Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 9679–9683, December 1986
Immunology

Purification and NH_2-terminal amino acid sequence of a T-cell-derived lymphokine with growth factor activity for B-cell hybridomas

(helper T lymphocyte/HPLC)

JACQUES VAN SNICK*, SYLVIE CAYPHAS†, ANNE VINK†, CATHERINE UYTTENHOVE*, PIERRE G. COULIE†, MICHAEL R. RUBIRA‡, AND RICHARD J. SIMPSON‡

*Ludwig Institute for Cancer Research, Brussels Branch, and †Unit of Experimental Medicine, International Institute of Cellular and Molecular Pathology and Université Catholique de Louvain, B-1200 Brussels, Belgium; and ‡Joint Protein Structure Laboratory, Melbourne Tumor Biology Branch, Ludwig Institute for Cancer Research and Walter and Eliza Hall Institute, P.O. Royal Melbourne Hospital, Parkville, 3050 Victoria, Australia

Communicated by C. de Duve, September 2, 1986

ABSTRACT **A T-cell-derived lymphokine was identified by its ability to support the growth of a subset of B-cell hybridomas. Hybrids that failed to survive in the absence of this molecule represented a major proportion of rat–mouse hybridomas but were very rare among mouse–mouse B-cell hybrids. Stable factor-dependent B-cell hybridomas were used to monitor the purification of the growth factor from the supernatant of a clonotypically stimulated mouse helper T-cell clone. Sequential fractionation using gel filtration, anion-exchange chromatography, and reversed-phase HPLC resolved the factor from other B-cell growth factors and yielded a single-chain protein characterized by a major charge (pI = 5–7) and molecular mass (22- to 29-kDa) heterogeneity, probably due to variations in glycosylation. The NH_2-terminal amino acid sequence of this protein, which is active on B-cell hybridomas in the 0.1 pM range, showed no significant homology with that of known lymphokines. Because the purified factor also supported the growth and survival *in vitro* of murine plasmacytomas (to be published elsewhere), it was provisionally designated interleukin-HP1 (where H stands for hybridoma and P stands for plasmacytoma).**

Although a wide variety of cells have been shown to promote the growth of B-cell hybridomas (1–6), little is known about the way such feeder cells operate. In some cases, the involvement of soluble growth factors has been demonstrated (6–8) but these molecules have never been purified to homogeneity.

Recently, we noticed that rat–mouse hybridomas could easily be grown in the absence of feeder cells if small amounts of rat or mouse spleen cell Con A supernatants (Con A sup) were added to the medium. As some of these hybridomas remained factor-dependent over long periods of time, we used them to measure the corresponding growth factor activity in a number of T-cell supernatants. This approach allowed us to identify a mouse helper T-cell clone that produced very large amounts of one such factor and to monitor this activity during its purification.

The present report describes the identification of this factor as a T-cell-derived 22- to 29-kDa protein that shows no NH_2-terminal amino acid sequence homology with previously described lymphokines.

MATERIALS AND METHODS

Hybridomas. Lewis rats and C57BL/6 mice were injected i.p. with *Escherichia coli* 055:B5 (Difco) lipopolysaccharide (400 and 50 μg, respectively) 3 days before fusion of their spleen cells with mouse fusion partners SP2/0 Ag14 (9), SP2 neo^R.1 (10), and NS1 (11). After selection in the presence of hypoxanthine/aminopterin/thymidine, hybridomas were grown in Iscove's medium containing 10% fetal bovine serum, L-arginine (0.55 mM), L-asparagine (0.24 mM), L-glutamine (1.5 mM), 2-mercaptoethanol (50 μM), hypoxanthine (0.1 mM), and thymidine (16 μM). For factor-dependent clones, this medium was supplemented further with conditioned media prepared as described below.

