Brief Definitive Report

HUMAN EOSINOPHILS EXPRESS CD4 PROTEIN AND BIND HUMAN IMMUNODEFICIENCY VIRUS 1 gp120

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The CD4 molecule, an ~55-kD glycoprotein initially detected with specific mAbs on the surface of a helper/inducer subset of human T lymphocytes (1, 2), is encoded by a single gene and is of invariant structure (3, 4). The function of CD4 on T cells is not completely understood; CD4 can mediate interactions of T cells with cells bearing class II MHC proteins (4, 5) and can function as a signal-transducing protein that modulates T cell activation (6, 7). CD4 binds the gp120 protein of HIV-1, and serves as a cellular receptor for HIV-1 (8) and HIV-2 (9). In addition to its expression on subsets of T lymphocytes, CD4 is expressed on monocytes and cells of monocyte/macrophage lineage, on which CD4 also functions as a receptor for HIV (2, 10, 11).

Eosinophils and neutrophils are distinct classes of bone marrow–derived granulocytic leukocytes; in contrast to neutrophils, eosinophils are longer lived and are predominantly tissue-dwelling cells (12). Eosinophils localize primarily to tissues that interface with the external environment, including the respiratory, gastrointestinal, and genitourinary tracts (12). Numbers of eosinophils increase in the blood and tissues in association with certain disease processes, and eosinophils are prominent in immunologic responses during allergic (13) and helminthic parasitic diseases (14, 15). In this report we demonstrate that human eosinophils express CD4.

Materials and Methods

Eosinophil Isolation and Culture. Eosinophils were obtained with informed consent from five eosinophilic (the hypereosinophilic syndrome, donors 1 and 2; *Loa loa* filariasis, donors 3 and 4; asymptomatic infection with HIV, donor 5) and four normal donors. For donor 1 eosinophils from leukapheresis were enriched by sedimentation over Hypaque-Ficoll (Pharmacia Fine Chemicals, Piscataway, NJ); eosinophils from other donors were purified by Percoll (Pharmacia Fine Chemicals) density gradient centrifugation (16) to the percentages indicated, as assessed by staining with fast green/neutral red and phloxine/methylene blue (13). Contaminating cells before culture (day 0) were neutrophils. 1.5×10^6 cells were cultured in 3 ml RPMI 1640, 10% FCS, and 50 pM recombinant human granulocyte/macrophage

This work was supported by grants AI-20241, AI-23826, and HL-33768 from the National Institutes of Health. A. N. -Weller is a Scholar of the Leukemia Society of America and P. F. Weller is an Established Investigator of the American Heart Association. Address correspondence to D. R. Lucey, Division of Infectious Diseases, Wilford Hall Medical Center, Lackland Air Force Base, TX 78236.

Opinions expressed herein are those of the authors and do not purport to express views of the Department of the Air Force or any other Department or Agency of the United States government.

CSF (rhGM-CSF) (Genzyme, Boston, MA) on a monolayer of Swiss 3T3 fibroblasts, as described (17). 1.5 ml of fresh RPMI/rhGM-CSF were exchanged on days 2, 5, and 7. CD4 expression on 10^4 cells was measured by flow cytometry (FACScan; Becton Dickinson & Co., Mountain View, CA) with fluoresceinated anti-Leu-3a and -b (Becton Dickinson & Co.) and is expressed relative to a subclass control mAb. Eosinophil granule staining and nuclear morphology were evaluated by staining with fast green/neutral red and phloxine/methylene blue (13). The absence of both lymphocytes and monocytes was confirmed by finding no more than background cellular staining by flow cytometric analyses with two fluoresceinated mAbs (anti-Leu-1, for CD5 on T cells and anti-Leu-M3 for monocytes/macrophages; both from Becton Dickinson & Co.).

