CD4-mediated Stimulation of Human Eosinophils: Lymphocyte Chemoattractant Factor and Other CD4-binding Ligands Elicit Eosinophil Migration

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Summary

Lymphocyte chemoattractant factor (LCF) is a tetrameric glycoprotein of 56,000 relative molecular mass produced by activated T lymphocytes. LCF binds to CD4 and has previously been found to stimulate migration of $CD4^+$ lymphocytes and monocytes. Because human eosinophils, like T cells and monocytes, express CD4, we examined functional responses of eosinophils to LCF. Recombinant LCF (rLCF) expressed in COS cells was purified on a CD4 affinity column. Migration of eosinophils was elicited by rLCF at low concentrations: the 50% effective dose (ED_{50}) was 10^{-12} to 10^{-11} M, concentrations 100- to 1,000-fold lower than the ED_{50}s for the recognized eosinophil chemoattractants C5a and platelet-activating factor. Two other ligands which bound to CD4, human immunodeficiency virus-1 envelope glycoprotein gp120 and monoclonal antibody OKT4, also stimulated eosinophil migration. Monovalent OKT4 F_{ab} competitively inhibited eosinophil responses to rLCF. rLCF did not influence other functional responses of eosinophils tested, including degranulation, superoxide generation, leukotriene C_4 production, in vitro survival, or surface expression of the adherence receptor CR3 (CD11b), human histocompatibility leukocyte antigen DR, or interleukin 2 receptor p55 (CD25). We conclude that CD4 on eosinophils is capable of transducing a migratory stimulus and serves as a receptor for a chemoattractant lymphokine LCF. T cell-derived LCF may contribute to recruitment of eosinophils and $CD4^+$ mononuclear cells concomitantly at inflammatory reactions.

Eosinophils are a distinct granulocyte lineage derived from bone marrow which transit through the circulation and are distributed predominantly to tissues adjacent to epithelial surfaces. Tissue eosinophilia is seen in a restricted number of pathologic conditions, suggesting that selective immunologic mechanisms result in eosinophil infiltration. Experimental models have demonstrated that production and localization of eosinophils is selectively regulated by T lymphocytes (1). Among the mechanisms by which T lymphocytes regulate eosinophils are three lymphokines with colony-stimulating activities, granulocyte-macrophage CSF (GM-CSF),[1] IL-3, and IL-5, which not only expand eosinophil progenitors but also stimulate functions of mature eosinophils (2–4).

The recent demonstration (5) that eosinophils express CD4 may help elucidate the interactions between eosinophils and other immunologic cells. The functions of CD4 on eosinophils are undefined, but several mechanisms of functioning have been postulated for CD4 on T lymphocytes. First, CD4 binds MHC class II antigens (6) and consequently enhances the avidity of interaction between $CD4^+$ lymphocytes and accessory cells presenting antigen with MHC class II (7). Second the cytoplasmic tail of CD4 is associated with a tyrosine kinase $p56^{lck}$ (8). The substrates for $p56^{lck}$ phosphorylation include the ζ-subunit of the TCR/CD3 complex (9). CD4 has been demonstrated to colocalize with the TCR during lymphocyte activation (10, 11) which would allow cooperation between CD4, $p56^{lck}$, and the TCR in signal transduction. Third, CD4 is the receptor for the lymphokine lymphocyte chemoattractant factor (LCF) (12, 13). LCF is a basic glycoprotein of M_r 56,000, consisting of a tetramer of identical subunits. Lymphocytes elaborate LCF after stimulation with either histamine or specific antigen (14, 15). In $CD4^+$ lymphocytes, responses to LCF include enhanced migration and expression of class II MHC antigens and IL-2 receptors.

In addition to $CD4^+$ T lymphocytes, other blood leukocytes which express CD4 are monocytes (16) and eosinophils

[1] *Abbreviations used in this paper:* GM-CSF, granulocyte-macrophage CSF; HES, idiopathic hypereosinophilic syndrome; LCF, lymphocyte chemoattractant factor; LTC_4, leukotriene C_4; MFI, mean fluorescence intensity by flow cytometry; PAF, platelet-activating factor; rs, recombinant soluble.

(5). As monocytes and eosinophils do not express either the TCR or $p56^{lck}$ (17), currently defined mechanisms of CD4 signaling which involve cooperation between the TCR and CD4 would not be operative in these $CD4^+$ nonlymphoid cells. Monocytes do respond to LCF with stimulated migration and MHC class II expression, and upregulation of CD4 during short-term culture of monocytes enhanced their responses to LCF (12). In the current study, we demonstrate that LCF is a potent eosinophil chemoattractant. Eosinophil migration elicited by LCF, like other agents which bind CD4 (specifically, HIV envelope glycoprotein [18] gp120 and CD4 mAbs), is mediated by CD4 expressed on eosinophils.