Conditioned Media. Rat and mouse Con A sup were prepared as described (12). Helper T-cell clone TUC2.15 was derived from C57BL/6 mice immunized with keyhole limpet hemocyanin (KLH, Calbiochem) according to the procedure described by Corradin *et al.* (13). For mass production of this clone, 5×10^6 T cells were cultured with 2×10^8 syngeneic spleen cells [irradiated with 2000 rads (1 rad = 0.01 gray) from a cesium source] in 50 ml of Dulbecco's modified Eagle's medium containing 25 μg of KLH per ml and supplemented with amino acids, 2-mercaptoethanol, and fetal bovine serum as described for hybridoma cells. After 10–15 days, these cultures yielded 40–50 $\times 10^6$ TUC2.15 cells. For the preparation of TUC2.15 supernatant (TUC2.15 sup), 10-day-old cultures were washed in medium without serum and the cells were incubated for 3–4 days with clonotypic monoclonal antibody 3D7 in medium with a low protein concentration (0.5% fetal bovine serum or 10 μg of human transferrin per ml, Calbiochem). The 3D7 antibody, which was derived from 129/Sv mice immunized with TUC2.15, stimulated lymphokine secretory activity and proliferation of TUC2.15 but of no other helper T-cell clones. It was used here as a 1:10,000 diluted ascites fluid. Supernatant was also prepared from TUC2.15 cells rigorously depleted from any remaining macrophages by passage through a nylon wool column followed by centrifugation over Ficoll-Paque (Pharmacia). This procedure reduced the number of contaminating macrophages to about 0.2%, as estimated by the proportion of cells positive for nonspecific esterases.

Lymphokines. Human recombinant interleukin 2 (IL-2) (14) was a gift of W. Fiers (State University, Ghent, Belgium), human interleukin 1 (IL-1) (15) was a gift of J. Van Damme (Catholic University, Leuven, Belgium), mouse recombinant IL-1 (16) was a gift of P. Lomedico (Roche Diagnostics) to J. Van Damme, and mouse purified multicolony-stimulating

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: BCGF-II, B-cell growth factor II; BSF-1, B-cell stimulatory factor 1; Con A sup, spleen cell Con A supernatant(s); TUC2.15 sup, TUC2.15 supernatant; HGF, hybridoma growth factor; RP-HPLC, reversed-phase HPLC; IL-1, IL-2, and IL-3, interleukin 1, 2, and 3, respectively; U, unit(s); KLH, keyhole limpet hemocyanin.

factor or interleukin 3 (IL-3) was a gift of A. W. Burgess (Ludwig Institute for Cancer Research, Melbourne Branch).

Lymphokine Assays. IL-1 was measured in the thymocyte costimulation assay (17) and IL-2 was measured in the CTLL assay (18). IL-3 was measured in a soft agar colony-stimulating factor assay with C57BL/6 bone marrow cells according to ref. 19.

B-cell stimulatory factor 1 (BSF-1) and B-cell growth factor II (BCGF-II) titers were determined with small B cells (density between 1.074 and 1.086) prepared by panning on anti-Ig antibodies, as described (20), followed by centrifugation through a discontinuous Percoll gradient. These B-cell populations contained <1% T cells, as determined on an ATC 3000 flow cytometer (Odam, Wissembourg, France), and did not proliferate in response to Con A. For the BSF-1 assay, B cells were incubated in the presence of 0.5 μg of affinity-purified goat anti-mouse IgM antibodies per ml (21) and thymidine incorporation was measured after 36 hr (0.5 μCi of [*methyl*-^{3}H]thymidine per well; 1 Ci = 37 GBq). For the BCGF-II assay (22), the cells were incubated in the presence of 10 μg of dextran sulfate per ml (Pharmacia) and thymidine incorporation was measured on day 5. BSF-1 and BCGF-II concentrations were evaluated either by measuring thymidine incorporation levels at a fixed dilution or by calculating half-maximal proliferation U/ml.

