IMMUNO-SUPPRESSIVE LYMPHOCYTE FACTORS

I. Purification of Inhibitor of DNA Synthesis to Homogeneity*

By BRIAN V. JEGASOTHY and DONALD R. BATTLES

From the Department of Medicine, Division of Dermatology, Duke University Medical Center, and the Veterans Administration Medical Center, Durham, North Carolina 27710

Inhibitor of DNA synthesis (IDS),[1] a lymphocyte product, has been previously shown to be released into supernates of concanavalin A (Con A)-stimulated normal rat spleen or lymph node lymphocytes and lymphocytes sensitized in vivo with antigen and later challenged with the same antigen in vitro (1, 2). IDS inhibits the mitogen-induced proliferative response of T and B lymphocytes and the proliferation of virally transformed mouse fibroblasts (1, 3). The inhibitory activity of IDS is mediated through the activation of adenylate cyclase in target cell membrane and the elevation of intracellular cAMP (4, 5).

IDS is released in large amounts when nonspecific immune suppression is brought about by the intravenous injection of large tolerogenic doses of protein antigen (2). IDS may therefore play a significant role in immunology in the area of nonspecific suppression mediated by a specific population of suppressor cells (6, 7). These cells, possibly through IDS, appear to be involved in the nonspecific immune suppression seen with antigenic competition (8), desensitization (6), parasitic infections (9), and tumors (10–12).

Partially purified IDS has been shown to be a glycoprotein, mol wt 80,000, with an isoelectric point between 2.5 and 3.0 (1). In this study we will describe the purification of IDS to homogeneity and further characterization of the properties of the pure product.

Materials and Methods

Animals. Inbred male Lewis rats, 8–10 wk old, purchased from Microbiological Associates, Bethesda, Md., were used throughout.

Reagents. Con A, ovalbumin (OA), bovine serum albumin (BSA), soybean trypsin inhibitor (SBTI), RNAse, phytohemagglutinin-P (PHA-P), and trypsin were purchased from Difco Laboratories, Detroit, Mich., RPMI-1640 with penicillin and streptomycin from Associated Biomedic Systems, Inc., Buffalo, N. Y., Heat-inactivated fetal calf serum and fungizone from Grand Island Biological Co., Grand Island, N. Y., [^{3}H]thymidine, ^{14}C-amino acid mix, [^{3}H]-dansyl chloride, and Aquasol from New England Nuclear, Boston, Mass., Sephadex G 100 and Blue Dextran from Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.. Ampholines pH 2.5–4 from LKB, Rockville, Maryland, polyacrylamide, 2-mercaptoethanol, dithiothreitol (DTT), sodium dodecyl sulfate (SDS), bromphenol blue, trypan blue, Coomassie Blue, and amido black from Bio-Rad Laboratories, Richmond, Calif. and potassium metaper-

* Supported by the General Medical research service of the Veterans Administration.

[1] *Abbreviations used in this paper*: BSA, bovine serum albumin; Con A, concanavalin A; DTT, dithiotriol; FCS, fetal calf serum; IDS, inhibitor of DNA synthesis; IEF, isoelectric focusing; L cells, transformed mouse fibroblasts; LNC, lymph node cells; OA, ovalbumin; PAGE, polyacrylamide gel electrophoresis; SBTI, soybean trypsin inhibitor; SDS, sodium dodecyl sulfate.

 J. Exp. Med. © The Rockefeller University Press · 0022-1007/79/09/0622/11 $1.00
Volume 150 September 1979 622–632

iodate from Fisher Scientific Co., Pittsburgh, Pa. Tissue culture flasks used were from Falcon Labware, Div. of Becton, Dickinson, & Co. Oxnard, Calif., Microwell culture plates from Linbro Chemical Co., Hamden, Conn., the 110-ml water-jacketed electrofocusing column from LKB Instruments, Inc., Rockville, Md. and the semi-automatic multiple-well cell harvester was purchased from Otto Hiller Co., Madison, Wis.

