Multiple Glycosylated Forms of T Cell-derived Interleukin 3 (IL-3)

HETEROGENEITY OF IL-3 FROM PHYSIOLOGICAL AND NONPHYSIOLOGICAL SOURCES*

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Interleukin 3 (IL-3) derived from mouse T cells was biosynthetically labeled with either [35S]methionine or [3H]mannose, affinity-purified using various anti-IL-3 antibodies, and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Autoradiography revealed the same three major bands with Mr values of 21,500–22,500, 27,000–31,000, and 32,000–36,000, irrespective of whether the anti-IL-3 antibody had been directed to the N or C termini of the IL-3 polypeptide. Bioassay of eluates from the gels confirmed that all three bands exhibited IL-3 bioactivity. IL-3 produced from two nonphysiological sources, the myelomonocytic leukemia WEHI-3B or Cos 7 cells that had been transfected with an IL-3 cDNA clone, had in each case a different pattern of microheterogeneity. Treatment with either tunicamycin or N-glycanase resulted in IL-3 running as one band with Mr 16,000, corresponding to its 140-amino acid polypeptide chain. No evidence for proteolytic processing was detected. These results show that the Mr heterogeneity of IL-3 was highly dependent on the cellular source and is due to N-linked glycosylation.

Activation of T lymphocytes by specific antigen or mitogen stimulates the synthesis and secretion of a number of highly potent factors termed lymphokines. The amino acid sequences of the primary translation products of these factors have been deduced from their cDNA sequences. In no case, however, are there extensive data about the nature and function of the various post-translational modifications that are known to occur. For example, there is evidence for post-translational modification of the polypeptide chain at the N termini of interleukin 2 (IL-2) and interleukin 3 (IL-3)1 (2). Glycosylation is known to be a feature of many of these lymphokines and cytokines, although the functional significance of this is not well understood. For example human interferon-γ has been reported to exist in two different glycosylated forms with Mr 25,000 and 20,000 and a nonglycosylated 15,500 form (3), whereas two murine forms with Mr 21,800 and 20,600 have been described (4).

IL-3 has been purified from medium conditioned by cultured cells by several research groups using chromatographic methods. Two groups purified IL-3 obtained from a nonphysiological source, the myelomonocytic leukemia WEHI-3B, with yields of 8.4 (5) or 4% (6). Other groups used either the T cell lymphoma LBRM-33-A4 (7) or mitogen-stimulated spleen cells (8) as the source and obtained IL-3 with yields of 10 (7) and 4%, respectively (8). Only microgram quantities of purified material were obtained in each case, making further structural characterization difficult. Moreover, the low yields made studies of the heterogeneity of the IL-3 molecule impossible, because selective losses of different forms of IL-3 molecules could not be ruled out. It seems likely that some of the disparities for the Mr of WEHI-3B-derived IL-3 reported in various studies (for example Mr 41,000 (9), Mr 28,000 (5, 10), Mr 25,000 (11), and Mr 32,000 (6)) could be due to such losses during the many biochemical purification steps that were necessary. The availability of specific antibodies directed against synthetic peptides (12, 13) offers an approach to the study of the molecular heterogeneity of IL-3. Previously we showed that polyclonal (14) and monoclonal (15) antibodies to IL-3 have facilitated quantitative purification of the native molecule with yields of up to 97%. In this paper, we describe the use of antipeptide antibodies to characterize IL-3 produced by three different sources: activated T cells, the only known physiological source of IL-3; the myelomonocytic leukemia WEHI-3B in which IL-3 is produced as a result of aberrant activation of the IL-3 gene (16); and as a model for the systems that are being used to produce IL-3 in large quantities from mammalian cells, Cos 7 cells which have been transfected with a plasmid expressing an IL-3 cDNA clone.

MATERIALS AND METHODS

Conditioned Media—Medium conditioned by high density cultures (5 × 105–106 cells/ml) of the myelomonocytic leukemia WEHI-3B containing approximately 50 units/ml IL-3 were collected by centrifugation and concentrated 10-fold using an Amicon hollow fiber system (Mr cutoff >20,000).

1 The abbreviations used are: IL-3, interleukin 3; ConA, concanavalin A; mAb, monoclonal antibody; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; HPLC, high performance liquid chromatography.