The hybridoma growth factor (HGF) assay was performed by incubating 2×10^3 factor-dependent hybridoma cells (rat–mouse hybrid 6H8 or mouse–mouse hybrid 7TD1) in 200-μl microcultures containing serial dilutions of growth factor. After 4 days, the number of cells was evaluated by colorimetric determination of hexosaminidase levels as described by Landegren (23). The concentration of HGF was expressed in U/ml arbitrarily defined as the dilution that produced half-maximal growth of the cells. A selected Con A sup containing about 1000 U/ml was used as an internal standard in all HGF assays.

Adsorption to Silicic Acid. TUC2.15-conditioned medium was incubated with 3 g of silicic acid per liter (Merck) for 3 hr at 4°C. After two washes in 124 mM NaCl buffered with 12 mM phosphate at pH 7.2 (PBS), the adsorbed material was eluted with a 1:1 mixture of ethylene glycol and PBS made up to 1.4 M NaCl. The eluate was either lyophilized after dialysis against 50 mM NH_4HCO_3 in the presence of a 10^{-4} (vol/vol) dilution of Tween 20 or concentrated by ultrafiltration on an Amicon YM-10 membrane, also in the presence of Tween 20.

Gel Filtration. Concentrated silicic acid eluates were fractionated at 4°C on an Ultrogel AcA 54 (LKB) column equilibrated with 18% ethylene glycol in 1 M NaCl buffered with 12 mM phosphate (pH 7.2).

Table 1. Differential growth requirements of rat–mouse and mouse–mouse B-cell hybridomas

Exp.	Spleen cell origin	Fusion partner	Hybrids tested, no.	Growing hybrids, %	
				Control	Con A sup
1	Rat	SP2/0 Ag14	48	6	96
2	Rat	SP2/0 Ag14	19	5	58
3	Rat	SP2 neo^R.1	27	4	78
4	Rat	SP2 neo^R.1	48	8	98
5	Mouse	SP2/0 Ag14	32	100	100
6	Mouse	SP2 neo^R.1	46	100	100

Spleen cells polyclonally activated by injection of lipopolysaccharide were fused with the indicated cell lines as described (26). After a 1-week culture in microtiter wells, spent medium was carefully removed and hybridomas were expanded without addition of exogenous feeder cells in 1-ml duplicate cultures either in regular medium or in medium supplemented with 1% mouse Con A sup. The percentage of hybridomas that proliferated under these conditions was scored 3 days later.

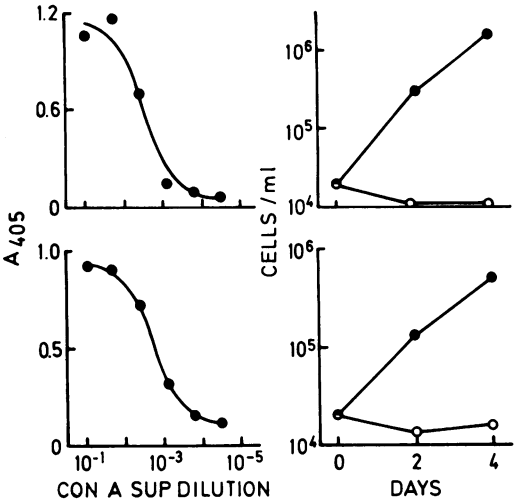

FIG. 1. Growth of factor-dependent B-cell hybridomas. (*Left*) Cell numbers evaluated by hexosaminidase levels after a 4-day culture in the presence of decreasing concentrations of mouse spleen cell Con A sup. (*Right*) Growth curves in the absence (○) or presence (●) of 4% (vol/vol) mouse Con A sup. (*Upper*) Mouse–mouse hybridoma 7TD1. (*Lower*) Rat–mouse hybridoma 6H8.

Anion-Exchange Chromatography. Pooled active fractions from the gel filtration were concentrated by lyophilization or ultrafiltration and applied on a MonoQ column (Pharmacia) in 20 mM Tris·HCl (pH 8.0) containing 20 mM NaCl and 10^{-4} (vol/vol) Tween 20. After a 5-min wash in starting buffer, the adsorbed material was eluted at 0.8 ml/min with a 30-min linear gradient of NaCl in 20 mM Tris·HCl (pH 8.0).

