FORMATION OF CHARCOT-LEYDEN CRYSTALS
BY HUMAN BASOPHILS*

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Charcot-Leyden crystals (CLC) are characteristically associated with diseases involving tissue or blood eosinophilia and are considered to be a hallmark of eosinophil involvement in the tissue reactions of asthma, myeloid leukemia, and allergic and parasitic diseases (1–3). The protein comprising the CLC has been shown to possess lysophospholipase activity (lysolecithin acylhydrolase, EC 3.1.1.5), and purified human eosinophil lysophospholipase crystallizes to form the hexagonal bipyramidal crystals typical of CLC (4). The CLC protein is a slightly acidic molecule of 13,000 mol wt (5, 6), with one free sulfhydryl group (6) that is required for its enzymatic activity (7).

Although CLC are currently thought to be distinctive to eosinophils (1, 2, 8), the uniqueness of CLC to eosinophils has been questioned. Archer and Blackwood (9) reported that CLC could be prepared from human basophils, and they described the formation of CLC from both basophil extracts and intact basophils suspended in hypotonic sodium chloride. Because the basophils they studied were obtained from a patient with tropical eosinophilia and their identity as basophils was not demonstrated histochemically, their findings have been dismissed in the literature as due to contamination of basophil preparations with eosinophils, which in hypereosinophilic syndromes may show aberrant staining of the granules (1). Bessis and Tabuis (10) also reported that basophils form small, fusiform, nonstaining crystals similar to CLC after exposure to a hypotonic sodium chloride solution.

The present study was conducted to test the hypothesis that CLC and the protein comprising the crystal may be derived from human basophils. Using a radioimmunoassay specific for the human eosinophil CLC protein (6) and a double-antibody immunofluorescence procedure, we showed that human basophils contain a protein immunochemically identical to that of the eosinophil CLC protein. In addition, we

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1 Abbreviations used in this paper: CLC, Charcot-Leyden crystal(s); FACS, fluorescence-activated cell sorter; HBSS-EDTA-BSA, Hanks' balance salt solution with 3 mM EDTA and 0.1% bovine serum albumin; HSA, human serum albumin; MBP, eosinophil granule major basic protein; PBS, Dulbecco's phosphate-buffered saline.
demonstrated the formation of crystals in basophils that are morphologically indistinguishable from eosinophil CLC.

Materials and Methods

Preparation of Basophil, Eosinophil, Neutrophil, and Mononuclear Rich Cell Suspensions. Highly enriched basophil suspensions for observation of spontaneous CLC formation and for immunofluorescence localization of CLC protein were prepared by flow microfluorometry using a fluorescence-activated cell sorter (FACS IV, B-D FACS Systems, Becton, Dickinson & Co., Sunnyvale, CA) (G. J. Weil and T. M. Chused, manuscript in preparation). Briefly, mononuclear cells (11) from normal venous blood were labeled with a 1/50 dilution of the IgG fraction of fluoresceinated sheep anti-human IgE (N. L. Cappel Laboratories Inc., Cochranville, PA) in Hanks' balanced salt solution (HBSS) (Gibco, Grand Island, NY) with 5 mM EDTA and 0.1% bovine serum albumin (HBSS-EDTA-BSA) (Sigma Chemical Co., St. Louis, MO) at 0°C for 30 min. After washing in HBSS-EDTA-BSA, the cells were sorted using the FACS IV. Sorted cells were stained for basophils with alcian blue and counted in a hemocytometer, as described by Gilbert and Ornstein (12). Differential cell counts were performed on both cytocentrifuge slides fixed with absolute methanol and stained with Wright's stain or fixed with Mota's fixative and stained with toluidine blue (13). 400 cells were counted at a magnification of × 1,000.

