INTERLEUKIN-HP1, A T CELL–DERIVED HYBRIDOMA GROWTH FACTOR THAT SUPPORTS THE IN VITRO GROWTH OF MURINE PLASMACYTOMAS

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A large number of plasmacytomas have been induced by injection of mineral oil into the peritoneal cavity of certain mouse strains (1), and numerous attempts have been made to grow these tumors in vitro. With a few exceptions (2, 3), successful adaptation to primary cultures was only achieved either in the presence of feeder cells (4, 5) or after repeated passage between culture and mouse (2, 6–8). For some plasmacytomas, feeder cells could be replaced by soluble factors secreted by macrophages (3, 4, 9), T lymphocytes (10), or a variety of other cells (4). However, so far, none of these factors has been clearly identified.

We have recently described a T cell–derived factor that was absolutely required for the in vitro growth of certain B cell hybridoma lines and appeared to be distinct from interleukin 1 (IL-1), IL-2, IL-3, interferon γ (IFN-γ), B cell–stimulatory factor 1 (BSF-1),[1] and B cell–growth factor II. This factor was produced in high titers by a C57BL/6 helper T cell clone upon stimulation with a clonotypic antibody. After purification by sequential gel filtration, anion exchange, and reversed-phase high performance liquid chromatography (HPLC), the growth factor appeared to copurify with a single chain glycoprotein that was very heterogeneous both in charge (pI 5–7) and molecular mass (22–29 kD in SDS polyacrylamide gels). Sequencing of this material generated a single NH_2-terminal amino acid sequence that clearly identified this factor as a new lymphokine, which we provisionally designated interleukin-HP1 (IL-HP1) (11).

In the present report, we show that IL-HP1 acts not only on B cell hybridomas, but is also a major growth factor for many murine plasmacytomas.

Materials and Methods

Primary Plasmacytoma Cultures. Plasmacytomas originally obtained from Dr. Michael Potter (National Institutes of Health, Bethesda, MD) were maintained as ascitic tumors in pristane-treated BALB/c mice (12). The animals were irradiated with 500 rad from a cesium source just before tumor transplantation. After 7–10 d, the peritoneal cavity of the mice was flushed with 5 ml Iscove's medium containing heparin (50 U/ml) and 10% FCS. Nucleated cells, separated from erythrocytes by centrifugation on Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden), were seeded in 24-well plates at 2.5–5 ×

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[1] *Abbreviation used in this paper:* BSF, B cell–stimulatory factor.

10^5 cells/ml in Iscove's medium supplemented with 10% FCS, 2-ME (50 μM), L-arginine (0.55 mM), L-asparagine (0.24 mM), and L-glutamine (1.5 mM). Medium was changed every other day, and cell concentrations were maintained at 1–5 × 10^5 cells/ml. Factor-dependent plasmacytoma and hybridoma cell lines were maintained in the same medium supplemented with appropriate concentrations of T cell supernatant (usually 2% for plasmacytomas and 0.01% for hybridomas).

Preparation of TUC2.15 Supernatant. Helper T cell clone TUC2.15 was derived from C57BL/6 mice immunized with keyhole limpet hemocyanin (KLH). For mass production of this clone, 5 × 10^6 T cells were cultured with 2 × 10^8 syngeneic spleen cells (irradiated with 2,000 rad from a cesium source) in 50 ml Dulbecco's modified Eagle's medium containing 25 μg/ml KLH and supplemented with amino acids, 2-ME, and FCS as described above for plasmacytoma cells. After 10–15 d, these cultures yielded 4.0–5.0 × 10^7 TUC2.15 cells. For the preparation of TUC2.15 supernatant, 10-d-old cultures were washed in medium without serum and the cells were incubated for 3–4 d with clonotypic monoclonal antibody 3D7 in medium with a low protein concentration (0.5% FCS or 10 μg/ml human transferrin). 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 10,000-fold diluted ascites.