Reversed-Phase HPLC (RP-HPLC). Pooled active fractions from the MonoQ column were concentrated down to 0.2 ml by means of a Centricon 10 microconcentrator (Amicon). After addition of 1.8 ml of 0.05% trifluoroacetic acid in water, this material was injected on a C1 250-Å pore-size TSK TMS-250 column (LKB) equilibrated with 0.05% trifluoroacetic acid. Elution was carried out at 0.8 ml/min with a gradient of acetonitrile. Gradients were generated with a model 720 system controller and two model 6000A solvent delivery pumps (Waters Associates). One-minute fractions were collected in Eppendorf tubes containing 10 μl of 1 M NH_4HCO_3 and 5 μl of Tween 20 (1% in water) and lyophilized. Active fractions from the C1 column were resolubilized in 0.05% trifluoroacetic acid just before chromatography on a C8-ProRPC column (Pharmacia) and eluted at 0.3 ml/min with acetonitrile in 0.05% trifluoroacetic acid.

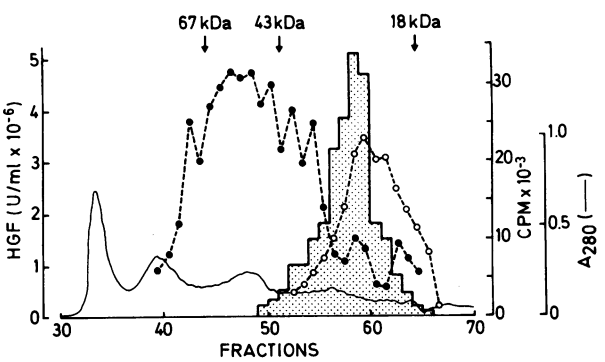

FIG. 2. Gel filtration of TUC2.15 sup on an AcA 54 column. Fractions were titrated for HGF (stippled area) and tested for BSF-1 (○, cpm × 10^{-3}) and BCGF-II (●, cpm × 10^{-3}) at 1:5000 and 1:25,000 dilutions, respectively. Bovine serum albumin (67 kDa), ovalbumin (43 kDa), and sperm whale myoglobin (18 kDa) were used as molecular mass markers.

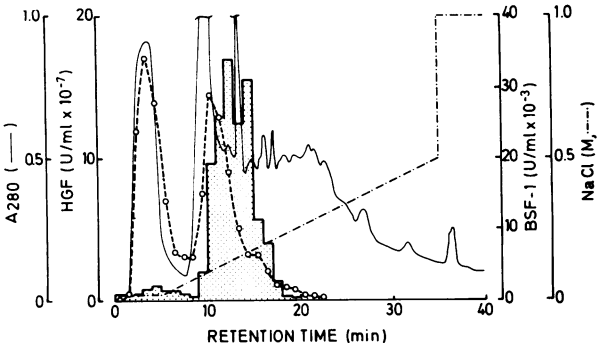

FIG. 3. Anion-exchange chromatography on a MonoQ column of HGF-containing fractions from gel filtration. Titrations of BSF-1 (○) and HGF (stippled area) are shown.

Electrophoresis. Purity of HGF preparations was checked by $NaDodSO_4$/PAGE with 15% polyacrylamide gels prepared according to Laemmli (24) and silver-stained as described by Morissey (25). Before electrophoresis, samples run under nonreducing conditions, which were used for biological activity assays, were incubated at room temperature in sample buffer containing 2.3% $NaDodSO_4$ but no 2-mercaptoethanol. Two-millimeter gel slices were eluted by overnight incubation in PBS with 0.1% polyethylene glycol (PEG-6000, Merck). Analytical isoelectric focusing was performed on a flat-bed Pharmacia FBE-3000 system in 5% polyacrylamide gels containing 2.5% LKB Ampholines (pH range, 3.5–10).