Assay for IDS Activity. 5×10^5 rat lymph node cells (LNC) are cultured in each well of microwell culture plates containing a total volume of 0.2 ml/well consisting of 0.1 ml of supernates to be tested for IDS activity (see preparation of supernates), and 0.1 ml of RPMI-1640 with 2 μl/ml PHA and 10% fetal calf serum (FCS) (final concentration). After incubation for 44 h at 37°C and 5% CO_2, 1 μCi of [^{3}H]thymidine (6.7 μCi/mM) is added to each well. After a further 4 h, cells from each well are harvested onto disks of filter paper using the semi-automatic multiple-well cell harvester and trichloroacetic acid-precipitable ^{3}H counts dissolved in Aquasol are determined in an Intertechnique liquid scintillation counter (IN/US Corp., Fairfield, N. J.). All results are expressed as an average of triplicate values. Standard errors of the mean are calculated from these values. The percentage of inhibition of DNA synthesis is calculated using background counts (no PHA) as 100% inhibition and PHA-stimulated (no supernates added) 0% inhibition.

Purification of IDS

STEP 1, PREPARATION OF SUPERNATES. Control and active IDS containing supernates were made as described previously (2). Briefly, single cell suspensions of rat LNC are obtained by harvesting rat inguinal, axillary, and cervical node. These are teased between rakes and filtered through sterile nylon mesh to remove extraneous tissue. The filtered cells are pelleted by centrifuging at 250 *g* for 10 min at 4°C in an Sorvall RC5 centrifuge (DuPont Instruments-Sorvall, DuPont Co., Newtown, Conn.) and washed twice in RPMI-1640 medium with penicillin and streptomycin, 5×10^6 viable LNC/ml (viability determined by trypan blue exclusion) are cultured in a total volume of 50 ml in plastic tissue culture flasks with added 10% FCS, and 2 μg/ml Con A in an incubator at 37°C with 5% CO_2. Control supernates have Con A added, at the same concentration, just before harvesting supernates. After 48 h the cells are harvested by centrifugation and the supernates are discarded. The cells are washed three times and recultured in plain RPMI-1640 with penicillin and streptomycin. Subsequent 24- and 48-h supernates are collected by centrifugation, sterile filtered, pooled, and stored at −20°C till further use.

STEP 2, CONCENTRATION OF SUPERNATE. Active and control supernates (starting vol, 3,000 ml) are concentrated ≅35-fold at 4°C, in an Amicon Diaflo concentrator using a PM 10 membrane (Amicon Corp. Scientific Sys. Div., Lexington, Mass.).

STEP 3, ISOELECTRIC FOCUSING. Isoelectric focusing at 4°C was performed on the concentrated supernates (80-ml vol) according to the method of Olof Vesterberg (13), in a 110-ml electrofocusing column, using a sucrose gradient with a 1% concentration of ampholines, pH 2.5–4. Isoelectric focusing was carried out for 18–24 h at constant power of 1.5 W and a maximum voltage of 1,600 V using an LKB constant power source. Fractions of volume 2.5–3.0 ml were collected and the pH of each fraction measured. The fractions are individually dialysed against RPMI-1640 to remove Ampholines and assayed for inhibitory activity.

STEP 4, GEL CHROMATOGRAPHY. Fractions from isoelectric focusing containing IDS activity are pooled and chromatographed on Sephadex G 100 (2.5 × 100-cm columns) equilibrated with 0.5 M NaCl, 0.01 M phosphate buffer at pH 7.4. 2- to 3-ml fractions were collected every 15 min, dialyzed overnight, and assayed for IDS activity. Standard proteins used to calibrate the column were blue dextran, BSA, OA, and SBTI.

Polyacrylamide Gel Electrophoresis. Disk gel electrophoreses were run according to the method of Davis (14). 7.5% polyacrylamide gels (100 mm) with a 3-mm stacking gel were used. 100 liters of active or control samples were loaded and electrophoresis was carried out in an upper Tris-glycine buffer pH 8.9 and lower Tris-HCl buffer pH 8.1. Bromphenol blue (tracking dye) was not used in the sample containing gels. At the end of the run, the gels were sliced longitudinally and one-half were stained routinely for protein with a mixture of Coomassie Blue and amido black.

The remaining gel half was immediately sliced into 3-mm widths and each slice macerated separately in 0.5 ml RPMI-1640. Elution was carried out on a rotating platform at 4°C, then

centrifuged to remove remaining gel pieces and the supernate dialyzed extensively to remove any unpolymerized polyacrylamide. The dialyzed samples were sterilized using individual Millipore (Millipore Corp., Bedford, Mass.) filters and assayed for activity.

