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Immunology

Selective growth of a population of human basophil cells *in vitro*

(basophil development/morphological characteristics/immunoglobulin E receptors)

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ABSTRACT **An initially homogeneous population of basophilic polymorphonuclear leukocytes was derived from human fetal liver cells grown in culture for 5–7 days. The cells were characterized as basophils by their morphology, histologic staining characteristics, and histamine content, and by the presence of IgE receptors on their surface. In this culture system the basophils were viable for up to 10 days.**

Several important discoveries of the past few decades have led to a resurgence of interest in basophil and mast cell function. These include the discovery of IgE as the major pathologic initiator of immediate hypersensitivity reactions, the identification of basophils and mast cells as cells with specific surface receptor sites for IgE (1–3), and the demonstration that both basophils and mast cells store and release histamine and other mediators of immediate hypersensitivity—e.g., serotinin and bradykinin (4). A major problem in studying basophil and mast cell function is the extreme difficulty in obtaining large numbers of purified cells uncontaminated by other cell types.

Schrader *et al.* (5) and we (6) have recently, independently, grown pure populations of mouse mast cells from bone marrow precursors. Success in this endeavor appeared to be attributable to use of conditioned medium from splenocytes treated with concanavalin A. Thus far, to our knowledge, neither basophils nor mast cells have been grown from human tissues. In this paper we describe the growth in culture of human basophilic cells from human fetal liver cell precursors.

MATERIALS AND METHODS

Human Fetal Liver. Fetal liver was obtained from human abortuses after abortion by intraamniotic or intravaginal installation of prostaglandins. Abortuses ranged between 13 and 18 weeks gestational age, as determined by measurements of crown–rump and crown–heel length.

Cell Cultures. Cells were separated by gentle mechanical disaggregation and filtered through gauze. The cells that passed through the gauze were collected and washed three times in culture medium (see below) and separated into different fractions by gradient centrifugation in lymphocyte separation medium (Bionetics, Kensington, MD). The cells in the upper layer of the gradient were collected and washed three times in culture medium. The cells were suspended (1.5×10^7 cells in 30 ml of culture medium) in 75-cm^2 tissue culture flasks (Falcon) and maintained at 37°C in a humidified atmosphere of 5% CO_2 in air.

Culture Medium. The cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, 2-mercaptoethanol (50 μM); L-glutamine (2 mM), nonessential amino acids (0.1 mM), and penicillin and streptomycin (100 units/ml). The pH of the culture medium was 7.1–7.3. All reagents were purchased from GIBCO.

Light Microscopy. The viability of the cells was measured at various stages in the procedure by trypan blue dye exclusion. Staining of the cells by toluidine blue (0.1% in ethanol, 50% vol/vol, pH 3.0) was examined before culture and again after 5 days in culture.

Electron Microscopy. Cells in suspension cultured for 5 days were fixed by the addition of an equal volume of Karnovsky's aldehyde fixative (7) containing paraformaldehyde (4%, vol/vol) and glutaraldehyde (5%, vol/vol) in sodium cacodylate buffer (0.1 M, pH 7.4) without calcium. After 45 min the cells were washed several times in cacodylate buffer and were postfixed for 1 hr in osmium tetroxide (1%) in 0.1 M cacodylate buffer. The cells were then prestained for 1 hr with uranyl acetate (0.5%) in sodium Veronal buffer (0.04 M, pH 7.5) and thereafter with tannic acid (1%) for 30 min. The cells were dehydrated in graded ethanol solutions and were embedded in Epon 812. Ultrathin sections were cut, stained with saturated uranyl acetate (7.5% wt/vol) at 60°C for 30 min, counterstained with Reynold's lead citrate at room temperature for 5 min, and then viewed in a transmission electron microscope (Philips 301).

Myeloma IgE (M-IgE). IgE purified from the serum of a patient with IgE multiple myeloma was a gift of H. Metzger (National Institutes of Health, Bethesda, MD).