NH_2-Terminal Sequence Analysis. NH_2-terminal sequence determination was carried out on a gas-phase sequencer (model 478, Applied Biosystems, Foster City, CA) equipped with an on-line phenylthiohydantoin analyzer (model 120A).

Enzymatic Treatment. Silicic acid eluates were treated for 4 hr at 37°C with 0.02 U of neuraminidase per ml from *Vibrio cholera* (Calbiochem).

RESULTS

Factor-Dependent B-Cell Hybridomas. In the absence of feeder cells, the growth of rat–mouse hybridomas was considerably enhanced by mouse or rat Con A sup. By contrast, the majority of mouse–mouse hybridomas established under similar conditions grew optimally in the absence of exogenous growth factors. The results obtained with mouse fusion partners SP2/0 Ag14 and SP2 neo^R.1 are shown in Table 1. Similar results were obtained with hybridomas derived from NS1 (data not shown).

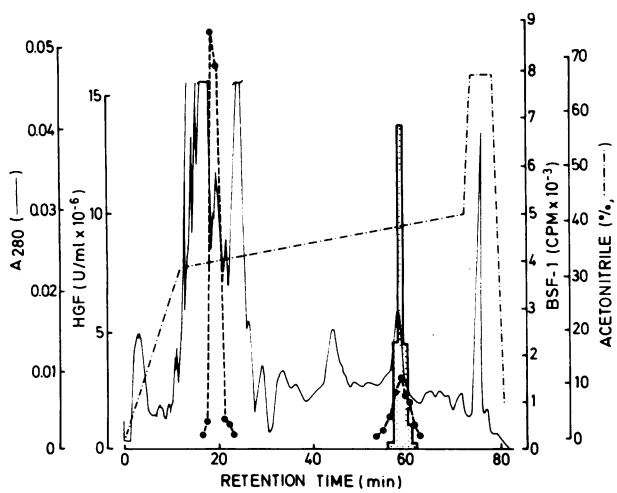

FIG. 4. RP-HPLC on a TSK TMS-250 (C1) column of HGF-containing fractions from anion-exchange chromatography. Eluted material was titrated for HGF (stippled area) and tested for BSF-1 (●) at a 1:1000 dilution.

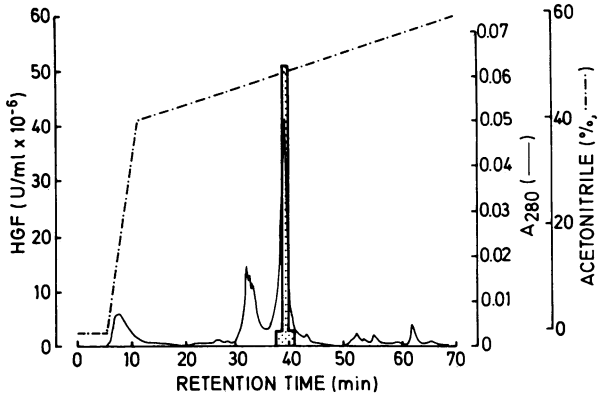

FIG. 5. RP-HPLC on a C8-ProRPC column of HGF-containing fractions from the C1 RP-HPLC. Stippled area represents HGF titers.

Several rat–mouse hybrids and one rare mouse–mouse hybrid, which retained this growth factor dependence as a stable trait, were cloned and subsequently used as indicator cells. Typical growth and dose–response curves are shown in Fig. 1 for rat–mouse hybrid 6H8 and mouse–mouse hybrid 7TD1. In the presence of an optimal concentration of Con A sup, the doubling time of these cell lines was about 15 hr and half-maximal growth was obtained with 0.1–0.5% conditioned medium.

