Cellular and Molecular Biology of Lymphokines

International Lymphokine Workshop 1984
at Schloss Elmau, West Germany

Edited by

Clemens Sorg
Department of Experimental Dermatology
Universitäts-Hautklinik
Münster, West Germany

Anneliese Schimpl
Institute of Virology and Immunobiology
Universität Würzburg
Würzburg, West Germany

Discussion editor

Maurice Landy
La Jolla, California

1985

ACADEMIC PRESS, INC.
(Harcourt Brace Jovanovich, Publishers)
Orlando San Diego New York London
Toronto Montreal Sydney Tokyo

ACADEMIC PRESS, INC.
Orlando, Florida 32887

United Kingdom Edition published by
ACADEMIC PRESS INC. (LONDON) LTD.
24–28 Oval Road, London NW1 7DX

LIBRARY OF CONGRESS CATALOGING-IN-PUBLICATION DATA

International Lymphokine Workshop (4th : 1984 : Schloss Elmau)
Cellular and molecular biology of lymphokines.

Workshop held at Schloss Elmau, West Germany, 1984.
Includes index.
1. Lymphokines—Congresses. 2. Molecular biology—Congresses. I. Sorg, Clemens. II. Schimpl, Anneliese. III. Landy, Maurice. IV. Title. [DNLM: 1. Immunity, Cellular—congresses. 2. Lymphokines—congresses. W3 IN729 4th 1984 c / QW 568 I595 1984c]
QR185.8.L93I57 1984 616.07'9 85-47816
ISBN 0-656160-5 (alk. paper)

PRINTED IN THE UNITED STATES OF AMERICA

85 86 87 88 9 8 7 6 5 4 3 2 1

THE MULTILINEAGE HEMOPOIETIC GROWTH FACTOR (MultiHGF/BPA/MCGF/IL-3)

N.N. Iscove

Basel Institute for Immunology
Basel, Switzerland

C. Roitsch
Transgene SA
Strasbourg, France

I. INTRODUCTION

A number of glycoprotein growth factors active on cultured nouse hemopoietic cells have been identified and characterized. Most of them act individually on cells in only one or two of the various hemopoietic lineages (reviewed in 1}. The best known examples in this category are erythropoietin, macrophage growth factor (CSF—1} and neutrophil/macrophage growth factor (GM-CSF). This chapter is concerned with a distinct factor - MultiHGF — which, unlike the others, supports the growth of cells in all the hemopoietic lineages including early precursors which are still pluripotent. Because of this broad spectrum of targets, a variety of different names were applied to the factor — principally "burst promoting factor/activity" (BPA), "mast cell growth factor" (MCGF) and "interleukin-3" (IL-3) — before it was appreciated that all these activities were attributable to a single factor.

In this chapter, the history of MultiHGF is reviewed, our own effort at its characterization and purification is summarized, and information bearing on its possible biological role is discussed.

ISBN 0-12-656160-5

II. AN HISTORICAL PERSPECTIVE

The record of discovery/rediscovery of MultiHGF begins with the in vitro cloning of hemopoietic cells. The seminal observations can be grouped in three major waves:

A. "CSF"

In 1965 (2), growth of granulocyte and macrophage colonies from mouse bone marrow was first achieved over feeder layers of living cells. Feeder layers were soon replaced by their conditioned medium which contained "colony-stimulating factor" (CSF). Later it became apparent that there was more than one biochemically distinct type of CSF, and a wide variety of sources. By the early '70's, medium conditioned by WEHI-3B myelomonocytic leukemia cells or by activated T lymphocytes had been found to contain CSF activity for granulocytes, macrophages and megakaryocytes. Today we know that most of this CSF activity from WEHI—3B cells, and part of the activity from stimulated lymphocytes, were distinct from the other CSF's and due in fact to MultiHGF.

1969 Medium conditioned by WEHI-3B cells contained granulocyte/macrophage "CSF" (3).

1973 Lymphocyte populations activated by lectin or antigen released "CSF" (4).

1974 Conditioned medium from WEHI—3B cells or lectin-activated spleen cells also stimulated eosinophil colonies (5).

1975 Lectin-activated spleen conditioned medium stimulated growth of megakaryocyte colonies (6).

1976 Granulocyte/macrophage "CSF" was found in conditioned medium from the Ralph subline (7) of WEHI-3B(D-) cells.

1978 Conditioned medium from the Ralph line of WEHI—3B(D-) cells stimulated growth of megakaryocyte colonies (8).

1978 A predominantly granulopoietic CSF species ("G-CSF") from the Ralph line of WEHI-3B(D-) cells ran unretarded on DEAE columns at alkaline pH (9).

B. "BPA"

In 1974, colonies, called "bursts" because of their dispersed configuration, were grown from primitive erythroid precursors in cultures containing erythropoietin (10). Today it is clear that the growth of such colonies can be supported initially by MultiHGF, while later steps including terminal erythroid differentiation, depend instead on erythropoietin. That growth of bursts was originally obtained without intentional addition of MultiHGF probably reflected the action of as yet unclarified mechanisms involving marrow accessory cells and bovine serum (11,12).

1977 Experiments in the whole mouse suggested that regulation of the proliferative activity of primitive erythroid precursors occurs independently of erythropoietin (13). The observation raised the perplexing question of what was supporting the growth of these early precursors in culture. The parallel responses of pluripotential and early committed erythroid, granulocyte and macrophage progenitors in the whole mouse in response to system perturbations led to the suggestion that mechanisms regulating early committed cells in the system may not have lineage—specificity (13).

1977 Experiments with serum-substituted medium suggested that initial steps in colony formation in culture by early erythroid progenitors do not require erythropoietin, but do depend on an activity provided by serum (14).

1977 Growth of colonies was obtained from pluripotential as well as committed erythroid progenitors in cultures of fetal liver cells. Growth was dependent on conditioned medium from lectin-stimulated spleen cells. Adult marrow responded poorly (15).

1978 Growth of early erythroid precursors from adult marrow was dependent on a "burst-feeding" activity ("BPA") which could be supplied by a marrow cell sub-population (11).

1978 A high efficiency of pluripotential colony growth was obtained in cultures of adult mouse bone marrow. Growth and survival of pluripotential and early committed erythroid precursors depended on "burst—promoting" activity ("BPA") present in conditioned medium from lectin-stimulated spleen cells. Erythropoietin was required but only at late stages of colony development. The respon-

siveness of pluripotential cells to EPA led to the prediction that BPA might also support colony formation by committed granulocyte and macrophage progenitors (16).

1979 Conditioned medium from WEHI-3B(D-) cells contained BPA (17).

1980 Antigen—dependent release of EPA occurred in cultures of cloned antigen-specific helper T lymphocytes and accessory cells matched at the I-A region of the major histocompatibility complex (18).

1980 Colony-stimulating species from lectin-stimulated spleen cells active on pluripotential, erythroid, megakaryocyte, eosinophil and granulocyte/macrophage precursors were similar in size, charge and hydrophobicity (19).

1982 The active principle from WEHI—3B(D-) cells was purified sequentially by methods including HPLC on reverse phase and gel permeation columns. Extensively purified material continued to support growth of pluripotential as well as committed precursors in the granulocyte, macrophage, erythroid and megakaryocyte lineages (20).

