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Lymphocyte chemotaxis in inflammation

IX. FURTHER CHARACTERIZATION OF LYMPHOCYTE CHEMOTACTIC LYMPHOKINES PRODUCED BY PURIFIED PROTEIN DERIVATIVE-STIMULATION IN VITRO AND IN VIVO

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Summary. As recently reported, one lymphocyte chemotactic factor (β-LCF, mol. wt. about 27,000) released from activated guinea-pig lymphocytes appeared to be identical to one of the LCFs (LCF-d) isolated from extract of purified protein derivative (PPD)-induced delayed-type hypersensitivity skin reaction sites in guinea-pigs with respect to antigenicity and chemotactic effect for T cells. However, the mol. wt. of LCF-d (about 300,000) was clearly distinct from β-LCF. The experiments were undertaken to clarify the problem. β-LCF appeared to be bound to some protein of normal guinea-pig serum (GPS) because the chemotactic activity was revealed in the fraction corresponding to that of LCF-d when the mixtures of β-LCF with GPS were applied to a Sephadex G-200 column. Additionally, binding experiments using fluorescein isothiocyanate (FITC)-labelled β-LCF were performed; fluorescence was only detected in the chemotactic fraction. It was thus assumed that the lymphokine (β-LCF) would be released from activated lymphocytes around the inflammatory tissue, then bound with serum protein exuded in the site and function as LCF-d. The possibility was supported by the evidence that β-LCF like-chemotactic substance (mol. wt. about 27,000) was dissociated from LCF-d under acid conditions. The factor dissociated from LCF-d was also bound with GPS protein under neutral conditions and converted to high molecular substance resembling LCF-d physiochemically. Furthermore, the chemotactic activity of LCF-d was almost completely absorbed by antibody against GPS. It is thus considered that the chemotactic activity of LCF-d may be attributed to β-LCF released from activated lymphocytes and that some serum protein which binds β-LCF may function as a carrier protein in the DTH sites.

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INTRODUCTION

When lymphocytes are stimulated with specific antigen or mitogen, they synthesize and release various biologically active substances including migration inhibitory factor (MIF), macrophage chemotactic factor (MCF), lymphotoxin and lymphocyte chemotactic factor (LCF); they are collectively called lymphokines and appear to be involved in some phases of inflammation resulting from delayed type hypersensitivity (DTH) response (Adelman *et al.*, 1979).

In our previous reports (Shimokawa *et al.*, 1982a, b; Harita, Shimokawa & Hayashi, 1983) and the preced-

ing communications (Shimokawa, Harita & Hayashi, 1984a; Shimokawa *et al.*, 1984b), we have shown that four types of LCF (LCF-a, -b, -c and -d) can be isolated from purified protein derivative (PPD)-induced DTH reaction skin sites in guinea-pigs and have characterized them. Among these LCFs, LCF-c and LCF-d, which were similarly highly purified, distinct antigenically from each other and effective for T cells, were considered to be products from stimulated lymphocytes, i.e. lymphocyte chemotactic lymphokines; LCF-c (mol. wt. about 160,000) shared common antigenicity with one of the lymphokine (α-LCF, mol. wt. about 160,000) and LCF-d (mol. wt. about 300,000) shared that with another lymphokine (β-LCF, mol. wt. about 27,000). Although LCF-c resembled α-LCF biochemically and biologically, LCF-d was clearly distinct from β-LCF in mol. wt.

The present experiments were undertaken to clarify the biochemical difference of the *in-vitro* lymphokine (β-LCF) from *in-vivo* lymphokine (LCF-d), and we will show that β-LCF may form a complex with serum protein exuded and function as LCF-d in DTH reaction sites.

MATERIALS AND METHODS

Animals and induction of DTH reaction

Male Hartley guinea-pigs weighing 300–350 g were used in the experiments. DTH reaction was induced by intradermal injection of PPD in BCG-sensitized animals (Shimokawa *et al.*, 1982a; 1984a).

Isolation and purification of lymphocyte chemotactic factors (LCFs)

As described in the acompanying communication (Shimokawa *et al.*, 1984a), skin extract of 24-hr-old DTH lesions contained four types of LCF (LCF-a, -b, -c and -d); LCF-c and LCF-d were highly purified by a complex series of steps.