Materials and Methods

Recombinant LCF. Cloning and expression of the cDNA for LCF are the subject of a separate report (W. Cruikshank, H. Kornfeld, K. Jacobs, S. Clark, A. Theodore, J. Berman, J. Bernardo, D. Beer, and D. Center, manuscript in preparation). Briefly, rLCF was generated from a transient expression system in which presence of the LCF gene was identified by screening a cDNA library of PHA-activated PBMC transfected into monkey COS cells (provided by Drs. K. Jacobs and S. Clark, Genetics Institute, Cambridge, MA). Screening was performed by assay of transfected COS supernatants for LCF activity by lymphocyte migration and IL-2 receptor expression as described (12), and specificity of activity was confirmed by blocking with F_{ab} of CD4 mAb or recombinant soluble (rs) CD4 (gift of American Biotechnologies, Cambridge, MA). A single clone with a single cDNA insert, as determined by DNA sequencing, was derived by sequential subcloning of COS transfectants with LCF bioactivity. The concentration of rLCF in COS supernatants was estimated by comparison of the ED_{50} with that of affinity-purified rLCF of known concentration. For comparison in preliminary experiments, purified natural LCF was generated as described in modifications (13) of our established methods (12).

Affinity Purification of rLCF. A CD4 affinity column was prepared by coupling 400 μg rsCD4 to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ). For each preparation of purified rLCF, 5 ml of transfected COS supernatant containing 0.1% FCS was adjusted to pH 8, repeatedly applied to the column, washed with 0.1 M $NaHCO_3$, 0.5 M NaCl, pH 8, and eluted with the same buffer adjusted to pH 4. SDS-PAGE analysis of the eluted 56,000 homotetrameric protein revealed a single major silver-stained band at M_r 14,000, and LCF activity was found in the corresponding gel slice of a parallel, unstained lane after elution and dialysis. Activities of rLCF before and after affinity purification were the same. Calculation of the molarity of rLCF assumed that all the protein in the affinity-purified preparation was rLCF; SDS-PAGE analysis indicated that purity was at least 90%, and this calculation might lead to a slight underestimation of the potency of rLCF. When a supernatant of vector mock-transfected COS cells was applied to the column, the eluant did not contain LCF activity.

Eosinophil Isolation. Eosinophil-rich granulocytes were isolated from two blood donors with idiopathic hypereosinophilic syndrome (HES) (19) by dextran sedimentation and centrifugation through Ficoll-Paque (Pharmacia Fine Chemicals). These HES granulocyte preparations contained 74 ± 13% eosinophils (mean ± SD, 26 isolations) with neutrophils as the only contaminating cells. Eosinophils from normal blood donors (initial leukocytes 2–6% eosinophils and taking no medications) were enriched on Percoll gradients by modification of a published method (20). Percoll (Pharmacia Fine Chemicals), 91.5 ml, was mixed with 2 ml 1 M Hepes buffer, pH 7.3, and 6.5 ml 10× HBSS to yield the lower layer of density 1.123 g/ml, pH 7.45, and 280 mOsm/kg H_2O. Middle layer was prepared by diluting 80 ml lower layer with approximately 40 ml HBSS until a density 1.083 g/ml was attained, as measured by density meter (Mettler/Paar, Graz, Austria). The upper layer was prepared by mixing 2 ml of granulocyte suspension at approximately 2×10^8 ml in HBSS with 10 ml middle layer Percoll to yield density 1.063 g/ml. Discontinuous gradients were formed in 50 ml conical plastic centrifuge tubes by layering 3 ml lower layer, 15 ml middle layer, and 2 ml upper layer containing granulocytes and were centrifuged at 1,000 *g* for 20 min at room temperature. Gradient fractions containing eosinophils were pooled. These normal eosinophil preparations contained 66 ± 20% eosinophils (15 isolations) with neutrophils as the only contaminating cells. Viability by trypan blue exclusion was greater than 98%.