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For the preparation of supernatants containing maximal amounts of IL-3, the T cell clones P41.1 (2) and E9.D4 (17) were cultured at 3 x 10^6/ml in RPMI supplemented with 1% fetal calf serum in 100-ml dishes; lymphokine production was induced by adding concanavalin A (ConA, Calbiochem) at 5 μg/ml. Supernatants removed after 18 h were harvested and 10% of the eluting material was applied to a column of IL-3.

**Transfection of Cos 7 Cells**—Cos 7 cells were grown to 50% confluence and then transfected with plasmid DNA using a calcium phosphate transfection method (18). The plasmid construct consisted of a full-length IL-3 cDNA clone (a gift from Dr. A. Dunn, Ludwig Institute of Cancer Research, Melbourne, Australia) inserted into the EcoRI site of the murine expression vector, p2103 (the gift of Dr. G. Wong, The Genetics Institute, Cambridge, MA) (19). Supernatants were collected after 4 days and contained approximately 15,000 units/ml IL-3.

Biologically Active IL-3—Prior to labeling with [35S]methionine, T cells were washed three times in methionine-free RPMI 1640 containing 5 μg/ml ConA and 1% fetal calf serum that had been dialyzed extensively against phosphate-buffered saline to remove free methionine. [35S]Methionine (specific activity, 800 Ci/mmol, ICN) was added to give a final concentration of 0.1 μCi/ml. In some experiments tunicamycin (10 μg/ml, Sigma) was added at the same time as the [35S]methionine. Labeling with [3H]mannose (specific activity, 25 Ci/mmol, ICN) was added to give a final concentration of 0.1 μCi/ml.

**Polyclonal and Anti-IL-3 Antibodies**—Peptides corresponding to murine IL-3 residues 1-29 and 1-6 and full-length IL-3 (1-140) were synthesized by solid phase methods and were gifts from Dr. Ian Clark-Lewis (20, 21).

Polyclonal antiserum to IL-3 were generated in rabbits immunized with synthetic IL-3 peptides corresponding to amino acid residues IL-3 (1-29) and IL-3 (1-6) coupled to keyhole limpet hemocyanin (14). Antibodies were affinity-purified on peptides coupled to Sepharose. The antibodies were then coupled to CNBr-activated Sepharose beads (10 μg of antibody/ml of gel) to form affinity columns recognizing specific peptide sequences within IL-3 (14). Monoclonal antibodies for native IL-3 were raised by the fusion of myeloma cells with spleen cells from mice immunized with a mixture of IL-3 peptides coupled to keyhole limpet hemocyanin, followed by a boost with synthetic IL-3 as described fully elsewhere (15). Monoclonal anti-IL-3 antibodies were partially purified from ascites fluid by differential centrifugation and were coupled to CNBr-activated Sepharose beads.

Affinity Purification of IL-3—Conditioned media from the different cell sources were applied to the immunosorbent columns made with polyclonal (14) or monoclonal (15) anti-IL-3 antibodies. These were then washed with at least 10 times their bed volumes of phosphate buffered saline. IL-3 was eluted in 0.1 m NaCl, pH 7.0, from the immobilized ConA-Sepharose column (Du Pont) with 5 times the bed volume of 0.1 M glycine (pH 2.5) and subsequently neutralized with a 25% volume of 1 M Tris (pH 8.0).

**Iodination of IL-3**—IL-3 derived from ConA-stimulated T cells or from the myeloid leukemia WEHI-3B was purified once using an anti-IL-3 (1-29) antibody-Sepharose column (14). The column eluate was iodinated by the chloramine-T method (22) to a specific activity of approximately 60 μCi/μmol. The iodinated IL-3 samples were then further affinity-purified using the anti-IL-3 monoclonal antibody 2E11 (15).