While screening a number of mouse T-cell lines for HGF production, we found a C57BL/6 KLH-reactive helper T-cell clone (TUC2.15) that, when stimulated with a clonotypic antibody in the absence of accessory cells, produced about 2000-fold more growth factor on a per cell basis than Con A-stimulated spleen cells. The T-cell origin of this HGF activity was demonstrated by the finding that passage of TUC2.15 cells over a nylon wool column to remove any remaining accessory cells did not diminish HGF titers.

Lymphokine Activities in TUC2.15 sup. TUC2.15 sup had a low IL-1 titer (1:8 in the thymocyte costimulation assay) and contained no detectable IL-2 or IL-3. The possibility that one of these lymphokines could nevertheless be responsible for the HGF activity was further excluded by the finding that purified human IL-1, which is active in the mouse IL-1 assay, mouse recombinant IL-1, human recombinant IL-2, and purified mouse IL-3 were all unable to sustain the growth of our factor-dependent hybridomas (data not shown). TUC2.15

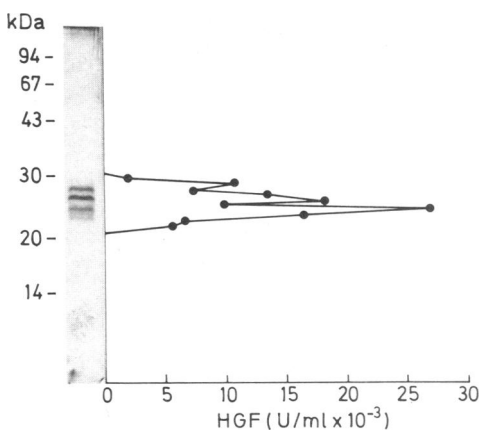

FIG. 6. $NaDodSO_4$/PAGE of purified HGF under nonreducing conditions. Silver staining of and biological activity eluted from duplicate lanes are shown.

sup was also devoid of detectable interferon-γ activity. By contrast, it contained high titers of BSF-1 ($\pm 5 \times 10^3$ U/ml) and BCGF-II ($\pm 2 \times 10^4$ U/ml).

Purification of TUC2.15 HGF. TUC2.15 sup was first concentrated by adsorption onto silicic acid. The eluted material was then fractionated by gel filtration on an Ultrogel AcA 54 column. This chromatography clearly separated BCGF-II from BSF-1 and HGF, which both eluted in the 20- to 35-kDa range. There was, however, no strict coincidence between the elution profiles of BSF-1 and HGF with, as shown in Fig. 2, a more pronounced trailing of the BSF-1 peak. Fractions containing most of the HGF and about 50% of the BSF-1 activity were submitted to chromatography on a MonoQ column. HGF eluted as a single peak, whereas BSF-1 was evenly distributed into two well-defined peaks: one that was not retained on the column and one that emerged slightly before but again overlapped extensively with HGF (Fig. 3). The fractions containing the HGF peak and about half of the second BSF-1 peak were applied on a C1 column equilibrated with 0.05% trifluoroacetic acid. The bulk of the remaining BSF-1 activity eluted with the major protein peak at 33% acetonitrile, whereas HGF eluted much later at about 40% acetonitrile (Fig. 4). This HGF peak still displayed a minor but significant BSF-1 activity. Finally, the HGF-containing fractions were chromatographed on a C8 column, which yielded one major protein peak that eluted with 50% acetonitrile and contained all HGF activity (Fig. 5). This HGF preparation was purified 3000-fold, with a final recovery of about 10%, and induced half-maximal proliferation of hybridoma 7TD1 in the 0.1 pM range, as estimated on the basis of silver-stained gels and of HPLC peak heights. Its purity was confirmed by the finding of a unique NH_2-terminal amino acid sequence that read as follows:

$$\overset{1}{\text{Phe}}\text{-Pro-Thr-Ser-}\overset{5}{\text{Gln}}\text{-Val-Arg-Arg-Gly-}\overset{10}{\text{Asp}}\text{-Phe-Thr-Glu-Asp-}\overset{15}{\text{Thr}}\text{-Xaa-Pro-Asn-Xaa-}\overset{20}{\text{Asn}}\text{-Val.}$$

A minor but significant BSF-1 activity could still be detected in this material but only at a concentration that was 10,000-fold higher than that required for the HGF assay (data not shown).

