Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 1857–1861, March 1986
Immunology

Characterization of a murine lymphokine distinct from interleukin 2 and interleukin 3 (IL-3) possessing a T-cell growth factor activity and a mast-cell growth factor activity that synergizes with IL-3

(synergy)

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Communicated by Kimishige Ishizaka, November 1, 1985

ABSTRACT **Murine mast-cell and T-cell growth factor activities, distinct from interleukins 3 and 2 (IL-3 and IL-2), have been identified and partially purified from the supernatant of the activated helper T-cell line Cl.Ly1$^+$2$^-$/9. This mast-cell growth factor (MCGF) activity supports only low levels of proliferation of several IL-3-dependent mast-cell lines and synergistically enhances the growth of mast cells in the presence of IL-3. The T-cell growth factor (TCGF) stimulates the proliferation of several T-cell lines, but to a lesser extent than recombinant IL-2. The MCGF and TCGF activities were not separable despite multiple biochemical fractionations, suggesting that both activities reside in the same protein. The MCGF/TCGF was separated from endogenous IL-3 by cation-exchange chromatography at neutral pH and could be distinguished from IL-2 by unique elution conditions from reverse-phase columns. Two bands of MCGF/TCGF activity were eluted from gels after sodium dodecyl sulfate/PAGE; under nonreducing conditions, the activities corresponded to molecular masses of 20 and 15 kDa, while after reduction, the molecular masses were 21 and 16 kDa. Thus, both activities may correspond to single polypeptide chains. The majority of the MCGF/TCGF activity appears to reside in the 20-kDa species, which displays a pI of 6.2 on chromatofocusing.**

T lymphocytes produce a number of soluble factors that regulate the growth and differentiation of other lymphoid and myeloid cells. One murine helper T-cell line, Cl.Ly1$^+$2$^-$/9 (hereafter designated Cl.1), produces a mast-cell growth factor (MCGF), T-cell growth factor (TCGF), B-cell growth and differentiation factors, and colony-stimulating activity (1). Recently cDNA clones encoding the inducible T-cell products interleukin 2 (IL-2) (2, 3), interleukin 3 (IL-3) (4, 5), and granulocyte–macrophage colony-stimulating factor (GM-CSF) (6, 7), were isolated and expressed in Cos-7 monkey kidney cells. A comparison of the biological activities of the proteins encoded in these cDNA clones with those in the supernatant of activated Cl.1 T cells revealed that recombinant IL-3 (IL-3^R) was consistently less active for mast cell proliferation than the T-cell supernatant was (8). Identical results were obtained with IL-3 purified from WEHI 3 supernatant (9). These findings indicate that IL-3, originally derived from a Cl.1 T-cell cDNA library, did not account for all the MCGF activity produced by this T-cell line. Moore and co-workers also found a discrepancy between the activities of purified IL-3 and their partially purified MCGF (10), and similar observations have been made with various fractionated T-cell supernatants (T. Mosmann, personal communication). A comparison of the TCGF activities of recombinant IL-2 (IL-2^R) and Cl.1 supernatant suggested differences as well (unpublished data).

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In light of these findings, we have examined the possibility that Cl.1 T cells produce MCGF and TCGF activities distinct from IL-3 and IL-2. Here we report that fractionation of Cl.1 T-cell culture supernatant by cation-exchange and reverse-phase chromatography separates IL-3 and IL-2 from previously unidentified MCGF and TCGF activities.

MATERIALS AND METHODS

Preparation of Conditioned Medium. Cells of T-cell lines Cl.1 (1) and GK15-1 (supplied by M. Giedlin, DNAX Research Institute) were resuspended at 5×10^5 per ml in RPMI 1640 medium containing 50 μM 2-mercaptoethanol, 1% fetal calf serum, and Con A at 2 μg/ml for 24 h. The supernatants were collected and stored at −70°C.

Cell Lines and Tissue Culture. A cloned mast cell line, MC/9 (11), was obtained from G. Nabel (Dana Farber Cancer Institute). The mast cell lines MM3 (provided by R. Coffman, DNAX Research Institute) and DX-2 (derived in this laboratory) were characterized by the absence of myelomonocyte-associated markers and by the presence of IgE receptors and histamine levels greater than 250 ng per 10^6 cells. The myeloid NFS-60 cell line (12) was provided by J. Ihle (Frederick Cancer Research Facility). The NFS-60 cell line was subcloned in this laboratory to obtain an IL-3-dependent clone. The T-cell line HT2 was obtained from S. Strober (Stanford University); CTLL-2 was supplied by W. Farrar (Frederick Cancer Research Facility); and Ly 23/4 was supplied by G. Nabel. The mast-cell and T-cell lines were grown in RPMI 1640 medium containing 10% fetal calf serum and 50 μM 2-mercaptoethanol, supplemented with IL-3^R or IL-2^R.