Assay of Eosinophil Migration. Eosinophil migration assays employed our previously described techniques by which eosinophils are selectively stained with aniline blue and enumerated at several distances in nitrocellulose membranes by fluorescence microscopy and image analyzer (21). Briefly, purified eosinophils ($1–2 \times 10^5$) were placed in the upper wells of 48-well microchemotaxis chambers (Neuro Probe, Cabin John, MD) separated from the material to be tested in the lower wells by a Toyo nitrocellulose filter (150 μm thickness, 5 μm pore size; Neuro Probe). After incubation at 37°C for 60 min, the filter was fixed, stained with aniline blue (Sigma Chemical Co., St. Louis, MO), cleared in cedar oil (Polysciences Inc., Warrington, PA), and mounted. For each well, eosinophils were quantified in four 25× fields at 10 μm intervals beginning 10 μm from the upper surface of the filter by fluorescence microscopy on a Leitz Laborlux S with a 50W mercury arc lamp and wide-band blue H3 excitation/emission filters. Data were collected by an Optomax V image analyzing system (Analytical Instruments, Shaffron Walden, Essex, England), and results were tabulated by a chemotaxis computer program (Optomax/Microvideo Systems, Hollis, NH). Migration index is calculated as the sum of the products of cell number and distance migrated at the sequential 10 μm levels enumerated. Net migration refers to the difference between migration index for the tested substance and that for medium alone (HBSS supplemented with 1 mg/ml OVA; Sigma Chemical Co.). Neutrophil migration was assessed by the same techniques, except that the filters were stained with Congo red, as described (21).

Standard eosinophil chemoattractants used were rC5a (HPLC purified; gift of Dr. Norma Gerard, Beth Israel Hospital, Boston, MA) (22) and platelet-activating factor (PAF) L-α-phosphatidylcholine β-acetyl-γ-O-hexadecyl (Sigma Chemical Co.) (23). Purified HIV-1 r gp120 was obtained from MicroGeneSys, Inc. (West Haven, CT). Purified CD4 mAbs OKT4A to 4F were a gift of Dr. Patricia Rao, Ortho Diagnostics (Raritan, NJ) (24).

Other Assays of Eosinophil Function. Superoxide generation was determined by spectrophotometric measurement of cytochrome C reduction as described (25). Eosinophils were preincubated for 60 min at 37°C with stimuli tested for priming followed by 10 min incubation in medium containing cytochrome C with stimuli tested for directly eliciting superoxide generation. Degranulation was measured by arylsulfatase B release as described (26). Ionophore A23187-triggered leukotriene C_4 (LTC_4) generation was performed as described (27). Briefly, duplicate samples of purified eosinophils at 2×10^6 ml were preincubated for 60 min at 37°C in HBSS in the presence of the cytokines tested, A23187 to 2 μM and L-serine to 50 mM (final concentration) were added in an equal volume

of HBSS adjusted to pH 7.4, incubations were continued for 10 min and were terminated by chilling and centrifugation. LTC_4 in the supernatant was quantified by RIA according to the manufacturer (New England Nuclear, Boston, MA). In two experiments, cell-associated LTC_4 after A23187 stimulation measured on methanol extracts of cell pellets after evaporation to dryness and reconstitution in water was 1.5–2.5 ng/10^6 eosinophils. LTC_4 production was ≤0.2 ng/10^6 eosinophils in the absence of A23187 stimulation for all conditions tested. Stimuli used as positive controls for eosinophil functional assays included rGM-CSF (2.5 × 10^5 CFU/μg; Genzyme Corp., Boston, MA), rIL-3 (5 × 10^7 U/mg, Genzyme Corp.), PMA (Sigma Chemical Co.), and opsonized zymosan prepared as described (26).

Flow Cytometry Analysis of Eosinophil Surface Antigens. Purified eosinophils were stained with mAb anti-CR3 PE (Leu-15, CD11b, clone D12), anti-HLA-DR FITC (clone L243), anti-IL-2 receptor FITC (CD25, clone 2A3), Leu-3a+3b FITC (CD4, clones SK3 and SK4), and appropriate IgG subclass mAb controls according to the manufacturer (Becton Dickinson and Co., Mountain View, CA) and analyzed on a FACStar® Plus. Eosinophils were selectively gated by the combination of forward light scatter, orthogonal light scatter, and autofluorescence. Data were collected on 5,000 gated cells per sample and analyzed by the program Consort 30 (Becton Dickinson & Co.). ΔMFI refers to the difference in mean fluorescence intensity (MFI) between the histogram of interest and control. Percentage positive was derived by subtraction of control histogram from the histogram of interest.

Preparation of F_{ab}. OKT4 (CD4; Ortho Diagnostics) or W6/32 (anti-MHC class I; Sera-Lab/Accurate, Westbury, NY), 50 μg of each mAb, were digested with 2.5 U insoluble papain (Sigma Chemical Co.) in 1 ml 10 mM 2-ME, 4 mM EDTA, 1 mg/ml sodium azide, 0.1 M phosphate buffer, pH 7.5, at 37°C for 14 h. Undigested IgG and Fc fragments were removed by two incubations with protein G Sepharose (Zymed Labs Inc., San Francisco, CA). Protein concentration (Biorad, Richmond, CA) was determined after dialysis. Absence of intact IgG was verified by SDS-PAGE under reducing and nonreducing conditions. Presence of functional F_{ab} was verified by flow cytometry after staining PBMC with the individual F_{ab} preparations followed by goat anti-mouse IgG FITC (Tago Inc., Burlingame, CA).