Biosynthetic Labeling of Eosinophil CD4. Eosinophils (8.7×10^6, >99% eosinophils, 97% $CD4^+$) from a hypereosinophilic donor were harvested after 15 d of culture with rhGM-CSF and 3T3 fibroblasts, as described above. After washing in PBS eosinophils were resuspended in 1 ml HBSS and pulse labeled for 1 h with 700 μCi ^{35}S-methionine (Tran^{35}S-label; ICN Radiochemicals, Costa Mesa, CA) at 37°C, 5% CO_2. Eosinophils were lysed in RIPA buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS) for 10 min at 4°C. After centrifugation (16,000 *g*, 15 min, 4°C), the soluble extract was immunoabsorbed in parallel with 0.6 μg control antibody (UPC 10; Cappel Laboratories, Malvern, PA) or 0.5 μg anti-OKT4 or anti-OKT4A (both from Ortho Diagnostic Systems, Inc., Westwood, MA). After 2 h at 4°C, 50 μl protein A-Sepharose (Genzyme) was added for 45 min. Protein A-Sepharose complexes were washed three times with RIPA buffer and once with water and then boiled for 3 min in Laemmli sample buffer (18). Proteins were resolved on SDS-PAGE gels (1% SDS, 10% polyacrylamide) and visualized by fluorography of gels for 3 d at −70°C with Kodak X-OMAT film.

HIV-1 GP120 Binding. Cells from donor 1 were maintained in culture for 10 d with 50 pM rhGM-CSF/3T3 fibroblasts, as described above, and then collected (>99% eosinophils, >99% viable). 1.5×10^6 eosinophils in 1 ml of Ca^{2+}, Mg^{2+}-free HBSS were incubated at 37°C for 30 min with or without 10 μg of partially purified HIV-1 gp120, which had been immunoaffinity purified from culture fluids of HTLV-IIIB-infected H9 cells. Eosinophils were pelleted, resuspended, and stained for 30 min at 4°C with 10 μl of anti-OKT4, anti-OKT4A (both from Ortho Diagnostic Systems, Inc.) or control UPC-10 (Cappel Laboratories) mAb. After two washes in HBSS with 0.1% BSA, cells were stained at 4°C for 30 min with fluoresceinated goat anti-mouse Ig (Becton Dickinson & Co.), washed twice with HBSS/0.1% BSA, fixed with 1% paraformaldehyde, and analyzed by flow cytometry.

Results and Discussion

Eosinophils were isolated from the blood of nine donors (Table I). After depletion of mononuclear leukocytes and further purification, eosinophil-enriched leukocytes were incubated with fluoresceinated mAbs anti-Leu-3a and -b, which recognize epitopes on CD4. By flow cytometry, CD4 expression was demonstrable on the eosinophil-enriched cells (Table I, Fig. 1). Eosinophils derived from the blood of all donors, both normal and those with heightened eosinophilia due to parasitic infections and idiopathic diseases, expressed CD4. Greater percentages of eosinophils from eosinophilic donors, in comparison with normal donors, were $CD4^+$. Cytochemical staining demonstrated neutrophils to be the sole contaminant of the eosinophil-enriched granulocytes, and flow cytometric analyses with fluoresceinated anti-T cell and anti-monocyte/macrophage mAbs confirmed the absence of lymphocytes and monocytes.

The eosinophil-enriched cells were placed in culture with rhGM-CSF on monolayers of 3T3 fibroblasts, and residual contaminating neutrophils died in the early days of culture, as previously reported (17). By day 5 of culture >99% of all cells, except the fibroblasts, were eosinophils, and these eosinophils from all donors expressed

TABLE I

Blood Eosinophilia of Donors and CD4 Expression of Eosinophils Before and During Culture

Donor	Diagnosis	Eosinophilia of donor blood		Percent eosinophils on day 0	Percent $CD4^+$ eosinophils				
		No. of eosinophils/mm^3	Percent eosinophils		0*	2	3	5	7
1a	Hypereos	10,430	74	86	83	78	76	78	92
1b		12,260	63	90	46		81	85	
2a	Hypereos	13,800	46	93	11	36	41	66	54
3a	Filariasis	960	17	73	48	18	19	26	20
4a	Filariasis	7,300	52	71	15			47	50
4b		10,300	63	94	23	45		49	
5a	Idiopathic	1,870	11	67	9	11		21	
6a	Normal	500	5	67	13	8	14	9	18
7a	Normal	500	5	90	14	13	8	18	29
8a	Normal	200	2	71	10	13	9	9	6
9a	Normal	200	2	80	5	36	10	12	7