SDS disk gels were run according to the method of Laemimli and Favre (15). A 0.1% concentration of SDS was present in the 7.5% gels and in the Tris-glycine buffer, pH 8.0, bromphenol blue was used as the tracking dye. Samples for application, of total 200-μl vol were prepared to contain 1% SDS, 5% 2-mercaptoethanol, DTT, and glycerol in 0.5 M Tris-HCl, pH 6.5. These were boiled for 4 min at 100°C and further SDS is added to a concentration of 4% and then boiled again at 100°C for 4 min. Electrophoresis was carried out at constant voltage of 120 V. Standards BSA, OA, and SBTI were treated in an identical manner.

Dansylation of IDS. Dansylation of active and control samples were carried out according to the method of Gray (16). Briefly, 200 μl of the sample is lyophilized and dissolved in 15 μl of 0.2 M $NaHCO_3$. After lyophilizing again and reconstituting with 190 μl of deionized water, 10 μl of [^{3}H]dansyl chloride (sp act 10–20 Ci/m mol) was added and incubated at 4°C for 4 h. The product is then diluted to 1 ml with deionized water and dialyzed for 24 h against distilled water. The sample is lyophilized again, reconstituted to 100 μl, and loaded onto disk gels. Electrophoresis was performed as described earlier.

At the completion of electrophoresis, the gels are sliced into 3-mm widths and prepared for counting tritium uptake in individual slices as follows: gel slices are placed in 22-ml screw cap glass vials (Kimble Products Div., Owens-Illinois, Inc. Toledo, Ohio), 0.2 ml of 60% perchloric acid and 0.4 ml 30% H_2O_2 are added, and the vials capped tightly. These are then placed in a shaking water bath and heated at 60°C till the gel dissolves. After cooling, 10 ml of Aquasol is added and samples are counted in a liquid scintillation counter.

Protein Assay. Assays for protein in samples were done using a commercially available kit from Bio-Rad Laboratories. Standard calibration proteins are available in the kit.

Tests for Cytotoxicity

VIABILITY. Samples of IDS and the control were incubated at different concentrations with PHA-stimulated and nonstimulated LNC in RPMI-1640, and the viability was determined at 0 and 16 h using trypan blue exclusion.

^{14}C-AMINO ACID UPTAKE. IDS or the control was cultured in the usual assay system for 16 h. 2.5 μCi/well of ^{14}C-amino acid mix is then added for a further 8 h. Cells are harvested and ^{14}C incorporation is determined.

(a) Digestion with Trypsin, RNAse, and DNAse. Trypsin digestion was carried out by incubating the sample for 3 h at 37°C with 5 μg/ml trypsin (final concentration). The reaction was stopped by adding SBTI 20 μg/ml (final concentration). Nuclease treatments were performed by adding RNAse (heat treated) and DNAase 1 and DNAse 2, 5 μg/ml (final concentration) for 3 h. at 37°C.

(b) Treatment with Periodate. Samples dialyzed overnight against acetate buffer (0.2 M pH 5.0) were treated with potassium metaperiodate to a final concentration of 5 mM. After incubation for 4 h in the dark, sucrose, to a final concentration of 5%, was added for further 30 min and the sample was extensively dialyzed before assay.

Results

Isoelectric Focusing. Fig. 1 depicts the results of electrofocusing carried out on Con A-stimulated crude concentrated supernate. A single peak of almost complete inhibitory activity is seen in the fraction with a pH of 2.66. The adjacent fraction of pH 2.80 shows partial but nevertheless significant inhibitory activity suggesting the isoelectric point for IDS to be in between these values, i.e., ≅2.73. Control supernate, where Con A was added just before harvest, showed no inhibitory activity. In other experiments using Ampholines of pH 4.0–10, IDS activity was not detected above pH 4.0.

Sephadex Gel Fractionation. Sephadex G 100 chromatography of active pooled

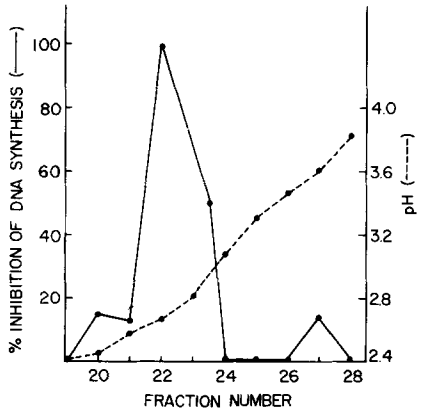

FIG. 1. Isoelectric focusing of crude, concentrated Con A-stimulated supernate. Percentage of DNA synthesis is calculated as in materials and methods. Standard errors of triplicate values <10% of mean.