C. Factor-Dependent Cell Lines and IL-3

1979 Growth of granulocytic cell lines obtained from retrovirus-infected bone marrow cultures was enhanced by conditioned medium from WEHI-3B{D-) cells (21).

1980 Growth of a line of mast cells obtained from Friend virus—infected spleen cells was dependent on conditioned medium from lectin—stimulated spleen cells. The active principle co—chromatographed with granulocyte/macrophage colony-stimulating activity on Con A-Sepharose and Sephadex G-150 (22).

1980 Growth of granulocytic cell lines derived without virus infection from bone marrow cultures was dependent on conditioned medium from either WEHI—3B(D-) cells or lectin—stimulated spleen cells (23).

1981 Mast cell lines derived without virus infection from bone marrow or spleen depended on conditioned medium from WEHI—3B(D-) or spleen cells for growth (24-28).

1981 Conditioned medium from lectin-stimulated spleen cells induced a rise in 20-α-hydroxysteroid dehydrogenase in cultured spleen cells from nu/nu mice. The responsible activity was named interleukin-3 (IL-3) (29).

1982 Conditioned medium from WEHI-3B(D-) cells contained IL-3. The activity was exhaustively purified and found not to separate from activity supporting the growth of factor-dependent granulocytic cell lines (30,31).

1982 20-α-hydroxysteroid dehydrogenase was present in granulocytic cell lines (32,33).

1983 Highly purified hemopoietic cell growth factor (HCGF,34) or IL-3 (35) suported growth of normal hemopoietic precursor cells and factor—dependent granulocytic or mast cell lines.

III. THE ACTIVITIES IN CONDITIONED MEDIUM FROM SPLEEN OR WEHI-3B(D-) CELLS

The studies referenced above document the presence in these conditioned media of the following biological activities on non—transformed mouse hemopoietic cells in culture:

1. Support of survival and growth of pluripotential, erythroid, megakaryocyte, macrophage, neutrophil, eosinophil and mast cell precursors in semi-solid medium. Pluripotential and early committed erythroid precursors die with a half-life of about 12 hr in the absence of factor (16). Growth of macrophage, granulocyte and mast cell precursors is supported through to terminal maturation. Completion of maturation to erythrocytes and megakaryocytes, on the other hand, depends on the additional presence in the cultures of erythropoietin (16) and a megakaryocyte differentiation factor (36,37), respectively. In some early reports, erythropoietin was supplied serendipitously as part of the serum component of the medium (e.g. 15).

2. Support of survival and extended growth of mast cells in suspension culture (24-28). Mast cell lines can be initiated reproducibly from bone marrow or spleen. With serial passage of non-adhering cells in the presence of MultiHGF, the cultures soon consist almost entirely of mast cells. After the

initial 4 weeks, doubling times progressively lengthen, and the typical untransformed line ceases growth by about 12 — 16 weeks.

3. Support of survival (38) or net increase (20, 39-42} in the number of pluripotential, erythroid, granulocyte and macrophage precursors present in 3 — 7 day suspension cultures of adult bone marrow. Numbers decline with subsequent passage despite the presence of factor (unpublished observations).

In the sections to follow, biochemical purification establishes that these diverse activities are attributable to a single qlycoprotein factor, MultiHGF.

IV. PURIFICATION OF MultiHGF

Extensive purification of MultiHGF from WEHI-3B{D-) conditioned medium has been reported by Ihle and co—workers (31) and more recently by Clark—Lewis et al. (65) on a microgram scale, and by Bazill and coworkers (34) on a smaller scale. We have purified the factor from the same source on a submicrogram scale to a coparable specific activity. In our experiments, biological activity was monitored at each stage both by support of pluripotential, erythroid, granulocyte and macrophage colony formation by adult mouse marrow cells in semi-solid cultures, and by support of growth of non—transformed factor-dependent 4 - 12 week old mast cell lines in liquid culture. The essential observation was that sequential procedures which separate polypeptides on the basis of size, charge, hydrophobicity and affinity for hydroxyapatite failed to dissociate the activities monitored.

The following sequential purification was performed on a 10—litre batch of cogditioned medium. WEHI—3B(D-) cells were grown to 5 — 10 x 10 cells/ml in IMDM containing 1% newborn calf serum, then split 1 in 5 in IMDM alone (resulting serum concentration 0.2%) for the final expansion and conditioning. Maximum cell densities (1 - 2 x 10^6 /ml) and activity concentrations required that the depth of the medium not exceed 2 mm. Medium was harvested when acid and when about 50% of the cells appeared non—viable. It was filtered (0.45μ) and concentrated 100-fold by ultrafiltration using an Amicon HP10 hollow fibre cartridge.

A. Step I. Ion Exchange Chromatography

Despite its mildly acidic isoelectric point (5.0 - 6.5, see below), MultiHGF interacts very weakly with DEAE columns even at pl-I 8.5 — 9.0. This unusual property can be exploited to give about 100—fold purification from crude conditioned

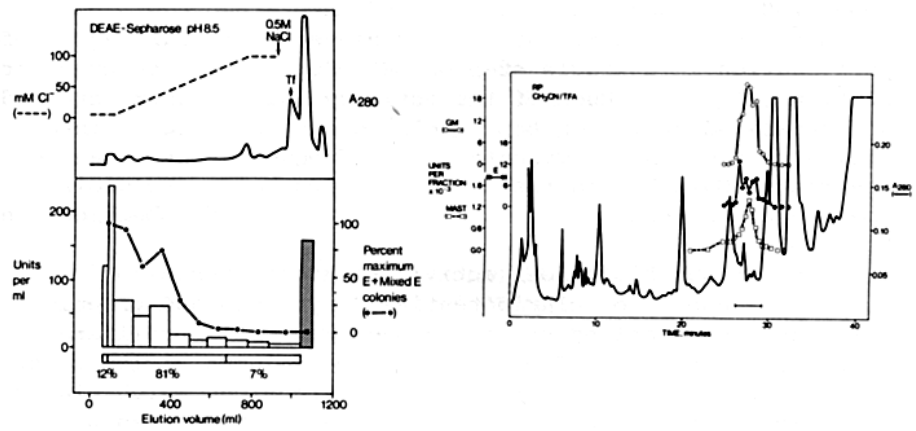


Fig. 1. (Left) Separation on DEAE-Sepharose. Crude conditioned medium (25 ml, concentrated from 2 litres by ultrafiltration) was applied to a 2.5 x 39 cm column equilibrated in Tris.HCl 15 mM total Cl- 6mM, pH 8.5, and eluted with a linear gradient rising to 100 mM Cl- (NaCl 60 mM, Tris.HCl 100 mM, pH 8.5). Transferrin (from the bovine serum), identified by its salmon pink colour, emerged as indicated (Tf) at the top of the gradient. One MultiHGF activity unit gives 1/2 maximum colony count (all categories) from adult mouse marrow cells in 1 ml methyl cellulose cultures containing erythropoietin (20). The hatched bar indicates predominantly macrophage colony-stimulating activity eluted with high salt. Percents of total eluted MultiHGF activity are indicated.