**Polyacrylamide Gel Electrophoresis**—Labeled ZL-3-derived IL-3 samples were applied to a 12% polyacrylamide gel containing 0.1% SDS in a horizontal slab gel apparatus. The gel was run under conditions essentially identical to those of Goding (25). The molecular weight standards (Bio-Rad) were: lysomysoz, M, 14,400; soybean trypsin inhibitor, M, 21,500; carbonic anhydrase, M, 31,300; ovalbumin, M, 45,000; bovine serum albumin, M, 66,000; phosphorylase, M, 92,500. Samples were concentrated by methanol precipitation as follows: 9 volumes of methanol were added to 1 volume of the IL-3 sample that was in the elution buffer used either in affinity or HPLC columns, or the buffer used in eluting material from SDS gels (see below) and held overnight at -20°C. Bovine serum albumin (50 μg) was added to the samples obtained from the affinity or HPLC columns, prior to the addition of methanol in order to obtain a complete precipitation of IL-3. Precipitates were collected by centrifugation at 16,000 x g for 30 min in SDS-saturated buffer, boiled for 5 min, and then loaded onto the gel. Gels were routinely stained with Coomassie Blue. The autoradiographic detection of [35S] was enhanced by soaking the gels in Amplify (Amersham Corp.) for 30 min before drying and exposing to Kodak XAR-5 or Amersham Hyperfilm MP for between 1 and 28 days.

**Elution of IL-3 from SDS-PAGE Gels**—After appropriate autoradiographic exposure, gel tracks were cut into 5-mm slices, and each slice was homogenized in 0.5 M NaCl containing 1 mg/ml of bovine serum albumin and 0.1% SDS as described (6). The homogenate was agitated for 24 h at room temperature, spun for 5 min at 15,000 x g, and the supernatant precipitated in methanol as described above. Precipitated samples were resuspended in medium and assayed for IL-3 bioactivity at a starting dilution of 1:2.

**High Performance Gel Permeation Chromatography**—P41.1-derived [35S]-labeled IL-3/IL-3 was added to give a specific activity of 10 μCi/ml. In some experiments tunicamycin (10 μg/ml, Sigma) was added at the same time as the [35S]methionine. Labeling with [3H]mannose (specific activity, 25 Ci/mmol, ICN) was added to give a final concentration of 0.1 μCi/ml.

**RESULTS**

**SDS-PAGE Analysis of Affinity-purified T Cell-derived IL-3**—P41.1, a BALB/c-derived L3T4+ positive T cell clone, that consistently produced high levels of IL-3 after stimulation with ConA, was chosen for the biosynthetic labeling of IL-3 using [35S]methionine. Labeled IL-3 was affinity-purified by two passages over a polyclonal anti-IL-3 (1-29) affinity column (14). The yield was about 95% based on biological activity. Samples of this material containing approximately 10,000-40,000 cpm were further analyzed on a 13% polyacrylamide gel run under reducing conditions. Fig. 1 shows an autoradiograph showing the starting T cell-conditioned medium (lane A), the eluate after two passages through the anti-IL-3 (1-29) column (lane E), and the breakthrough fraction following a single passage over the anti-IL-3 (1-29) column (lane F). lane E exhibited three broad labeled bands with apparent M, 32,000-36,000, M, 27,000-31,000, and M, 21,500-22,500.

In order to confirm the specificity of the polyclonal anti-IL-3 (1-29) antibody column, IL-3 was also passed over immobilized monoclonal anti-IL-3 antibodies (15). [35S]Labeled IL-3 from the P41.1 T cell clone was purified twice using either mAb 2E11 or mAb 1A3 (15). The yields were approximately 90% (15). Once again three major bands were seen after SDS-PAGE and autoradiography (Fig. 1, lanes C (mAb 1A3) and D (mAb 2E11)). None of these bands corresponded to any major band in the starting conditioned medium (lane A) nor did passage of T cell material over the affinity columns result in a detectable loss of a major band (lane B). An aliquot of [35S]IL-3 was also run over a control column composed of CNBr-activated Sepharose coupled with glycine in order to
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FIG. 1. Anti-IL-3 antibodies recognize several Mr forms of T cell-derived IL-3. T cells were stimulated with ConA in the presence of [35S]methionine as described. [35S]-Labeled IL-3 was affinity-purified twice on the column coupled with the appropriate antibody. The fractions were then analyzed by SDS-PAGE run under reducing conditions. Lane A shows crude T cell supernatant. Lane B shows T cell supernatant depleted of IL-3 using mAb 1A3; lanes C–E show IL-3 affinity-purified twice using mAb 1A3 (lane C), mAb 2E11 (lane D), or polyclonal anti-1–29 antibody (lane E), and lane F represents T cell supernatant depleted of IL-3 using anti-IL-3 (1–29) antibody.

rule out nonspecific binding of IL-3 to Sepharose beads. No radioactivity bound to the glycine-Sepharose column (data not shown).