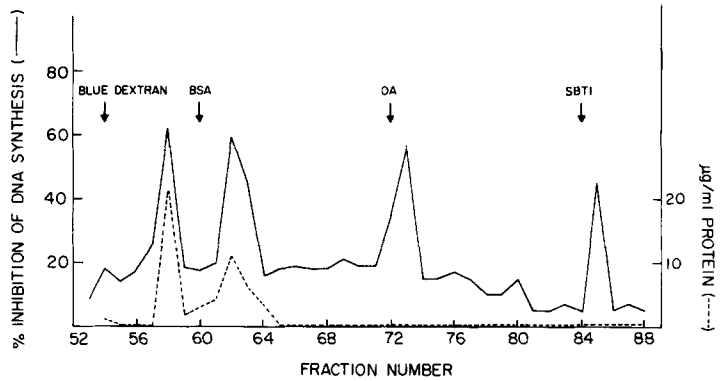

FIG. 2. Sephadex G 100 gel chromatography of active fractions from IEF. Standard errors of triplicate values <5% of the mean.

fractions from above were carried out using a buffer containing high salt concentration (0.05 M) Fig. 2 shows 4 distinct peaks of activity, corresponding to approximate mol wt of 80,000, 60,000, 40,000, and 20,000, respectively.

Purification of IDS. This is shown in Table I. Maximum inhibitory activity is seen with crude concentrate and fractions from IEF. Because >100% activity is obviously not possible, active supernates were diluted until ≅50% inhibition was obtained with each sample. Protein measurements made on these showed that, with activity remaining the same, a >238-fold purification was produced in terms of protein quantity.

Our protein assay is unable to measure protein amounts of <10 µg with consistent accuracy. Although there is a loss of total activity after Sephadex chromatography, these fractions are significantly more biologically active per milligram of protein.

Polyacrylamide Gel Electrophoresis. Purity of samples was analyzed at each stage by polyacrylamide gel electrophoresis (PAGE). Fig. 3 shows gel electrophoresis of IEF-fractionated active samples. Because molecules of similar charge have been resolved by isoelectric focusing, separation on these gels occurs on the basis of molecular size. At least four distinctly stained protein bands are seen with the major band slightly

TABLE I
Purification of IDS

Stage of purification	Percentage of inhibition of DNA synthesis (total)	Total protein concentration	Protein concentration with 50% inhibition	Fold purification
		mg/ml	*mg/ml*	
Crude concentrate	99.3	5.68	2.38	
Isoelectric focusing	98.7	0.43	0.22	× 11
Sephadex gel fractionation.				
Peak I	64	0.024	0.019	× 125
Peak II	60	0.012	0.012	× 198
Peak III	58	<0.010	<0.010	>× 238
Peak IV	44	<0.010	<0.010	>× 238

Samples diluted until they produce an ≅50% inhibition of DNA synthesis.

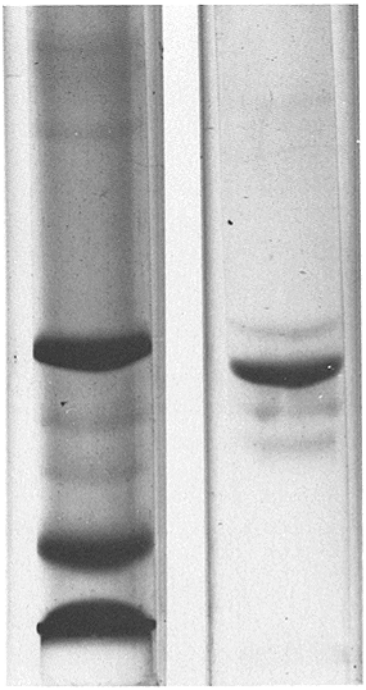

FIG. 3. PAGE of active fractions from IEF stained with Coomassie-amido black. 7.5% gels are used. Standard protein markers on the left are BSA, OA, SBTI, and chymotrypsinogen.

smaller than BSA, ie, ≅60,000 mol wt. However, elution of material from corresponding unstained gel slices shows the activity to be present only in the gel slices which corresponds to the stained band of mol wt ≅80,000 (Fig. 4). PAGE of the four active samples from Sephadex chromatography previously, dialyzed against RPMI-1640, on staining shows single protein bands. The R_F value for the stained protein bands were identical and each corresponded to a mol wt of 80,000 when compared with simul-