Results

LCF as a Chemoattractant for Eosinophils. LCF was originally identified as a chemoattractant for $CD4^+$ lymphocytes (12, 15, 28). Since eosinophils also express CD4 (5), we examined whether LCF is a chemoattractant for eosinophils. In preliminary experiments, both natural LCF and unpurified rLCF expressed in COS cells induced eosinophil migration in chemotaxis chambers. Subsequent experiments used rLCF affinity-purified by binding to immobilized rsCD4. With eosinophils from four normal donors, ED_{50}s for rLCF ranged from 0.3 to 5 × 10^{-12} M (Fig. 1). Supraoptimal concentrations of rLCF elicited migration less than that at optimal concentrations, a finding termed "high-dose inhibition." In a total of 27 experiments, eosinophils from all donors tested (11 total) showed enhanced migration in response to rLCF.

Comparative Potency of LCF as an Eosinophil Chemoattractant. The activity of rLCF as an eosinophil chemoattractant was compared to rC5a and PAF (Fig. 2). rLCF was 100- to 1,000-fold more potent than either rC5a or PAF (ED_{50}s

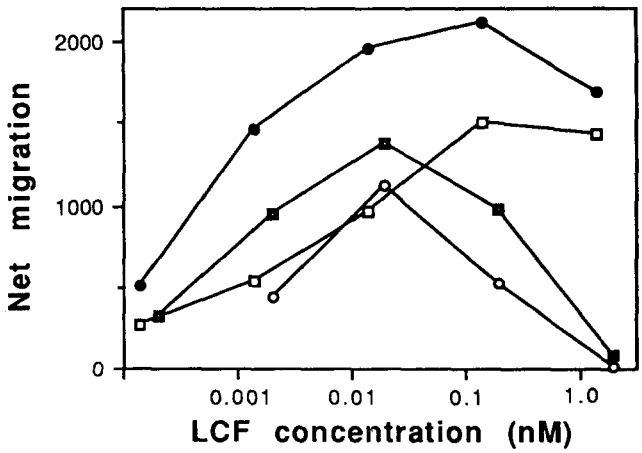

Figure 1. Concentration-dependent eosinophil chemoattractant activity of rLCF. Purified eosinophils from four normal donors were used in migration assays with the indicated concentrations of rLCF. Results are expressed as net migration: the difference between migration index for the condition tested and that for medium in the same experiment. Migration indices with medium for the individual experiments shown were 580 ± 99, 729 ± 84, 759 ± 106, and 915 ± 8. Each point is the mean of triplicates.

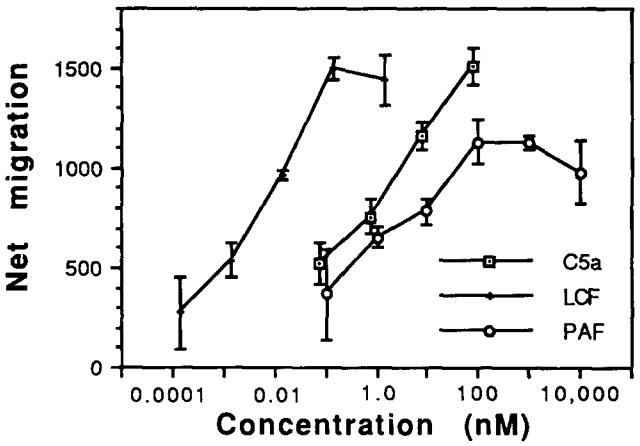

Figure 2. Eosinophil chemoattractant activity of rLCF compared to rC5a and PAF. The migration of eosinophils purified from a normal donor were measured in response to the indicated concentration of rC5a, rLCF, and PAF. Net migration was calculated as above. The migration index with medium was 915 ± 8 for the experiment shown. Error bars indicate SEM of triplicates.

10^{-9} M). When optimal concentrations of each agent were compared, similar net migration was achieved with 10^{-10} M rLCF, 10^{-7} M rC5a, and 10^{-6} M PAF in each of seven experiments with eosinophils from normal or HES donors (Fig. 3), indicating that the three agents elicited comparable numbers of migrating eosinophils. In each experiment, we plotted the number of eosinophils migrating to sequential 10 μm levels within the filter to determine whether the migratory response was elicited in the entire population or only in a subpopulation of eosinophils; these analyses did not identify a restricted LCF-responsive subpopulation (data not shown).

