Clin Exp Immunol 1993; 91:257–265

Identification and purification of a receptor on macrophages for the dengue virus-induced suppressor cytokine

R. MUKHERJEE, P. CHATURVEDI & U. C. CHATURVEDI *Postgraduate Department of Microbiology, K. G. Medical College, Lucknow, India*

(Accepted for publication 5 October 1992)

SUMMARY

Dengue type 2 virus (DV)-induced suppressor cytokine (SF) binds to macrophages to transmit the suppressor signal to recruit the second subpopulation of suppressor T cells. The present study was undertaken to identify and purify the receptor for SF (SF-R) on macrophages. The binding of ^{125}I-SF to macrophages was saturable and reversible. Scatchard analysis showed the presence of both high (54 000/cell) and low ($1{\cdot}78 \times 10^6$/cell) affinity receptor sites. The binding of ^{125}I-SF to macrophages was inhibited by pretreatment of macrophages with anti-SF antiserum but not by a heterologous antiserum. Normal mouse peritoneal macrophage membrane was solubilized with Triton-X-100 and the components separated by low pressure liquid chromatography (LPLC) to purify SF-R. The presence of SF binding moiety (SF-R) was screened at each step of purification. The purified SF-R resolved into two bands of 45–50 kD mol. wt on SDS–PAGE. ^{125}I-SF + SF-R complex run on SDS–PAGE showed a single band at about 55–60 kD mol. wt by autoradiography. Anti-SF-R antiserum reacted with SF-R in a Western blot test; the reaction was abolished by pretreatment of the blots with proteinase K, but not by pretreatment with periodic acid. SF-R was composed of two polypeptide chains (α and β) which were obtained in pure form by high performance liquid chromatography (HPLC) of dithiothreitol- and iodoacetamide-treated SF-R. Only the β chain bound SF.

Keywords dengue virus suppressor cytokine macrophages receptor specific binding

INTRODUCTION

Cascades of suppressor T cells consisting of sequential generations of two or more subpopulations of suppressor T cells have been described in several models using synthetic antigens. These cascades consist of suppressor T cells, their soluble products and macrophages, the latter being essential for transmission of the signal (reviewed in [1]). Recently, evidence has been presented for suppressor T cells being a distinctive cell type and being induced by suppressor determinants on the antigen [2]. Dengue type 2 virus (DV) induces a suppressor pathway in mice (reviewed in [3]) consisting of a subpopulation of suppressor T cells (Ts1) in the spleen [4], which produce a soluble suppressor cytokine (SF) [5]. The suppressor signal of SF is transmitted via syngeneic macrophages to recruit a second subpopulation of suppressor T cells (Ts2) which produce another soluble, prostaglandin-like, suppressor cytokine (SF2) [6–8]. SF2 recruits the third set of suppressor T cells (Ts3) which finally mediate antigen-specific suppression of humoral immune response [9,10]. SF is a low molecular weight, heat-labile, acid and alkaline pH-labile, highly potent protein and acts in a dose-dependent manner (reviewed in [3,11]).

Correspondence: Professor U. C. Chaturvedi, Head of the Postgraduate Department of Microbiology, K. G. Medical College, Lucknow -226 003, India.

The presence of live macrophages is obligatory for the transmission of the suppressor signal by SF between Ts1 and Ts2 both *in vivo* and *in vitro* [6,8]. Further, SF adsorbed on macrophages remains at the surface and can be retrieved completely by DV-stimulated spleen cells kept in contact with them [12]. The present study was undertaken to identify and purify the specific receptor for SF (SF-R) on macrophages.

MATERIALS AND METHODS

Animals

Inbred adult Swiss albino mice, aged 3–4 months, obtained from the colony maintained in this Department were used.

Virus

Dengue type 2 virus (DV), strain P23085, was used in the form of infected mouse brain suspension. The infectivity of the virus was titrated by intracerebral (i.c.) inoculation in groups of mice as described [13].

Preparation and purification of SF

DV-induced SF was prepared by the technique described previously [5]. Briefly, mice were inoculated intracerebrally with 1000 LD_{50} of DV. Spleens of infected mice were harvested

8–11 days after inoculation and were homogenized in chilled PBS pH 7·2. The homogenate was dialysed (cut off value of membrane was 12 kD) against distilled water for 24 h with constant shaking at 4°C. The dialysed preparation of SF was vacuum dried in a Speed Vac (Savant Instruments Inc., NY) and stored at −70°C. SF was purified by LKB Pharmacia high performance liquid chromatography (HPLC) using reversed phase super pac columns packed with silica gel C18. Elution was performed with 20% methanol in water at a flow rate of 0·5 ml/min and the elution fluid was monitored at 206 nm. The chromatographic peaks were collected, vacuum dried, protein content estimated [14] and stored at −70°C.