Preparation of lymphocyte chemotactic factors from LN cells

This was done essentially according to the previously described method (Harita *et al.*, 1983). Details were given in the accompanying communication (Shimokawa *et al.*, 1984b).

Preparation of antisera and antibody fractions

Antisera against normal guinea-pig serum (anti-GPS) and LCF-d (anti-LCF-d) were respectively prepared in rabbits by repeated intradermal injections of GPS (4 ml, once a week, for 4 weeks) and LCF-d (1–2 mg, once a week, for 5 weeks) with Freund's complete adjuvant (C57Ra, Difco Lab., Detroit, MI). Antibody fractions were separated from each antiserum at 33% saturation with ammonium sulphate. For immunoadsorbent chromatography, antibodies were coupled with BrCN activated Sepharose 4B (Pharmacia, Uppsala, Sweden) according to the method of Porath, Axen & Ernback (1967).

Labelling with fluorescein isothiocyanate (FITC)

After dialysis against 0·1 M sodium carbonate–bicarbonate buffer pH 9·0, chemotactic materials were labelled with FITC by stirring for 8 hr at room temperature (Maeda, 1979). The labelled materials were separated from the reaction mixtures by gel filtration on Sephadex G-25 equilibrated with 0·067 M PB, pH 7·4. Furthermore, dialysis against the same buffer for 24 hr was carried out in order to ascertain the removal of free fluorescein chromatrope. The fluorescence of labelled materials was measured at 475 nm (excitation 390 nm) using a fluorescence spectrophotometer (Model RF-502, Shimazu Seisakusho Ltd., Kyoto); data were represented by the concentration (μM) of fluorescein chromatrope.

The increase in the mol. wt. of FITC-labelled materials by binding with normal guinea-pig serum (GPS) or other substance was determined by the increase of fluorescence polarization value (Dandlicker, 1977). It was measured at 520 nm (excitation 490 nm) at 30° using a fluorescence spectropolarimeter (Model Mac-2, Type HR-1, Japan Immunoindustries Corp., Takasaki).

Chromatography

Molecular sieve chromatography was performed using a 2·5 × 46 cm bed of Sephadex G-200, fine (Pharmacia). Immunoadsorbent chromatography was done by using a column (1·5 × 5 cm) packed with anti-GPS or anti-LCF-d antibody conjugated Sepharose 4B.

Chemotactic assay

This test was performed *in vitro* by previously described method using cellulose nitrate filters (pore size 5 μm; Sartorius, Göttingen, Germany) and a chamber containing two 1- or 0·2-ml compartments (Higuchi, Honda & Hayashi, 1975; Shimokawa *et al.*, 1983, 1984a).

RESULTS

Complex formation of lymphocyte chemotactic lymphokine with serum protein

As noted above, β-LCF, released from activated LN cells and absorbed completely by anti-LCF-d antibody, had a smaller mol. wt. than LCF-d isolated from the DTH reaction sites. Some possible reasons can be considered. One is that LCF-d may be isolated as aggregated form of β-LCF in the extraction procedure. However, this is unlikely because LCF-d behaved as a single protein band when estimated by sodium dodecyl sulphate polyacrylamide electrophoresis under alkylating conditions (Shimokawa *et al.*, 1984a). It is well known that serum proteins are demonstrably exuded into the extravascular spaces, resulting from the increased vascular permeability in DTH reaction site (Voisin & Toullet, 1960; Morley *et al.*, 1972; Hayashi, 1975). However, in our experiments *in vitro* on LCF production, no serum was added to the culture medium. This raised the possibility that the lymphokine (β-LCF) released from LN cells were present in a free form in the culture medium, while it would be present as complex form with serum protein exuded into the inflamed sites.

To investigate the possibility, the following experiments were carried out. When β-LCF recovered from immunoadsorbent column coupled with anti-LCF-d antibody in acid elution was applied to a column of Sephadex G-200, the chemotactic activity was detected near the α-chymotrypsinogen A marker (Shimokawa *et al.*, 1984b; Fig. 1A). Then, β-LCF (3 ml, absorption 0·04 at 280 nm) was incubated with 5% (v/v) freshly prepared normal guinea-pig serum (GPS) at 37° for 30 min and the mixtures were applied to the same column under the same conditions; the chemotactic activity was clearly detected in void volume fractions and it appeared little in other fractions (Fig. 1B). It is thus suggested that β-LCF can be bound with some GPS protein and the chemotactic substance is converted to large mol. wt., corresponding to that of LCF-d.

