parallels between the previously reported data on developmental equations (*1, 2*) and those reported here concerning postural tremor. First, well-fitting regression equations are available that accurately describe normal development. Second, brain disorder decreases the dominant frequency of both the EEG and postural tremor. Hence, it now appears possible to obtain two separate physiological measures of development that are sensitive to organic impairment. Perhaps more such measures will soon be reported.

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Migration Inhibition of Endothelial Cells by Lymphokine-Containing Supernatants

Abstract. *Many of the reactions of cellular immunity are mediated by soluble lymphocyte-derived factors (lymphokines). One important category of lymphokine action involves effects on cell motility. These effects have been described mainly with respect to inflammatory cells. In this report, we describe the ability of a lymphocyte product to inhibit the migration of endothelial cells in a system in vitro. The responsible factor is distinct from a previously described mediator that inhibits the migration of tumor cells. The ability of lymphocytes to influence the migration properties of endothelial cells is consistent with data of others showing a relation between the immune system and processes involving neovascularization.*

Many of the reactions of cellular immunity are mediated by lymphocyte-derived soluble factors known collectively as lymphokines. These mediators exert profound effects on inflammatory cells such as monocytes, granulocytes, and lymphocytes themselves. Such actions fall into three main categories: effects on cell motility (migration inhibition, chemotaxis, and chemokinesis), effects on cell proliferation or cellular viability, and effects on cellular activation for specific specialized functions [reviewed in (*1*)]. Of these, migration inhibition has been considered the prototype, in part because a migration inhibition factor was the first lymphokine to be described, and in part because such factors could play important roles in vivo by virtue of their capacity to immobilize or retain cells at critical tissue sites.

Migration inhibitory factors for macrophages (MIF) and neutrophils have been studied extensively (*1*). More recently, we have described a lymphokine, TMIF, that inhibits the migration of a variety of tumor cells including P-815 mastocytoma, Ehrlich ascites, hepatoma 129, sarcoma 37, and the Walker carcinosarcoma (*2*). TMIF is distinguishable from both neutrophils and MIF on the basis of molecular weight, monosaccharide inhibition profiles, and enzyme inactivation spectra. In addition, it is possible to dissociate the production of these factors by appropriate experimental conditions (*2, 3*).

The present study was undertaken in part to explore the specificity of TMIF for tumor cells and in part to determine whether lymphokines can influence the migration of cells that participate in reparative processes in a manner similar to their well-known effects on the migration of inflammatory cells. In this report we present evidence that human lymphokine-containing supernatants from a variety of sources have the capacity to inhibit the migration of endothelial cells. This activity is separable from TMIF but not from MIF activity by Diaflo ultrafiltration (Amicon).

For these studies, the human lymphoblastoid line RPMI 8392 was the source of most of the supernatants. Such culture fluids, which we have previously characterized, are known to contain lymphokines that are indistinguishable from those obtained from antigen- or mitogen-stimulated normal human lymphocytes (*4*). In addition, normal human lymphocytes were cultured with concanavalin A, or, when obtained from a suitably sensitized individual, purified protein de-

Table 1. Migration inhibition of endothelial cells by lymphokine-containing supernatants. Confluent cultures of endothelial cells were harvested by incubation with warmed trypsin-EDTA for 10 minutes at 37°C. The reaction was stopped by the addition of cold Hanks buffered salt solution (HBSS) supplemented with 10 percent FCS. The cells were washed three times with HBSS and resuspended in RPMI 1640 medium supplemented with 10 percent FCS and anitbiotics, placed on a rocker (Uni-Mixer, Lab-Line Biomedical Products), and incubated at 37°C for 3 to 4 hours before use in the agarose microdroplet assay (*8*). Migration indices (MI ± standard error of the mean) are calculated as follows: $MI = (A_E/A_C) \times 100$, where A_E is the area in the experimental supernatant and A_C is the area in the control supernatant. MI ≤ 80.0 represents significant migration inhibitory activity. Each MI value is the average of at least six determinations.