Biochemical Procedures. Crude TUC2.15 supernatant was concentrated by adsorption on silicic acid (3 g/liter for 3 h at 4°C) and elution with 50% ethylene glycol and 50% 1.4 M NaCl containing 12 mM phosphate buffer, pH 7.2. Aliquots of this material were analyzed by gel filtration, isoelectric focusing, or reversed-phase HPLC. Gel filtration was carried out on an Ultrogel AcA 54 column (LKB, Bromma, Sweden) equilibrated with 18% ethylene glycol in 1 M NaCl containing 12 mM phosphate buffer pH 7.2. For isoelectric focusing, samples were applied to a prefocused (600 V for 1 h) flat-bed polyacrylamide (5%) gel equilibrated with pH 3.5–10 ampholines (2.5%, LKB) and focused for 2 h at 1,000 V. Material eluted from the gel was tested for hybridoma and plasmacytoma growth factor activities after dialysis against culture medium. Reversed-phase HPLC was performed on a Cl-column (TSK TMS 250; LKB) equilibrated in 0.05% trifluoroacetic acid (TFA) and eluted at 0.8 ml/min with a gradient of acetonitrile containing 0.05% TFA. 1-min fractions were collected. Pure IL-HP1 was obtained from crude TUC2.15 supernatant by sequential gel filtration, anion exchange, and reversed-phase HPLC as described (11). Upon SDS-PAGE, material prepared according to this procedure is resolved in multiple bands (Fig. 1), which all display HGF activity. The purity of this material has been further demonstrated by the finding of a single NH_2-terminal amino acid sequence (11). Semipurified BSF-1 was obtained after gel filtration, anion-exchange and Cl reversed-phase HPLC as reported (11). This material, which was active on anti-IgM-stimulated B cells, was found to be free from IL-2 and IL-HP1.

Growth Factor Assays. Hybridoma growth factor (HGF) titrations were performed by incubating mouse factor–dependent B cell hybridoma 7TD1 (2,000 cells/microwell) with serial dilutions of the fractions to be tested in a final volume of 0.2 ml. Cell numbers were evaluated after 4 d at 37°C by measuring hexosaminidase levels as described (11). A titer of 1 U/ml was arbitrarily assigned to material that produced half-maximal growth of hybridoma 7TD1. The plasmacytoma growth factor assay was carried out on appropriately diluted fractions with factor-dependent plasmacytoma cell lines (3,000 cells/microwell). Cells were incubated in triplicate cultures and pulsed with methyl-[^{3}H]thymidine (0.5 μCi/well) during the last 12 h of a 72-h incubation. For clarity, standard errors, which did not exceed 10% of the means, are not shown. IL-2 was measured with the CTLL assay (13).

Results and Discussion

Of six plasma cell tumors that failed to grow in vitro in normal primary cultures, five readily gave rise to vigorous cell lines in the presence of medium conditioned by helper T cell clone TUC2.15, which was previously shown to support the growth of B cell hybridomas (11). In the absence of supernatant, not

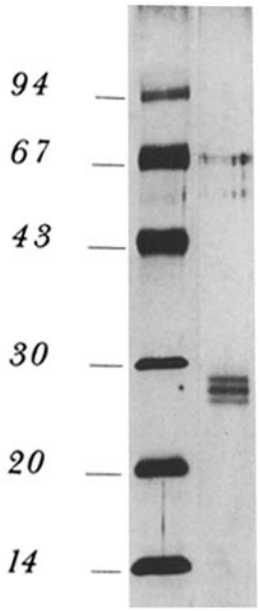

FIGURE 1. Silver-stained SDS-PAGE of purified IL-HP1. Sample was run under nonreducing conditions in a 15% polyacrylamide gel as described by Laemmli (14) and stained according to Morrissey (15). Bands at 67 and 59 kD are staining artefacts.

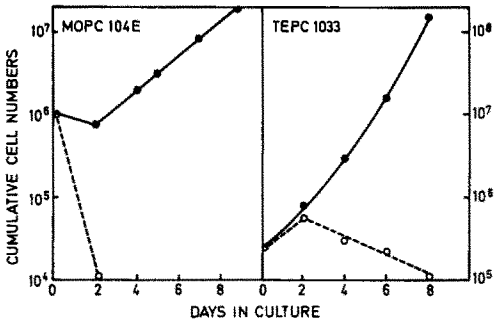

FIGURE 2. Growth curves of MOPC 104E and TEPC 1033 in primary cultures. Cultures were set up either in the absence (○) or in the presence (●) of 2% supernatant of helper T cell clone TUC2.15.

a single cell line could be established in this experiment. By extrapolating initial growth curves (shown in Fig. 2 for MOPC 104E and TEPC 1033) to time 0, the frequency of cells capable of proliferation in primary cultures supplemented with supernatant could be evaluated for four of the five successfully adapted tumors (Table I). These frequency estimates, which ranged from 0.03 to 0.5, were at least five orders of magnitude above those of cultures set up in the absence of T cell supernatant.