Additionally, FITC-labelled lymphokine was used to confirm the results described above. The lymphokine was mixed with 5% GPS or 0·067 M PB, pH 7·4, in the same ways. Table 1 shows that the factor certainly binds to some GPS protein because the fluorescence polarization value clearly increases in comparison with the control value. Then, the mixtures were applied to the Sephadex G-200 column under the same conditions. As shown in Fig. 1C, fluorescence was only detected in the same fraction as the one in which chemotactic activity of the mixtures was found.

Table 1. Increase in fluorescence polarization value of FITC-labelled β-LCF

Incubated with	Polarization value*
None†	195
Buffer‡	193
GPS‡	262

* Fluorescence polarization value was measured at 30°.

† Preincubation.

‡ FITC-labelled β-LCF was incubated with 0·067 M PB, pH 7·4 or 5% (v/v) freshly prepared normal guinea-pig serum at 37° for 30 min.

LCF-d as a complex of β-LCF like substance with serum protein

The above results suggested that LCF-d would be a complex of the lymphokine (β-LCF) with some GPS protein exuded in the inflammed sites. The following experiments were undertaken to clarify the problem.

Immunoadsorption of chemotactic activity of LCF-d by anti-GPS antibody. We first examined whether LCF-d activity was absorbed by an immunoadsorbent column conjugated with anti-GPS antibody. LCF-d and LCF-c were respectively applied to the column and respective effluent fractions were collected for chemotactic assay. Figure 2 shows almost the complete adsorption of LCF-d activity by the antibody. In contrast, anti-GPS antibody was little influenced on LCF-c activity. It is thus suggested that LCF-d, which had common antigenicity with β-LCF, also has common antigenicity with some GPS protein and that LCF-d may be complex form of β-LCF with some GPS protein.

Dissociation of β-LCF like substance from LCF-d complex. Secondly, we examined whether chemotactic substance corresponding to β-LCF was dissociated from LCF-d. LCF-d sample (absorption 1·8 at 280 nm) was dialysed against 1 M acetic acid for about 3 hr and applied to a Sephadex G-200 column previously equilibrated with 1 M acetic acid. As shown in Fig. 3, most of chemotactic activity was eluted near the