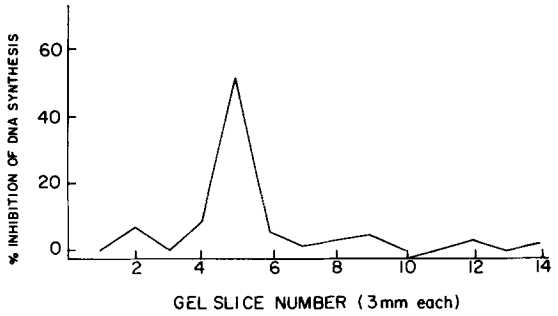

FIG. 4. Activity eluted from 3-mm gel slices of active IEF fractions on PAGE. Slices 5, 6, 8, and 10 correspond to mol wt 80,000 60,000, 40,000, and 20,000, respectively. Standard errors of triplicate values were <10% of the mean.

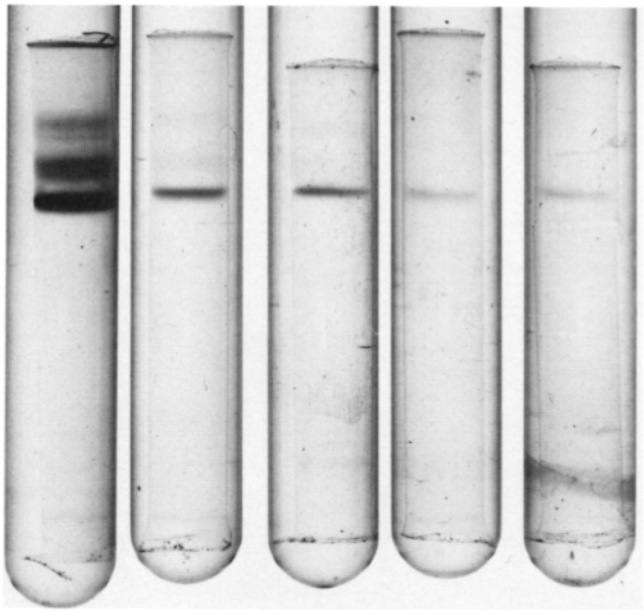

FIG. 5. PAGE of active peaks from Sephadex chromatography, stained with Coomassie-amido black. Gels in order from the left are standard (BSA) and peaks 1–4, respectively. The R_F values for the four stained protein bands were identical.

taneously run standards. (Fig. 5). Elution of material from gel slices has activity only in slices that correspond to the protein bands (data not shown). However, SDS gel electrophoresis (samples prepared with vigorous treatment with SDS, DTT, and 2-mercaptoethanol) show a single protein bands of mol wt ≅20,000 (Fig. 6) in each of the four active peaks. Treatment with SDS destroys IDS activity and hence, elution experiments could not be performed on SDS gels.

Dansylation of IDS. Attempts were made to dansylate IDS as another means of accurately estimating the molecular weight of purified IDS monomer and of identifying any other nonstaining contaminating proteins which may be present in the purified IDS preparation. Fig. 7 shows [^{3}H]dansyl chloride-treated purified IDS,

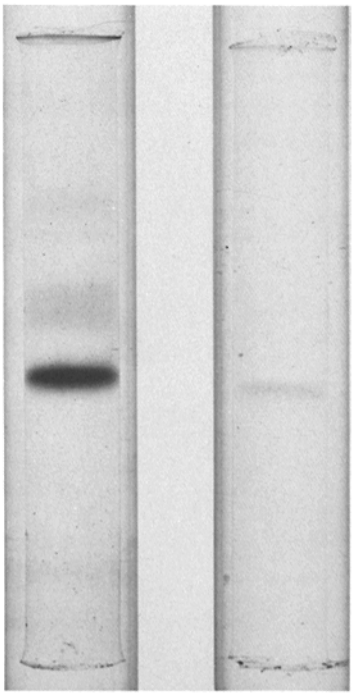

FIG. 6. SDS gel electrophoresis of peak 1 from Sephadex chromatography stained as before. Standard on left is SBTI. Peaks 2–4 showed the same band.