Radiolabelling of SF

Radioiodination of SF was carried out using lactoperoxidase as a catalyst [15,16]. SF (10 mg) was added to 1 mCi radiolabelled iodine (^{125}I) (Amersham, UK) in the presence of 20 mM Tris-HCl buffer pH 7·4, 50 μg lactoperoxidase and 0·1 mM cold sodium iodide solution. The reaction was accelerated by addition of 0·8 mM H_2O_2 in five aliquots of 15 μl each and terminated by addition of β-mercaptoethanol (final concentration of 10 mM). Radioiodinated SF was dialysed against 2 mM sodium phosphate buffer pH 9. Radioactivity was measured in a LKB-mini gamma counter. The biological activity of the radiolabelled SF was not diminished when assessed for the suppressor activity. Purity and molecular weight determination of the labelled SF was performed by SDS–PAGE using 12·5% slab gels [17] followed by autoradiography. The specific activity was calculated to be 9213 ct/min per nmole.

Preparation of peritoneal macrophages

Heparinized Eagle's minimum essential medium (MEM) containing 10% fetal calf serum (FCS) was inoculated into mice intraperitoneally in doses of 5 ml and the peritoneal lavage was aspirated onto a 5-cm glass Petri dish. After 2 h of incubation at 37°C in the presence of 5% CO_2-air, the glass-non-adherent cells were washed off and the glass-adherent macrophages were gently scraped off with a rubber policeman. The cells were suspended in normal saline and viable cells were counted using exclusion of trypan blue dye.

Binding assay

Macrophages (1×10^6) were suspended in 500 μl of MEM with 10% FCS, pH 7·2. Various concentrations of ^{125}I-SF (0·005–20 nM/ml) were added to the cells in the presence or absence of a 100-fold excess of unlabelled SF to assess specific binding, with a final incubation volume of 1000 μl in 1·8 ml Nunc cryotubes (Nunc, Denmark). After incubation at 37°C for 1 h the cells were centrifuged and the supernatant was aspirated. Radioactivity of the cells was counted in a LKB-mini-gamma counter with an efficiency of *ca* 60%. Non-specific binding, determined in the presence of a 100-fold excess of unlabelled SF, was subtracted from the total binding to give specific binding. Data from the binding experiments were subjected to Scatchard analysis.

Detection of SF-R

The protocol for detection of SF-R was based on the principle of allowing SF to bind to the putative receptor at 37°C for 1 h. The preparation was dialysed against an equal volume of PBS at 4°C overnight so that the unbound SF flowed out of the dialysis bag. The suppressor activity of the dialysed and non-dialysable portions was assayed by inhibition of DV-specific IgM antibody plaque forming cells (PFC) in the spleen of mice [7]. The presence of suppressor activity in the non-dialysable portion was considered to indicate binding of SF, thereby showing presence of SF-R.

Study of the structure of SF-R

The purified SF-R protein (5 μg in 0·5 ml) was incubated with equal volume of 5 mM dithiothreitol (DTT; Sigma Chemical Co., St Louis, MO) in the presence of 0·15 M Tris-HCl buffer pH 8·0 for 45 min at room temperature. To this, 1 ml of 20 mM iodoacetamide (Sigma) was added and incubated for 20 min at room temperature. The preparation was analysed by HPLC as described above.

Preparation of antisera

Antisera against SF-R were raised in mice by injecting 5 μg of purified SF-R emulsified in Freund's complete adjuvant (FCA; Sigma) intraperitoneally at the day 0, followed by a second dose of 5 μg SF-R in Freund's incomplete adjuvant (FIA) intraperitoneally 15 days later. Blood was collected on day 30 and the sera separated were pooled and stored at −20°C. A similar protocol was used to prepare anti-SF antisera (SFAs) and anti-helper cytokine-antisera (HFAs) [18]. The HFAs were used as irrelevant antisera to study the specificity of the reaction with SFAs.

PAGE and autoradiography

Electrophoresis was done on 12·5% PAGE or SDS–PAGE [17] at constant current of 30 mA in a vertical slab gel system (Pharmacia, Sweden). Gels were stained with silver nitrate [19] or with coomassie brilliant blue R250 stain (Sigma). Some of the gels were run along with molecular weight markers [20]. For autoradiography the gels were vacuum dried (LKB slab gel dryer) and exposed to Kodak x-ray film. The films were developed after exposure for 15 days at −70°C.

Immunoblotting

Samples were resolved on 12·5% SDS–PAGE and electroblotted (Novablot Electrophoretic Transfer Kit, Pharmacia) onto nitrocellulose paper. The blot was blocked overnight with blocking buffer containing 3% bovine serum albumin (BSA) in 10 mM Tris-HCl, pH 7·4, followed by extensive washing with PBS containing 0·05% Tween-20. The blots were incubated for 1 h with the anti-SF-R antisera at room temperature and after three washings incubated with anti-mouse IgG horseradish peroxidase conjugate for 1 h at room temperature. After extensive washing, blots were developed using diaminobenzidine and hydrogen peroxide [21]. For control, the blots were treated with irrelevant antisera like anti-DV-antisera [22] and HFAs. All chemicals and reagents used were purchased from Sigma.