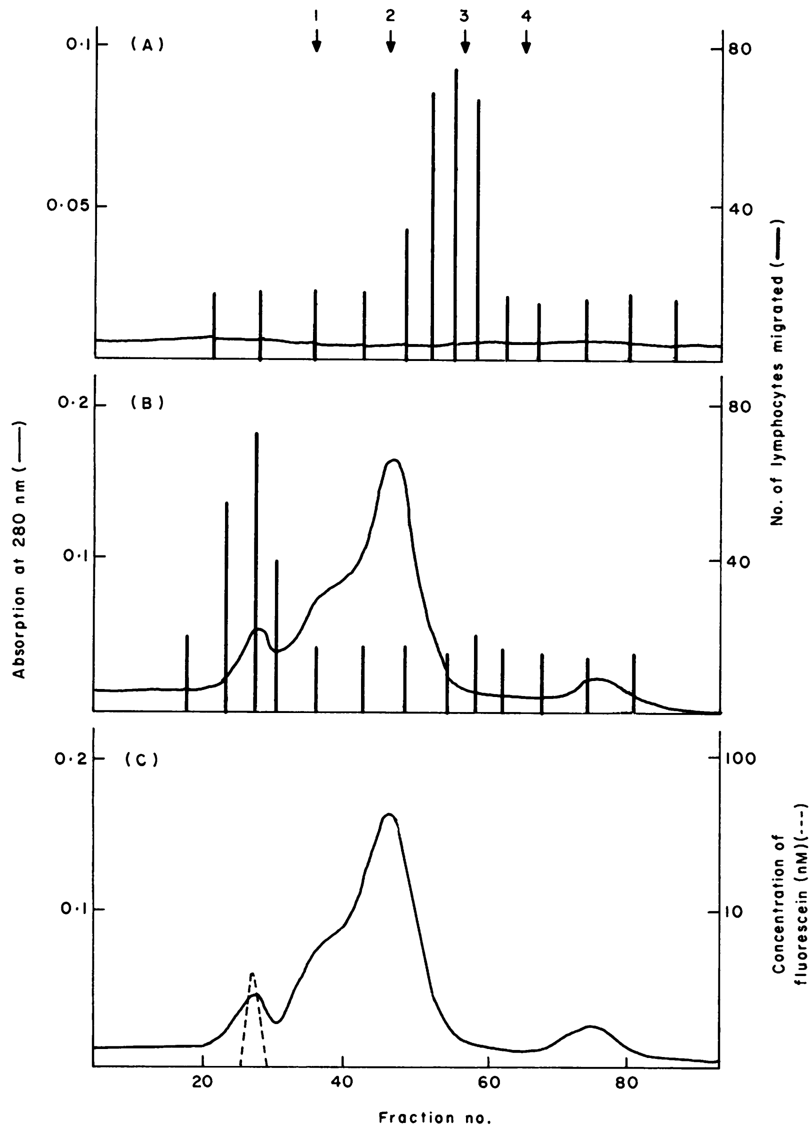

Figure 1. Complex formation of β-LCF with GPS protein.

(A) Gel filtration of β-LCF on Sephadex G-200. The factor was recovered from the immunoadsorbent column coupled with anti-LCF-d antibody by acid elution after culture fluids from PPD-stimulated LN cells had been applied to the column, and then applied to a column of Sephadex G-200 (2·5 × 46 cm); each 3-g effluent fraction was collected for chemotactic assay.

(B) Gel filtration of the mixtures of β-LCF with 5% normal GPS on the Sephadex G-200 column. Each 3-g-effluent fraction was collected for chemotactic assay.

(C) After β-LCF had been labelled with FITC, the mixtures of labelled β-LCF with 5% GPS were applied to the same column under the same conditions. Fluorescence was measured at 475 nm. Data were experessed as concentration of fluoescence.

1, IgG; 2, BSA; 3, α-chymotrypsinogen A; 4, cytochrome c.

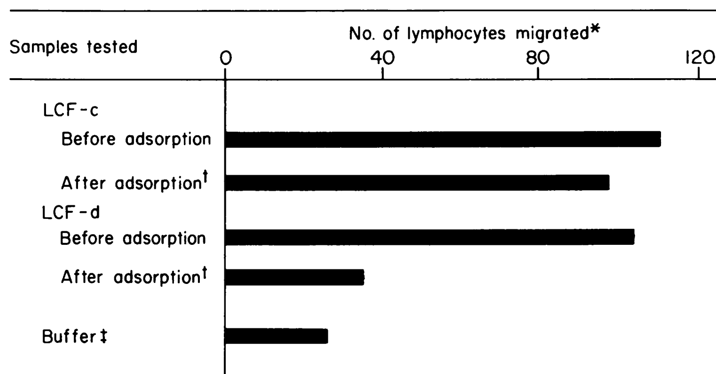

Figure 2. Immunoadsorption of LCF-d by anti-GPS antibody.

* Chemotactic activity is expressed as mean number of lymphocytes migrated of two experiments in three duplicate assays.

† LCF-c obtained after CM-Sephadex chromatography or LCF-d obtained after CM-Sephadex chromatography was applied to the immunoadsorbent column coupled with anti-GPS antibody and each effluent fraction (unadsorbed fractions) was assayed for chemotactic activity for lymphocytes after concentration to applied volume.

‡ 0·067 M PB, pH 7·4.

α-chymotrypsinogen A marker (mol. wt. 25,700). In contrast, the activity of LCF-d itself was only demonstrated in the void fraction, when the sample was applied to the same column equilibrated with 0·067 M PB, pH 7·4 (data not shown). These results suggest that the chemotactic activity of LCF-d may be attributed to a low molecular substance. Its activity was little abolished by heating (Table 2); its physicochemical properties were virtually identical to β-LCF.