Assays. Histamine levels of several IL-3-dependent cell lines were generously assayed by M. Siegel (Schering–Plough, Kenilworth, NJ), using the method of Shore *et al.* (13).

TCGF and MCGF activities were determined by [^{3}H]thymidine incorporation (8) or by a colorimetric assay (14) as described.

Source of Lymphokines. IL-3 purified from WEHI 3 supernatant was a gift of J. Ihle. DNAX Research Institute provided IL-2^R, IL-3^R, GM-CSF, and γ interferon (IFN-γ) in the form of supernatant from Cos-7 monkey kidney cells transfected with the corresponding cDNA clones (2, 4, 6). One unit (U) of IL-2, IL-3, or GM-CSF was defined as the amount of factor that stimulated 50% maximum [^{3}H]thymidine incorporation by factor-dependent cell lines (8). One unit

Abbreviations: MCGF, mast-cell growth factor; TCGF, T-cell growth factor; IL-3, interleukin 3; IL-3^R, recombinant IL-3; IL-2, interleukin 2; IL-2^R, recombinant IL-2; IL-1, interleukin 1; GM-CSF, granulocyte–macrophage colony-stimulating factor; IFN-γ, γ interferon; U, lymphokine unit.

of IFN-γ protects 50% of murine L cells from the cytopathic effect of vesicular stomatitis virus (15).

Biochemical Methods. Protein determinations were based either on UV absorption (280 nm or 220 nm) or on a dye-binding assay (16). Supernatants were initially concentrated 20-fold with a Pellicon cassette unit (Millipore) or, after reverse-phase chromatography, by solvent evaporation in a Speedvac (Savant). $NaDodSO_4$/PAGE was performed by using the Laemmli system (17) with a 12% separating gel. Marker proteins were Pharmacia low molecular weight standards (Pharmacia, Uppsala, Sweden). Gels were stained with silver as described (18). To assess directly MCGF and TCGF activity as a function of molecular weight, samples with or without prior reduction with 50 mM dithiothreitol were electrophoresed, the gels were sliced to 1-mm sections and crushed, and protein was eluted overnight at 4°C into 0.5 ml of assay medium, supplemented with ovalbumin (Sigma) at 5 mg/ml.

Chromatography was performed at 18°C on a Pharmacia FPLC system equipped with a Kratos Spectroflow 773 UV detector (Kratos, Ramsey, NJ). Cation-exchange chromatography utilized a Pharmacia Mono S column (0.5 × 5 cm) equilibrated with 50 mM $NaPO_4$/1 mM EDTA, pH 7.0. Supernatants in the same buffer were applied to the column and eluted with a NaCl gradient to 1 M. For reverse-phase chromatography, a Pharmacia ProRPC (C_8) column (0.5 × 2 cm) was used. The sample, diluted with 0.1% trifluoroacetic acid in water to pH 2, was loaded onto the column and eluted with acetonitrile gradients containing 0.1% trifluoroacetic acid.

Isoelectric points of MCGF and TCGF were estimated by chromatofocusing on a Pharmacia Mono P column (0.5 × 20 cm). The sample, in 0.025 M [bis(2-hydroxyethyl)amino]tris-(hydroxymethyl)methane, pH 7.1, was loaded onto the Mono P column equilibrated with the same buffer. Gradient elution was effected with Pharmacia Polybuffer 74, pH 4.0 (1:10 dilution, pH adjusted with 0.2 M iminodiacetic acid). Effluent pH was continuously determined with a Pharmacia pH monitor. Prior to injections, all samples were filtered through a Millex GV 0.2-μm-pore unit (Millipore).

Fractions from $NaDodSO_4$/PAGE or chromatographic runs were assayed for their ability to support proliferation of three cell lines, NFS-60 (IL-3), MC/9 (MCGF), and HT2 (TCGF).