An effort was made to understand the chemical nature of the antigen reactive in immunoblots. Before treatment with the specific antibody, the blots were treated with proteinase K (5 μg/ml) for 1 h at 37°C or periodic acid (100 nM) in sodium acetate buffer 50 nM, pH 4·6, followed by submersion in 1 M glycine in PBS for 30 min in the dark [23]. A comparison with untreated blot indicates the chemical nature of the antigen.

Statistical analysis

Student's *t*-test was used for statistical analysis of the data. A P value of less than 0·05 was considered significant.

RESULTS

The preparation containing ^{125}I-SF was pooled and subsequently tested for purity by SDS–PAGE. The autoradiograph of the pooled preparations presented in Fig. 1 shows presence of a single band with an approximate mol. wt of 10 kD corresponding to that of SF.

Saturation of binding

The saturability of binding of ^{125}I-SF to macrophages was examined. The data presented in Fig. 2a show that the binding of SF increased in proportion to the amount of added labelled material and the specific binding was saturated with 15 nM ^{125}I-SF. Scatchard analysis of the data was performed [24]. High affinity receptors were detected on macrophages for SF when binding assays were performed with low concentrations (0·005–0·05 nM) of ^{125}I-SF while low affinity receptors were detected with higher concentrations (0·5–20 nM). The high affinity receptors showed an equilibrium dissociation constant (KD) of 0·0067 nM and the number of receptor sites was calculated as 54 000/cell (Fig. 2b). The low affinity receptors showed a larger number of receptor sites, $1{\cdot}78 \times 10^6$/cell, and a KD of 8·47 nM (Fig. 2c). Conventional linear regression techniques were used to calculate the line of best fit in the Scatchard plots (Fig. 2b, c).

Effect of anti-SF antisera on the binding of ^{125}I-SF to macrophages

The specificity of the binding of the radiolabelled SF to macrophages was investigated using SFAs. For control a heterologous antiserum (HFAs) was used. It was observed (Fig. 3) that SFAs blocked the binding of ^{125}I-SF to macrophages (74% inhibition) while HFAs had no significant effect (5% inhibition).

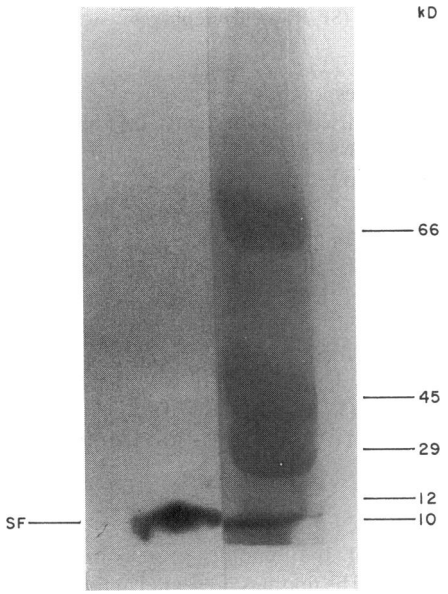

Fig. 1. Autoradiograph of ^{125}I-suppressor cytokine (SF) analysed by SDS–PAGE. The molecular mass (kD) of protein standards run on a parallel gel are also indicated.

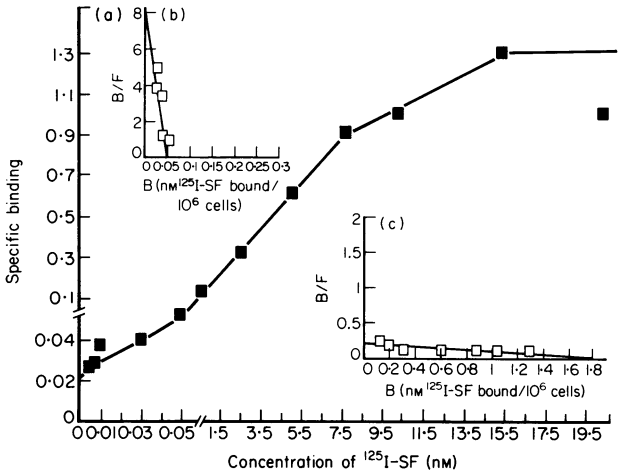

Fig. 2. Specific binding of ^{125}I-suppressor cytokine (SF) to normal mouse peritoneal macrophages. Macrophages (1×10^6) were incubated with various concentrations of ^{125}I-SF for 60 min at 37°C. Non-specific binding was measured in the presence of 100-fold excess of unlabelled SF. The results are expressed as the amount of specifically bound SF in nmoles/10^6 cells (a). Insets show Scatchard plot of the binding data from (a) showing high affinity (b) and low affinity (c) receptors. Each point was calculated by determining the moles of specifically bound (B) and free (F) SF and plotting them as B/F *versus* B.