Complex formation of β-LCF like substance with serum protein. Additionally, we studied whether the chemotactically active substance dissociated from LCF-d in an acid condition (Fig. 3) would be bound with GPS protein. First, the concentrated sample (3 ml) after dialysis against 0·067 M PB, pH 7·4, was incubated with 5% (v/v) GPS at 37° for 30 min and applied to a Sephadex G-200 column equilibrated with the same buffer. As shown in Fig. 4A, the chemotactic

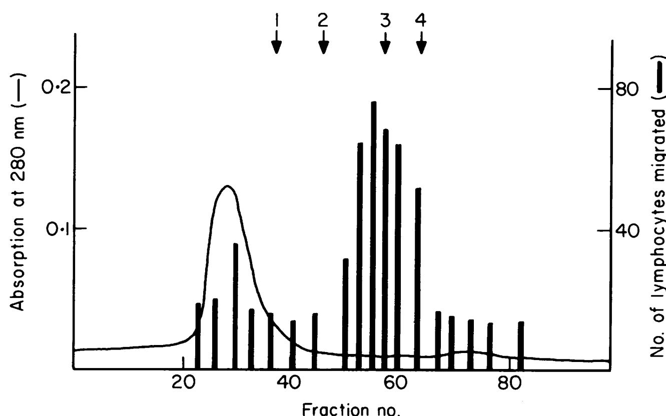

Figure 3. Dissociation of chemotactic substance from LCF-d. After LCF-d obtained after CM-Sephadex chromatography had been dialysed against 1 M acetic acid, the sample was applied to a Sephadex G-200 column (2·5 × 46 cm) previously equilibrated with 1 M acetic acid; the effluent fractions were vigorously dialysed against 0·067 M PB, pH 7·4, for chemotactic assay for lymphocytes.

1, IgG; 2, BSA; 3, α-chymotrypsinogen A; 4, cytochrome c.

Table 2. Effect of heating of dissociated chemotactic substance from LCF-d

Samples tested	Chemotactic activity in Exp.*	
	Exp. 1	Exp. 2
Active substance†		
Non-treated	83	71
Heated at 56° for 30 min	78	75
β-LCF‡		
Non-treated	70	75
Heated at 56° for 30 min	73	68
Buffer§	21	18

* Chemotactic activity is expressed as mean number of lymphocytes migrated of two duplicate assays in each experiment.

† Chemotactically active substance was obtained after gel filtration of LCF-d by acid elution (Fig. 3).

‡ β-LCF was obtained after gel filtration on Sephadex G-200 (Fig. 1A).

§ 0·067 M PB, pH 7·4.

activity was clearly demonstrated in the void fraction; it corresponded to elution profile of LCF-d itself. Secondly, the sample dissociated from LCF-d was labelled with FITC and incubated with GPS or 0·067 M PB, pH 7·4, in the same ways. Table 3 shows that the sample certainly binds to GPS protein. The mixture of FITC-labelled sample and GPS was then applied to the same column in the same conditions. As shown in Fig. 4B, fluorescence was only detected in the chemotactic fractions. It was not shown in other fractions.

These results clearly support the possibility that LCF-d exists in the form of a complex of chemotactically active substance corresponding to β-LCF with serum protein.

Table 3. Increase in fluorescence polarization value of FITC-labelled β-LCF-like substance

Incubated with	Polarization value*
None†	196
Buffer‡	194
GPS‡	266

* Fluorescence polarization value was measured at 30°.

† Preincubation.

‡ FITC-labelled β-LCF-like substance (Fig. 3) was incubated with 0·067 M PB, pH 7·4 or 5% (v/v) freshly prepared normal guinea-pig serum at 37° for 30 min.

DISCUSSION

As stated earlier, we have demonstrated that two types of LCF (α-LCF and β-LCF) were released *in vitro* from specific antigen (PPD)- or Con A-stimulated guinea-pig LN cells; α-LCF (mol. wt. about 160,000) was specifically adsorbed by antibody against LCF-c, which was one of four types of LCF (LCF-a, -b, -c, and -d) isolated from PPD-induced DTH skin reaction sites in guinea-pigs, and virtually identical to LCF-c (mol. wt. about 160,000) with respect to mol. wt., heat stability and chemotactic effect on T cells. In contrast, the other LCF (β-LCF, mol. wt. about 27,000) was specifically adsorbed by antibody against LCF-d (mol. wt. about 300,000) although these factors were clearly different from each other with respect to mol. wt. (Harita *et al.*, 1983; Shimokawa *et al.*, 1984a, b). In this report, we show that LCF-d also shares common antigenicity with some GPS protein, because the chemotactic activity of the factor is completely adsorbed by anti-GPS antibody while that of LCF-c is not influenced by the antibody (Fig. 2). The results suggest that when the lymphocyte chemotactic lymphokines were released from activated lymphocytes around inflammatory tissue, α-LCF exists as free form and functions as LCF-c while β-LCF exists as complex form with serum protein exuded and functions as LCF-d in inflammed site.