Eosinophils from five eosinophilic and four normal donors were isolated and enriched to the percentages indicated before culture on day 0. Eosinophils were maintained in culture with rhGM-CSF and 3T3 fibroblasts, as described in Materials and Methods. CD4 expression on 10^4 cells was measured by flow cytometry and is expressed relative to a subclass control mAb.

* Day of culture.

CD4 (Table I, Fig. 1). The lack of other contaminating leukocytes during culture was established by cytochemical staining and again by an absence of staining with the fluoresceinated anti-T cell and anti-monocyte/macrophage mAbs. Cultured eosinophils were pulse labeled with ^{35}S-methionine, and detergent extracts of these cells were immunoprecipitated with anti-OKT4 and anti-OKT4A, which react with different epitopes on CD4 (19, 20). Both mAbs precipitated a ^{35}S-labeled 55-kD polypeptide (Fig. 2). In separate immunoprecipitation studies with anti-OKT4, the

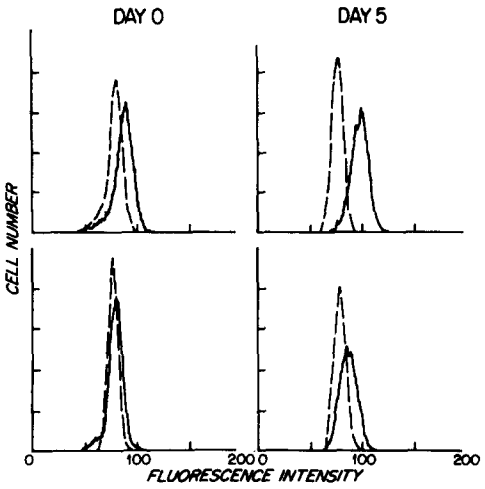

FIGURE 1. Flow cytometric analysis of CD4 expression on eosinophils. Eosinophil-enriched leukocytes were obtained and analyzed on day 0 before culture (*left panels*) and after 5 d in culture with rhGM-CSF/3T3 fibroblasts (*right panels*). The top panels are from cells from donor 1b and the bottom panels from donor 4b. Flow cytometry of 10^4 cells was performed after cells were stained with either anti-CD4 mAbs (anti-Leu-3a and -b) (*solid lines*) or a subclass control mAb (*dashed lines*). Linear fluorescence intensity is in arbitrary units.

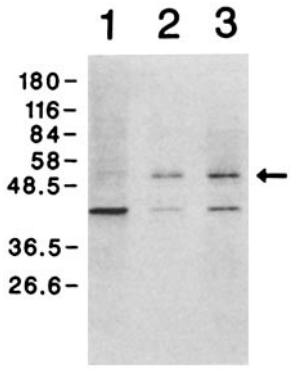

FIGURE 2. SDS-PAGE of CD4 (*arrow*) from cultured, ^{35}S-methionine-labeled eosinophils immunoprecipitated with either nonspecific control (lane *1*) or specific anti-OKT4 (lane *2*) or anti-OKT4A (lane *3*) mAbs. M_r ($\times 10^{-3}$) standards are on the left.

CD4 precipitated from eosinophils comigrated with ^{35}S-labeled PBMC CD4 on SDS-PAGE (data not shown) with an M_r identical to that previously determined for CD4 on monocytes and lymphocytes (2). Immunoprecipitation from eosinophils of metabolically-labeled molecules with an M_r of 55,000 confirmed the identity of the protein recognized by the anti-CD4 mAbs and also established that mature eosinophils, often considered end-stage terminally differentiated cells, retain the capacity to synthesize new proteins, including CD4.