Fig. 2. (Right) Reverse phase HPLC on a Brownlee Aquapore RP-300 C8-silica column equilibrated with 0.1% trifluoracetic acid (TFA), pH 2, and eluted with a 30 min gradient (25 - 40%) of acetonitrile in 0.1% TFA. Ehe starting sample was purified by ion exchange (CM + DEAE) and concentrated by ultrafiltration. Before application to the column, it was further purified by discarding the precipitate brought down by 40% saturated ammonium sulfate at room temperature, pH 8.8. Fractions pooled for further purification are indicated by the horizontal bar. GM granulocyte/macrophage colonies, E erythroid colonies.

medium. Initial purification protocols entailed two ion exchange steps (not shown). In the first step, concentrated conditioned medium was run on CM-Sepharose at pH 5.8 (succinate.NaOH 12.5 mM: NaCl 40 mM; total Na+ 46 mM) to remove proteins with isoelectric points substantially above this pH. MultiHGF eluted unretarded with most of the protein. The active fractions were pooled, concentrated and then run on DEAE—Sepharose at pH 9.0 (Tris.HCl 25 mm; NaCl 50 mM; PEG 6000 0.005%). Activity again eluted unretarded.

More recent experiments have achieved a similar degree of purification in a single step on DEAE-Sepharose with gradient elution (Fig. 1). Much of the activity was somewhat retarded and separated away from both excluded and more highly interactive contaminants. Transferrin, a frequent contaminant of the eluted MultiHGF in the earlier ion exchange protocols, emerged at the end of the salt gradient after the bulk of the MultiHGF.

The various biological activities attributable to MultiHGF (support of pluripotential, erythroid and granulo—cyte/macrophage colony growth, and of mast cell proliferation) eluted together from ion exchange columns at low salt. On the other hand, the strongly bound fractions which eluted with high salt from DEAE columns regularly stimulated growth of colonies which were mainly macrophage. The observation has been made before (9) and serological evidence suggests that this activity is attributable to macrophage growth factor (CSF-1} [51].

B. Step II. Reverse-phase HPLC

Ion exchange-purified material (CM + DEAE) was applied to a C8-silica column and eluted with a gradient of acetonitrile. The various biological activities again eluted together, reproducibly at 35% acetonitrile (Fig. 2). Typical gain in specific activity was about 20-fold.

C. Step III. Reverse-phase HPLC with HTBA

Active fractions from the previous step were pooled and re-applied to the same C8 column now equilibrated with heptafluorobutyric acid. HFBA is a strong "ion pairing" agent which can add additional selectivity on the basis of charge. The column was developed with a dual gradient of n—propanol and acetonitrile (66). The various activities again eluted together (Fig. 3), and the step gave an additional purification of about 5-fold.

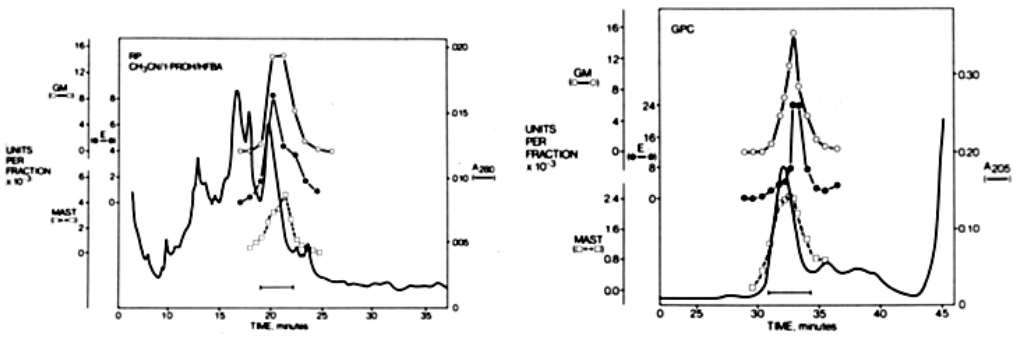


Fig. 3. (Left) Reverse phase HPLC of Step II material on a RP-300 C8-silica colwnn equilibrated in 10 mM HFBA and eluted with a 30 min dual gradient of n-propanol (14 - 21%) and acetonitrile (30 - 46%) in 10 mM HFBA (66). Fractions pooled are indicated.

Fig. 4 (Right) Gel permeation HPLC of Step III material on tandem 60 cm TSK G2000SW and G-3000SW columns running in 10 mM Tris.HCl pH 7.0, 150 mM NaCl and 0.05% PEG 6000. Fractions pooled are indicated.

D. Step IV. Gel Permeation HLC

Pooled active fractions from the previous step were concentrated by ultrafiltration and applied to a gel permeation column. All activities ran at an apparent MW of 33,000. The bulk of the contaminating protein was of only slightly greater MW and consequently little useful separation was achieved (Fig. 4).

E. Step V. SDS-PAGE

Pooled activity from the gel permeation step was run on SDS-PAGE (Fig. 5). All activities migrated together in a broad band with an apparent nolecular weight of 26-28,000. Major contaminants had apparent weights of 40,000 and higher. A small amount of silver-stainable material, estimated to be no sore than 10 ng, was coincident with the biological activity.

Table I shows the quantitative considerations leading to an estimate of 7 x 10^{-13} M for the concentration of silver-stainable protein required for 1/2 maximum colony formation by pluripotential and erythroid precursors. The specific activity is within a credible range for a completely purified polypeptide growth factor.

TABLE I. Activity recovered from SDS—PAGE

Total activity[a] recovered	130	units
Estimated visible protein	10	ng
Fraction loaded activity recovered	0.25	
Estimated protein recovered	2.5	ng (10 x 0.25)
Specific activity	$5x10^{7}$	units/mg
Molarity of 1 unit/ml[b]	$7x10^{-13}$	M
Mass equivalent of 1 unit	20	pg

[a]1 unit/ml is the amount giving half maximum numbers of pure and mixed erythroid colonies in cultures of adult bone marrow containing erythropoietin.

[b]assuming a MW of 27000

The SDS-PAGE step can therefore be used preparatively to give final purification on the basis of size. It has the advantage of high resolution, but the disadvantage, as performed, of relatively low recovery (typically 25%},

From the results of two sequential purifications utilizing steps I to V, it is estimated that the original WEHI—3B(D-) conditioned medium contained no more than about 1 μg of MultiHGF per litre.

F. HPLC on Hydroxyapatite

Because material purified by charge, hydrophobicity and gel permeation still required final purification on SD5-PAGE, an additional HPLC step operating on an independent principle seemed desirable. HPLC on hydroxyapatite was therefore tried on material already purified by DEAE ion exchange, two cycles of reverse-phase HPLC, and gel permeation HPLC. As shown in Fig. 6, it proved effective in separating biological activity away from contaminating protein of similar size.

Our preliminary experience suggests that the purification sequence outlined in Table II may suffice to obtain pure MultiHGF without resort to SDS-PAGE. The yields with relatively small amounts of starting material (e.g. 5 litres of conditioned medium) at each of the listed steps are sufficiently high (at least 50%) to permit complete purification of sub-microgram quantities. The sequence has been chosen to permit optical detection (205 nm) of nanogram quantities of factor and contaminants at the last stage.