When neutrophils were used in migration experiments in which cells were stained with Congo red and analyzed by

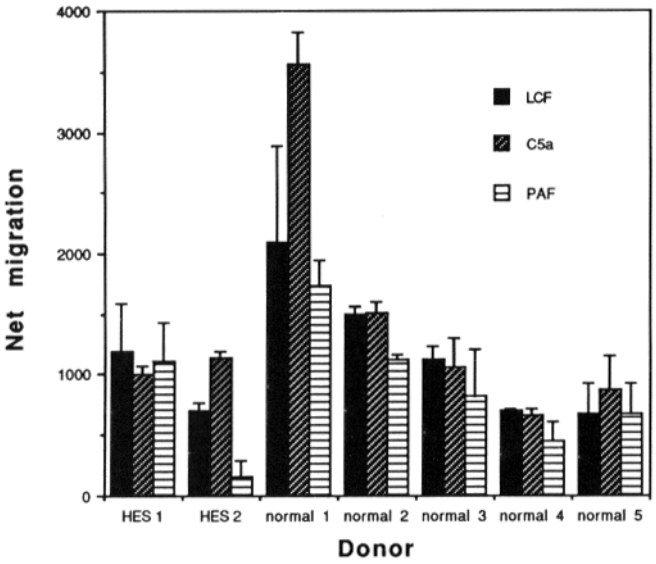

Figure 3. Migration responses of eosinophils from different donors to optimal concentrations of rLCF, rC5a, and PAF. The migration of eosinophils purified from five normal and two HES donors was measured in response to rLCF (10^{-10} M), rC5a (10^{-7} M), and PAF (10^{-6} M). Net migration was calculated as above. Migration indices with medium alone were 1,196 ± 70 for HES donor 1, 560 ± 87 for HES donor 2, 915 ± 8 for normal donor 1, 356 ± 131 for normal donor 2, 1,197 ± 111 for normal donor 3, 236 ± 92 for normal donor 4, and 1,380 ± 232 for normal donor 5. Error bars indicate SEM of triplicates.

the same fluorescence techniques, chemoattractant responses were readily demonstrated with C5a, FMLP, or PAF. In contrast, rLCF (10^{-13} to 10^{-10} M) did not elicit chemoattractant responses with neutrophils (data not shown, three experiments). Although neutrophils contaminate the eosinophil preparations used, neutrophils do not respond to rLCF and are not detected by aniline blue staining for quantitation of eosinophil migration.

Random (Chemokinesis) and Directed (Chemotaxis) Eosinophil Locomotion Stimulated by LCF. We performed checkerboard analyses (29) to evaluate whether eosinophil migration to LCF was dependent on a concentration gradient (Table 1). The predominant effect on eosinophil locomotion was chemokinesis (that is, gradient-independent migration), as demonstrated by the increase in migration at increasing concentrations along the diagonal of the table. The highest concentration of rLCF elicited less migration than an optimum concentration, a property referred to as high-dose inhibition. In addition to the chemokinetic activity of rLCF, chemotaxis was observed: rLCF stimulated quantitatively greater migration in the direction of a concentration gradient, as demonstrated by greater migration to the left of the diagonal than to the right of the diagonal in the table.

Involvement of CD4 in the Eosinophil Chemoattractant Response to LCF. Based on previous studies with LCF-responsive mononuclear cells (12, 13) we postulated that LCF chemoattractant activity depended upon interaction with CD4 on the eosinophil. To evaluate this hypothesis, we first investigated whether other CD4-binding agents, which have been shown to induce migration of $CD4^+$ lymphocytes (30), influenced the migration of eosinophils. Both HIV-1 r gp120 and the CD4 mAb OKT4 elicited chemoattractant responses which were comparable in magnitude to rLCF and to the control chemoattractant PAF (Fig. 4). In contrast to the activity of OKT4, two control antibodies of the same IgG subclass did not elicit eosinophil chemoattractant responses: the myeloma protein UPC10, which does not bind to eosinophils, and mAb W6/32, which binds to class I MHC antigens expressed on eosinophils. OKT4 was the most active among a series of seven CD4 mAbs (24) in eliciting eosinophil migration: net migration was 1,590 with OKT4, 1,120 with OKT4A, 870 with OKT4B, 1,260 with OKT4C, 1,190 with OKT4D, 1,310 with OKT4E, 1,220 with OKT4F, compared to 1,770 with 10^{-10} rLCF and 160 with UPC10 as IgG subclass control (means of two experiments, antibodies were tested at 0.5 μg/ml).

Monovalent F_{ab} fragments of OKT4 did not stimulate eosinophil motility. Binding of OKT4 F_{ab} competitively blocked the response of eosinophils to rLCF (Fig. 4). In contrast, W6/32 F_{ab}, which binds to another cell surface antigen on eosinophils, did not inhibit rLCF activity. These experiments suggest that LCF stimulates motility by binding to CD4 on eosinophils.