In silver-stained $NaDodSO_4$/PAGE run under nonreducing conditions, purified HGF was resolved in multiple regularly spaced bands that migrated between 22 and 29 kDa and coincided with the biological activity eluted from the gels (Fig. 6). This migration pattern was not changed under reducing conditions. The isoelectric focusing profile of HGF revealed a major charge heterogeneity with activity being recovered between pH 5 and 6.9. However, after treatment with neuraminidase, HGF focused as a much narrower peak between pH 6.5 and 7.2 (data not shown).

DISCUSSION

The present report describes the purification of a T-cell-derived factor with considerable growth factor activity for certain B-cell hybridomas. This HGF was purified from the supernatant of a mouse helper T-cell clone stimulated with a clonotypic antibody in the absence of accessory cells. After sequential gel filtration, anion-exchange chromatography, and RP-HPLC, the HGF activity copurified with a 22- to 29-kDa single-chain protein that was heterogeneous in silver-stained $NaDodSO_4$ gels yet proved to be pure on the basis of NH_2-terminal amino acid analysis. HGF also had a marked charge heterogeneity that was considerably reduced upon treatment with neuraminidase, which suggests that variations in glycosylation may be responsible for the charge and molecular mass heterogeneity of this molecule. Based on the intensity of silver stainings and on HPLC peak heights, HGF appeared to support the growth of B-cell hybridomas in the 0.1 pM range.

The NH_2-terminal amino acid sequence of HGF showed no significant homology with that of known lymphokines. Moreover, IL-1, IL-2, and IL-3 were all devoid of HGF activity and HGF was clearly resolved from BSF-1 and BCGF-II in the course of its purification. However, our purest HGF preparations still induced some cell proliferation in the BSF-1 assay. It is difficult on the basis of our current data to interpret this effect because of the very high concentrations of HGF that are required to produce it and because of the heterogeneity of the cell populations used in this assay.

Comparison of the HGF described here with other factors that promote the growth of B-cell hybridomas is rather difficult because these factors have not yet been purified to homogeneity. Astaldi and colleagues have described a human endothelial cell supernatant that contains a very strong HGF but little is known about the molecular characteristics of this factor (6). More recently, another HGF has been identified in crude human IL-2 preparations (7). This factor, which is of monocytic origin, has a molecular weight that is not significantly different from murine HGF and also acts at a very low concentration. Its isoelectric point is, however, much more acidic than that of murine HGF.

Although we know very little about the biological function of HGF, it is clear, already, that its growth factor activity will not be limited to B-cell hybridomas. For example, several primary mouse plasmacytomas have been found to require HGF for *in vitro* growth (unpublished data) and, as stated above, anti-IgM-stimulated B cells respond to HGF by increased proliferation in a BSF-1 assay. Because the term HGF does not properly describe these multiple effects and because our amino acid sequence analysis identifies the factor described here as a distinct lymphokine, we propose to provisionally designate it interleukin-HP1 (where H stands for hybridoma and P stands for plasmacytoma).

We are indebted to Drs. C. Chatelain, D. Godelaine, and J. Van Damme for lymphokine determinations and to Dr. L. Van Rompuy (Plant Genetic Systems, Ghent, Belgium) for a computer-assisted search for amino acid sequence homology. The expert technical assistance of Mrs. B. de Lestré, Mrs. D. Donckers, and Mrs. M. Stevens is gratefully acknowledged. This work was supported in part by grants from Fonds de la Recherche Scientifique Médicale (F.R.S.M.), Fonds National de la Recherche Scientifique (F.N.R.S.), and Loterie Nationale, Belgium. P.G.C. is a research assistant with F.N.R.S. and S.C. is the recipient of a fellowship from I.R.S.I.A., Belgium.