After several months in culture, four of the five plasmacytoma cell lines remained strictly dependent on the continuous presence of TUC2.15 supernatant in the culture medium. The exception was the MOPC 315 line, which was able to proliferate, although at a reduced rate, in the absence of supernatant. The

TABLE I

Facilitation of Primary Cultures of Mouse Plasmacytomas by Supernatant of Helper T Cell Clone TUC2.15

Plasmacytomas	Frequency of proliferating cells		Doubling time*
	Without TUC2.15	With TUC2.15	
			h
MOPC 104E	$<4 \times 10^{-6}$	2×10^{-1}	36
MOPC 173	$<4 \times 10^{-6}$	3×10^{-1}	20
MOPC 315	$<4 \times 10^{-6}$	3×10^{-2}	25
RPC 20	$<10^{-6}$	ND	25
TEPC 1033	$<4 \times 10^{-6}$	5×10^{-1}	16
FLOPC 21	$<4 \times 10^{-6}$	$<4 \times 10^{-6}$	—

Culture conditions were as described under Materials and Methods. The frequency of cells capable of in vitro proliferation was calculated by extrapolating growth curves to time 0.

* Calculated for established cell lines grown in the presence of 2% TUC2.15 supernatant.

four lines that failed to survive without supernatant were used in subsequent attempts to identify T cell–derived plasmacytoma growth factors.

Screening of a panel of murine helper T cell supernatants showed a strong correlation between hybridoma and plasmacytoma growth factor titers ($n = 12$, $r = 0.97$ for MOPC 173), suggesting that the same factor could be responsible for both hybridoma and plasmacytoma growth. This idea was further supported by the finding that both activities copurified in analytical fractionations of TUC2.15 supernatant. As illustrated in Fig. 3 for MOPC 173, it was impossible to separate plasmacytoma growth factor from hybridoma growth factor by either gel filtration, isoelectric focusing, or reversed-phase HPLC. Identical results were obtained with MOPC 104E, RPC 20, and TEPC 1033. The heterogeneity of the plasmacytoma and hybridoma growth factor activities in isoelectric focusing was most probably due to variations in glycosylation because it was considerably reduced upon treatment with neuraminidase (results not shown). Collectively, these observations provided strong evidence for the idea that the hybridoma growth factor previously identified as IL-HP1 (11) represented the major and probably the only plasmacytoma growth factor present in TUC2.15 supernatant. Further proof of this hypothesis was obtained with IL-HP1 purified to homogeneity, which, at optimal concentration, induced maximal plasmacytoma proliferations that were comparable with those induced by crude TUC2.15 supernatant. From the dose-response curves shown in Fig. 4, the concentrations of IL-HP1 required for half-maximal growth of plasmacytomas were evaluated at approximately 30 pM. Noteworthy, two IL-HP1-dependent hybridomas, which we have used as indicator cells in the IL-HP1 assay, required 200-fold less growth factor for half-maximal proliferation than the plasmacytoma lines. Although the significance of this difference is presently not clear, it is conceivable that some complementation occurs in hybridomas, resulting in an abnormally high sensitivity to IL-HP1. The fact that IL-HP1-independent cells emerge much more frequently from IL-HP1-dependent hybridoma lines than from plasmacytoma lines (our unpublished observation) seems to support this view.