Conjugation of M-IgE to Fluorescein Isothiocyanate (FITC). M-IgE was conjugated to FITC (yielding FITC-IgE) according to a described method (8).

Measurement of FITC-IgE Binding to Cells. Suspensions of cells before culture or after culture for 5–7 days (1×10^6 cells in 0.5 ml of phosphate-buffered saline) were incubated with 1 μg of FITC-IgE. Control tubes contained a 100-fold excess of unlabeled M-IgE, which was mixed with the cell suspensions prior to the addition of FITC-IgE. After incubation at 37°C for 15 min the samples were examined by fluorescent incident illumination.

Assay of Histamine. Cells (1×10^6) were examined for histamine content after 5–7 days in culture. The cells were washed three times in phosphate-buffered saline and then homogenized in 0.7 ml of distilled water. After centrifugation at $1000 \times g$ for 15 min, the supernatant was stored frozen until assayed for histamine content by the spectrophotofluorometric technique of Anton *et al.* (9).

RESULTS

At the initiation of culture, not more than 0.5% of the cells were lightly stained with toluidine blue, suggesting that a few basophils or mast cells were present in the culture inoculum. After 5 to 7 days in culture, almost all of the cells present in the flasks contained cytoplasm filled with metachromatic granules as

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Abbreviations: M-IgE, myeloma IgE; FITC-IgE, M-IgE conjugated to fluorescein isothiocyanate.

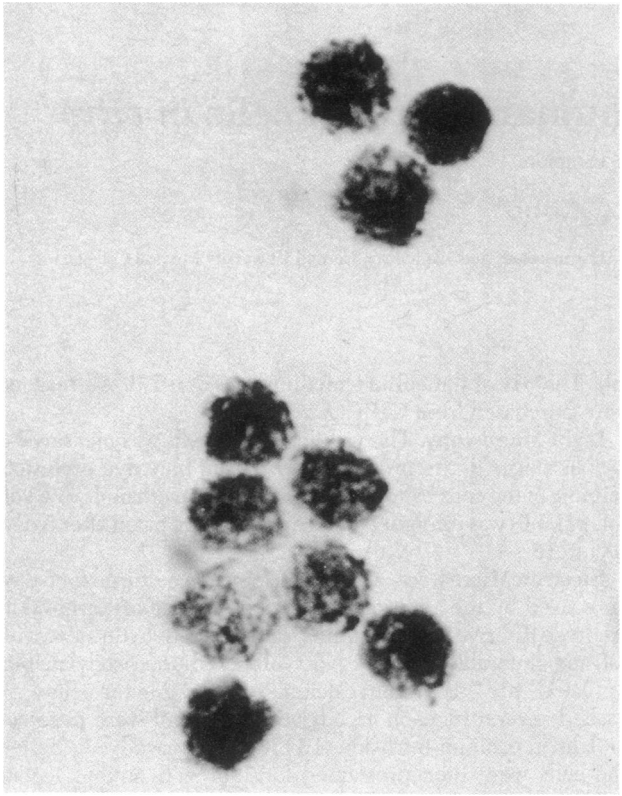


FIG. 1. Basophils obtained from human fetal liver culture. Note the pure basophil culture after 6 days of incubation. (Toluidine blue; ×1100.)

shown by toluidine blue staining. The nuclei were obscured by the granules (Fig. 1). Most of the cells floated free in the culture medium. Almost all the cells appeared to be round but were heterogeneous with respect to size, ranging between 3 and 10 μm in diameter. They also were heterogeneous with respect to the number of metachromatic granules per cell and with respect to the apparent intensity of the granules after toluidine blue staining. After 5 days in culture, the most successful cul-