RESULTS

MCGF Activity of Cl.1 Supernatants. Purified IL-3 and supernatant from Cos-7 cells transfected with an IL-3 cDNA clone ($IL\text{-}3^R$) stimulated proliferation of the mast cell line MC/9 to the same extent (Fig. 1*A*). However, this level was a third to a half of that induced by Cl.1 T-cell supernatant. The presence of Con A in the T-cell supernatant (2 μg/ml) does not contribute significantly to its MCGF activity (8). One explanation for the effect noted above is that IL-3 does not account for all of the MCGF activity produced by Cl.1 cells. Alternatively, our mast cell line could be contaminated with a second factor-dependent population of cells. This latter possibility is excluded, however, because recloning the MC/9 cell line yielded subclones each of which responded to IL-3 and the Cl.1 T-cell supernatant in the same way as the original MC/9 clone (data not shown).

Since Cl.1 supernatant also contains lymphokines other than IL-3, several factors were tested for their ability to promote the growth of MC/9 cells. MC/9 cells were unresponsive to various concentrations of recombinant IL-2, IFN-γ, and GM-CSF, as well as to IL-1-containing-supernatant (P388D1 cells) (Fig. 1*A*). Moreover, supplementation of cultures containing various concentrations of recombinant IL-3 with IL-2, GM-CSF, IFN-γ, or IL-1 did not stimulate proliferation of MC/9 cells above the level obtained with IL-3 alone. This indicates that the increased MCGF activity of the T-cell supernatant is not due to its content of these other known factors. Our experiments pursued the possibility that the enhanced MCGF activity of the T-cell supernatant is due to one or more additional factors acting alone or in conjunction with IL-3.

Comparative Studies with Other Factor-Dependent Cell Lines. Two other mast cell lines, DX-2 and MM3, also gave higher proliferative responses to Cl.1 supernatant than to IL-3 (representative data for DX-2 are shown in Fig. 1*B*). These cells also did not respond to IL-2, IFN-γ, GM-CSF, or supernatant of P388D1 cells. Thus, the enhanced proliferative response of MC/9 cells to the T-cell supernatant may be typical of mast cells in general. In contrast, the granulocyte-type cells NFS-60 (12) were stimulated almost equally by IL-3 and Cl.1 supernatant (Fig. 1*C*). Although NFS-60 cells were unresponsive to IL-2, IFN-γ, and P388D1 supernatant, there was a small but reproducible stimulation by GM-CSF.