Isolation of Three IL-3 Forms—Two different techniques were used to show that each of the three Mr species contained IL-3 bioactivity. In the first experiment, 6,400 units of twice 2E11-purified [35S]IL-3 (corresponding to approximately 145,000 cpm) were applied to a TSK 3000 SW HPLC column and run at low ionic strength. The IL-3 bioactivity eluted as a broad peak with a trailing shoulder that coincided with the profile of radioactivity (Fig. 2). Total recovery of radioactivity was 95%, the peak fraction being at an elution volume of 16.6 ml. Fractions were pooled as indicated in Fig. 2 and analyzed by SDS-PAGE and autoradiography. Comparison of the radioactivity showed patterns that fractions 3, 6, and 9–13 contained only forms corresponding to apparent Mr values of 21,500–22,500, 27,000–31,000, and 32,000–36,000, respectively. Each of the fractions contained significant amounts of bioactivity.

The second approach involved the elution of bioactivity directly from SDS-polyacrylamide gels that were run under nonreducing conditions to avoid the adverse effect of reduction on the recovery of bioactivity (6). Fig. 3 shows [35S]IL-3 purified twice on the 2E11 column produced either by P41.1 (lane B) or by a second L3T4 positive T cell clone, E9.D4 (lane A). The pattern of bands was very similar in the two preparations. The three species ran at a lower apparent Mr, under nonreducing conditions, suggesting the presence of intrachain disulfide bonds. The profile on the right of Fig. 3 shows the bioactivity eluted from lane B after Coomassie Blue staining, drying, and autoradiography for 3 weeks to obtain an adequate exposure. Total recovery of both bioactivity and radioactivity was approximately 90%. Each of the three bands clearly contained IL-3 bioactivity.

Analysis of IL-3 with Anti-1–6 Antibody—Two forms of IL-3 which differ at the N terminus have been identified (2). An antibody specific for the first 6 amino acids can distinguish and divide these forms into binding and nonbinding fractions. The fraction of IL-3 binding to the anti-IL-3 (1–6) antibody varies between 70 and 98%, depending on the cellular source and conditions of preparation. Repeated application of nonbinding material to the affinity column ruled out a saturation or equilibrium mechanism (2). The molecular basis for the failure of a fraction of molecules to bind to the IL-3 (1–6) antibody is not known. To help clarify this issue, the binding and nonbinding material was analyzed for the various Mr forms by SDS-PAGE. T cell-derived [35S]IL-3 was purified
shown in Fig. 5 demonstrated that the increased $M_c$ of the three major bands in P41.1 T cell-derived IL-3 above that of the unmodified IL-3 polypeptide resulted principally from $N$-linked glycosylation. Tunicamycin, a potent inhibitor of $N$-linked glycosylation, was added at 10 $\mu$g/ml before stimulation with ConA. The $[^{35}S]IL-3$ was purified twice on a mAb 2E11 column. Yields of biosynthetically labeled IL-3 were approximately 5% of those from nontunicamycin-treated cells. An aliquot was run on a 13% gel under reducing conditions in parallel with nonglycosylated chemically synthesized (21) IL-3 (Fig. 5, lanes A and C). Tunicamycin treatment reduced the $M_c$ of most of the T cell-derived IL-3 to approximately 16,000, corresponding exactly to the apparent $M_c$ of the synthetic IL-3. There was some material running at a lower $M_c$ than the major band in both the tunicamycin-treated and synthetic preparations; however, this may have resulted from incomplete reduction. There were also minor bands in the tunicamycin-treated preparation that ran at slightly higher $M_c$ (up to approximately 23,000 under reducing conditions). A second sample of $[^{35}S]IL-3$ from tunicamycin-treated P41.1 cells was run under nonreducing conditions (Fig. 5, lane D), and bioactivity was eluted from the gel after autoradiography. The bioactivity profile corresponded exactly to the bands seen on the autoradiograph.