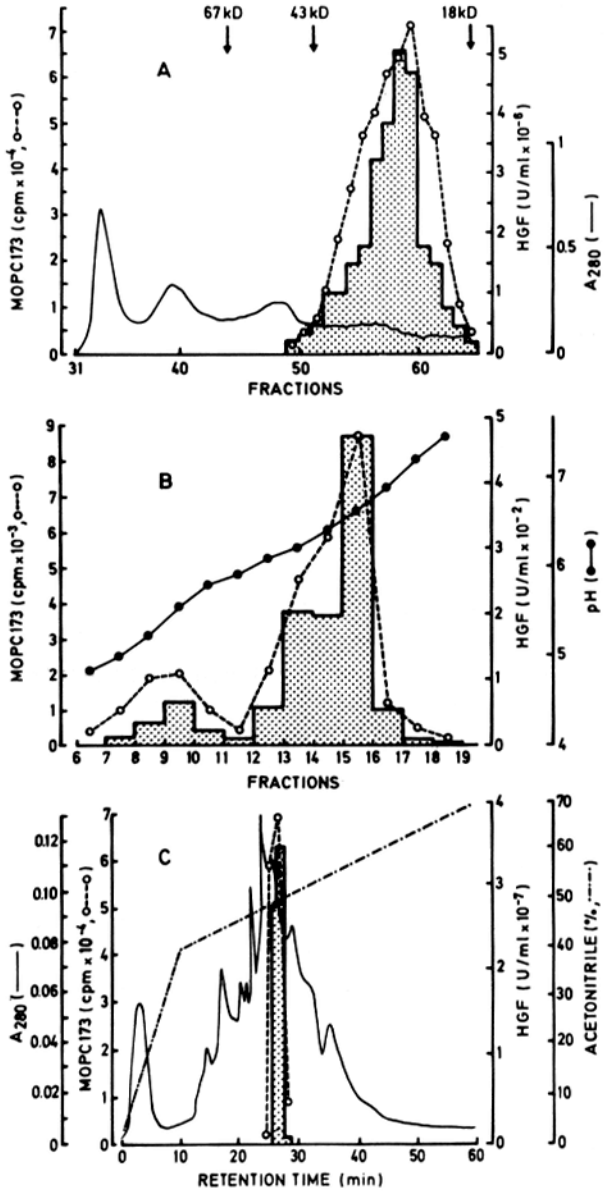

FIGURE 3. Copurification of hybridoma and plasmacytoma growth factors. Fractions were titrated for hybridoma growth factor (stippled area) and tested at a selected dilution for plasmacytoma growth factor. In the absence of supernatant, [^{3}H]thymidine incorporation levels of plasmacytoma MOPC 173 amounted to about 350 cpm. (*A*) Gel filtration on Ultrogel AcA 54. The plasmacytoma growth factor assay was performed on fractions diluted 10,000-fold. (*B*) Isoelectric focusing. Proliferation of the MOPC 173 line was tested in the presence of fractions diluted 1,000-fold. (*C*) Reversed-phase HPLC on a Cl-column. Plasmacytoma growth factor was assayed on fractions diluted 10,000-fold.

Three IL-HP1-dependent plasmacytoma cell lines (MOPC 104E, RPC 20, and TEPC 1033) that had been maintained in culture for several months were tested for tumorigenicity in pristane-treated, irradiated (500 rad) BALB/c mice. After

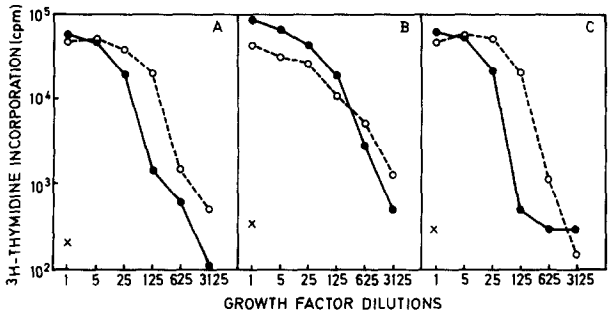

FIGURE 4. Comparison of plasmacytoma growth factor activity of crude TUC2.15 supernatant and of purified IL-HP1. Plasmacytoma cell lines (*A*, MOPC 104E; B, MOPC 173; C, RPC 20) established and maintained in vitro in the presence of 2% TUC2.15 supernatant were washed free of T cell factors and incubated with decreasing concentrations of crude TUC2.15 supernatant (●) or of purified IL-HP1 (○). Proliferation in the absence of growth factors is represented by (×). Initial concentration of TUC2.15 supernatant was 10% (vol/vol) and that of purified IL-HP1 was estimated at about 2 nM on the basis of silver-stained gels.

i.p. injection of 5×10^5 cells, the three lines were found to form tumors that killed the animals in less than 2 wk, indicating that IL-HP1-dependent plasmacytomas are still able to proliferate in vivo. Whether this means that these cells do not need IL-HP1 in vivo or that there is enough IL-HP1 or related molecules in the peritoneal cavity of pristane-treated mice is not known.