Experiment No.	Migration index
RPMI 8392	
1	66.2 ± 2.9
2	61.8 ± 2.2
3	67.0 ± 3.0
4	63.1 ± 2.8
5	72.8 ± 2.7
*Concanavalin A activated lymphocytes**	
6	75.2 ± 1.8
7	76.5 ± 4.3
8	70.0 ± 3.6
PPD activated lymphocytes	
9	71.0 ± 5.0
10	68.3 ± 4.1
Unstimulated lymphocytes†	
11	98.4 ± 3.2
12	102.7 ± 4.6
13	96.5 ± 5.1

*Concanavalin A was removed from experimental and control supernatants by incubation with 0.1*M* α-methyl-D-mannoside for 30 minutes at 37°C prior to assay. †Lymphokine-free control.

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Table 2. The migration inhibition effects of fractions of culture supernatants from RPMI 8392 cells on various cell types. The RPMI 8392 culture supernatants were subjected to Diaflo ultrafiltration with PM 10 and YM 5 membranes (Amicon) in succession to obtain estimates of molecular weight. The retentates were concentrated three times before testing. N.D., not done.

Experiment No.	Treatment of supernatants*	Migration index		
		Endothelial cells	Macrophages	P-815 mastocytoma
1	Untreated	56.5 ± 2.5	65.8 ± 3.2	48.0 ± 4.1
	PM 10	64.5 ± 2.9	65.2 ± 3.9	84.8 ± 3.1
	YM 5	98.8 ± 4.2	88.0 ± 3.2	59.7 ± 1.3
2	Untreated	65.3 ± 3.2	N.D.	N.D.
	PM 10	69.0 ± 4.3	N.D.	N.D.
	YM 5	97.5 ± 5.2	N.D.	N.D.
3	Untreated	54.9 ± 2.5	N.D.	N.D.
	PM 10	47.7 ± 3.0	N.D.	N.D.
	YM 5	83.7 ± 4.4	N.D.	N.D.
4	Untreated	54.9 ± 3.2	N.D.	N.D.
	PM 10	61.1 ± 3.5	N.D.	N.D.
	YM 5	97.5 ± 5.1	N.D.	N.D.

*Fractions were obtained sequentially; that is, after removal of material of molecular weight ≥ 10,000 on PM 10, the fluid passed through that filter was used for the YM 5 filtration.

rivative (PPD). The procedures for preparing supernatants and controls have been described (*5*). The target cells were endothelial cells, macrophages, and P-815 mastocytoma cells. Endothelial cells were obtained from calf pulmonary arteries and cultured in RPMI 1640 medium with 10 percent fetal calf serum (FCS) by standard techniques (*6*). In some cases, cells were grown in minimum essential medium with D-valine (Gibco) for one or two passages for removal of fibroblasts (*7*). Cells were obtained for assay by dissociation of confluent cultures with trypsin-EDTA. Their identity as endothelial cells was established by the detection of factor VIII antigen by indirect immunofluorescence (*6*). Macrophages were obtained from guinea pig peritoneal exudates induced by oil over a 4-day period. P-815 mastocytoma cells were obtained from a line maintained in our laboratory by serial intraperitoneal passage in DBA/2 mice.

Migration inhibition activity against the various target cells was determined by the capillary tube assay or by our modification of the agarose microdroplet assay (*8*). Controls for lack of cytotoxicity and reversibility of effect on prolonged incubation of target cells with mediators were performed in all experiments.

First we examined the effect of unfractionated lymphokine preparations on endothelial cells (Table 1). In every instance significant migration inhibitory activity was observed. The migration indices are indicative of approximately 25 to 40 percent inhibition of migration relative to control preparations. For the antigen- or mitogen-stimulated culture supernatants, controls consisted of supernatants from unstimulated cells, reconstituted with the stimulating agent. For the RPMI 8392 cultures, the control was medium alone, which supports migration to the same extent as the other control preparations. As is the case for the traditional studies of macrophage migration inhibition, such inhibition was reversible after 48 to 72 hours of culture, demonstrating that the results obtained were not due to cytotoxicity. Viability was also confirmed by dye exclusion studies with erythrosin B. Morphologically, the individual cells did not show cytopathic effects.