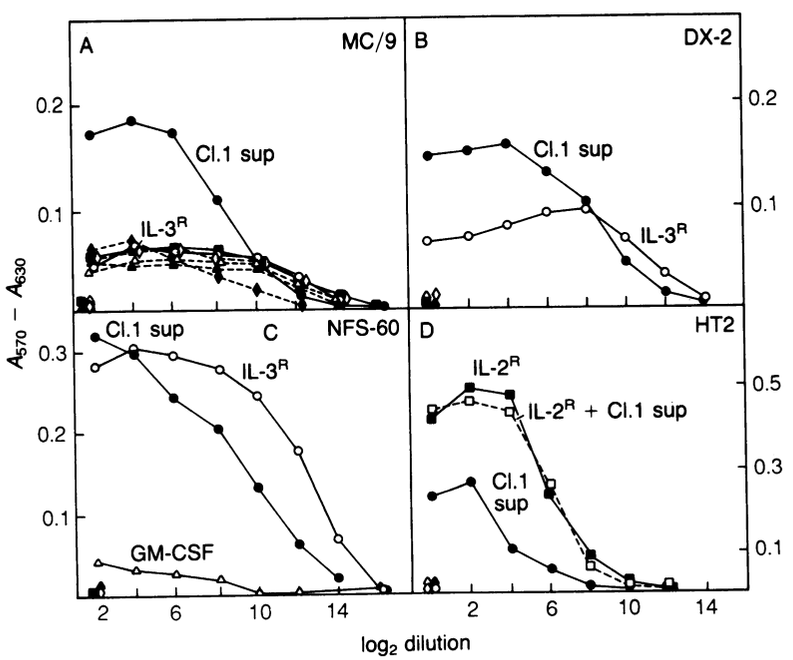

FIG. 1. Growth of factor-dependent cell lines: MC/9 mast cells (*A*), DX-2 mast cells (*B*), NFS-60 cells (*C*), and HT2 T cells (*D*). In each case, 5 × 10^3 cells were cultured with various concentrations of Cl.1 supernatant (●—●), $IL\text{-}2^R$ (■—■), $IL\text{-}2^R$ + Cl.1 supernatant (□---□), $IL\text{-}3^R$ (○—○), recombinant GM-CSF (△—△), recombinant IFN-γ (▲—▲), or P388D1 supernatant (◇—◇). MC/9 cells were also cultured with various concentrations of purified IL-3 (◆---◆) or $IL\text{-}3^R$ mixed with >200 U of $IL\text{-}2^R$ (■---■), recombinant GM-CSF (△---△), recombinant IFN-γ (▲---▲), or P388D1 supernatant (◇---◇). Growth factor activity was measured after 24 hr by a colorimetric assay (11). The absorbance at 570 nm (reference 630 nm) was measured on a Dynatek Micro ELISA reader.

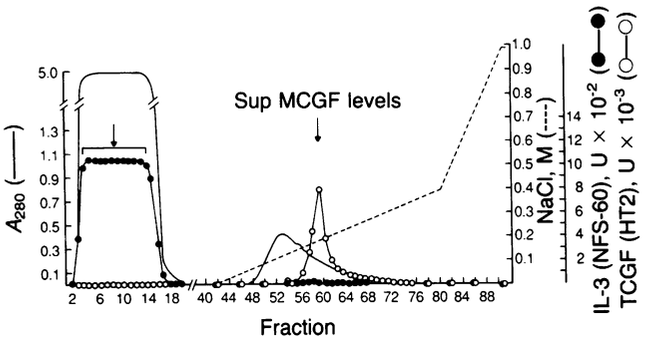

FIG. 2. FPLC cation-exchange chromatography of Cl.1 supernatant. Seven milliliters (35 mg of protein) of concentrated supernatant was dialyzed into 50 mM sodium phosphate/1 mm EDTA, pH 7.0 (buffer A; 7.8 mS/cm) and applied to a Pharmacia Mono S column (0.5 × 5 cm) equilibrated with the same buffer. Elution conditions: 0.5 ml/min flow rate; 0.5 ml per fraction; 0–40% buffer B in 40 min, 40–100% buffer B in 10 min (buffer B = buffer A + 1 M NaCl). Aliquots of each fraction were assayed for proliferation activity on NFS-60 (IL-3, ●), HT2 (TCGF, ○), and MC/9 (MCGF) cells. MC/9 response not shown; arrows denote positions where MC/9 proliferation levels reached those of Cl.1 supernatant.

The fact that IL-3 and the T-cell supernatant induce comparable levels of proliferation suggest the NFS-60 cell line is relatively insensitive to the uncharacterized factor present in the T-cell supernatant. Thus, NFS-60 cells were used to assay IL-3 during attempts to separate IL-3 from other MCGF activities by chromatographic fractionations.

The TCGF activity of Cl.1 supernatant was assessed by using the IL-2-dependent T-cell line HT2. Saturating concentrations of Cl.1 supernatant invariably stimulated cell proliferation well below the maximal level achieved with IL-2^R (Fig. 1*D*). Similar results were obtained with two other T-cell lines, CTLL-2 and Ly 23/4 (19) (data not shown). The supernatant does not contain an inhibitory substance since its addition to HT2 cultures containing IL-2 did not reduce proliferation (Fig. 1*D*). These results suggest Cl.1 supernatant contains a TCGF activity distinct from IL-2. Biochemical evidence in support of this conclusion is presented below.

Chromatographic Fractionation of Cl.1 Supernatant. Cl.1 supernatant was fractionated by various chromatographic methods with the immediate goal of separating IL-3 from other MCGF activities. Proliferation of NFS-60 cells was used to track IL-3 specifically against the background of total MCGF activity revealed by MC/9 proliferation. TCGF activity was assessed by HT2 cell proliferation.

When Cl.1 supernatant was fractionated by cation-exchange chromatography at neutral pH, approximately 98% of the loaded protein, 97% of the IL-3 units (assessed by NFS-60 proliferation), and 1% of the TCGF units appeared in the flow-through (Fig. 2). Elution of bound protein with a gradient of NaCl released TCGF activity at ≈0.19 M NaCl (fraction 60). A small but reproducible peak of TCGF activity also appeared at 1 M NaCl. No further TCGF activity could be eluted at higher NaCl concentrations (up to 3 M). A small amount of IL-3 also eluted in approximately the same region as the TCGF peak (detectable NFS-60 response). Only fractions corresponding to peak TCGF activity (fractions 59–61) and the flow-through (fractions 1–20) stimulated high levels of MC/9 proliferation, comparable to the unfractionated supernatant (arrows, Fig. 2). Since titration curves suggested fractions 59–61 contained more of the high-level MCGF activity than did the flow-through (not shown), we attempted to deplete further the IL-3 activity co-eluting with the TCGF peak and again assess MC/9 proliferation levels. Fractions 59–61 were therefore rechromatographed twice more under identical conditions.