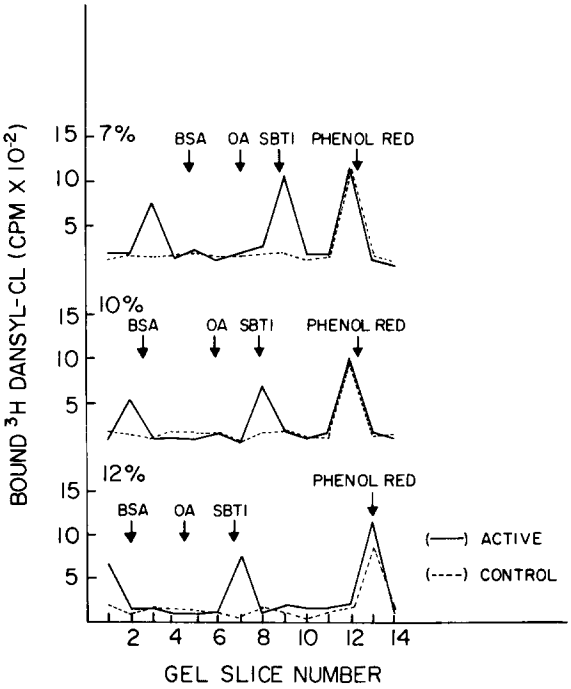

FIG. 7. PAGE on sephadex-fractionated sample. [^{3}H]dansyl chloride-treated IDS on polyacrylamide gels of three concentrations, 7.5, 10, and 12%. Gels sliced into 3-mm widths. Tritium counts in individual gel slices determined after dissolving gels. Standard error of duplicate values <5% of the mean.

TABLE II
Test for Cytotoxicity of IDS

Supernate added		Viability*		^{14}C-amino acid mix uptake	
		0 h	16 h	24 h‡	(SE)
		%			
Control		95	92	17,662	(1491)
IDS	50%	95	91	22,256	(449)
	25%	95	90	17,848	(206)
	12.5%	95	92	20,033	(346)

* Trypan blue exclusion.
‡ ^{14}C-amino acid mix added for final 8 h of culture.

TABLE III
Heat and Enzyme Treatment of IDS

Treatment of IDS	Inhibition of DNA synthesis
	% of control
None	92
Heat-60°C-30 min	86
80°C-30 min	24
RNAse	82
DNAse 1 & 2	87
Trypsin	21
Periodate	12

electrophoresed on gels of different polyacrylamide concentrations. Only two distinct peaks of [^{3}H]dansyl bound protein are seen, one corresponding to a mol wt of 80,000 and another one of 20,000 (Fig. 7), ie, the tetrameric and monomeric forms of IDS, respectively. These comigrate as the gel concentration increases along with the standards (Fig. 7) again confirming molecular weight. No other dansyl-bound protein peaks are seen suggesting purity. Dansylation of IDS also destroys its activity.

Cytotoxicity Tests. IDS has no capacity to kill stimulated or unstimulated LNC as determined by ability to exclude trypan blue dye and by estimation of ^{14}C-amino acid uptake into newly formed proteins (Table II). IDS is similarly not toxic to normal human fibroblasts or to mouse L cells (data not shown).

Heat and Enzyme Treatment. IDS is resistant to heating at 60°C for 30 min but loses partial activity at 80°C (Table III). Inhibitory activity of IDS was destroyed by trypsin and periodate but not by RNAse or DNAse (Table III).

Discussion

Purification of lymphocyte mediators has been technically difficult for many years because a given factor, although being biologically very active, is present in extremely low concentrations in active supernates. A compounding problem is the multiplicity of factors released into such supernates by very diverse cell populations used in preparing these supernatants.

Fortunately, the kinetics of IDS release help eliminate some of the above problems. IDS is secreted by lymphocytes into supernates during the second phase of lymphocyte activation (17), ie, that associated with DNA synthesis, and is therefore present in supernates after 48 h of stimulation. Discarding supernates from the first 48 h of

stimulation eliminated a plethora of factors that would interfere with purification. Again, the ability to use only unattached cells to produce IDS after 48 h removes certain populations of cells, eg, plastic adherent cells, ie, macrophages which may secrete factors. Finally, because IDS can be made adequately in cultures in the absence of serum, a further complication is avoided, because serum interferes in purification. We attempted to remove a further hindrance ie, that of low concentration, by starting out with large volumes of IDS-containing supernate.