Effects of LCF on Other Eosinophil Functions and Surface Antigens. We evaluated the capacity of rLCF to stimulate responses of eosinophils in other in vitro assays. In some ex-

Table 1. *Checkerboard Analysis of Eosinophil Chemoattractant Activity of rLCF*

rLCF in lower chamber	in upper chamber:	Eosinophil migration index*			
		0	2×10^{-12} M	6×10^{-12} M	18×10^{-12} M
0		**211** ± 24	273 ± 61	491 ± 44	474 ± 79
2×10^{-12} M		1,336 ± 119	**1,197** ± 163	1,246 ± 38	1,421 ± 198
6×10^{-12} M		3,397 ± 174	2,833 ± 829	**3,459** ± 347	2,321 ± 129
18×10^{-12} M		2,448 ± 317	2,332 ± 459	2,365 ± 565	**2,041** ± 272

Results are representative of two similar experiments from a normal donor.
* mean ± SEM of triplicates.

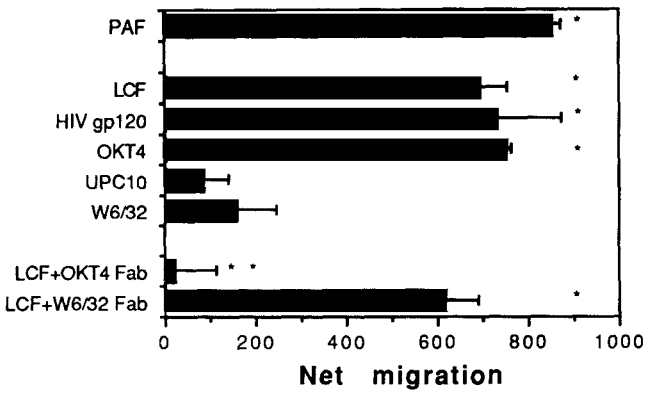

Figure 4. Effect of agents binding CD4 on eosinophil migration. Migration of eosinophils purified from a normal donor were measured in response to PAF (10^{-6} M), rLCF (4×10^{-12} M), HIV-1 r gp120 (1 μg/ml), OKT4 (CD4 mAb, 0.5 μg/ml), UPC10 (IgG2a myeloma protein, 0.5 μg/ml), W6/32 (mAb against class I MHC, 0.5 μg/ml), rLCF mixed with OKT4 F_{ab} (0.5 μg/ml), and rLCF mixed with W6/32 F_{ab} (0.5 μg/ml). Net migration was calculated as above. Migration index with medium alone was 899 ± 64 in the experiment shown. Error bars indicate SEM of triplicates. (*) indicates result significantly different from medium control ($p < 0.005$, Student's t test). (**) indicates result significantly different from rLCR ($p < 0.005$) and not significantly different from medium control. Results which are not marked by an asterisk are not significantly different from medium control ($p > 0.05$). Optimum concentrations of gp120, OKT4, and OKT4 F_{ab} were determined in three, six, and two prior experiments, respectively.

periments shown, rLCF-containing supernatant was used at high concentrations (up to 5×10^{-10} M, 300-fold greater than the ED_{50} in chemotaxis) in order to be certain that negative findings were not due to inadequate amounts of LCF. Other experiments used purified rLCF at lower concentrations (10^{-13} to 10^{-10} M) with similar results.

In vitro assays which relate to generation of inflammatory mediators by eosinophils include superoxide as a measure of oxidative burst activity, LTC_4 as a potently active arachidonate product selectively produced by eosinophils, and degranulation. rLCF did not stimulate eosinophil superoxide production (2.9 ± 0.1 nmol/10^6 cells with medium or rLCF tested up to 2×10^{-10} M), in contrast to rC5a (23.2 ± 1.1 with 10^{-8} M rC5a, compared to 38.2 ± 0.4 with 10^{-7} M PMA; results are mean ± SEM of triplicates, representative of six similar experiments). rLCF did not prime eosinophils for enhanced rC5a-triggered superoxide production, in contrast to rGM-CSF (the responses to 10^{-9} M rC5a were 5.4 ± 0.4 nmol/10^6 cells after preincubation with medium, 5.7 ± 0.1 after preincubation with 2×10^{-10} M rLCF, and 12.3 ± 0.1 after preincubation with 10^{-10} M rGM-CSF). The inability of rLCF to influence superoxide generation was found with eosinophils from HES and a normal donor. Ionophore A23187-triggered LTC_4 production was not significantly enhanced by preincubation with rLCF, in contrast to rGM-CSF (the responses were 20.3 ± 7.8 ng/10^6 eosinophils after preincubation with medium, 40.0 ± 13.7 after preincubation with 2×10^{-11} M rGM-CSF, and 23.6 ± 7.8, 32.1 ± 9.5, 27.6 ± 6.4, and 28.1 ± 8.0 after preincubation with 10^{-12} M, 10^{-11} M, 10^{-10} M, and 10^{-9} M rLCF, respectively; results are mean ± SEM of seven experiments with eosinophils from HES and normal donors). Appropriate controls indicated that rLCF did not increase the residual cell-associated LTC_4. Degranulation measured by arylsulfatase B release was 87 ± 2, 144 ± 6, and 856 ± 30 pmol/min/10^6 eosinophils with medium, 10^{-8} M rC5a, and 20 mg/ml opsonized zymosan, respectively. With LCF arylsulfatase B release was 90 ± 3 with 4×10^{-10} rLCF, 144 ± 11 with rC5a and rLCF simultaneously, and 129 ± 6 with rC5a after 60 min preincubation with rLCF (mean ± SEM of triplicates, representative of two experiments).