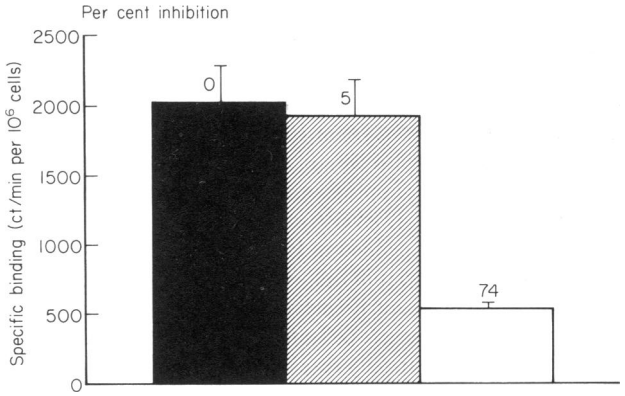

Fig. 3. Effect of anti-suppressor cytokine (SF) antisera (SFAs) on the binding of ^{125}I-SF to macrophages. Macrophages (1×10^6) were treated with 1:10 diluted SFAs or irrelevant antisera, the anti-HF antisera (HFAs) for 1 h at 37°C. Specific binding was calculated by incubating macrophages for 1 h at 37°C with 15 nM/ml ^{125}I-SF in the presence or absence of 100-fold excess of unlabelled SF. Data represent mean values from two experiments. ■, Control; ▨, anti-HFAs; □, anti-SFAs.

Effects of various concentrations of unlabelled SF on specific binding of ^{125}I-SF to macrophages

The results presented in Fig. 4 show that excess concentration of unlabelled SF was able to displace the bound ^{125}I-SF to macrophages in a dose-dependent manner.

Purification of SF-R from macrophages

Figure 5 presents the flow chart of the purification steps [25] of SF-R. Normal mouse macrophages were sonicated for 2 min. A

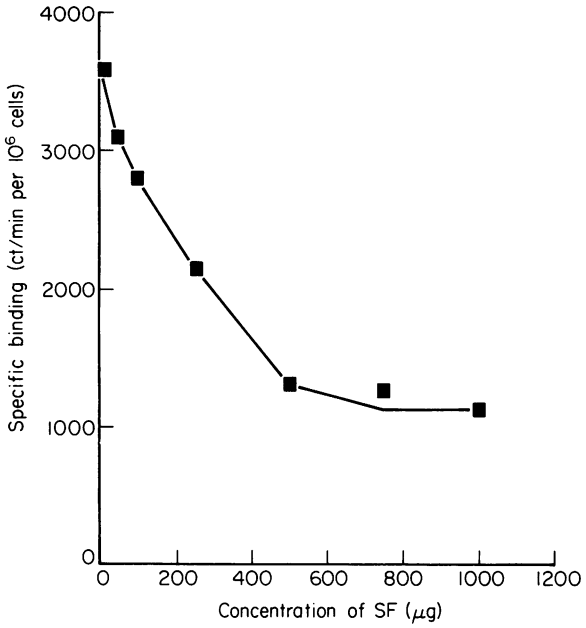

Fig. 4. Competition for the binding of ^{125}I-suppressor cytokine (SF) to macrophages by unlabelled SF. Macrophages (1×10^6) were incubated with various concentrations of unlabelled SF at 37°C for 1 h. The cells were washed and further incubated with 15 nM/ml of ^{125}I-SF for 1 h at 37°C and the radioactivity was counted.

drop of this preparation was examined under microscope to confirm disruption of all the cells. The preparation was centrifuged at 25 000 *g* for 90 min, the supernatant and pellet were separately collected (A in Fig. 5) in an aliquot and were assayed for binding with SF. It was observed that the pellet bound the SF (61% suppression; $P < 0{\cdot}001$) while the supernatant did not (Table 1, level A).

The pellet was suspended in PBS containing 1% Triton-X-100 and sonicated for 5 min followed by stirring in cold for 2 h. The preparation was centrifuged at 25 000 *g* for 30 min and the supernatant and pellet were collected separately (B in Fig. 5). An assay of the binding capacity revealed that the moiety present in the supernatant bound SF (60% suppression, $P < 0{\cdot}001$) while the pellet did not (Table 1, level B).