After the third column pass, greater than 95% of the TCGF activity consistently eluted at 0.19 M NaCl. There was no measurable IL-3 (NFS-60 response) in the flow-through or in any of the column fractions. However, still co-eluting with

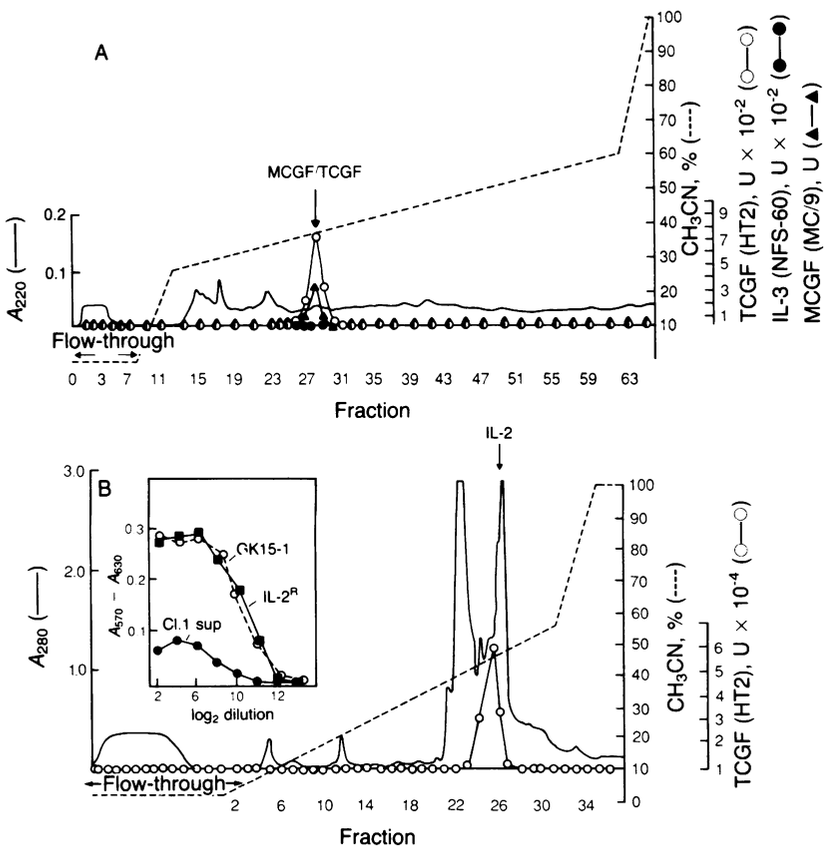

FIG. 3. Reverse-phase chromatography of cell supernatants. Chromatography was on a Pharmacia C_8 reverse-phase column (0.5 × 2 cm). Buffer A = 0.1% trifluoroacetic acid in water; buffer B = 0.1% trifluoroacetic acid in acetonitrile. Percent acetonitrile is vol/vol. Elution conditions: 0.5 ml/min, 0.5 ml per fraction, 0–25% buffer B in 4 min, 25–60% buffer B in 50 min, and 60–100% buffer B in 4 min. (*A*) IL-3-depleted Cl.1 supernatant; 100 μg of thrice Mono-S-passed supernatant (fractions 59–61, Fig. 2) in 0.1% trifluoroacetic acid was loaded onto the column. Aliquots of each fraction were assayed for proliferation activity on NFS-60 (IL-3, ●), HT2 (TCGF, ○), and MC/9 cells (MCGF, ▲). Arrow denotes fraction containing peak TCGF activity, which produced MC/9 proliferation levels similar to those of Cl.1 supernatant *after* addition of saturating IL-3^R levels to each fraction. (*B*) Chromatography of GK15-1 murine T-cell supernatant; 2 mg (0.5 ml) of concentrated GK15-1 supernatant in 0.1% trifluoroacetic acid was applied to the column and eluted as above. Aliquots of each fraction were assayed for TCGF activity on HT2 cells (○). Arrow marks the position where murine IL-2^R eluted under identical conditions. (*Inset*) Titrations of Cl.1 (●), IL-2^R (■), and GK15-1 supernatant (○) TCGF activities directly compared on the same plate.