We next examined cell surface antigen expression during

Table 2. *Surface Expression of HLA-DR, CD25, and CD4 on Eosinophils Cultured with rLCF or rGM-CSF*

Donors	Conditions	HLA-DR	CD25	CD4
		(% positive/ΔMFI)	*(% positive/ΔMFI)*	*(% positive/ΔMFI)*
Normal‡	Not cultured	4 ± 1/0.3 ± 0.3	9 ± 6/2.4 ± 0.6	19 ± 3/3.1 ± 0.6
	Medium	9 ± 2/1.7 ± 1.0	13 ± 3/3.2 ± 1.0	21 ± 3/5.8 ± 0.9
	rGM-CSF	34 ± 9*/11.6 ± 3.4*	19 ± 9/5.4 ± 3.5	23 ± 6/6.5 ± 2.0
	rLCF	12 ± 4/2.8 ± 1.5	22 ± 8/6.1 ± 4.5	19 ± 5/5.2 ± 1.6
HES‡	Not cultured	13 ± 2/3.4 ± 0.8	33 ± 6/10.2 ± 2.4	24 ± 4/6.7 ± 1.3
	Medium	17 ± 3/4.6 ± 1.7	35 ± 5/10.3 ± 1.6	34 ± 5/9.9 ± 1.7
	rGM-CSF	32 ± 9*/10.6 ± 3.7*	36 ± 10/10.5 ± 3.3	37 ± 5/11.0 ± 1.7
	rLCF	17 ± 5/4.7 ± 1.9	40 ± 10/12.1 ± 3.4	33 ± 6/9.7 ± 2.1

The indicated cell surface antigens were analyzed by flow cytometry with purified eosinophils either before culture or after culture for 24–48 h with medium alone (RPMI 1640 with 10% FCS), rGM-CSF (10^{-11} M), or rLCF (5×10^{-10} M as a dilution of a COS cell supernatant). ΔMFI and % positive are defined in Materials and Methods.

* Indicated result is significantly different ($p < 0.05$) than either medium or not cultured by analysis of variance followed by blocked Newman-Kuehl's test.

‡ Results from normal donors are mean ± SEM of three experiments; results from HES donors are mean ± SEM of five experiments.

culture with rLCF. The eosinophil surface markers chosen were CR3 (31), because it is upregulated by cytokines such as GM-CSF (32); HLA-DR (33) and p55 IL-2 receptor (T.H. Rand, D.S. Silberstein, H. Kornfeld, and P.F. Weller, manuscript submitted for publication) because these surface antigens are induced by LCF in $CD4^+$ lymphocytes (12); and CD4 (5), because this receptor is required for responses to LCF (12, 13). Culture with rGM-CSF, rIL-3, or PMA enhanced eosinophil surface expression of CR3 (CD11b/CD18, a receptor for the opsonin C3bi and for intercellular adherence), but rLCF did not have this effect: CD11b expression for eosinophils cultured 24 h were medium, ΔMFI 84.0; 5×10^{-10} M rLCF, ΔMFI 84.0; 10^{-11} M rGM-CSF, ΔMFI 99.4; 10^{-11} rIL-3, ΔMFI 94.5; 10^{-10} M PMA, ΔMFI 109.0 (results are representative of two similar experiments). A similar pattern to that shown with 24-h cultures was seen at 1 h and 4 h with these stimuli. rLCF did not significantly upregulate eosinophil expression of the class II MHC antigen HLA-DR (which was enhanced by rGM-CSF), p55 IL-2 receptor subunit (CD25), or CD4 during 24–48-h cultures (Table 2). In some experiments, there were increases in CD25 expression after culture with rLCF, but these increases did not attain statistical significance when each group of experiments with HES or normal eosinophils were analyzed. In contrast to rGM-CSF, rLCF did not maintain the viability of eosinophils in vitro: survival of eosinophils from an HES donor at 7 d was 22% in medium or 10^{-12} to 10^{-9} M rLCF compared to 89% in 10^{-11} M rGM-CSF. When 5-d cultures of eosinophils with medium, rGM-CSF, or rLCF were analyzed for cell surface antigen expression with exclusion of nonviable cells by staining with propidium iodide, results were similar to those in Table 2. Taken together, the findings with rLCF indicate that it is highly selective in its effect on eosinophil motility and that a chemotactic response can be stimulated independently of metabolic effector function and expression of selected "activation" antigens by eosinophils.