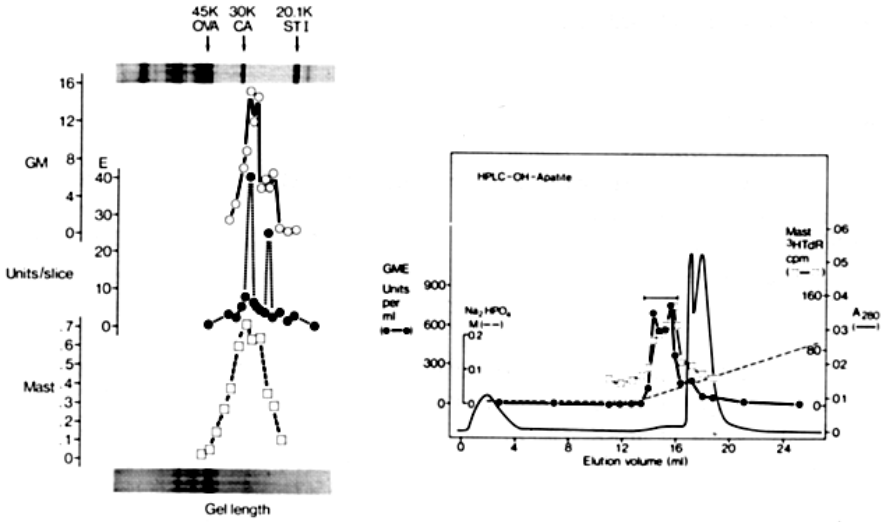


Fig. 5. (Left) SDS-PAGE of 100 ng of protein purified by DEAE ion exchange, 5 cycles of reverse-phase HPLC and finally gel permeation HPLC. The lane at the bottom was formalin fixed and stained with amnoniacal silver (67). An identical lane was sliced and eluted passively into buffer containing 1% BSA for assay of biological activity. Molecular weight standards are shown at the top (ovalbumin, carbonic anhydrase, soybean trypsin inhibitor).

Fig. 6. (Right) Hydroxyapatite HPLC of Step IV material on a BioRad Biogel HPHT column equilibrated in 10 rd-I sodium phosphate pH 6.8 and 0.3 mM CaCl2, and eluted with a linear 30 min gradient rising to 350 mM phosphate and 0-01 ml! CaCl2. GME total colonies including pluripotential, erythroid, granulocyte and macrophage.

Table II. Proposed sequence for complete purification of MultiHGF

1) DEAE—Sepharose {gradient elution)
2) HPLC — Reverse Phase with HFBA/AcN/n-propanol
3) HPLC - Reverse Phase with TFA/AcN
4) HPLC - Hydroxyapatite
5) HPLC — Gel Permeation

V. CHARACTERIZATION

A. Properties

A few elementary properties of MultiHGF that we have determined are summarized in Table III. Most entries in the Table confirm similar observations from other laboratories. The data are consistent with a singe—chain glycoprotein having internal disulfide links important for its active conformation.

The result of isoelectric focusing of extensively purified (step IV, text) material in a_polyacrylamide slab gel with 0.1% Triton-X is shown in Fig. 7, indicating charge heterogeneity over the pI range of 4.8 - 6.5.

Thus, all tested activities remained associated with one another in four separative steps, each of high resolving power and operating on independent principles - reverse phase HPLC, SDS-PAGE, hydroxyapatite HPLC and isoelectric focusing. Taken together, these results provide very strong evidence that the various activities are functions of a single factor.

TABLE III. Biochemical properties of MultiHGF

1.	Apparent MW 33000 (gel permeation, non—denatured} 27000 (SDS-PAGE)
2.	pI 4.8 - 6.5
3.	anomalously weak interaction with DEAE-Sepharose pH 8.5 — 9.0
4.	About 50% is retained on Con A—Sepharose and is eluted with α—methyl glucoside.
5.	Activity destroyed by trypsin chymotrypsin papain
6.	Activity 90% destroyed in 5% β-mercaptoethanol at 55°, 10 min. Residual activity is unchanged in size on SDS-PAGE.
7.	Stable to SDS guanidine.HCl heat 56°, 10 min.

Table IV. Protective effects of various agents on MultiHGF in dilute solution[a]

time stored	agent	concentration %	colony counts[b] (% BSA–protected control)
20 hr	0		0
	BSA	1.0	100
	gelatin	0.5	114
	Triton X-100	0.05	82
	PVP-15	0.1	91
	PVP–40	0.5	80
	PEG 400	0.01	0
		0.1	0
		1.0	0
	PEG 550	0.01	0
		0.1	7
		1.0	30
	PEG 3400	0.01	7
		0.1	43
		1.0	125
	PEG 6000	0.01	9
		0.1	88
		1.0	148
5-7 d	0		0
	BSA	1.0	100
	PVP-15	0.1	6
	PVP–40	0.5	3
	Pyrrolidone	1.0	2
	PEG 6000	1.0	46
	PEG 6000 + glycerol	0.1 10	77
	PEG 6000 + glycerol	1.0 10	138

[a]10 ng total protein/ml; 10 activity units/ml; purified by ion exchange (DEAE), reverse–phase HPLC and gel permeation HPLC. Buffer contained 20 mM Tris pH 9.0, 160 mM NaCl and 1 mM EDTA.

[b]total colonies in 7 day cultures of adult bone marrow containing 20% of the MultiHGF solution to be assayed.

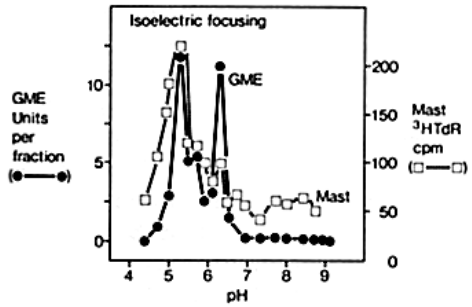
B. Stability

Biological activity of crude WEHI-3B(D-) conditioned medium, or of material partially purified on DEAE— Sepharose, slowly decayed at 4° C with a variable 1/2 life sometimes as short as 30 days. At purification stage III or beyond, little decay was observed at 4° C for periods as long as 4 months in solutions containing only nanograms of total protein per ml. These samples were stored at pH 9.0 in Tris 20 mM, NaCl 160 mM, EDTA 1 mm, polyethylene glycol (PEG 6000) 0.1 - 1.0% and glycerol 10%.

These latter conditions were determined in pilot experiments in which various additives were tested for effectiveness in preserving activity in solutions of highly purified MultiHGF containing only 10 - 20 ng total protein per ml. Each test group was membrane—filtered (Millipore 0.45μ, mixed cellulose acetate/nitrate), stored in polystyrene tubes at 4° C and assayed after 20 hr and again after 5 - 7 days (Table IV). Survival at 20 hr was better with 1% than with 0.1% PEG 6000, and better with PEG 6000 than with lower order polymers. Polyvinyl pyrrolidone (MW 15,000 or 30,000), but not pyrrolidone, was as effective as PEG 6000. Survival for 7 days was best with the additional presence of 10% glycerol.