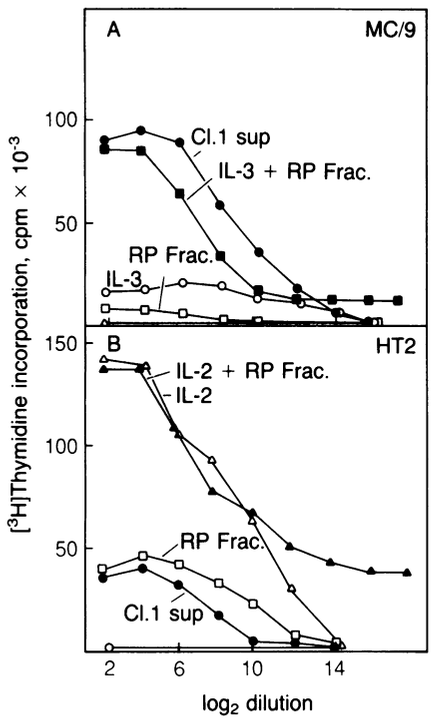

FIG. 4. MCGF and TCGF activities of partially purified factor from reverse-phase column (RP Frac.). Proliferation was measured by [^{3}H]thymidine incorporation after a 24-hr culture period. MC/9 mast cells and HT2 T cells were cultured with various concentrations of Cl.1 supernatant (●), IL-3^R (○), IL-2^R (△), RP Frac. (□), and dilutions of RP Frac. in the presence of >200 U of IL-3^R (■) or dilutions of IL-2^R in the presence of saturating levels of RP Frac. (▲).

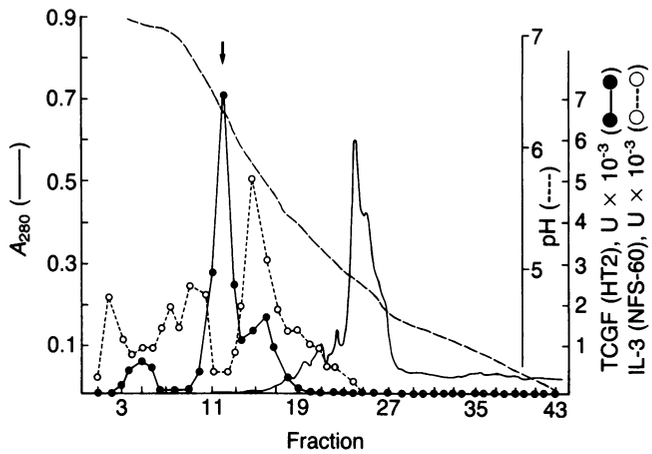

FIG. 5. Chromatofocusing of Cl.1 supernatant. Supernatant (1.5 ml, 3.5 mg) in 25 mM [bis(2-hydroxyethyl)amino]tris(hydroxymethyl)-methane, pH 7.1, was loaded onto a Pharmacia Mono P column (0.5 × 20 cm) and eluted with Polybuffer 74 (1:10), pH 4.0. Flow rate 0.5 ml/min, 1 ml per fraction. Aliquots of each fraction were assayed by proliferation on NFS-60 (IL-3, ○), HT2 (TCGF, ●), and MC/9 (MCGF, not shown). Peak MCGF activity is indicated by arrow. The TCGF peak (fraction 12, pI = 6.2) coincides with a minimum in IL-3 activity; MCGF proliferation levels are highest here (arrow).

the TCGF peak was an MCGF activity that now stimulated a plateau level of MC/9 proliferation that was significantly lower than that obtained with Cl.1 supernatant or IL-3 (see below). Since these fractions were devoid of measurable IL-3 activity (absence of NFS-60 response), apparently the novel MCGF by itself was incapable of stimulating MC/9 cells to the same degree as the crude T-cell supernatant.