The possibility is supported by the evidence that β-LCF, which was obtained by gel filtration of active culture supernatants from LN cells on Sephadex G-200 or recovered from immunoadsorbent column conjugated with anti-LCF-d antibody, can form a high molecular complex with a GPS protein (Fig. 1, Table 1). Additionally, it is shown that LCF-d is dissociated under acid conditions to a chemotactically active substance, which is eluted near α-chymotrypsinogen A marker (mol. wt. 25,700); it resembled β-LCF on Sephadex G-200 elution profiles and heat stability (Fig. 3, Table 2). Furthermore, β-LCF like substance dissociated from LCF-d can bind to some GPS protein under neutral conditions and then it is converted to a high mol. wt. substance; the chemotactic activity of the complex is detected only in the void volume fraction (Fig. 4, Table 3). In elution profile on Sephadex G-200, LCF-d, complex of β-LCF with GPS protein and complex of β-LCF like substance from

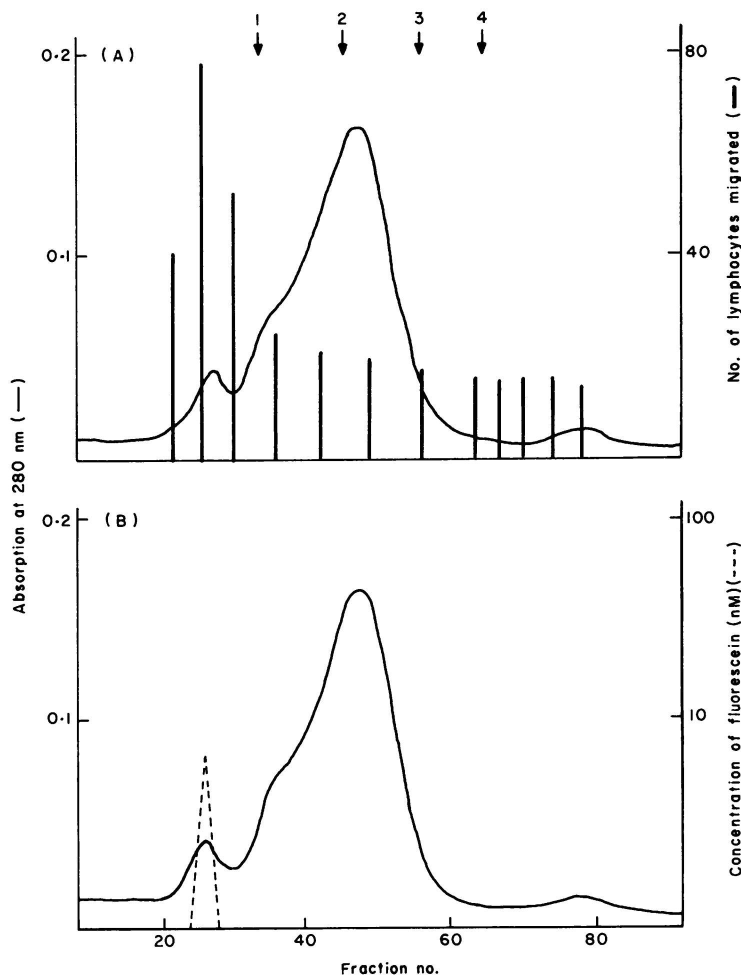

Figure 4. LCF-d as complex form of chemotactic substance with GPS protein.

(A) Gel filtration of the mixtures of the chemotactic substance dissociated from LCF-d (Fig. 3) with 5% normal GPS on Sephadex G-200 column (2·5 × 46 cm). Each 3-g effluent fraction was collected for chemotactic assay.

(B) After the factor (Fig. 3) had been labelled with FITC, the mixtures of FITC-labelled factor with 5% GPS were applied to the same column under the same conditions. The fluorescence was measured at 475 nm. Data were expressed as concentration of fluorescence.

1, IgG; 2, BSA; 3, α-chymotrypsinogen A; 4, cytochrome c.