The supernatant was run on low pressure liquid chromatography (LPLC) using Sephacryl S-200 gel packed in 650×16 mm column with a bed height of 450 mm (Pharmacia). Elution was performed with PBS at 40 cm^3/h and the elution fluid was monitored at 280 nm. Two chromatographic peaks were obtained which were collected separately and freeze dried and stored at −70°C (C in Fig. 5). The findings presented in Table 1, level C, show that the protein eluted in peak 2 bound SF (59% suppression, $P < 0{\cdot}001$) while that in peak 1 did not bind. At every step of purification the original volume was made up and the protein contents were estimated. The data summarized in Table 2 show that in the final step of purification the specific binding increased 14-fold.

PAGE of SF-R

The protein collected from the peak 2 of LPLC was freeze dried and further investigated for purity and binding capacity. The protein run on native PAGE yielded a single band, while under reducing conditions on SDS-PAGE it resolved into two closely

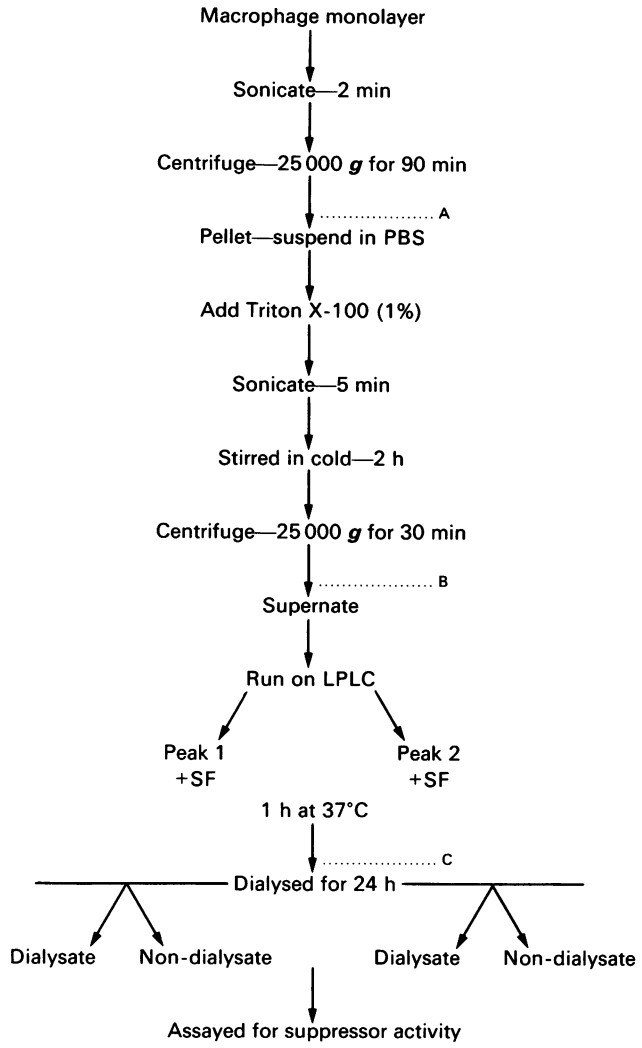

Fig. 5. Flow chart of the purification steps of the receptors for suppressor cytokine (SF-R) on macrophages.

placed bands corresponding to a position of 45–50 kD mol. wt (Fig. 6a). To identify the band which could bind SF, the gel was incubated with ^{125}I-SF at 37°C for 2 h, extensively washed, cut into slices and counted for radioactivity. The radioactivity was present mainly in the eighth fraction of the gel, ct/min being 1204 per slice of the gel. This fraction of the gel corresponded with the band at 45–50 kD position of the silver-stained gel.

The capacity of the purified peak 2 protein to bind SF was further investigated. The protein was mixed with ^{125}I-SF and incubated at 37°C for 2 h and was then run on the native PAGE followed by autoradiography. It showed a single band (Fig. 6b). A similar run on SDS-PAGE showed a single band at 55–60 kD position of the SF (10 kD mol. wt) + SF-R (45–50 kD) complex (Fig. 6c).

Immunoblotting

The specificity of the binding protein was confirmed by immunoblotting. The findings presented in Fig. 6d show that the anti-SF-R antisera reacted with both the bands resolved on SDS-PAGE. No reaction was observed with normal mouse sera or irrelevant antisera (HFAs and anti-DV antisera). Pretreat-

Table 1. Assay for the presence of suppressor cytokine receptor (SF-R) in different preparations during the course of purification

Groups	No. of PFC/2 × 10^6 cells	Per cent suppression
Level A		
Supernatant		
Non-dialysed	443 ± 36	11
Dialysed	204 ± 28	59
Pellet		
Non-dialysed	199 ± 27	60*
Dialysed	408 ± 19	18*
Level B		
Supernatant		
Non-dialysed	200 ± 37	60*
Dialysed	428 ± 47	15*
Pellet		
Non-dialysed	470 ± 38	6
Dialysed	277 ± 38	44
Level C		
Peak 1		
Non-dialysed	446 ± 36	11
Dialysed	229 ± 15	54
Peak 2		
Non-dialysed	217 ± 24	57*
Dialysed	462 ± 33	7*
SF alone	207 ± 34	59
Control (DV alone)	498 ± 22	0