Cell suspensions used for the preparation of freeze-thaw extracts were prepared in the following manner: eosinophils and neutrophils were separated with an FACS (FACS II, Becton, Dickinson & Co.), using differential cell autofluorescence as previously described (14). Highly enriched basophil suspensions were prepared by flow microfluorometry in a similar manner as described above. Briefly, mononuclear cells were labeled with a 1/100 dilution of fluoresceinated goat anti-human IgE (N. L. Cappel Laboratories Inc.). After washing, the cells were sorted using the FACS II, counted, and samples were frozen at −70°C in HBSS-EDTA-BSA. Cells were counted with a Coulter Counter (Coulter Electronics Inc., Hialeah, FL), and basophils were stained with alcian blue (12). Sorted basophils contained normal amounts of histamine and were >90% viable, as determined by trypan blue exclusion (G. J. Weil and T. M. Chused, manuscript in preparation). Differential cell counts of eosinophils, neutrophils, and mononuclear cells were performed on smears stained with 1% fast green (Harleco, American Hospital Supply Corp., Gibbstown, NJ) in methanol for 1 min, followed by 1% neutral red (Harleco) in distilled water for 5 min. 200 cells were counted at a magnification of × 400.

Immunofluorescence Localization of CLC Protein in Eosinophils and Basophils. Cytocentrifuge slides of human peripheral blood buffy coat cells, Ficoll-Hypaque interface cells, or FACS-purified preparations of basophils were stained for CLC protein by an immunofluorescence procedure previously described (15). Briefly, slides were fixed for 10 min in absolute methanol, washed three times with Dulbecco's phosphate-buffered saline (PBS) (5 min per wash) in staining dishes on a rocker platform, and incubated overnight in 10% heat-inactivated normal goat serum in PBS at 4°C. Slides were washed three times with PBS, placed in a humidified chamber, overlaid with a 1/40 dilution of either control normal rabbit sera, preimmunization sera from rabbits immunized with CLC protein, or rabbit anti-CLC protein, and incubated overnight at 4°C. Slides were washed three times with PBS, stained for 30 min with a 1% solution of chromotrope 2R (15670; Harleco) at room temperature, and washed again with PBS. In the second stage of the immunofluorescence assay, the slides were overlaid with a 1/40 dilution of IgG fraction of fluorescein-conjugated goat anti-rabbit IgG (13823; N. L. Cappel Laboratories Inc.) and incubated for 30 min at 37°C. Slides were washed three times with PBS, mounted in 50% 0.01 M Tris and 50% glycine solution, coverslipped, and sealed with nail polish. Slides were examined with a Zeiss standard microscope (Carl Zeiss, Inc., Oberkochen, West Germany) equipped with Zeiss IV FL vertical illumination for epifluorescence and fluorescein filter system (487710; Zeiss). Immunofluorescence was photographed using Kodak Ektachrome 400 ASA slide film (Eastman Kodak Co., Rochester, NY) and exposure times of 60–120 s. The background fluorescence resulting from the treatment of Ficoll-Hypaque interface cells with fluoresceinated sheep anti-human IgE (Fc epsilon specific) before cell sorting in the FACS was minimal and did not interfere with the bright specific staining for CLC protein.

Specificity Controls for Immunofluorescence Staining of CLC Protein. Two types of specificity
controls were used for immunofluorescence staining of CLC protein in eosinophils and basophils. First, preimmunization sera from individual rabbits were compared with the immunofluorescence obtained with sera from the same rabbits after immunization with CLC protein. Second, rabbit anti-CLC sera were absorbed with human serum albumin-Sepharose (HSA), human eosinophil granule major basic protein-Sepharose (MBP), CLC protein-Sepharose, or Sepharose alone before their use as the first stage reagent for immunofluorescence. Sepharose 4B and CL-6B were activated by cyanogen bromide; 2 mg of HSA or MBP was coupled to 1 ml of Sepharose 4B, and 4.4 mg of CLC protein was coupled to 10 ml Sepharose CL-6B. Uncoupled sites were blocked with 0.1 M lysine monohydrochloride. Protein-Sepharose conjugates were washed with 0.05 M glycine-HCl buffer, pH 3.0, and used to absorb sera (1 ml conjugate to 2 ml of a 1:10 serum dilution).

Preparation of Cell Extracts. Basophil, eosinophil, neutrophil, and mononuclear cell-enriched suspensions in HBSS-EDTA-BSA were subjected to five successive freeze-thaw cycles using dry ice acetone and 37°C water bath, with vortexing between each cycle. Extracts were centrifuged at 12,000 g for 10 min. The resulting clear supernatants were stored on ice, and CLC protein levels were measured immediately by radioimmunoassay.