Next, we subjected the RPMI 8392 preparation to Diaflo ultrafiltration and examined the effect of the resulting preparations on tumor cells, endothelial cells, and macrophages (Table 2). In agreement with our previous studies (*2*), MIF activity was found in the fraction (PM 10) corresponding to a molecular weight above 10,000 and TMIF in the fraction (YM 5) corresponding to a molecular weight of 5,000 to 10,000. The migration inhibitory activity against endothelial cells was found in the fraction with MIF, but not TMIF activity.

These results provide further evidence for the specificity of TMIF for tumor cells by demonstrating that a fraction with such activity cannot influence the migration of a nonneoplastic cell, namely, the endothelial cell. This does not rule out the possibility that TMIF might represent a subunit of a larger molecule that is responsible for migration inhibition of other cell types. A more important and definitive conclusion is that lymphokine preparations can inhibit the migration of a noninflammatory cell involved in normal processes of repair. Endothelium was chosen for these studies since revascularization is an essential feature of such processes. The contention that cells of the immunologic system play a role in such events has been based on indirect evidence such as the observation that capillary proliferation occurs in local graft-versus-host reactions as well as other immunologically induced reactions (*9*). Macrophages and their products (*10*) and mitogen-activated lymphocytes (*11*) were found capable of inducing neovascularization. Sidky and Auerbach in 1975 presented indirect evidence for a lymphocyte-derived factor capable of initiating angiogenesis in vivo (*12*). Angiogenesis involves not only endothelial proliferation but also regulated cell movement. Factors inhibitory to migration as well as factors directing and stimulating movement (chemotaxis, chemokinesis) presumably play a role in this process.

In addition to a regulatory effect in reparative processes there is another possible role for endothelial migration inhibitory factors. For example, it is of interest that the effect of lymphokines on endothelial cells we have demonstrated could be antagonistic to at least some of the effects of endogenous, tumor-derived angiogenesis factors such as TAF (*13*). Thus it is possible that lymphokines, in addition to their capacity for direct cytotoxic effect and their inhibitory effect on tumor cell migration, could also play a protective role in neoplastic disease by interfering with the tumor-induced vascular response.

The present study provides no information as to whether the factor that inhibits endothelial cell migration in vitro is the same as or different from MIF. In either case, however, it demonstrates the potential of lymphocyte-derived mediators to influence the endothelial cell in a way that could have significance in vivo. Further studies of the capacity of lymphokines to influence endothelial cell proliferation and activation and to function as chemotactic agents for such cells, as well as studies of the direct detection of these effects in vivo, will be necessary to delineate precisely the biologic importance of this new category of lymphokine effect.

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Stephanopogon, a Phylogenetically Important "Ciliate," Shown by Ultrastructural Studies to Be a Flagellate

Abstract. *A benthic marine protist* (Stephanopogon) *with a homokaryotic nucleus has long been considered to be a gymnostome ciliate. It has been important in hypotheses concerning the origin of ciliates, the evolution and origin of the dual nuclear apparatus of contemporary species of the Ciliophora, and the origin of the multicellular Eumetazoa. Ultrastructural observations reveal that the organism should be reclassified as a flagellate, despite its superficial resemblance to ciliates.*

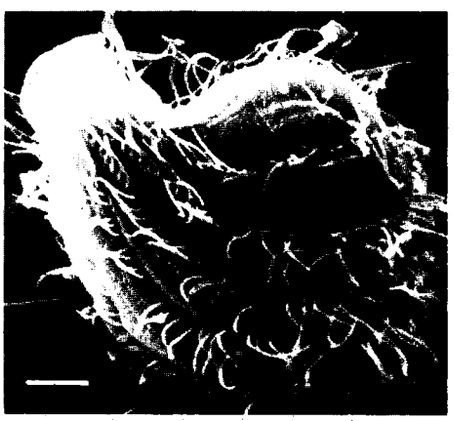
Protistologists have long been confronted with the challenges of explaining both the origin of the allegedly highly evolved ciliated protozoa, presumably from a flagellate progenitor, and the subsequent evolution of their dual nuclear apparatus (micro- plus macronucleus). Several theories implicate *Stephanopogon* not only in the phylogenetic origin of ciliates from a flagellate ancestry and in the evolvement of the duplex ciliate nucleus but even in the origin of the metazoa from a ciliate protozoan line.