To detect the presence of SF-R, each preparation at different levels of purification (as shown in Fig. 5) was treated with SF followed by dialysis to remove the unbound SF. Then the suppressor activity of the dialysed and non-dialysed fractions was assayed. Presence of suppressor activity in non-dialysed part indicated existence of SF-R (*$P < 0.001$). At each step the original volume was maintained. For the assay of suppressor activity mice were primed with 1000 LD_{50} of dengue type 2 virus (DV) intraperitoneally followed 48 h later with the i.v. inoculation of the test fraction. IgM plaque-forming cells (PFC) were counted in the spleen on the sixth day after DV inoculation and the findings were compared with those in control mice given only DV intraperitoneally after deducting the background value in normal mice. The suppression was calculated as follows:

$$\text{Suppression (\%)} = 100 - \frac{\text{PFC in test} - \text{background value}}{\text{PFC in control} - \text{background value}} \times 100$$

Each group consisted of six-to-eight mice and from each mouse spleen four-to-six smears were prepared. The mean value ± s.d. has been presented.

ment of the blots with proteinase K abolished the immunoblot reaction while treatment with periodic acid had no effect.

SF-R has two polypeptide chains

This experiment was done to discover the structure of SF-R protein molecule. The findings presented in Fig. 7 show that DTT- and iodoacetamide-treated SF-R protein resolved into two peaks, the first termed α and the second β chain. Both the chains were incubated separately with SF and then dialysed overnight. The dialysate and non-dialysate of both the chains were assayed for suppressor activity. The results presented in Table 3 show that the α chain of SF-R did not bind SF (14% suppression), while the β chain of SF-R bound SF (56% suppression).

Table 2. Protein concentration and scaling up of the binding capacity at different steps of purification

Purification steps	Samples	Amount of protein (μg/100 μl)	Increase in binding of SF
Starting material	Total sonicate	162·5	
Level			
A	Supernatant	75	—
	Pellet	83·3	1·95-fold
B	Supernatant	60·8	2·67-fold
	Pellet	28·3	—
C	Peak 1	10	—
	Peak 2	11·6	14-fold

At different steps of purification of suppressor cytokine receptor (SF-R) (see Fig. 5) protein contents of different preparations were estimated, keeping volumes of all the preparations exactly the same as the starting material. Increase in the binding capacity was calculated in terms of reduction in the amount of protein required for the similar per cent of suppressor activity.

DISCUSSION

The results of the experiments reported here establish the presence of a receptor, specific for DV-induced SF on mouse peritoneal macrophages. It has been observed that macrophages are obligatory for the transmission of suppressor signal by SF to Ts2 cells in DV-induced suppressor pathway [6,8]. A crucial role of macrophages in the transmission of suppressor [26,27] and helper [28] signal has been reported in other models also. The present study may indicate that uptake of SF by macrophages and subsequent presentation of the signal to the T lymphocytes is mediated by the presence of SF-specific cell surface receptors on macrophages.

The interaction between SF and the receptor was specific as it fulfilled all the criteria [29] of a specific peptide–receptor interaction. It was observed that the binding approached saturation at a concentration of 15 nM of labelled SF; the binding was displaced with excess unlabelled SF and was reversible. Further, the binding was pH-, time- and temperature-dependent (Mukherjee *et al.*, unpublished data). Scatchard analysis of the binding data revealed the presence of both high (54 000/cell) and low (1·78 × 10^6/cell) affinity receptor sites. This analysis indicated that interaction of SF with its receptors fits with a bimolecular association of the peptide and a population of non-interacting receptor sites. This is consistent with dissociation experiments which indicate simple first-order kinetics. That the binding was specific, was further supported by the finding of a marked inhibition of the binding by pretreatment with SFAs but not by a heterologous antisera (HFAs) and affinity cross-linking experiments (unpublished data). The find-