In spite of using large quantities of starting materials eg, 3 liters, total protein in concentrated supernates was still low 5–6 mgs, whereas complete purification resulted in amounts of protein <10 μg in some fractions. To express the degree of purity of the final product in terms of protein concentration we determined an arbitrary level of inhibitory activity ie, 50% to be the standard (because $>100\%$ inhibition is obviously impossible) and then the protein concentrations at each step of purification, that produced 50% inhibition of DNA synthesis, were compared. A >238-fold purification was obtained in the final stage.

Isoelectric focusing confirmed previous findings that the pI of IDS is between 2.5 and 3.0 (1) and can now be estimated to be in the range of 2.7. However, only one peak of activity was found in contrast to earlier reports where two peaks of activity were defined in the pH range 2.5–4 (1). The extremely low pI of IDS would suggest a highly charged acidic macromolecule which is easily aggregated (18). This led us to use a high salt buffer in Sephadex gel chromatography, which would aid in disaggregation (19). Four peaks of activity obtained corresponded to mol. wt. 20,000, 40,000, 60,000, and 80,000, suggesting the monomer of IDS to be 20,000 mol wt but existing usually in the tetrameric 80,000 mol wt form. Rapid reaggregation was again seen when taken out of high salt buffer, dialyzed against medium (to determine activity) and run on polyacrylamide gels. However, SDS gel electrophoresis reduced the aggregated forms to single protein bands of mol wt 20,000 with no other contaminating protein bands visible. Activity in this band, however, cannot be determined because SDS treatment inactivated IDS.

Dansylation of the protein was done to confirm the above findings. Again, using disk gels of three different concentrations, [^{3}H]dansyl chloride incorporation into protein occurred in two peaks, one corresponding to the tetrameric form and the other to the monomeric forms, respectively. Complete breakdown of the tetrameric form did not occur, however, with dansylation. The protein peaks comigrated in the higher concentration gels along with standards confirming molecular weights. No other protein peaks were present again confirming purity. IDS activity is also destroyed by dansylation. We have confirmed homogeneity of IDS using the following three criteria: (*a*) A single staining protein band on PAGE using two different gel concentrations (7.5% and 10%) and performing electrophoresis in buffers of different pH (8.9 and 6.8); (data not shown). (*b*) A single staining protein band on SDS gel electrophoresis, and (*c*) Protein peaks corresponding only to the tetrameric and monomeric forms of IDS when purified product is reacted with [^{3}H]dansyl chloride (dansyl chloride reacts with free amino groups of proteins). No contaminating protein peaks are seen when pure IDS is reacted with dansyl chloride.

Purified IDS activity is not susceptible to RNAse or DNAse but is completely destroyed by trypsin or periodate, the latter reconfirming its glycoprotein nature and

that activity is dependent on the carbohydrate moiety. It is of course possible that periodate inactivation might occur from protein modification. Again, it is heat stable at 60°C and at 80°C it is partially inactivated. It is not cytotoxic to lymphocytes, normal human, or transformed mouse fibroblasts (L cells).

An unexpected observation was that the smaller molecular forms, especially the monomer was much more active biologically per milligram of protein although IDS appears to exist mainly in its tetrameric form. However the preponderance of the tetrameric form in culture may be an artifact of laboratory conditions whereas in vivo, the very active monomer may predominate. The loss of total activity in each peak in the Sephadex fractionation was probably a result of the dilution factor in the column. Combination of the peaks showed no loss of activity when compared with that of samples from isoelectric focusing (IEF).

We have therefore purified a lymphokine, IDS using standard biochemical techniques. A big factor in aiding our process of purification was the ability to use large quantities of serum-free starting material. IDS has been previously shown to be different from other suppressive lymphokines and lymphotoxin (20) and to be secreted by a specific subpopulation of suppressor T cells (6, 7). A number of inhibitory lymphokines have now been described in a partially purified state, most of them being glycoprotein in nature and many molecular weights ranging from 500 to 100,000 (17). IDS monomer certainly falls within this range. It differs from any other such factors however in being a very highly charged molecule, which accounts for its rapid reaggregation into the commonly detected tetrameric form.

Summary

Inhibitor of DNA synthesis (IDS) is a T-lymphocyte factor, whose role in immunoregulation might be to nonspecifically suppress the immune system especially in situations where very high, prolonged tolerogenic doses of antigens are present.