To further attempt separation of MCGF from TCGF, the thrice-fractionated material (fractions 59–61) was diluted 1:10 with 0.1% trifluoroacetic acid in water to pH 2 and loaded directly onto a C_8 reverse-phase column. Fig. 3*A* shows the elution profile of bound protein released with a gradient of acetonitrile, 0.1% in trifluoroacetic acid. No MCGF or TCGF activity appeared in the flow-through; both activities co-eluted at 37% acetonitrile (fractions 26–29). However, the saturating MCGF response was now only about half that produced by IL-3 alone (Fig. 4*A*). When all fractions were reassayed in the presence of saturating levels of IL-3, only fractions 26–29 showed an MC/9 proliferation response in excess of IL-3 alone (arrow, Fig. 3*A*). In fact, the magnitude of the response was similar to that of the unfractionated supernatant itself (Fig. 4*A*). Further, proliferation levels equivalent to those of unfractionated supernatant were also observed when fraction 26–29 was assayed in the presence of IL-3 containing flow-through or IL-3 purified from WEHI 3 (not shown). Thus, the combination of IL-3 and the unique MCGF/TCGF stimulates levels of MC/9 proliferation characteristic of the original T-cell supernatant.

Although reverse-phase chromatography fails to resolve the MCGF from the TCGF, the TCGF was shown to be distinct from IL-2 by two criteria. First, when IL-2^R and supernatant from an IL-2-producing (Con-A stimulated) murine T-cell line (GK15-1) are both chromatographed on the same column under identical conditions, the TCGF activity elutes at 45% acetonitrile in a sharp peak that does not overlap with the 37% acetonitrile position marking the Cl.1 TCGF (Fig. 3*B*). Second, the saturating response of HT2 cells to IL-2^R is much different from that which characterizes the Cl.1 supernatant (Fig. 3*B Inset*). Further, we could detect no obvious synergy between the partially purified TCGF and IL-2 (Fig. 4*B*).

The chromatofocusing pattern of Cl.1 supernatant is shown in Fig. 5. IL-3 (NFS-60 response) displayed a very heterogeneous profile, including activity with pI values higher than 7.1, but the TCGF activity appeared more homogeneous, with the major TCGF species (fraction 12) showing an approximate pI of 6.2. This coincides with the maximal MCGF activity (MC/9 response) after addition of saturating levels of IL-3.

NaDodSO$_4$/PAGE of Cl.1 Supernatant and of Partially Purified MCGF/TCGF. Fig. 6 compares the electrophoretic profiles of unfractionated Cl.1 supernatant (Fig. 6*A*) with that of the cation-exchanged material, fractions 59–61 (Fig. 6*B*). Protein was eluted directly from gel slices and activities were determined by proliferation. Both the cation-exchange fraction and Cl.1 supernatant show TCGF peaks at 20 and 15 kDa under nonreducing conditions and 21 and 16 kDa under reducing conditions. Since the cation-exchanged material was depleted of IL-3, only low levels of MC/9 proliferation were induced and this MCGF activity was still coincident with TCGF peaks. Addition of saturating amounts of IL-3^R to all fractions raised the MC/9 proliferation response to Cl.1 supernatant levels only in the peak MCGF/TCGF fractions (arrows, Fig. 6*B*). IL-3 activity of the supernatant was greatest at the 24-kDa position, but it also appears in broad peaks around the 20-kDa region. Significantly, MC/9 proliferation levels well above the level of IL-3 occur at the 20-kDa position of electrophoresed supernatant (arrow, Fig. 6*A*). A silver-stained NaDodSO$_4$ gel of the most highly purified MCGF/TCGF material (fraction 28 of the reverse-phase column) also showed prominent protein bands at 20 and 15 kDa (data not shown).

DISCUSSION

Fractionation of supernatant from a Con A-activated murine T-cell line Cl.1 indicates the existence of MCGF and TCGF activities distinct from IL-3 and IL-2. The novel MCGF was