Like the CD4 molecule on human lymphocytes and monocytes, eosinophil CD4 also binds the HIV-1 gp120 envelope glycoprotein. HIV-1 gp120 competitively blocked the staining of eosinophils with mAb OKT4A, which recognizes a gp120 binding epitope on CD4, but not the staining with OKT4, an mAb to a region of CD4 not involved in gp120 binding (11, 19, 20) (Fig. 3). Eosinophils staining with anti-OKT4A decreased from 85.4% to 26.6% after gp120 binding but anti-OKT4 staining was unchanged from 90.8% to 89.6% with gp120. Similar results were obtained at two concentrations of gp120 (10 and 17 μg/ml).

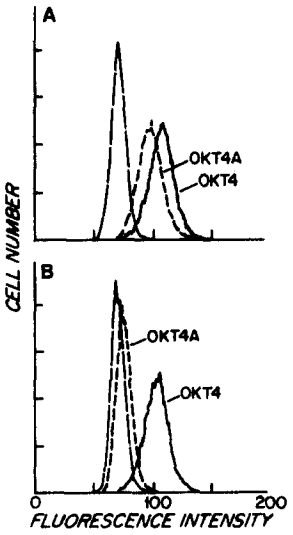

FIGURE 3. HIV-1 gp120 binding to CD4$^+$ eosinophils. *A* represents eosinophils not incubated with gp120 and *B* represents those that were incubated with gp120. Staining with mAb OKT4A, which recognizes a gp120 binding epitope on CD4, and with OKT4, an mAb to a region of CD4 not involved in gp120 binding, are presented relative to a subclass control mAb.

These findings demonstrate that human eosinophilic leukocytes express CD4 protein, previously recognized on leukocytes belonging to subsets of T lymphocytes or to monocyte/macrophage lineage. The expression of CD4 by eosinophils could allow these $CD4^+$ eosinophils to interact with class II MHC-bearing cells and the CD4 molecule could function as a signal-transducing ligand on these cells, as it can on other $CD4^+$ cells (8). Moreover, the expression on eosinophils of CD4 with its capacity to bind HIV gp120 may render the eosinophil susceptible to infection with HIV (21). Indeed, the eosinophil's predominant localization in tissues of the gastrointestinal and genitourinary tracts could expose this cell in these sites to inocula of sexually transmitted HIV. Further, increased blood and tissue eosinophilia are usual concomitants of infections with metazoan parasites, as in donors 3 and 4 with *L. loa* infections acquired in Africa. Many individuals from areas of the world, including tropical Africa, where intestinal and extra-intestinal helminthic parasitic infections are endemic, would have augmented numbers of eosinophils. The heightened numbers of $CD4^+$ eosinophils in patients with chronic helminthic infections could provide an increased population of cells susceptible to HIV infection, which would have bearing on the epidemiology, transmission, and clinical manifestations of these retroviral infections.

Summary

The CD4 glycoprotein, expressed on leukocytes belonging to subsets of T lymphocytes and to cells of monocyte/macrophage lineage, participates in the functioning of T cells and serves as a receptor for HIV-1 and HIV-2. Human eosinophils, a class of granulocytic leukocytes, have been found to express CD4. With anti-CD4 mAbs CD4 was demonstrable on eosinophils from both normal and eosinophilic donors. Eosinophils synthesized a 55-kD CD4 polypeptide immunoprecipitable with two anti-CD4 mAbs. Eosinophil CD4 bound HIV-1 gp120 as assessed by competition for anti-OKT4A, but not anti-OKT4, mAb binding. Eosinophils, normally rich in gastrointestinal and genitourinary tract tissues, increase in numbers in patients with metazoan parasitic infections. In these sites and diseases, CD4 expression by eosinophils may be pertinent to their immunologic functions and could make these cells susceptible to HIV infection.

We thank Drs. Stephen W. Pyle and Larry O. Arthur of Program Resources Inc., National Cancer Institute, Frederick Cancer Reseach Facility, for providing the gp120; Bridget Greaves, Susanne Picard, Lorna Muramoto, and Gail Raymond for technical assistance; and Michael Verronneau for his contributions.

Received for publication 14 September 1988.

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