We have purified IDS-contained supernates of stimulated lymphocytes to homogeneity, through isoelectric focusing and Sephadex gel chromatography. IDS has an isoelectric point of 2.73–2.75 and in its monomeric form has a mol wt of 20,000 but exists in the supernate usually as an aggregated tetrameric form. Di- and trimeric forms are also seen. All forms are biologically active. Purity was confirmed by SDS gel electrophoresis and the binding of dansyl chloride to terminal or free amino groups of proteins and peptides.

We have, further confirmed that pure IDS is not cytotoxic and is probably a glycoprotein whose activity depends on an intact carbohydrate moiety.

Received for publication 6 June 1979.

References

1. Namba, Y., and B. H. Waksman. 1975. Regulatory substances produced by lymphocytes. Inhibitor of DNA synthesis in the rat. *Inflammation.* **1**:5.
2. Namba, Y., B. V. Jegasothy, and B. H. Waksman. 1977. Regulatory substances produced by lymphocytes. V. Production of inhibitor of DNA synthesis (IDS) by proliferating T lymphocytes. *J. Immunol.* **118**:1379.
3. Wagshal, A. B., B. V. Jegasothy, and B. H. Waksman. 1978. Regulatory substances

produced. V1. Cell cycle specificity of Inhibitor of DNA synthesis action in L cells. *J. Exp. Med.* **147:**171.

4. Jegasothy, B. V., A. R. Pachner., and B. H. Waksman. 1976. Cytokine inhibition of DNA synthesis: effect on cyclic adenosine monophosphate in lymphocytes. *Science (Wash. D.C.).* **193:**1260.
5. Jegasothy, B. V., Y. Namba, and B. H. Waksman. 1978. Regulatory substances produced by lymphocytes. V11. IDS (Inhibitor of DNA synthesis) inhibits stimulated lymphocyte proliferation by activation of membrane adenylate cyclase at a restriction point in late G_1. *Immunochemistry.* **15:**551.
6. Gershon, R. K. 1973. T cell control of antibody production. *Contemp. Top. Immunobiol.* **3:**1.
7. Pierce, C. W., and J. A. Kapp. 1976. Regulation of immune responses by suppressor T cells. *Contemp. Top. Immunobiol.* **5:**91.
8. Liacopoulos, P., and S. Ben-Efraim. 1975. Antigenic competition. *Prog. Allergy.* **18:**97.
9. Jayawardene, A. N., and B. H. Waksman. 1977. Suppressor cells in experimental Trypanosomiasis. *Nature (Lond.).* **265:**539.
10. Kilburn, D. G., J. B. Smith, and T. M. Gorozunski. 1974. Nonspecific suprression of lymphocyte responses in mice carrying progressively growing tumors. *Eur. J. Immunol.* **4:** 784.
11. Thomas, D. W., W. K. Roberts, and D. W. Talmadge. 1975. Regulation of the immune response. Production of a soluble suppressor by immune spleen cells in vitro. *J. Immunol.* **114:**1616.
12. Thomas, D. W., D. W. Talmadge, and W. K. Roberts. 1975. Inhibitor of Tumor cell proliferation. A second role for suppressor cells. *J. Immunol.* **115:**1366.
13. Vesterberg, O. 1971. Isoelectric focusing of proteins. *Methods Enzymol.* **22:**389.
14. Davis, B. J. 1964. Disc Electrophoresis - 11. Method and application to human serum proteins. *Ann. N.Y. Acad. Sci.* **112:**404.
15. Laemmli, U. K., and M. Favre. 1973. Maturation of the Head of Bacteriophage T_4 1. DNA packaging events. *J. Mol. Biol.* **80:**575.
16. Gray, W. R. 1972. End group analysis using dansyl chloride. *Methods Enzymol.* **25:**126.
17. Waksman, B. H. 1979. Modulation of immunity by soluble mediators. *J. Pharmacol. Exp. Therapeutics.* In press.
18. Lee, J. C., and S. N. Timasheff. 1977. In vitro reconstitution of calf brain microtubules; Effects of solution variables. *Biochemistry* **16:**1754.
19. Josephs, R., and W. F. Harrington. 1968. On the stability of myosin filaments. *Biochemistry.* **7:**2834.
20. Namba, Y., and B. H. Waksman. 1975. Regulatory substances produced by lymphocytes. 11. Lymphotoxin in the rat. *J. Immunol.* **115:**1018.