VI. MultiHGF-RESPONSIVE CELLS

The domain of activity of MultiHGF is depicted in Fig. 8. The scheme is based on the MultiHGF-dependent growth of marrow- or spleen-derived mast cells, and the cellular content of MultiHGF—dependent hemopoietic colonies. The responsive set is shown to include pluripotential hemopoietic progenitors, early committed precursors in all the hemopoietic lineages, and members of the granulocyte, macrophage and mast cell lineages all the way down to their terminally maturing forms. The only hemopoietic cells demonstrated to be unresponsive to MultiHGF are those in the distal limbs of the erythroid (16) and megakaryocyte (36,37) pathways. Thus, whereas MultiHGF can support production of mature macrophages, granulocytes and mast cells starting from cells as far back as the pluripotential precursors, it can not support production of mature erythrocytes and platelets in the absence of other factors specifically required by late cells in these lineages, namely erythropoietin and megakaryocyte differentiation factor.



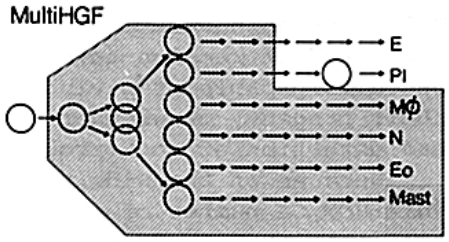


Fig. 7. (Left) Isoelectric focusing in a 5% polyacrylamide slab gel with 0-1% Triton-X of I00 ng of protein previously purified by DEAE ion exchange, 3 cycles of reverse phase HPLC and 2 cycles of gel permeation HPLC. The gel was sliced and eluted passively into 1% BSA.

Fig. 8. (Right) Hemopoietic lineages responsive to MultiHGF are shown in the shaded area. Furthest to the left in the shaded area is a pluripotential cell giving rise to oligopotential cells which give rise in turn to committed unipotential precursors. E erythroid, Pl platelet preceded by a megakaryocyte, Mp macrophage, N neutrophil, Eo eosinophil.

Table V. Names for the same factor

Old	References	Proposed (1)
BPA	16,20	
IL-3	29,31	
BFA	11	
E—CSF	15,19	
SAF	39,41	MultiHGF
BEF	43	
MK-CSF	36,37	
MCGF	28	
PSF	24,55	
HCGF	34	
Hemopoietin-2	44	
Multi-CSF	45	

Little is known about the factor requirements of the earliest cells of the hemopoietic system. We do know that pluripotential cells can increase in number over a period of days in liquid culture {20,39-42}, and that this increase is MultiHGF-dependent. However, other factors are involved (42), and it remains to be seen whether MultiHGF itself acts on cells capable of giving rise to pluripotential cells among their progeny, or whether it is simply required for survival and detection of such progeny after they arise.

Because of the wide variety of cells responsive to MultiHGF, a number of operational names accumulated before it was clear that the various assays were detecting the same factor. Some of the names are listed and referenced in Table V.

Immortal and presumably "transformed" cell lines have been described which are dependent on MultiHGF for growth and survival in culture. Some of these have possessed markers also associated with normal T (46) or B (47) lymphocytes. Extrapolation of these observations of MultiHGF responsiveness to untransformed lymphocyte precursors has its hazards (1,48) and does not seem warranted on the basis of the available evidence. Experiments which would be decisive on this issue await the development of suitable assays for normal lymphocyte precursors in culture. In one such assay, MultiHGF, GM—CSF and CSF—1 all appeared to have similar stimulatory effects on the growth of clonogenic pre-B cells from mouse fetal liver (49). Because these three factors share macrophage precursors as targets in common, the observation was interpreted as an indirect, possibly macrophage-mediated, effect on the pre-B cells rather than a direct effect. More recent experiments, in which purified clonogenic pre—B cells did not respond to these factors, have now confirmed this interpretation (50).

VII. DIRECT ACTION OF MultiHGF ON HEMOPOIETIC PRECURSORS

Bone marrow cultures contain many different kinds of cell, only a minority of which can form colonies. It was therefore necessary to ask whether MultiHGF might exert its action indirectly by causing "accessory" cells to release other factors directly active on hemopoietic precursors. The cleanest test is to ask whether MultiHGF can support the growth of single micromanipulated precursor cells cultured in isolation. To render the experiment practicable, it was first necessary to enrich the marrow for early precursor

cells. The enrichment was accomplished biologically, in two stages. First, mice were injected with 5-fluorouracil to kill the actively cycling, majority marrow population. After two days, bone marrow cells from treated mice were plated in methyl cellulose. Most of the growth~competent precursors in such cultures go on to form multilineage colonies. The second enrichment step involved identifying nascent clones after 4 days of incubation. Pilot experiments had previously indicated that certain morphological features of such nascent clones correlated strongly with eventual development into large multilineage colonies. Accordingly, clones showing these features (non-adhesiveness, heterogeneity of cell shape, size, and motility) were selected, and cells from them were deposited singly in methyl cellulose either containing or lacking MultiHGF (step II, text). The result, shown in Table VI, formally establishes growth—competent hemopoietic precursors, pluripotential as well as app-arently committed ones, to be primary responders to MultiHGF.

Table VI. Response of isolated micromanipulated cells to MultiHGF.

MultiHGF[a]	Colonies	
	content[b]	number
-		0/60[c]
+	EMegM±G	8
	EMeg	5
	E	1
	Meg	1
	GM	1
	G	3
	M	2
	?	1
		22/60[c]

[a]Purified on CM-sepharose, DEAE-Sepharose and reverse-phase HPLC.

[b]E erythroid; Meg megakaryocyte; G granulocyte; M macrophage.

[c]Total of 60 cells cultured without and 60 with MultiHGF.

VIII. THE SOURCES OF MultiHGF

The WEHI-3B(D-) line used in our studies was subcloned from the WEHI-3B(D-) myelomonocytic leukemia line originally adapted to culture by Ralph (7). The cells are macrophages phenotypically. This line is the only known macrophage source of this factor. Conditioned media from normal macrophages and a variety of other mouse macrophage cell lines have been consistently negative for MultiHGF (unpublished observations).

Lectin- or antigen-stimulated lymphocyte populations, and a number of T cell lymphoma and hybridoma lines release activity with biological and chemical characteristics essentially identical to those of the WEHI-3B(D-) factor (16,35,38,55, 65). The identity of the concanavalin A-induced product of a cloned Ly1+2- T cell line with the wEHI—3 product has now been formally shown by cDNA cloning (53,54).

The most physiological situation known in which MultiHGF is released occurs when helper T cells are stimulated with antigen. The cleanest of the published experiments showed that MultiHGF was released when functional, cloned antigen-specific T helper cells were exposed to antigen in the presence of accessory cells. These had to match the T cells at the I—A locus of the major histocompatibility complex (18).