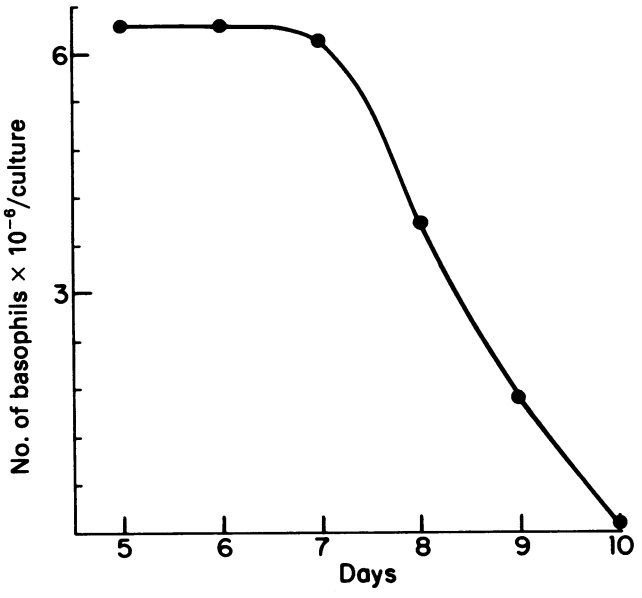
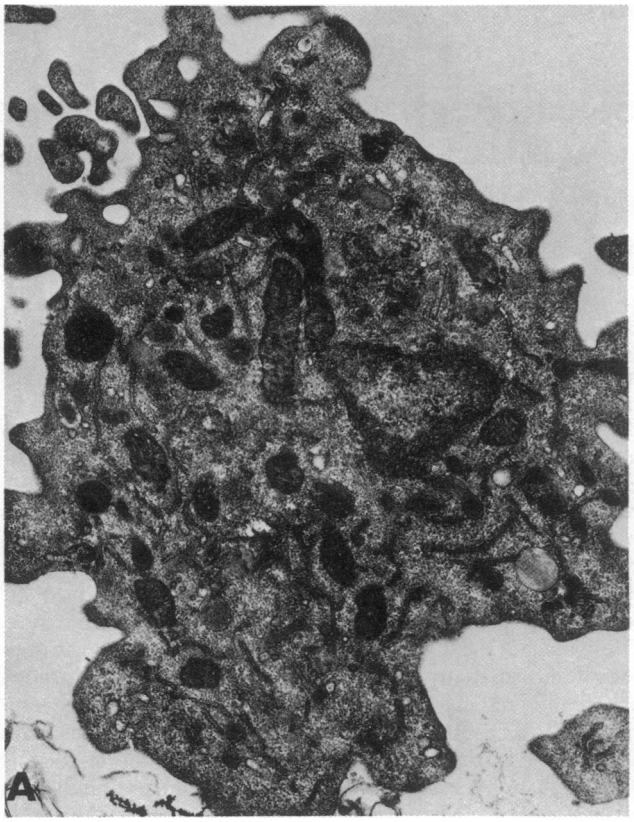


FIG. 2. Number of basophils in culture at different time intervals after initiation of the culture of fetal liver cells.