LCF-d with GPS protein are indistinguishable. It is thus concluded that the activity of LCF-d may be attributed to β-LCF and serum protein bound with β-LCF may function as a carrier protein in the inflammatory sites. The findings that lymphocyte chemotactic lymphokine (β-LCF) forms a complex with serum protein under neutral conditions appears to be reasonable because it is well known that the leucocyte reaction is followed by extravascular exudation of serum protein resulting from increased vascular permeability (Hayashi, 1975) and that the pH of inflammed tissue or inflammatory exudates is around neutral (Hutchins & Sheldon, 1972; Hayashi, 1975). However, we do not know why α-LCF exists as free form and β-LCF, as complex form; it is assumed that these lymphokines have different binding sites against serum protein. Studies of the protein bound with β-LCF are in progress.

As noted above, it is of importance to demonstrate lymphokine-like substance(s) in DTH reaction sites. However, the literature dealing with lymphocyte chemotactic lymphokines presented here is limited and most available studies have focused on the other materials including MIF and MCF. Postlethwaite & Snyderman (1975) have found MCF activity in the peritoneal exudate of sensitized guinea-pigs exhibiting DTH following intraperitoneal challenge with horseradish peroxidase; it was eluted near the cytochrome c marker and its mol. wt. resembled that of MCF produced *in vitro* by activated lymphocytes. Postlethwaite, Townes & Kang (1976) further demonstrated that MIF activity was also shown in the same peritoneal exudate and it was virtually identical to MIF released from the cells *in vitro* with respect to mol. wt., heat stability and trypsin sensitivity. Similar findings on MIF from inflammatory sites were shown (Yamamoto, Dunn & Willoughby, 1976, Yamamoto & Ishikura, 1979).

In respect to complex formation of lymphokine, it is important to note that one (MCF-c, mol. wt. about 110,000) of MCFs isolated from PPD-induced DTH skin reaction sites in guinea-pigs is thought to be complex of macrophage chemotactic lymphokine (mol. wt. about 12,500) with GPS protein corresponding to α-globulin (Honda & Hayashi, 1982; Honda *et al.*, 1982). They demonstrated that MCF activity in culture fluids from specific antigen (PPD)- or PHA-stimulated LN cells was specifically absorbed by anti-MCF-c antibody and that *in-vitro* MCF was bound with PGS protein. They have further shown that MCF-c was dissociated to macrophage chemotactic lymphokine-like substance under acid condition and that the dissociated substance was bound with GPS protein. Additionally, MCF-a (mol. wt. about 150,000 was completely adsorbed by anti-IgG antibody or anti-light chain antibody while MCF-b (mol. wt. about 14,000) was specifically adsorbed by anti-C5 antibody. Evidence for the presence of a carrier protein of serum thymic factor (mol. wt. 867) in normal human or mouse serum was obtained from the binding experiments using ^{3}H-labelled titrated serum thymic factor; the factor was eluted near the albumin marker (mol. wt. 40,000–60,000) on Sephadex G-150 (Dardenne, Pleau & Bach, 1980).

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REFERENCES

Adelman N.E., Hammond M.E., Cohen S. & Dvorak H.F. (1979) Lymphokines as inflammatory mediators. In: *Biology of the Lymphokines* (eds S. Cohen, E. Pick and J. J. Oppenheim), p. 13. Academic Press, New York.

Dandlicker W.B. (1977) Investigation of immunochemical reactions by fluorescence polarization. In: *Immunochemistry of Proteins* (ed. M. Z. Atassi), Vol. 1, p. 231. Plenum Press, New York.

Dardenne M., Pleau J.M. & Bach J.F. (1980) Evidence of the presence in normal serum of a carrier of the serum thymic factor (FTS). *Eur. J. Immunol.* **10,** 83.

Harita S., Shimokawa Y. & Hayashi H. (1983) Production of two lymphocyte chemotactic factors by antigen-stimulated guinea-pig lymphocytes. *Int. Archs Allergy appl. Immun.* **70,** 118.

Hayashi H. (1975) The intracellular neutral SH-dependent protease associated with inflammatory process. *Int. Rev. Cytol.* **40,** 101.

Higuchi Y., Honda M. & Hayashi H. (1975) Production of chemotactic factor for lymphocytes by neutral SH-dependent protease of rabbit PMN leucocytes from immunoglobulins, especially IgM. *Cell. Immunol.* **15,** 100.