In other experiments IL-3 was biosynthetically labeled with either $[^{35}S]$methionine or $[^3H]$mannose and purified by sequential affinity adsorption on antibodies recognizing the N terminus (anti-IL-3 (1–29)) and the C-terminal region (mAb 2E11, recognizing residues 130–135). Aliquots of the purified samples were concentrated by methanol precipitation. One aliquot of each of the $[^{35}S]$- or $[^3H]$-labeled samples was treated with $N$-glycanase. SDS-PAGE of untreated and $N$-glycanase-treated samples showed that this enzymatic treatment reduced the apparent $M_c$ of $[^{35}S]IL-3$ from the higher $M_c$ forms to one single band with an apparent $M_c$ of 16,000 (Fig. 6, lane A). SDS-PAGE does not detect differences between forms of IL-3 that bind to anti-IL-3 (1–6) antibodies and those that do not. $[^{35}S]$-Labeled IL-3 affinity-purified twice on mAb 1A3 was applied to an affinity column recognizing the first 6 amino acids of IL-3. The breakthrough representing 5% of the bioactivity was cut into slices after appropriate autoradiographic exposure. The gel slices were homogenized, and IL-3 was eluted by diffusion and tested in the standard bioassay using the IL-3-dependent cell line, R6-X.E4.8.9. Values are given as total units of IL-3 eluted per gel slice.

**Fig. 4.** SDS-PAGE does not detect differences between forms of IL-3 that bind to anti-IL-3 (1–6) antibodies and those that do not. $[^{35}S]$-Labeled IL-3 affinity-purified twice on mAb 1A3 was applied to an affinity column recognizing the first 6 amino acids of IL-3. The breakthrough representing 5% of the bioactivity (lane A) and the glycine eluate representing 95% of the bioactivity (lane B) were then analyzed by SDS-PAGE run under reducing conditions.

**Fig. 3.** Gel elution after SDS-PAGE establishes that all three $M_c$ forms of IL-3 are active. $[^{35}S]IL-3$ produced by two different L3T4 positive T cell clones was affinity-purified twice on mAb 2E11 and analyzed by SDS-PAGE run under nonreducing conditions (lane A, T cell clone E9.D4; lane B, T cell clone P41.1). Lane B was cut into slices after appropriate autoradiographic exposure, the gel slices were homogenized, and IL-3 was eluted by diffusion and tested in the standard bioassay using the IL-3-dependent cell line, R6-X.E4.8.9. Values are given as total units of IL-3 eluted per gel slice.
whether the difference in form with an apparent and with N-glycanase as described. SDS-PAGE was run under nonreducing conditions and shows the M, pattern of untreated [3H]mannose IL-3 (lane A) and [3H]mannose IL-3 (lane B) and the corresponding samples after N-glycanase treatment (lane C, [3H]methionine IL-3, and lane D, [3H]mannose IL-3). Cos 7 cell-derived [3H]methionine IL-3 was also affinity-purified twice using the anti-IL-3 (1-29) column, and one aliquot was treated with N-glycanase. Lane E was loaded with nontreated IL-3 and lane F with IL-3 treated with N-glycanase.

C) and removed all the 3H from the IL-3 labeled with [3H]mannose (Fig. 6, lane D). In both cases IL-3 bioactivity was eluted from gel slices corresponding to the 35S-labeled M, 16,000 band (data not shown).

Analysis of the M, Forms of WEHI-3B-derived IL-3—Unlike affinity-purified T cell-derived IL-3 (Fig. 3), chromatographically purified IL-3, derived from WEHI-3B cells, gave a broad band around M, 32,000 when analyzed by SDS-PAGE (6). WEHI-3B-derived material was examined to determine whether the difference in M, patterns reflected differences in the cellular sources or the techniques used in their purification. We radioiodinated the WEHI-3B-derived material because the low amounts of IL-3 bioactivity in medium conditioned by WEHI-3B cells (50 units/ml compared to 10,000 units/ml for the T cell clones P41.1) made 35S labeling very difficult. Concentrated (10 ×) supernatant from WEHI-3B, containing about 500 units/ml IL-3, was purified in parallel with an unlabeled P41.1 supernatant on the anti-IL-3 (1-29) antibody column. The two column eluates were then iodinated to a high specific activity by the chloramine-T method and repurified on the 2E11 mAb column. SDS-PAGE analysis under nonreducing conditions revealed that the WEHI-3B-derived material had a similar electrophoretic pattern to the chromatographically purified IL-3 (6), but a strikingly different pattern to that of T cell-derived IL-3 (Fig. 7). Because iodination by this method destroyed over 95% of the bioactivity of the IL-3, a preparation of unlabeled WEHI-3B-derived IL-3 was run in parallel on the same gel, and the bioactivity profile was determined by elution of the unlabelled track. Samples of labeled IL-3 were run under reducing conditions (data not shown) to determine the M, values given below. The bioactivity was contained in a broad band between M, 29,000 and 45,000 as well as in a very faint band migrating at exactly the position of the smallest (21,500-22,500) T cell-derived band. Two other iodinated bands were apparent at M, 27,000 and 17,500 but had no detectable bioactivity associated with them and could therefore be impurities.