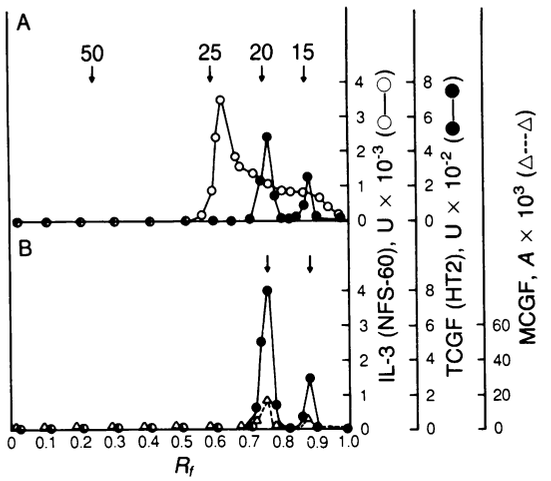

FIG. 6. NaDodSO$_4$/PAGE of Cl.1 supernatant and partially purified MCGF/TCGF. (*A*) Nonreducing NaDodSO$_4$/PAGE of unfractionated supernatant. Prior reduction with 50 mM dithiothreitol (60°C for 5 min) shifts TCGF peaks to slightly higher molecular masses (21 and 16 kDa) and is accompanied by a drastic loss in activity. (*B*) Nonreducing NaDodSO$_4$/PAGE of peak MCGF/TCGF fractions from cation-exchange chromatography (fractions 59–61, Fig. 2). Prior reduction with 50 mM dithiothreitol (60°C for 5 min) shifts MCGF and TCGF peaks to slightly higher molecular masses (21 and 16 kDa) and is accompanied by a drastic loss in activity. Arrows mark the only fractions to which addition of saturating amounts of IL-3 raises MCGF activity to supernatant levels. ○, IL-3; ●, TCGF; △, MCGF (colorimetric assay).

separated from IL-3 by strong cation-exchange chromatography at neutral pH. By itself, the factor has only slight activity for supporting MC/9 proliferation. However, together with IL-3, it produced a response greater than the sum of either factor alone. This enhancing activity is not mediated by lymphokines IL-1, IL-2, GM-CSF, or IFN-γ.

The same partially purified material that enhances IL-3 activity also has a TCGF activity. The novel TCGF and IL-2 are different, since they eluted at different positions from C_8 reverse-phase columns (37% acetonitrile for the novel TCGF, 47% for IL-2). Moreover, the Cl.1-derived TCGF supports HT2 proliferation to only one-third the level of IL-2. It is unlikely the TCGF fraction contains a suppressive activity, since saturating levels of the material obtained by reverse-phase chromatography do not affect the HT2 response to IL-2. Additionally, there is no obvious synergy between the novel TCGF and IL-2 for several T-cell lines. It is premature, however, to conclude that their response is characteristic of normal T cells.

It seems likely that the MCGF and TCGF activities each reside in single polypeptide chains; reduction with dithiothreitol prior to electrophoresis resulted in activity of only higher apparent molecular weight. Further, simultaneous NaDodSO$_4$ and dithiothreitol exposure drastically lowered both activities, suggesting the presence of critical intrachain disulfide bonds. In fact, failure to separate these two activities through seven fractionation methods, including anion-exchange, gel-filtration, and C_{18} reverse-phase chromatography, argues that the activities reside in the *same* polypeptide. The relationship between the 20-kDa and the 15-kDa MCGF/TCGF activities resolved by NaDodSO$_4$/PAGE is unclear. Proteolysis or differential glycosylations would account for the result.

While the chromatographic fractionations in Fig. 3 indicate the novel MCGF/TCGF activity can be distinguished from IL-2 by hydrophobicity differences, it also suggests that Cl.1 supernatant does not contain IL-2. In fact, blot analysis of Cl.1 poly(A)$^+$ RNA probed with a full-length mouse IL-2 cDNA clone failed to detect IL-2 transcripts (J. Culpepper and F. Lee, personal communication). The results in Fig. 3 also demonstrate the absence of the novel MCGF/TCGF activity in supernatants of GK15.1 T-cell line. These observations are consistent with results of a comprehensive study showing that only a subset of T-cell lines produces the MCGF/TCGF activity described here (T. Mosmann, personal communication).

The physiological role of this novel MCGF/TCGF is of interest since lymphocytes and mast cells are induced in several physiological responses (e.g., delayed-type hypersensitivity, chronic inflammation, and parasitic infections). It is well documented that T cells can regulate the growth of mast cells through the production of IL-3 (9, 11, 20). In turn, mast cell products such as histamine may influence the function of certain T lymphocytes (21, 22). These findings suggest that mast cells and T cells are linked in a complex immunoregulatory circuit. The activities described here may add another dimension to this circuit since the factor may coordinately regulate aspects of mast-cell and T-cell growth and differentiation. Furthermore, the ability of the TCGF/MCGF to enhance IL-3-dependent growth of mast cells demonstrates that an interaction between two factors may be required for optimal mast-cell growth.

We thank Patricia Meyerson and James Kami for excellent technical assistance. We also thank Gary Burget and Jill A. Zahner for typing the manuscript and Frank Lee, Tim Mosmann, and Ken-ichi Arai for helpful discussions and critical reading of the manuscript.

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