Discussion

Our studies indicate that CD4 expressed on eosinophils can transduce stimulatory signals resulting in enhanced eosinophil migration and that eosinophils respond to the T lymphocyte product, LCF. For $CD4^+$ lymphocytes, LCF stimulates migration and induces expression of HLA-DR and p55 IL-2 receptors (12). Several lines of evidence indicate that CD4 is the receptor for LCF: (*a*) all responses elicited by LCF can be blocked by F_{ab} fragments of the CD4 mAb OKT4; (*b*) LCF binds to rsCD4; and (*c*) transfection of human CD4 into murine lymphoid cells confers responsiveness to LCF (12, 13).

Both natural and recombinant LCF stimulated eosinophil migration. Evidence that LCF-elicited responses of eosinophils were mediated by CD4 include the capacity of two other CD4-binding ligands, HIV-1 gp120 and the CD4 mAb OKT4, to stimulate eosinophil migration, the capacity of monovalent OKT4 F_{ab} to competitively inhibit LCF-elicited migration, and the inability of LCF to stimulate migration of neutrophils, which lack CD4.

rLCF was a potent eosinophil chemoattractant with ED_{50} 10^{-12} to 10^{-11} M, 100- to 1,000-fold less than ED_{50}s for two currently recognized eosinophil chemoattractants, rC5a and PAF. rLCF with eosinophils had both chemokinetic and chemotactic activities, and high-dose inhibition was seen at supraoptimal rLCF concentrations as seen with other leukocyte chemoattractants (34). Although eosinophil CD4 expression is less than that of $CD4^+$ lymphocytes, flow cytometric analyses of eosinophil surface antigens have consistently demonstrated a unimodal pattern of staining for CD4 without evidence for a distinct eosinophil subpopulation negative for CD4 (reference 5; T.H. Rand and P.F. Weller, unpublished results). Concordant with flow cytometric findings which suggest that all eosinophils express CD4, albeit at low levels, migration experiments are consistent with the possibility that most, if not all, eosinophils are responsive to LCF. Analyses of eosinophil migration by enumeration of the numbers of eosinophils at multiple levels in nitrocellulose filters suggest that rLCF elicited the migration of as many eosinophils as did rC5a or PAF.

Although LCF was a potent stimulus of eosinophil migration, LCF did not elicit or prime for metabolic effector responses in which inflammatory mediators are generated. Unlike $CD4^+$ mononuclear cells, which express HLA-DR and p55 IL-2 receptor following culture with LCF, no significant changes in surface markers of eosinophils cultured in LCF were found. Whether LCF might influence such responses in the presence of another agonist can only be answered by further study.

LCF is one of several lymphokines, including IL-2 and IL-5 (T.H. Rand, D.S. Silverstein, H. Kornfeld, and P.F. Weller, manuscript submitted for publication; reference 35) which are eosinophil chemoattractants in vitro and might participate in recruitment of eosinophils to T cell-dependent hypersensitivity reactions in vivo. Since the elaboration of LCF by T cells can be elicited by specific antigen or histamine stimulation (12, 15, 28), LCF may be a common mechanism in diverse immunologic reactions which culminate in the emigration of $CD4^+$ mononuclear cells and eosinophils from the circulation into sites of inflammation. Examples of pathologic lesions which involve T cell activation and eosinophil infiltration include granulomatous reactions to tissue-invasive helminth parasites, cutaneous and respiratory late-phase reactions to allergens, and chronic asthma (1, 36–39). Since eosinophils, like monocytes, lack the TCR and $p56^{lck}$, the capacity of LCF and other CD4-binding ligands to elicit migration of these nonlymphoid cell types indicates that CD4 can transduce signals independent of association with the TCR/CD3 complex and the tyrosine kinase $p56^{lck}$. CD4, expressed on eosinophils as well as mononuclear leukocytes, enables LCF to stimulate the migration of each of the $CD4^+$ leukocytes: eosinophils, monocytes, and T lymphocytes.

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