Analysis of the M, Forms of Cos 7-derived Recombinant IL-3—IL-3 produced by transient expression of an IL-3 cDNA clone in Cos 7 cells was biosynthetically labeled using [35S]methionine. The supernatant was harvested after 96 h, affinity-purified twice on anti-IL-3 (1-29) antibodies, and analyzed by SDS-PAGE run under reducing (lane A) and nonreducing (lane B) conditions. Lane B was cut into slices after autoradiography, and eluates of the slices were tested for bioactivity.

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FIG. 6. N-Glycanase treatment of IL-3 gives rise to one form with an apparent M, of 16,000. IL-3, biosynthetically labeled in clone P41.1 either with [3H]methionine or with [3H]mannose, was affinity-purified using anti-IL-3 (1-29) antibody followed by mAb 2E11. One aliquot of each of these samples was treated with N-glycanase as described. SDS-PAGE was run under nonreducing conditions and shows the M, pattern of untreated [3H]mannose IL-3 (lane A) and [3H]mannose IL-3 (lane B) and the corresponding samples after N-glycanase treatment (lane C, [3H]methionine IL-3, and lane D, [3H]mannose IL-3). Cos 7 cell-derived [3H]methionine IL-3 was also affinity-purified twice using the anti-IL-3 (1-29) column. The two column eluates were then iodinated (6), and analyzed by SDS-PAGE (run under nonreducing conditions). Unlabeled WEHI-3B-derived IL-3 purified on an anti-IL-3 (1-29) column was run in parallel with the 125I-labeled material, the gel track cut into slices, and eluates of the slices were analyzed for bioactivity.

FIG. 7. IL-3 released by the myeloid leukemia WEHI-3B has a different M, from T cell-derived material. IL-3 produced by T cells (lane A) and IL-3 produced by the myeloid leukemia WEHI-3B (lane B) was affinity-purified using anti-IL-3 (1-29) affinity columns. An aliquot of each of the column eluates was labeled with 125I, repurified on mAb 2E11, and analyzed by SDS-PAGE (run under nonreducing conditions). Unlabeled WEHI-3B-derived IL-3 purified on an anti-IL-3 (1-29) column was run in parallel with the 125I-labeled material, the gel track cut into slices, and eluates of the slices were analyzed for bioactivity.

FIG. 8. IL-3 produced by transient expression in Cos 7 cells has a M, of 26,000-45,000. IL-3 was produced by transient expression of IL-3 cDNA in Cos 7 cells in the presence of [35S]methionine. The supernatant was harvested after 96 h, affinity-purified twice on anti-IL-3 (1-29) antibodies, and analyzed by SDS-PAGE run under reducing (lane A) and nonreducing (lane B) conditions. Lane B was cut into slices after autoradiography, and eluates of the slices were tested for bioactivity.
Cos 7 cell-derived \(^{35}S\)IL-3 was also treated with N-glycanase in order to remove N-linked carbohydrate. Comparison of N-glycanase-treated \(^{35}S\)IL-3 with untreated material (Fig. 6, lane E versus lane F) established that approximately 50% of the IL-3 is converted to the \(M_r\) 16,000 form, whereas the other 50% of the \(^{35}S\)-labeled material appears as a smear between \(M_r\) 28,000 and 16,000 (as determined by scanning densitometry of the autoradiograph, data not shown).