Another problem that remains to be solved is that of the specificity of IL-HP1 for cells of the B cell lineage. The importance of this question is highlighted by the recent finding that BSF-1, a lymphokine formerly thought of as a B cell–specific factor, has now been found to stimulate the proliferation of certain T cell lines (16, 17). As a first step in the analysis of the specificity of IL-HP1, we therefore compared the effects of IL-2, BSF-1, and IL-HP1 on the proliferation of IL-2-dependent cytolytic T cell line (CTLL), helper T cell clone TUC2.15, and plasmacytoma TEPC1033. As shown in Table II, TUC2.15 cells, which responded very well to BSF-1 and to IL-2, failed to proliferate in the presence of IL-HP1 even at a concentration in 100-fold excess of that required by plasmacytomas. Similar results were obtained with several other helper T cell clones. Although these results do not prove that IL-HP1 is a B cell–specific growth factor, they clearly show that this molecule does not stimulate the growth of every established cell line. Moreover, the data in Table II also formally demonstrate the functional difference between BSF-1 and IL-HP1, which corroborates the physicochemical differences already noted between these molecules (11, 18, 19).

Many different cell types, including macrophages (3, 9, 20), endothelial cells (21), peripheral blood cells (22), fibroblasts, (23) and T lymphocytes (11 and this report), have now been reported to secrete growth factors for hybridomas and plasmacytomas. It is difficult for the time being to evaluate the structural relations between these factors because, in most cases, they have not been cloned or purified to homogeneity. This situation is complicated even further by the fact that the structural data that are available are related to factors derived from different species. This is the reason why we do not presently know whether IL-HP1 is the mouse homologue of the plasmacytoma growth factor that we recently identified in media conditioned by human fibroblasts, and which appeared to be

TABLE II

Response of T Cell– and Plasmacytoma Lines to IL-2, BSF-1, and IL-HP1

Growth factors	Dose*	[^{3}H]Thymidine incorporation by:		
		T cell lines		Plasmacytoma line TEPC1033
		CTLL	Tuc2.15	
	U/ml		*cpm*	
None	—	173	145	94
rIL-2‡	20	79,695	155,950	57
	4	78,397	142,530	52
	0.8	30,480	41,318	69
	0.16	9,293	1,011	51
BSF-1	125	96	107,103	490
	25	103	45,907	323
	5	139	3,717	93
	1	127	138	60
IL-HP1	10,000	146	262	34,300
	2,000	124	243	46,200
	400	155	346	28,600
	80	107	170	7,900

CTLL (2×10^3 cells), TUC2.15 (5×10^4 cells) and TEPC1033 (5×10^3 cells) were incubated in microtiter wells in the presence of the indicated growth factors. [^{3}H]Thymidine was added after 24–48 h, and incorporation was measured 16 h later.

* 1 U/ml is defined as the titer of a growth factor preparation that stimulates half-maximal proliferation of the appropriate target cell: CTLL, anti-IgM–stimulated B cells and hybridoma 7TD1 for IL-2, BSF-1, and IL-HP1, respectively.

‡ Human rIL-2 was a gift of Dr. W. Fiers (State University of Ghent, Ghent, Belgium).

identical with a molecule previously described as 26K factor, also known as interferon β2 (Van Damme, J., G. Opdenakker, A. Billiau, M. Rubina, R. Simpson, S. Cayphas, A. Vink, and J. Van Snick, manuscript submitted for publication.

Summary

We have recently described the purification and NH_2-terminal amino acid sequence of a T cell–derived hybridoma growth factor that was provisionally designated interleukin-HP1 (IL-HP1). Here we report that a T cell supernatant containing high titers of this hybridoma growth factor considerably facilitated the establishment of primary cultures of murine plasmacytomas. Most plasmacytoma cell lines derived from such cultures remained permanently dependent on IL-HP1-containing T cell supernatant for both survival and growth in vitro. These cell lines, however, retained their ability to form tumors in irradiated pristane-treated mice.

Analytical fractionation of a T cell supernatant rich in IL-HP1 by either gel filtration, isoelectric focusing, or reversed-phase HPLC revealed the existence of only one plasmacytoma growth factor activity that strictly copurified with IL-HP1, strongly suggesting the identity of both factors. This conclusion was further supported by the finding that IL-HP1 purified to homogeneity supported the

growth of both B cell hybridomas and plasmacytomas. For half-maximal growth, plasmacytomas, however, required a concentration of IL-HP1 of ~30 pM, which is ~200 times higher than that required by B cell hybridomas.

A clear difference in the specificity of IL-HP1 and B cell stimulatory factor 1 (BSF-1) was demonstrated by the finding that IL-HP1-dependent plasmacytomas did not survive in the presence of BSF-1, whereas helper T cell lines that proliferated in the presence of BSF-1 failed to respond to IL-HP1.

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