In summary, the only known physiological source of MultiHGF involves T lymphocytes. The release of MultiHGF by pure populations of immortalized T cells in vitro (38,55,56, unpublished observations) constitutes circumstantial evidence implicating normal T lymphocytes (rather than accessory cells) as the producing cells. Except for the singular instance of the WEHI-3 line, the bulk of the evidence indicates that normal macrophages do not produce this factor. It seems reasonable to speculate that a transformation event leading to “constitutive” production and release of MultiHGF by the precursor of the WEHI-3 line may have been at least partly responsible for the myelomonocytic leukemia in the original host animal.

IX. THE RELATIONSHIP OF MultiHGF TO OTHER HEMOPOIETIC GROWTH FACTORS

The chemical purification and most recently the cDNA cloning of MultiHGF have resolved some previously confusing questions.

First, the purification helped to show that MultiHGF was chemically and biologically distinct from the neutrophil/ macrophage growth factor (GM-CSF) purified earlier from mouse lung (52). The recent cDNA cloning of both factors now establishes formally the non~identity of their polypeptide backbones {45,53,54. Both are present in the conditioned medium of lectin-activated spleen cells (55). and, like MultiHGF, GM-CSF is also produced by T cell hybridomas and lymphomas (55,57). However, little or no GM-CSF is produced by WEHI-3B(D-) cells. Whereas both MultiHGF and GM—CSF can support macrophage and neutrophil precursor growth, GM-CSF differs in not supporting mast cell growth (55) or formation of eosinophil (55), megakaryocyte (20) or pure or mixed erythroid colonies (20). In spleen conditioned medium, GM—CSF is readily separated from MultiHGF because it is more strongly retarded than MultiHGF on DEAE columns (35,52,55).

MultiHGF also differs chemically and biologically from the macrophage growth factor CSF-1 (59), whose action is restricted to cells in the macrophage pathway. CSF-1 can be detected biologically and serologically in conditioned medium from both spleen and WEHI-3B(D-) cells (51). However, it is readily separated from MultiHGF on the basis of its stronger retardation on DEAE columns and its higher molecular weight when fully glycosylated.

MultiHGF is also distinct from the neutrophil growth factor G-CSF (60). The latter appears to act mainly on cells of the neutrophil lineage. It differs from MultiHGF in having stronger affinity for DEAE columns and higher hydrophobicity.

From the foregoing, it is clear that MultiHGF can mimic the action of other known growth factors on the neutrophil and macrophage lineages. However, no other growth factor known to date can replace MultiHGF in supporting growth of pluripotential and early committed cells in the erythroid and megakaryocyte lineages. Therefore, under appropriately defined conditions of culture (20), we consider the stimulation of large multilineage, erythroid and megakaryocyte colonies in semi-solid cultures of adult mouse bone marrow to be an unambiguous response to MultiHGF, and to be the most specific biological assay for it.

There are a number of immortalized granulocytic cell lines which are dependent on MultiHGF for survival and growth. Before it is assumed that these respond only to MultiHGF, it is necessary to establish that they do not also respond to other hemopoietic factors. Instances of response of such lines to more than one hemopoietic factor have recently been documented (61).

X. THE BIOLOGICAL ROLE OF MultiHGF

Consideration of the origin of MultiHGF from stimulated lymphocytes, and of the types of cell which respond to it, leads to the suggestion depicted schematically in Fig. 9. MultiHGF, and GM–CSF, are released together by an antigen-challenged immune system. They may act peripherally at sites of inflammation on relatively advanced cells in granulocyte, macrophage or mast cell lineages to yield a few terminal divisions, or they could act nore distantly on precursors in the bone marrow. There, responsiveness of very early progenitors of granulocytes and macrophages, including pluripotential precursors, permits the maximum possible exponential expansion in these lineages. On the other hand, late cells in the erythroid and megakaryocyte lineages are not responsive, so an inappropriate overproduction of red cells and platelets would not occur. This suggestion provides a possible basis for the well known granulocyte and monocyte responses to infection, and also suggests a mechanism to account for the increased proliferation of primitive bone marrow precursors which occurs in mice challenged with antigen {62,63) or bearing antigenic tumours (64).

Although it seems clear that the stimulated immune system is a source of MultiHGF, we do not know whether or not it is the only source. Primitive hemopoietic precursor cells die rapidly in the absence of MultiHGF in culture (16). Perhaps other nolecules acting on other receptors keep these cells alive and growing in situ in the animal. Alternatively, MultiHGF could be provided to hemopoietic precursors locally in hemopoietic tissues by non-lymphoid cells which transcribe the same gene but under different regulation relating more directly to hemopoietic function.

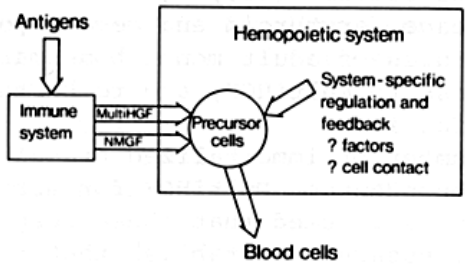


Fig. 9. A scheme indicating that MultiHGF and NMGF (neutnophil/macrophage growth factor, GM-CSF) arise from an activated immune system and suggesting that they may play a modulatory rather than essential role in normal blood cell production.

Answers to these questions will arrive when we have available monoclonal antibodies, radioimmunoassays, nucleotide probes for in situ analysis and quantities of MultiHGF sufficient for whole animal studies of its effects.

This article has charted the progress of work on MultiHGF from its initial detection in 1969 to its complete purification in the early 1980's. Its recognition now opens up important new directions of inquiry. The recent cDNA cloning of mouse MultiHGF opens the way toward identifying the human MultiHGF analogue, and from there to an understanding of its role in human health and disease. In the murine model, MultlHGF gives us a new capacity to assay and manipulate early cells in vitro. Its availability in partially purified form has led to the recognition of new factors required for the expression of early precursor function in culture (42). These in turn offer the promise of extending our analysis of function of early cells in vitro to more primitive cells than have been accessible until now.

ACKNOWLEDGMENTS

The authors thank M. Schweizer and M. Barnes for excellent technical assistance, and B. Fagg, G. Keller, G. Wu and C. Paige for critical readings of the manuscript. The Basel Institute for Immunology was founded and is supported by F. Hoffmann-La Roche & Co. Ltd., Basel, Switzerland.

REFERENCES

1. Iscove NH (1985) Specificity of hemopoietic growth factors. In: Leukemia. Dahlem Konferenzen, ed. I.L. Weissman, Springer–Verlag, in press.

2. Pluznik DH, Sachs L (1965) The cloning of normal "mast" cells in tissue culture. J. cell. comp. Physiol. 66, 319-324.

3. Metcalf D, Moore MAS, Warner NL (1969) Colony formation in vitro by myelomonocytic leukemic cells. J.N.C.I. 43, 983-1001.

4. McNeill TA (1973) Release of bone marrow colony stimulating activity during immunological reactions in vitro. Nature New Biology 244, 175-176.

5. Metcalf D, Parker J, Chester H, Kincade PW (1974) Formation of eosinophilic-like granulocytic colonies by mouse bone marrow cells in vitro. J. cell. Physiol. 84, 275-290.

6. Metcalf D, MacDonald HR, Odartchenko N, Sordat B (1975) Growth of mouse megakaryocyte colonies in vitro. Proc. Natl. Acad. Sci. USA. 72, 1744-1748.