Measurement of CLC Protein Levels in Cell Extracts by Radioimmunoassay. CLC protein concentrations in the cell extracts were measured by a double-antibody radioimmunoassay, as previously described (6). Briefly, the radioimmunoassay was performed by adding 0.1 ml of a 5 × 10⁻³ dilution of rabbit anti-CLC antibody, 0.5 ml assay buffer, and 0.1 ml of dilutions of the cell extracts or purified CLC protein to 10-× 75-mm glass tubes. The contents were incubated at 37°C for 30 min, after which 1 ng [¹³¹]CLC was added to each tube. Solutions were incubated overnight at 4°C, and the resulting immune complexes were precipitated by the addition of 0.1 ml of a 1:20 dilution of normal rabbit serum and 0.1 ml of a burro anti-rabbit IgG antiserum. Tubes were incubated at 4°C for 2 h and centrifuged for 20 min at 2,500 g at 4°C. The supernatants were decanted, and the sediments were counted in a Nuclear Chicago gamma scintillation counter (Searle Radiographs Inc., Des Plaines, IL). Data were analyzed by unweighted logit-log transformation using a Hewlett-Packard 9845B computer (Hewlett-Packard Co., Palo Alto, CA) with radioimmunoassay program 09845-14254 and regression analysis methods program 09845-15014.

Results

CLC Formation in Basophil-enriched Cell Suspensions. To determine whether human basophils formed crystals of similar morphology to eosinophil CLC, we prepared basophil-enriched cell suspensions using the FACS to enrich for surface IgE-positive leukocytes from peripheral blood mononuclear cells of normal individuals. These cell suspensions contained 68% (experiment 1) and 54% (experiment 2) basophils, as determined by alcian blue staining, and no eosinophils were present (Table I). Basophil-enriched cell suspensions were incubated on ice for 24 or 48 h in either PBS (experiment 1) or HBSS-EDTA-BSA (experiment 2) and examined for crystal formation (Fig. 1). At both 24 and 48 h, we observed the formation of crystals in many of the cells (Fig. 1 a). These crystals were morphologically similar to those previously described as forming from both eosinophils (9, 16–18) and basophils (9, 10). When basophil-enriched suspensions containing cells with crystals were pelleted onto microscope slides and stained with toluidine blue, we observed numerous intact basophils with characteristic metachromatic granules as well as many basophils that contained nonstaining, crystalloid inclusions typical of CLC (Fig. 1 b, c).

Detection of CLC Protein by Immunofluorescence. To determine whether basophils contained immunoreactive CLC protein, we stained cyt centrifuge slides of human buffy coat cells, Ficoll-Hypaque interface cells, or FACS-enriched preparations of basophils for CLC protein by immunofluorescence. When buffy coat cells were stained for CLC protein, a small percentage of the cells stained with bright fluorescence (Fig.
TABLE I
Analyses of Purified Basophil Preparations by Specific Histochemical Staining and by Immunofluorescence Staining for CLC Protein

| Differential cell counts* | Percent basophils | Percent positive cells |
|---------------------------|-------------------|------------------------|
|                           | Alcian blue‡      | Toluidine blue§        | Wright's stain§  | Immunofluorescence for CLC protein† |
| Experiment 1              | 68                | ND¶                    | 60              | 33                                      |
| Experiment 2              | 54                | 73                     | 64              | 66                                      |

* Differential cell counts of FACS IV-purified basophils by alcian blue staining of cells in suspension or staining of cytocentrifuge slides with toluidine blue, Wright's stain, or immunofluorescence for CLC protein. Only intact cells were counted. There were no eosinophils in these preparations, and contaminating cells consisted of surface IgE-positive small lymphocytes and monocytes.
‡ Hemocytometer counts.
§ Differential count on 400 cells.
¶ Differential count on 1,000 cells (experiment 1) or 300 cells (experiment 2).
† Not done.

2 a). These cells were identified as eosinophils by counterstaining the immunofluorescence slides with Wright's stain (Fig. 2 b). Eosinophils fluoresced brightly when stained with specific anti-CLC antibody but did not stain when normal rabbit sera or the preimmunization sera from CLC protein-immunized rabbits was used in the first stage of the immunofluorescence procedure. Polymorphonuclear neutrophils, erythrocytes, and mononuclear cells (lymphocytes and monocytes) did not fluoresce when specific anti-CLC antibody was applied. However, an occasional cell could be found that fluoresced with anti-CLC antibody but could not be subsequently identified as an eosinophil after the counterstain with Wright's stain. These cells showed diffuse cytoplasmic fluorescence (Fig. 2 c), and when counterstained with Wright's stain, they had a nuclear morphology characteristic of a polymorphonuclear leukocyte (Fig. 2 d).