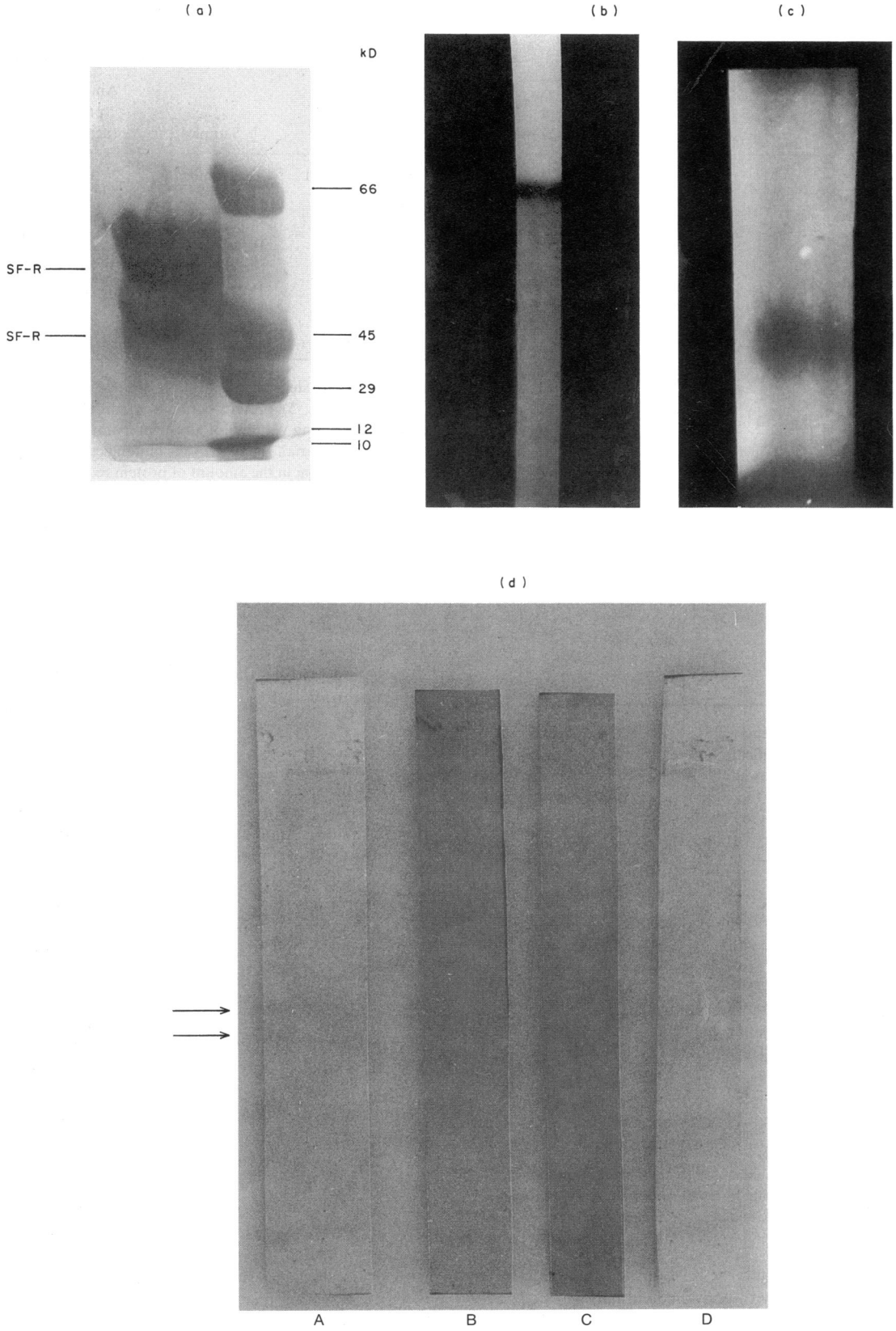

Fig. 6. PAGE and autoradiography of purified suppressor cytokine receptor (SF-R). (a) SDS–PAGE of purified SF-R along with the mol. wt marker proteins. Autoradiogram of ^{125}I-SF + SF-R complex on (b) native PAGE and (c) SDS–PAGE. (d) Western blot analysis was done by transfer of protein from SDS–PAGE. A, Treated with anti-SF-R antisera; B, anti-dengue type 2 virus (DV) antisera; C, proteinase K; D, periodic acid.

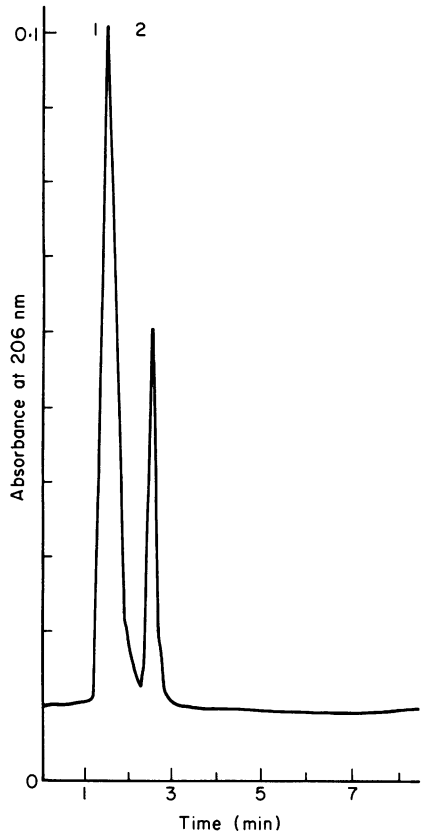

Fig. 7. High performance liquid chromatography (HPLC) elution profile of suppressor cytokine receptor (SF-R) treated with dithiothreitol (DTT) and iodoacetamide. Peak 1 was termed as α and peak 2 as β chain.