7. Ralph P, Moore MAS, Nilsson K (1976} Lysozyme synthesis by established human and murine histiocytic lymphoma cell lines. J. exp. med. 143, 1528-1533.

8. Williams N, Jackson H, Sheridan APC, Murphy MJ, Elste A, Moore MAS (1978) Regulation of megakaryopoiesis in long—term murine bone marrow cultures. Blood 51, 245-255.

9. Williams N, Eger RR, Moore MAS, Mendelsohn N (1978) Differentiation of mouse bone marrow precursor cells into neutrophil granulocytes by an activity separation from WEHI-3 cell—conditioned medium. Differentiation 11, 59-63.

10. Axelrad AA, McLeod DL, Shreeve M, Heath DA (1974) Properties of cells that produce erythrocytic colonies in vitro. In: Hemopoiesis in Culture, ed. W. Robinson, U.S. Government Printing Office, pp. 226-234.

11. Wagemaker G (1978) Cellular and soluble factors influencing the differentiation of primitive erythroid progenitor cells (BFU-e) in vitro. In: In Vitro Aspects of Erythropoiesis, ed. M.J. Murphy, Jr., Springer-Verlag, pp 44-57.

12. Eliason JP, Van Zant G (1983) Erythropoietin-dependent and erythropoietin-independent enhancement of colony formation by immature erythroid progenitors (BFUe). Cell Tissue Kinet. 16, 65-76.

13. Iscove NN (1977) The role of erythropoietin in regulation of population size and cell cycling of early and late erythroid precursors in mouse bone marrow. Cell Tissue Kinet. 10, 323-324.

14. Iscove NN (1977) Erythropoietin independent regulation of early erythropoiesis. (abstract) Exp. Hematol. 5 Suppl 2, 6.

15. Johnson GR, Metcalf D (1977) Pure and mixed erythroid colony formation in vitro stimulated by spleen conditioned medium with no detectable erythropoietin. Proc. Natl. Acad. Sci. (USA) 74, 3879-3882.

16. Iscove NN (1978) Erythropoietin-independent stimulation of early erythropoiesis in adult marrow cultures by conditioned media from lectin-stimulated mouse spleen cells. In: Hematopoietic Cell Differentiation. eds. D.W. Golde, M.J. Cline, D. Metcalf & C.F. Fox, Acad. Press. pp 37-52.

17. Iscove NN, Schreier M (1979) Involvement of T cells and macrophages in generation of burst—promoting activity. (abstract) Exp. Hematol. 7, 4.

18. Schreier MH, Iscove NN (1980) Haematopoietic growth factors are released in cultures of H—2—restricted helper T cells, accessory cells and specific antigen. Nature 287, 228-230.

19. Burgess AW, Metcalf D, Russell SHM, Nicola A (1980) Granulocyte/macrophage-, megakaryocyte—, eosinophil— and erythroid—colony—stimulating factors produced by mouse spleen cells. Biochem. J. 185, 301-314.

20. Iscove NN, Roitsch CA, Williams N, Guilbert J (1982) Molecules stimulating early red cell, granulocyte, macrophage, and megakaryocyte precursors in culture: Similarity in size, hydrophobicity, and charge. J. cell. Physiol. Suppl. 1, 65-78.

21. Greenberger JS, Davisson PB, Gans PJ, Moloney WC (1979) In vitro induction of continuous acute promyelocytic leukemia cell lines by Friend or Abelson murine leukemia virus. Blood 53, 987-1001.

22. Hasthorpe S (1980) A hemopoietic cell line dependent upon a factor in pokeweed mitogen-stimulated spleen cell conditioning medium. J. cell. Physiol 105, 379-384.

23. Dexter TM, Garland J, Scott D, Scolnick E, Metcalf D (1980) Growth of factor-dependent hemopoietic precursor cell lines. J. exp. Med. 152, 1036-1047.

24. Schrader JW, Lewis SJ, Clark-Lewis I, Culvenor JG (1981) The persisting (P) cell: Histamine content, regulation by a T cell-derived factor, origin from a bone marrow precursor, and relationship to mast cells. Proc. Natl. Acad. Sci. USA. 78, 323-327.

25. Nagao K, Yokoro K, Aaronson SA (1981) Continuous lines of basophil/mast cells derived from normal mouse hone marrow. Science 212, 333-335.

26. Razin E, Cordon-Cardo C, Good RA (1981) Growth of a pure population of mouse mast cells in vitro with conditioned medium derived from concanavalin A-stimulated splenocytes. Proc. Natl. Acad. Sci. USA. 78, 2559-2561.

2?. Nabel G, Galli SJ, Dvorak AM, Dvorak HF, Cantor H (1981) Inducer T lymphocytes synthesize a factor that stimulates proliferation of cloned mast cells. Nature 291, 332-334.

28. Yung Y—P, Eqer R, Tertian G, Moore MAS (1981) Long-term in vitro culture of murine mast cells. J. Immunol. 127, 794-799.

29. Ihle JN, Pepersack L, Rebar L (1981) Regulation of T cell differentiation. In vitro induction of 20α-hydroxysteroid dehydrogenase in splenic lymphocytes from athymic mice by a unique lymphokine. J. Immnnol. 126, 2184-2189.

30. Ihle JN, Keller J, Greenberger JS, Henderson L, Yetter RA, Morse HC (1982) Phenotypic characteristics of cell lines requiring interleukin 3 for growth. J. Immunol. 129, 1377-1383.

31. Ihle JN, Keller J, Henderson L, Klein F, Palaszynski E (1982) Procedures for the purification of interleukin 3 to homogeneity. J. Immunol. 129, 2431-2435.

32. Garland JM, Lanotte M, Dexter TM (1982) Expression of 20α-hydroxysteroid dehydrogenase in nonlymphoid hemopoietic cell lines. Eur. J. Immunol. 12,332-336.

33. Ihle JN, Keller J, Greenberger JS, Henderson L, Yetter RA, Morse HC (1982) Phenotypic characteristics of cell lines requiring interleukin 3 for growth. J. Immunol. 129, 1377-1383.

34. Bazill GW, Haynes M, Garland J, Dexter M (1983) Characterization and partial purification of a haemopoietic cell growth factor in WEHI-3 cell conditioned medium. Biochem. J. 210, 747-759.

35. Ihle JN, Keller J, Oroszlan S, Henderson LE, Copeland TD, Pitch F, Prystowsky MB, Goldwasser E, Schrader JW, Palaszynski E, Dy M, Lebel B (1983) Biologic properties of homogeneous Interleukin 3. J. Immunol. 131, 282-286.

36. Williams N, Jackson H, Ralph P, Nakoinz I (1981) Cell interactions influencing murine marrow megakaryocytes: Nature of the potentiator cell in hone marrow. Blood, 57, 157-163.

37. Williams N, Eger RR, Jackson HM, Nelson DJ (1982) Two-factor requirement for murine megakaryocyte colony formation. J. cell. Physiol. 110, 101-104.