When cytocentrifuge slides of Ficoll-Hypaque interface cells were stained with anti-CLC antibody, a small percentage of cells fluoresced brightly with diffuse cytoplasmic staining (Fig. 3 a, c). The Ficoll-Hypaque interface cell population was primarily mononuclear (lymphocytes and monocytes) and was slightly enriched for basophils (~3%), as determined by alcian blue staining of cells in suspension. This cell population did not contain any eosinophils or neutrophils, as determined by a differential cell count of Wright's stained cells. When these slides were counterstained with Wright's stain, the morphology of the fluorescent cells was not distinctive, although they possessed polymorphic nuclei (Fig. 3 b, d).

To determine whether enrichment for basophils also enriched for cells staining positively by immunofluorescence for CLC protein, immunofluorescence staining was performed on cytocentrifuge slides of FACS-purified basophil suspensions. As shown in Table I, >50% of the surface IgE-positive cell population in both experiments 1 and 2 were basophils, as assessed by staining with alcian blue or by differential counts performed on cytocentrifuge slides stained with toluidine blue or Wright's stain. Differential cell counts performed on duplicate slides stained for CLC protein by
Spontaneous CLC formation in normal human blood basophils. 10 µl of cell suspension was placed on a precleaned microscope slide, coverslipped, and sealed with nail polish. Cells were observed for crystal formation over a period of 1 h using a Zeiss standard microscope with phase-contrast optics. (a) Phase-contrast photomicrograph (X 1,000) of a normal human basophil suspended in PBS for 24 h, showing a CLC in the cytoplasm. (b) Photomicrograph of basophil on a cytocentrifuge slide prepared from the above cell suspension in part a, fixed with Mota's fixative for 1 min and stained with toluidine blue. Note the distinctive, nonstaining CLC in the cytoplasm (arrow), the granularity of the cytoplasm, and nuclear morphology typical of the blood basophil. (c) Same preparation as in part b, showing two basophils, one of which contains a typical, nonstaining CLC inclusion (arrow).
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Fig. 2. Immunofluorescence staining of human peripheral blood leukocytes for CLC protein. (a) Fluorescence photomicrograph (× 400) of a cytocentrifuge slide of peripheral blood buffy coat cells stained for CLC protein. Note the two cells with bright membrane-like staining around the periphery of the cells. (b) Identical field as in part a, counterstained with Wright's stain, showing that the two fluorescent cells in part a are eosinophils (arrows). Note that mononuclear cells and neutrophils do not stain. (c) Another field from the same slide as in part a, showing four brightly fluorescent cells. Three of these cells show the same pattern of fluorescent staining, while a fourth (arrow) shows a more diffuse cytoplasmic staining. (d) Identical field as in part c, counterstained with Wright's stain, showing that three of the fluorescent cells are eosinophils (closed arrows), whereas the fourth cell (open arrow) does not show the distinctive morphology of the eosinophil lacking the bilobed nucleus and granules.

Immunofluorescence showed that >50% of the cells in these preparations fluoresced brightly. Therefore, enrichment for surface IgE-positive cells (primarily basophils) by FACS also enriched for cells staining positively for CLC protein by immunofluorescence (Table I).