**Discussion**

In this study we describe the use of polyclonal and monoclonal antibodies to characterize murine IL-3 obtained from three different sources: T cell clones, the myelomonocytic leukemia WEHI-3B, and Cos 7 cells expressing an IL-3 cDNA. Affinity columns prepared from these antibodies bound 90–98% of IL-3 bioactivity (14, 15) and were used for purification and study of the heterogeneity of the molecules. SDS-PAGE analysis of affinity-purified IL-3, derived from T cell clones, revealed the presence of three broad bands, with apparent \(M_r\) of 32,000–36,000, 27,000–31,000, and 21,500–22,500. The three \(M_r\) forms were present in preparations purified using antibodies recognizing either N-terminal sequences (corresponding to amino acid residues 1–6, 1–29) or C-terminal sequences (amino acid residues 130–135) (Fig. 1). All three bands isolated using two independent separation techniques, gel permeation HPLC and preparative SDS-PAGE, contained bioactive IL-3.

Major proteolytic cleavage can be excluded as a cause of the multiple molecular weight forms, since IL-3 that was purified first on mAb 1A3 (recognizing amino acid residues close to the N terminus that would result in the loss of the 1–6 epitope (for example, loss of amino acids or O-linked glycosylation) could not be identified by our experiments.

IL-3 has been described as a glycoprotein (5, 28) and has four potential sites for N-linked glycosylation (29, 30). Our experiments using tunicamycin and N-glycanase (Figs. 5 and 6) clearly show that the \(M_r\) heterogeneity of T cell-derived IL-3 is principally due to N-linked glycosylation. No evidence for O-linked glycosylation was detected, although the present data cannot completely exclude a minor level of C-linked modification.

The pattern of WEHI-3B-derived IL-3 (Fig. 7) differed strikingly from that of the T cell clone (Fig. 3) in that distinct bands were not apparent, but rather a broad smear was present between \(M_r\) 29,000 and 45,000. As IL-3 is the product of a single gene and the major source of \(M_r\) heterogeneity of IL-3 is N-linked glycosylation (this study), it is likely that WEHI-3B glycosylates IL-3 differently from T cells, leading to the different patterns observed.

IL-3 produced by transient expression in Cos 7 cells was again different from that of the T cell clone or WEHI-3B-derived material. The \(M_r\) forms of IL-3 were larger than the T cell-derived material with strong bands of IL-3 bioactivity at \(M_r\) 26,000 and 30,000–21,000 and a broad smear up to \(M_r\) 45,000. In the case of Cos 7 cell-derived material only about 50% of IL-3 was converted to the \(M_r\) 16,000 form by N-glycanase. The remaining material was converted to a smear running between \(M_r\) 28,000 and 16,000 (Fig. 6). Neuraminidase treatment reduced the mean apparent \(M_r\) of this material further, indicating the presence of residual carbohydrate (data not shown). It is not clear whether this smear is due to incomplete removal of N-linked sugar or whether a portion of IL-3 from these cells undergoes partial O-linked glycosylation or other modification.

There are many precedents for variations in the glycosylation in different cell types. It is well documented that patterns of glycosylation may differ not only between different tissues (31, 32) and at different stages of development within the same cell lineage, but also between normal and tumorigenic cells of the same type (33, 34). In the case of murine interferon-\(\gamma\), one T cell lymphoma has been shown to make two species with \(M_r\) 16,800 and 17,800 rather than the normal forms of \(M_r\) 20,600 and 21,800 (4). The human "acute T cell lymphoma" line HUT 102B expresses IL-2 receptors from a normal gene but with an aberrant glycosylation pattern (35). However, there are also many cases in which glycosylation patterns are the same in normal and tumorigenic cells (36, 37).

The fact that most cell-surface or secreted proteins are glycosylated suggests that glycosylation has an important, although poorly understood, function. Deglycosylated forms of lymphokines such as IL-3, granulocyte-macrophage colony-stimulating factor, and interferon-\(\gamma\) can mediate biological effects of native molecules (21, 38, 39). However, it is not always clear whether the potencies of some recombinant lymphokines are as high as those of the native glycoproteins. Deglycosylation of granulocyte-macrophage colony-stimulating factor, produced by recombinant techniques in yeast or Chinese hamster ovary cells, has been reported to lead to an increase in biological activity (40), suggesting that abnormal glycosylation in these nonphysiological host cell types resulted in molecules with impaired activity. Clearly attention must be paid to glycosylation when secreted proteins like IL-3 or granulocyte-macrophage colony-stimulating factor are produced under unusual conditions or by nonphysiological cell types. As demonstrated here, affinity-purified and biosynthetically labeled lymphokines should prove a very useful system for further study of the effect of glycosylation on these factors.

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