Superficially, *Stephanopogon* species, which are small (20 to 50 μm by 10 to 20 μm) and relatively inconspicuous, resemble ciliates (Fig. 1), and they live and behave like various benthic marine gymnostomes (*1*). They move by means of "cilia," arranged in several rows; and, possessing a conspicuous cytostome-cytopharyngeal apparatus, they are actively phagotrophic, feeding on bacteria, various diatoms, and small flagellates. Lwoff's (*2*) discovery of their homokaryotic status, with its phylogenetic implication of primitiveness, was long ignored. Raikov and Corliss (*3*) revived and expanded Lwoff's idea, hypothesizing that the nuclear condition characteristic of ciliates today (diploid micronucleus plus independent polyploid macronucleus) evolved from the single-nucleated condition (with *Stephanopogon* as a lone survivor) via the intermediate (diploid micronucleus plus nondividing diploid macronucleus) "karyorelictid" ciliates. Corliss (*4*) further utilized this phylogenetic theory in his recent major revision of the classification scheme for the Ciliophora, the system essentially adopted by the international Society of Protozoologists (*5*). Hanson (*6*) identified *Stephanopogon* as his (homokaryotic) ciliate progenitor of the first Eumetazoa (for him, the acoelous turbellarians).

We discovered several nonciliate characteristics in *Stephanopogon apogon* Borror, 1965 (*7*), by use of electron microscopy. Our material was taken from Rehoboth Bay, Delaware, in 1977. Specimens were isolated in filtered (Millipore, 0.45 μm) seawater (30 per mil) with a micropipette and fed on unidentified bacteria grown in the culture dishes by addition of a split pea. We have substantiated Lwoff's observation that the organism, while having multiple nuclei (2 to 12 in our species; others may have as many as 16), is homokaryotic; all nuclei are vesicular, each having a single large endosome (Fig. 2). During mitosis an intranuclear spindle forms, the nuclear envelope remains intact, and the endosome divides without dedifferentiation or dissolution. The nucleus and its pattern of acentric mitosis are distinctly trypanosome-like (*8*).

As concerns the "infraciliature" underlying the eight sparsely distributed rows (six on the ventral surface plus two short ones dorsally) of flagella, the most significant aspect of our transmission electron micrograph sections is the complete absence of the kinetidal system characteristic of ciliates (*4*). While an unusually short (~ 0.25 μm) kinetosome exists at the base of every flagellum, neither a kinetodesma (or any homolog) nor ribbons of transverse and postciliary microtubules are present (Figs. 3 and 5). Furthermore, there are no pellicular alveoli, parasomal sacs, contractile vacuole (or pore), or cytoproct in *Stephanopogon*. Spherical microbodies, of unknown function, are found in the peripheral cytoplasm next to the cell membrane.

Although *Stephanopogon* has a functional and well-developed cytostome-cytopharyngeal apparatus (as do a number of flagellates and ciliates), the 32 bundles of microtubules supporting it show an unusual quadratic packing (Fig. 4) and a curious origin from fibrous material. The area enclosed by these long microtubules



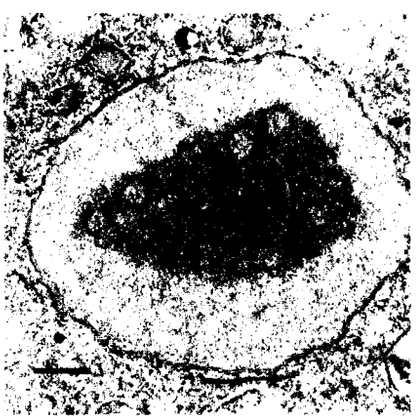


Fig. 1 (left). Scanning electron micrograph of *Stephanopogon apogon*. Ventral rows of flagella and the opening of the cytostome-cytopharyngeal complex have caused this unusual genus of flagellates to be incorrectly classified as a ciliate. Scale bar, 10 μm. Fig. 2 (right). Transmission electron micrograph of a section through the nucleus. There are two or more such identical nuclei in every organism, each measuring 3.5 to 4.0 μm in diameter, with a large central endosome. The chromatin appears finely granular and evenly dispersed. Scale bar, 1.0 μm.

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