Cytocentrifuge slides prepared from suspensions of FACS-purified basophils that had formed crystals were also stained for CLC protein by immunofluorescence. As shown in Fig. 4 a, cells containing crystals typical of CLC were observed under phase-contrast illumination. These same cells showed positive fluorescent staining for CLC protein, with bright glowing around the perimeter of the crystal inclusion (Fig. 4 b). In general, intact cells without crystals stained more brightly than did those that contained CLC, possibly because of depletion of CLC protein in the cell and concentration of the protein in the crystal (Fig. 4 c). When cells containing crystals
Fig. 3. Immunofluorescence staining of Ficoll-Hypaque interface cells for CLC protein. (a and c) Fluorescence photomicrographs (× 400, × 1,000, respectively) of a cytospin slide of Ficoll-Hypaque interface cells containing basophils, lymphocytes, and monocytes but no eosinophils. Note the bright fluorescent cells with diffuse cytoplasmic and perinuclear staining. (b and d) Identical fields as in parts a and c, counterstained with Wright's stain, showing the polymorphonuclear appearance of the cells (arrows), presumably basophils, the granules of which have been washed out and are no longer evident. Note that the mononuclear cells do not stain.
FIG. 4. Phase-contrast and immunofluorescence photomicrographs of purified basophils containing CLC. Slides were examined first by phase-contrast illumination to identify cells containing crystals. Cells containing crystals were photographed, and the same field was examined under fluorescent illumination for positive fluorescent staining of cells and crystals. (a and d) Phase-contrast photomicrographs (× 1,000) of cells from purified basophil suspensions incubated for 24 h on ice. Note the dark CLC inclusions in the cells (arrows). (b) Immunofluorescence staining for CLC protein of the same field as in part a, using rabbit anti-CLC in the first stage of the immunofluorescence assay. Note the characteristic polymorphonuclear appearance of the basophil with diffuse cytoplasmic staining and staining around the periphery of the CLC (arrow). (c) Fluorescent staining of two basophils, one of which contains a CLC. Note the bright cytoplasmic staining of the cell at the bottom, as compared with the lower intensity cytoplasmic fluorescence of the cell containing the CLC. Also note the bright staining around the periphery of the crystal (arrow). (e) Immunofluorescence staining of the same field as in part d, using the preimmunization serum in the first stage of the immunofluorescence assay. Note the absence of any fluorescent staining of the cell or its CLC.
were stained with preimmunization sera in the first stage, positive fluorescent staining was not observed (Fig. 4 d, e).

The specificity of immunofluorescence staining for CLC protein in basophils and eosinophils from Ficoll-Hypaque interface cells or buffy coat cells, respectively, was tested by absorbing rabbit antisera with various solid-phase protein-Sepharose conjugates (Table II) and using these absorbed sera in the first stage of the immunofluorescence assay. Absorption with HSA-Sepharose, MBP-Sepharose, or unconjugated

| Serum tested           | Absorption§ | Fluorescence‡ |
|------------------------|-------------|---------------|
|                        |             | Basophil      | Eosinophil   |
| Anti-CLC protein       | None        | +             | +            |
| Anti-CLC protein       | Unconjugated Sepharose | +     | +            |
| Anti-CLC protein       | HSA-Sepharose | +             | +            |
| Anti-CLC protein       | MBP-Sepharose | +             | +            |
| Anti-CLC protein       | CLC protein-Sepharose | -      | -            |
| Preimmunization rabbit serum | None       | -             | -            |

* The specificity of immunofluorescent staining for CLC protein in basophils and eosinophils was tested by staining cytocentrifuge slides of interface cells (primarily mononuclear cells and basophils) from Ficoll-Hypaque gradient centrifugation or of unseparated peripheral blood buffy coat cells, respectively. The identity of the fluorescent cells in buffy coat preparations as eosinophils was confirmed by counterstaining the slides with Wright's stain as in Fig. 2.

† Symbols: +, very bright fluorescent staining of cells; -, absence of fluorescent staining of cells.

§ Rabbit anti-CLC protein was absorbed with the various solid-phase immunosorbents before use as the first-stage reagent for immunofluorescence.

**Table III**

Measurement of CLC Protein Levels in Freeze-Thaw Extracts of Human Leukocytes by Radioimmunoassay *

| Cell extract tested§ | Total number of cells (× 10⁶) | Differential cell counts‡ | ng CLC/extract | pg CLC/cell | pg CLC/Baso | pg CLC/Eos |
|----------------------|-------------------------------|---------------------------|----------------|-------------|-------------|-----------|
| Experiment 1 Basophil| 1.50                          | 99                        | 0              | 1           | 0           | 448       | 3.0       | 2.98      | —         |
| Eosinophil           | 6.40                          | 0                         | 90             | 0           | 10          | 4921      | 7.7       | —         | 8.5       |
| Neutrophil           | 41.60                         | 0                         | 1.5            | 0           | 98.5        | 860       | 0.2       | —         | 13.8      |
| Experiment 2 Basophil| 0.35                          | 97                        | 0              | 3           | 0           | 1085      | 31.0      | 31.9      | —         |
| Unseparated Mononuclear| 36.50                    | 3                         | <1             | 97          | 0           | 1285      | 0.4       | —         | —         |

* CLC protein levels in supernatants of freeze-thaw extracts of basophil, eosinophil, neutrophil, and mononuclear cell-enriched preparations were determined by radioimmunoassay. Cells were extracted by five successive freeze-thaw cycles using dry ice acetone and 37°C water bath with vortexing between each cycle, followed by centrifugation for 10 min at 12,000 g.