38. Schrader JW, Clark-Lewis I (1982) A T cell-derived factor stimulating multipotential hemopoietic stem cells: Molecular weight and distinction from T cell growth factor and T cell-derived granulocyte-macrophage colony-stimulating factor. J. Immunol. 129, 30-35.

39. Cerny J, Waner EB, Rubin AS (1975) T cell products activating stem cells: Further studies on the origin and action of the factor(s). J. Immunol. 115, 513-517.

40. Garland JM, Crompton S (1983) A preliminary report: Preparations containing Interleukin-3 (IL-3) promote proliferation of multipotential stem cells (CFUs) in the mouse. Exp. Hematol. 11, 757-761.

41. Dorssers L, Burger H, Wagemaker G (1984) Identity of murine stem cell activating factor (SAF) and Interleukin-3 (IL-3) and common specificity for pluripotent hemopoietic stem cells. (abstract) Exp. Hematol. 12, 357.

42. Iscove NH, Keller G, Roitsch C (1985) Factors required by pluripotential hemopoietic stem cells in culture. In: Stem Cell Physiology, ed. J. Palek, Liss, in press.

43. Humphries RK, Eaves AC, Eaves CJ (1979) Characterization of a primitive erythropoietic progenitor found in mouse marrow before and after several weeks in culture. Blood 746, 746-763.

44. Bartelmez SH, Sacca R, Stanley ER Lineage-specific receptors used to identify a growth factor developmentally early hemopoietic cells: Assay of hemopoietin-2. J. cell. Physiol. in press.

45. Gough N, Gough J, Metcalf D, Kelso A, Grail D, Nicola NA, Burgess AW, Dunn AR (1984) Molecular cloning of cDNA encoding a murine haematopoietic growth regulator, granulocyte-macrophage colony stimulating factor. Nature 309, 763-767.

46. Hapel AJ, Lee JC, Farrar WL, Ihle JN (1981) Establishment of continous cultures of Thy1.2+, Lyt1+,2- T cells with purified Interleukin 3. Cell 25, 179-186.

47. Palacios R, Henson G, Steinmetz M, Mckearn JP (1984) Interleukin-3 supports growth of mouse pre-B-cell clones in vitro. Nature 309, 126-131.

48. Schrader JW, Battyle F, Scollay R (1982) Expression of Thy-1 antigen is not limited to T cells in cultures of mouse hemopoietic cells. Proc. Natl. Acad. Sci.USA. 79, 4161-4165.

49. Paige CJ, Gisler RH, McKearn JP, Iscove NN (1984) Differentiation of murine B cell precursors in agar culture. Frequency, surface marker analysis and requirements for growth of clonable pre—B cells. Eur. J. Immunol. 14, 979-987.

50. Paige CJ, Skarvall H, Sauter H (1985) Differentiation of murine B cell precursors in agar culture II. Response of precursor-enriched populations to growth stimuli and demonstration that the clonable pre-B cell assay is limiting for the B cell precursor. Submitted.

51. Stanley ER (1979) Colony-stimulating factor (CSF) radioimmunoassay: Detection of a CSF subclass stimulating macrophage production. Proc. Natl. Road. Sci. USA. 76, 2969-2973.

52. Burgess AW, Camakaris J, Metcalf D (1977) Purification and properties of colony—stimulating factor from mouse lung-conditioned medium. J. biol. Chem. 252, 1998-2003.

53. Fung MC, Hapel AJ, Ymer S, Cohen DR, Johnson RM, Campbell HD, Young IG (1984) Molecular cloning of cDNA for murine Interleukin—3. Nature 307, 233-237.

54. Yokota T, Lee F, Rennick D, Hall C, Arai N, Mosmann T, Mabel G, Cantor H, Arai KI (1984) Isolation and characterization of a mouse cDNA clone that expresses mast—cell growth factor activity in monkey cells. Proc. Natl. Acad. Sci. USA. 81, 1070-1074.

55. Clark-Lewis I, Schrader JW (1981) P cell—stimulating factor: Biochemical characterization of a new T cell-derived factor. J. Immunol. 127, 1941-1947.

56. Corbel C (1982) Different factors active in lymphoid and hematopoietic proliferation produced by single clones of helper T cell hybridomas. Current Topics in Microbiology and Immunology. Springer-Verlag. 100, 231-237.

57. Burgess AW, Bartlett PF, Metcalf D, Nicola NA, Clark-Lewis I, Schrader JW (1981) Granulocyte-macrophage colony-stimulating factor produced by an inducible murine T—cell hybridoma: Molecular properties and cellular specificity. Exp. Hematol. 9, 893-903.

58. Metcalf D, Johnson GR, Burgess AW (1980) Direct stimulation by purified GM—CSF of the proliferation of multipotential and erythroid precursor cells. Blood 55, 138-146.

59. Stanley ER, Heard PM (1977) Factors regulating macrophage production and growth. J. biol. Chem. 252, 4305-4312.

60. Nicola NA, Metcalf D, Matsumoto M, Johnson GR (1982) Purification of a factor inducing differentiation in murine mye—lomonocytic leukemia cells. J. biol. Chem. 258, 9017-9023.

61. Hapel AJ, Warren HS, Hume DA (1984) Different colony-stimulating factors are detected by the "interleukin-3"-dependent cell lines FDC-P1 and 32D c1-23. Blood 64, 786-790.

62. Frindel E, Leuchars E, Davies AJS (1975) Thymus dependency of bone marrow stem cell proliferation in response to certain antigens. Exp. Hematol. 4, 275-284.

63. Burstein SA, Erb SK, Adamson JW, Harker LA (1981) Immunologic stimulation of early murine hematopoiesis and its abrogation by cyclosporin A. Blood 59, 851-856.

64. Fache M–P, Lepault F, Zelick R, Frindel E (1979) Influence of factors derived from EMT_6 tumors and from bone marrow of tumor-bearing mice on tumor and bone marrow stem cell kinetics. Cancer Res. 39, 3959-3963.

65. Clark–Lewis I, Kent SBH, Schrader JW [1984] Purification to apparent homogeneity of a factor stimulating the growth of multiple lineages of hemopoietic cells. J. biol. Chem. 259, 7488-7494.

66. Roitsch C, Barnes M (1985) High performance liquid chromatography of biologically active proteins in the nanogram (picomole) range. In: Immunological Methods 3, eds. I Lefkovits, B Pernis, Acad. Press, in press.

67. Bürk RR, Eschenbruch M, Leuthard P, Steck G (1983) Sensitive detection of proteins and peptides in polyacrylamide gels after formaldehyde fixation. Methods in Enzymology 91, 247-254.

DISCUSSION

SCHRADER: I have two comments to offer on lscove's report: With regard to the history of T-cell lymphokines affecting multi—potential hemopoetic stem cells he has neglected the 1974 Nature paper by Cerny showing that medium conditioned by mitogen—stimulated spleen cells stimulates CFUs in liquid cultures. As regards the relationship of multi—HGF to cells involved in lymphopoiesis, we have shown that purified PSF supports directly or indirectly in liquid cultures of bone-marrow, cells that give rise in irradiated animals to thymo-cytes and B cells. Accordingly they must be hemopoietic stem cells or hypothetical lymphoid stem cells.