1. Oi, V. T. & Herzenberg, L. A. (1980) in *Selected Methods in Cellular Immunology*, eds. Mishell, B. B. & Shiigi, S. M. (Freeman, San Francisco), pp. 351–372.
2. Köhler, G. & Milstein, C. (1976) *Eur. J. Immunol.* **6**, 511–519.
3. Fazekas de st Groth, S. & Scheidegger, D. (1980) *J. Immunol. Methods* **35**, 1–21.
4. Brodsky, F. M., Parham, P., Barnstable, C. J., Crumpton, M. J. & Bodmer, W. F. (1979) *Immunol. Rev.* **47**, 3–61.
5. Long, W. J., McGuire, W., Palombo, A. & Emini, E. A. (1986) *J. Immunol. Methods* **86**, 89–93.
6. Astaldi, G. C. B., Janssen, M. C., Lansdorp, P. M., Willems, C., Zeijlemaker, W. P. & Oosterhof, F. (1980) *J. Immunol.* **125**, 1411–1414.
7. Aarden, L., Lansdorp, P. & De Groot, E. (1985) *Lymphokines* **10**, 175–185.
8. Walker, K. Z., Gibson, J., Axiak, S. N. & Prentice, R. L. (1986) *J. Immunol. Methods* **88**, 75–81.
9. Shulman, M., Wilde, C. D. & Köhler, G. (1978) *Nature (London)* **276**, 269–270.
10. Van Snick, J., De Plaen, E. & Boon, T. (1985) *Eur. J. Immunol.* **15**, 1148–1151.
11. Köhler, G., Howe, C. S. & Milstein, C. (1976) *Eur. J. Immunol.* **6**, 292–295.
12. Spiess, P. J. & Rosenberg, S. A. (1981) *J. Immunol. Methods* **42**, 213–222.

13. Corradin, G., Etlinger, H. M. & Chiller, J. M. (1977) *J. Immunol.* **119,** 1048–1053.
14. Devos, R., Plaetinck, G., Cheroute, H., Simons, G., Degrave, W., Tavernier, J., Remout, E. & Fiers, W. (1983) *Nucleic Acids Res.* **11,** 4307–4323.
15. Van Damme, J., De Ley, M., Opdenakker, G., Billiau, A., De Somer, P. & Van Beeumen, J. (1985) *Nature (London)* **314,** 266–268.
16. Lomedico, P. T., Gubler, U., Hellman, C. P., Dukovich, M., Giri, J. G., Pan, Y.-C. E., Collier, K., Semionow, R., Chua, A. O. & Mizel, S. B. (1984) *Nature (London)* **312,** 458–462.
17. Mizel, S. B., Oppenheim, J. J. & Rosenstreich, D. L. (1978) *J. Immunol.* **120,** 1497–1503.
18. Gillis, S., Ferm, M. M., Ou, W. & Smith, K. A. (1978) *J. Immunol.* **120,** 2027–2032.
19. Chatelain, C., Burstein, S. A. & Harker, L. A. (1983) *Blood* **62,** 172–176.
20. Wysocki, L. J. & Sato, V. L. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2844–2848.
21. Howard, M., Farrar, J., Hilfiker, M., Johnson, B., Takatsu, K., Hamaoka, T. & Paul, W. E. (1982) *J. Exp. Med.* **155,** 914–923.
22. Swain, S. L., Howard, M., Kappler, J., Marrack, P., Watson, J., Booth, R., Wetzel, G. D. & Dutton, R. W. (1983) *J. Exp. Med.* **158,** 822–835.
23. Landegren, U. (1984) *J. Immunol. Methods* **67,** 379–388.
24. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
25. Morissey, J. H. (1981) *Anal. Biochem.* **117,** 307–310.
26. Claflin, L. & Williams, K. (1979) *Curr. Top. Microbiol. Immunol.* **81,** 107–109.