‡ Differential cell counts were performed as described in Materials and Methods.

§ Cells were extracted in total volumes of 0.28, 0.19, and 0.4 ml for basophil, eosinophil, and neutrophil preparations in experiment 1, and volumes of 0.5 ml for the basophil and unseparated mononuclear cell preparations in experiment 2.
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Fig. 5. Inhibition of the binding of $[^{125}\text{I}]\text{CLC}$ to specific rabbit antibody by CLC protein standard and freeze-thaw extracts of human basophils, eosinophils, neutrophils, and mixed mononuclear leukocytes. Inhibition curves produced in experiment 1 (A) and experiment 2 (C) when increasing quantities of CLC protein standard (●) or increasing amounts of freeze-thaw extracts of purified human basophils (□), eosinophils (○), neutrophils (■), or unseparated mononuclear leukocytes (▲) were measured by radioimmunoassay. (B and D) Regression lines for logit-log transformed data from panels A and C, respectively. The coefficients of determination ($r^2$) for the CLC protein standard inhibition regression lines were 0.992 and 0.993 and for cell extracts averaged 0.98 ± 0.007 and 0.99 ± 0.004 for experiments 1 and 2, respectively. A statistical analysis of these logit-log regression lines revealed that the null hypothesis of common slope could not be rejected ($F_{3,12} = 1.86; \text{NS}$ and $F_{2,8} = 3.94; \text{NS}$ for experiments 1 and 2, respectively).

Sepharose did not abrogate fluorescence staining of the cells. In contrast, absorption with CLC protein-Sepharose completely eliminated fluorescence staining. No staining occurred with the preimmunization sera.

Measurement of CLC Protein in Freeze-Thaw Extracts of FACS-purified Basophils by Radioimmunoassay. To test whether basophils and eosinophils contained similar amounts of CLC protein, we measured CLC protein concentrations in the supernatants of freeze-thaw extracts of cell suspensions enriched for basophils, eosinophils, neutrophils, or mononuclear cells by radioimmunoassay (Table III). CLC protein was detected in all freeze-thaw cell extracts that contained eosinophils as well as in basophil extracts derived from highly purified basophils (99 and 97% pure, with no contaminating eosinophils, experiments 1 and 2, respectively). When the CLC protein concentrations on a per cell basis were calculated for the basophil and eosinophil extracts, they contained significantly more CLC protein than did the neutrophil or unseparated mononuclear cell extracts (Table III). We also calculated the CLC protein concentrations per eosinophil and basophil in these experiments, and roughly comparable picogram quantities of CLC protein per cell were extracted by the freeze-thaw procedure (Table III).

The radioimmunoassay measures CLC protein concentration by inhibition of the binding of radiolabeled $[^{125}\text{I}]\text{CLC}$ to specific antibody. Fig. 5a and c shows the radioimmunoassay inhibition curves obtained when increasing quantities of freeze-thaw extracts of the purified cell suspensions were reacted with specific antibody to CLC protein and 1 ng $[^{125}\text{I}]\text{CLC}$. The shapes of these inhibition curves were very
similar for both experiments. Fig. 5 b and d shows the logit-log transformed data for these assays. A statistical comparison of these regression lines failed to reveal any significant differences in their slopes. The similarity of the inhibition curves of the basophil extracts to those of the CLC protein standard and to other cell extracts containing eosinophils indicates that basophils contain a protein that is immunochemically indistinguishable from that of the eosinophil CLC protein.

Discussion

The CLC is currently believed to be distinct to the eosinophil and a hallmark of active eosinophilic inflammation or proliferation in a variety of conditions characterized by tissue or peripheral blood eosinophilia, including myeloid leukemias, eosinophilic granuloma of bone, asthma and other allergic diseases, and parasitic diseases (1, 2). The finding by Weller et al. (4) that the protein comprising the eosinophil CLC possesses lysophospholipase activity (lysolecithin acylhydrolase, EC 3.1.1.5) and that chromatographically purified human eosinophil lysophospholipase crystallizes to form the hexagonal bipyramidal crystals typical of CLC has stimulated interest in the possible importance of CLC protein to eosinophil function. The uniqueness of CLC to eosinophils has not been clearly substantiated in the scientific literature, and the possibility existed that CLC might also be derived from human basophils, as first proposed by Archer and Blackwood in 1965 (9).