Honda M. & Hayashi H. (1982) Characterization of three types of macrophage chemotactic factors from PPD-induced delayed hypersensitivity reaction site in guinea pig, with special reference to a chemotactic lymphokine. *Am. J. Pathol.* **108,** 171.

Honda M., Miura K., Kuratsu J. & Hayashi H. (1982). Characterization of macrophage chemotactic lymphokine produced by purified protein derivative-stimulation *in vitro* and *in vivo*. *Cell. Immunol.* **67,** 213.

Hutchins G.M. & Sheldon W.H. (1972) The pH of inflammatory exudates in acidotic diabetic rabbits. *Proc. Soc. exp. Biol. Med.* **140,** 623.

Maeda H. (1979) Assay of proteolytic enzymes by the fluorescence polarization technique. *Analyt. Biochem.* **92,** 222.

Morley J., Williams T.J., Slater A.J., Cubitt D. & Dumonde D.C. (1972) Simultaneous measurements of the accumulation of isotope-labelled protein and erythrocytes in skin reactions of allergic inflammation in the guinea-pig. *Immunology,* **23,** 113.

Porath J., A§en R. & Ernback S. (1967) Chemical coupling of proteins to agarose. *Nature (Lond.),* **215,** 1491.

Postlethwaite A.E. & Snyderman R. (1975) Characterization of chemotactic activity produced *in vivo* by a cell-mediated immune reaction in the guinea pig. *J. Immunol.* **114,** 274.

Postlethwaite A.E., Townes A.S. & Kang A.H. (1976) Characterization of migration inhibitory factor activity produced *in vivo* by a cell-mediated immune reaction in the guinea pig. *J. Immunol.* **117,** 1716.

Shimokawa Y., Harita S. Higuchi Y. & Hayashi H.

(1982a) Lymphocyte chemotaxis in inflammation. III. Demonstration of lymphocyte chemotactic activity in extract from PPD-induced delayed hypersensitivity skin reaction site in the guinea pig. *Br. J. exp. Pathol.* **63,** 355.

SHIMOKAWA Y., HARITA S., HIGUCHI Y. & HAYASHI H. (1982b) Lymphocyte chemotaxis in inflammation. IV. Isolation of lymphocyte chemotactic factors from PPD-induced delayed hypersensitivity skin reaction site in the guinea pig, with special reference to a factor chemotactic for B lymphocytes. *Br. J. exp. Pathol.* **63,** 362.

SHIMOKAWA Y., HARITA S. & HAYASHI H. (1984a) Lymphocyte chemotoaxis in inflammation. VII. Isolation and purification of chemotactic factors for T lymphocytes from PPD-induced delayed hypersensitivity skin reaction site in the guinea-pig. *Immunology,* **51,** 275.

SHIMOKAWA Y., HARITA S., MIBU Y. & HAYASHI H. (1984b) Lymphocyte chemotaxis in inflammation. VIII. Demonstration of lymphocyte chemotactic lymphokines in PPD-induced delayed hypersensitivity skin reaction site in the guinea-pigs. *Immunology,* **51,** 287.

SHIMOKAWA Y., MIURA K., HIFUMI M. & HAYASHI H. (1983) Lymphocyte chemotaxis in inflammation. VI. Lyt phenotype analysis of effector cells responsible for producing murine lymphocyte chemotactic factor. *Immunology,* **49,** 95.

VOISIN G.A. & TOULLET F. (1960) Modifications of capillary permeability in immunological reactions mediated through cells. In: *Ciba Foundation Symposium on Cellular Aspects of Immunity* (eds G. E. W. Wolstenholme and M. O'Connor), p. 373. Churchill, London.

YAMAMOTO S., DUNN C.J. & WILLOUGHBY D.A. (1976) Studies on delayed hypersensitivity pleural exudates in guinea-pigs. I. Demonstration of substances in the cell-free exudate which cause inhibition of mononuclear cell migration *in vitro. Immunology,* **30,** 505.

YAMAMOTO S. & ISHIKURA A. (1979) Delayed hypersensitivity reactions in guinea pigs using cotton pellets; demonstration of macrophage migration inhibition activity in the cell free exudate. *J. Pathol.* **127,** 1.