Table 3. Binding of suppressor cytokine (SF) to the polypeptide chains of SF-R

Groups	No. of PFC/2 × 10^6 cells	Per cent suppression
α-chain SF		
non-dialysed	465 ± 32	14
dialysed	222 ± 23	59
β-chain SF		
non-dialysed	241 ± 22	56
dialysed	481 ± 33	11
SF	228 ± 20	58
Control	542 ± 42	0

Purified SF-R was treated with dithiothreitol and then with iodoacetamide to cleave the S–S linkage between the two polypeptide chains. The preparation was resolved on high performance liquid chromatography (HPLC) and peak 1 (α chain) and peak 2 (β chain) as shown in Fig. 7 was assayed for the capacity to bind SF as described in Table 1. When SF binds to SF-R chain, it does not move out of the dialysis bag. All the details including the number of mice are as in Table 1. PFC, Plaque-forming cells.

ings, thus, present evidence of a specific receptor for SF on macrophages. In preliminary experiments some such receptor sites were found on T and B cells of mouse spleen (Mukherjee *et al.*, unpublished data). The purified SF-R had the capacity to bind SF, as shown by the presence of suppressor activity in SF + SF-R complex and demonstration of binding of ^{125}I-SF to SF-R by autoradiography of the complex. This is further supported by the findings of affinity cross-linking experiments (unpublished data).

Cytokines transmit the signal by binding to specific sites on cell membrane, the receptor molecules. The receptors distinguish and receive the specific signal and relay it in such a way that an appropriate cellular response follows. The binding of cytokines to the receptor may be of high, low or intermediate affinity. IL-2 has high, low and intermediate affinity receptors [30–35]. Both high and low affinity receptors have been reported for IL-5 [36,37] and IL-7 [38]. While the receptors for IL-3 are only of low affinity [39], those for GM-CSF and M-CSF [40] are only of high affinity.

Purified SF-R resolved as a single band on native PAGE but was split into two closely placed bands of 45–50 kD on SDS-PAGE. Both the bands reacted equally well in a Western blot test with anti-SF-R antisera. Pretreatment of the blot with proteinase K abolished the Western blot reaction, while treatment with periodic acid had no effect. This indicated that the antigenic site on SF-R was protein in nature.

The receptors for various cytokines have been shown to be composed of a single polypeptide chain, for example the receptors for G-CSF [41] and M-CSF [40]; while the receptors for IL-1 [42] and IL-8 [43] have two polypeptide chains. The receptor for IL-2 is composed of two non-covalently linked chains (α and β chains) forming high affinity binding sites; the β chain has no biological role except to cooperate with the α chain to make high affinity sites [44,45]. The SF-R was shown to be composed of two polypeptide chains (α and β) coupled by disulphide linkages and only the β chain was capable of binding SF, as shown by (i) the biological activity of SF (the SF bound to the SF-R chain did not move out of the dialysis bag), and (ii) the autoradiography following SDS-PAGE analysis of ^{125}I-SF + SF-R complex which showed binding to only one of the two bands (*P* 50) of SF-R. Presence of a second membrane-bound protein which complexes with the primary receptor to form high affinity binding sites has also been shown in IL-3, IL-6 and GM-CSF, which indicates that a receptor-ligand complex lacking intracellular sequences can interact with other cell surface molecules to transduce signals into cells (reviewed in [46]). Using the model of natural killer cell stimulatory factor (NKSF), a disulphide-linked heterodimer, it has been suggested that the regulation of cell growth and differentiation could be provided by variable combination of subunits in a receptor–ligand complex [46].

SF is composed of two polypeptide chains linked by disulphide bonds [47] and its α chain binds to the SF-R and the β chain (of SF) to H-2A gene product on macrophage membrane (Mukherjee *et al.*, manuscript in preparation). SF has both high and low affinity receptor sites on macrophages. It is not yet known whether the SF-R, purified in the present study, is responsible for high or low affinity binding and what factors determine it. Studies are underway to answer these questions and analyse the sequence of the SF-R to determine its homology to other cytokine receptors.

ACKNOWLEDGMENTS

The study has been carried out with financial assistance from the Government of India, Department of Science and Technology, New Delhi. We sincerely thank Professor B. Dev, Head of the Department of Zoology, Lucknow University, Lucknow; Dr P. K. Seth, Dr A. K. Agarwal, Ms Kavita Seth and Ms Renu Sah of the Industrial Toxicology Research Centre, Lucknow, and Dr Ravi Kumar of ASTRA Research Centre, Bangalore, for valuable advice and help in analysis of the data, and the Head, Division of Biochemistry and Shri R. K. Vaish, the Central Drug Research Institute, Lucknow, for help in radioisotope work.

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