In the present study, we tested the hypothesis that CLC form from basophils. Basophils possess considerable quantities of IgE on their surface, and we enriched for surface IgE-positive cells using the FACS to obtain preparations of cells containing up to 99% basophils, without any contaminating eosinophils (Tables I and III). We examined these basophil-enriched cell suspensions for the ability to spontaneously form crystals. In two separate experiments, cells in suspensions containing >50% basophils and no eosinophils spontaneously formed crystals with the characteristic morphology of CLC (Fig. 1). When cells from these suspensions were pelleted onto microscope slides and stained by a histochemical procedure specific for basophils, crystal-containing cells stained as basophils, and the nonstaining crystals had the typical appearance of CLC (Fig. 1). By using an immunofluorescence procedure specific for eosinophil CLC protein, we demonstrated the presence of immunoreactive CLC protein in Ficoll-Hypaque interface cell preparations containing mononuclear cells, basophils, and no eosinophils and in basophil-enriched cell suspensions prepared by FACS purification of IgE-bearing cells. In these experiments, basophil-enriched cell suspensions contained >50% basophils, as determined by three different staining procedures, and >50% of these cells also fluoresced brightly when stained for CLC protein (Table I). In addition, examination of basophil-enriched cell suspensions containing CLC by immunofluorescence showed the presence of CLC in cells staining with positive fluorescence (Fig. 4). Finally, we demonstrated the presence of CLC protein in basophils by preparing freeze-thaw extracts of highly purified suspensions of basophils, eosinophils, neutrophils, and mononuclear cells and comparing the ability of these extracts to inhibit the binding of radiolabeled CLC protein to specific antibody in a radioimmunoassay. The results of these experiments (Table III, Fig. 5) indicate that basophils contain a protein that is immunochemically indistinguishable from that of eosinophil CLC protein and that the quantity of CLC protein in the basophil approximates that of the eosinophil. In contrast, on a per cell basis,
neutrophils and mononuclear cells contained very small quantities of CLC protein, which presumably resulted from the presence of small numbers of contaminating basophils or eosinophils in these cell populations.

Based upon these findings, the CLC can no longer be considered unique to the eosinophil. Basophils obtained from normal individuals can spontaneously form CLC under appropriate conditions, and basophils can be shown to contain measurable quantities of CLC protein, as determined by radioimmunoassay and immunofluorescence. Therefore, we must now regard the presence of CLC in tissues, sputum, or stool as indicative of the presence of either eosinophils or basophils.

Summary

Charcot-Leyden crystals (CLC) are currently believed to be unique to the eosinophil and a hallmark of active eosinophilic inflammation or proliferation. The distinctiveness of the CLC to the eosinophil was questioned in 1965 by Archer and Blackwood (9), but their demonstration of CLC formation in basophils was ignored and later dismissed (1) as being the result of eosinophil contamination of basophil-enriched cell suspensions.

We reexamined this question and showed that basophils obtained from the peripheral blood of normal individuals form CLC and that basophils contain a protein that is immunochemically indistinguishable from eosinophil CLC protein. These conclusions are based upon the findings that (a) crystal formation in basophils was demonstrated by specific histochemical staining of crystal-containing cells in highly enriched basophil suspensions prepared by fluorescence-activated cell sorter (FACS) purification of surface IgE-positive cells, (b) that enrichment for surface IgE-positive cells (primarily basophils) by the FACS also enriched for cells staining positively by immunofluorescence for eosinophil CLC protein, and (c) that CLC protein was measured by radioimmunoassay in cell extracts prepared from purified basophil suspensions containing 97-99% basophils and absolutely no contaminating eosinophils. These basophil extracts contained a protein immunochemically indistinguishable from eosinophil CLC protein.

Based upon these findings, the CLC or the protein comprising the crystal (lysophospholipase) can no longer be considered as distinctive to the eosinophil. We must now consider the possibility that the presence of CLC in tissues, sputum, or stool may also represent basophil involvement in disease processes.

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