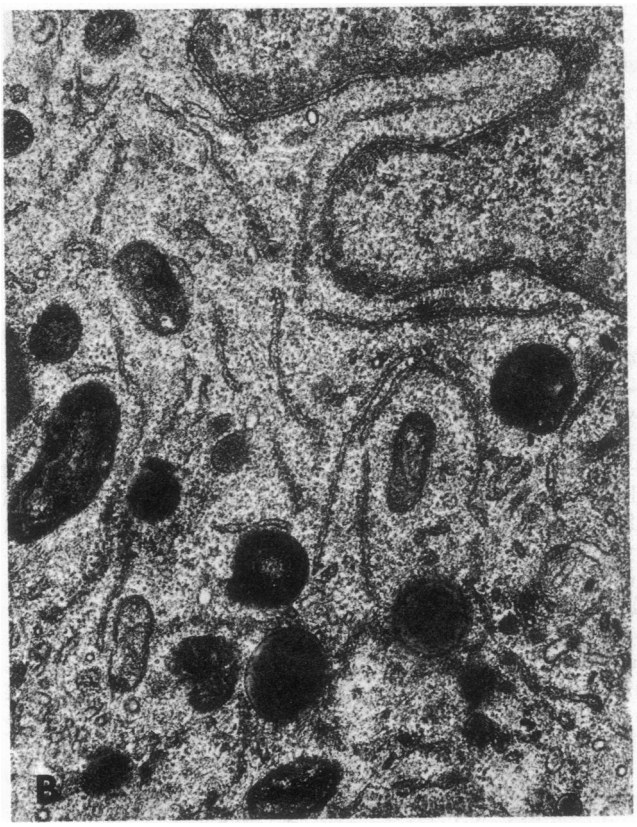


FIG. 3. Transmission electron microscopic view of basophils obtained after 6 days in culture of human fetal liver. Cells were fixed in Karnovsky's aldehyde fixative, postfixed in OsO_4, and stained in the block with uranyl acetate and on the grid with uranyl acetate and lead citrate. (*A*, ×9300; *B*, × 20,000.)

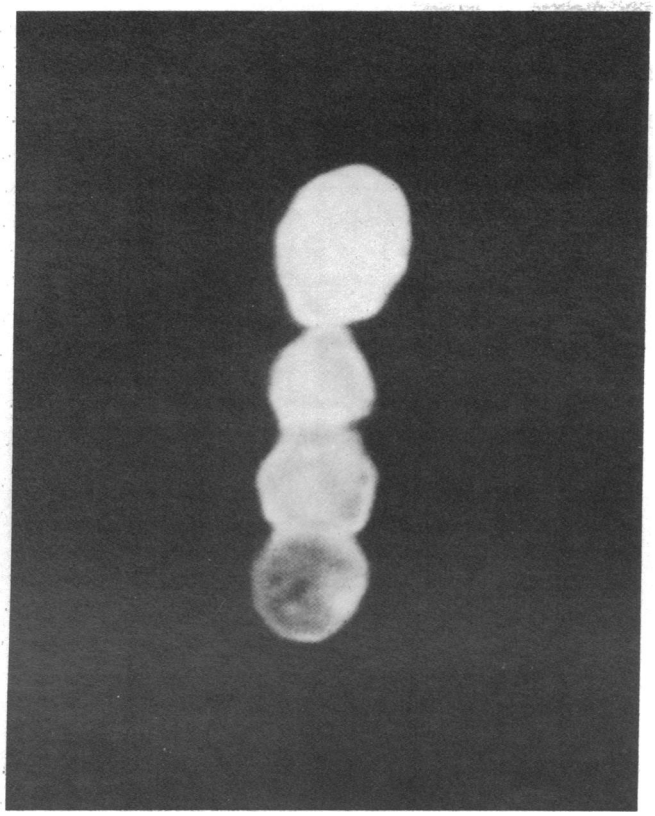


FIG. 4. Binding of fluorescent IgE to basophils obtained after 6 days in culture of human fetal liver. (×1200.)

tures contained more than 6×10^6 viable cells per flask, of which more than 90% were stained with toluidine blue. The number of viable cells decreased sharply when the cultures were maintained longer than 7 days. Virtually no viable cells remained after 10 days in culture (Fig. 2).

Examination of the cells by transmission electron microscopy showed that their cytoplasm contained granules (Fig. 3) with a particulate appearance. The nuclei of the cultured cells were segmented. Their chromatin patterns were similar to those of other granulocytes.

IgE receptors could not be detected on the surface of the fetal liver cells at the start of culture. However, IgE receptors were regularly present on the surface of the cells cultured for 5–7 days. Almost all of the cells were stained specifically by the fluorescent M-IgE (Fig. 4). The binding of the IgE did not decrease after the cells were washed three times with phosphate-buffered saline.

The histamine content of cells cultured 5–7 days ranged between 50 and 450 ng per 10^6 cells. When sedimented at 400 $\times$ *g* for 20 min on the lymphocyte separation medium gradient, the cells all settled in a pellet at the bottom of the gradient, as was the case with other granulocytes.

DISCUSSION

Basophils and mast cells have many common characteristics. They both contain histamine and other mediators of immediate hypersensitivity reactions, have IgE receptors on their cell surfaces, and degranulate by similar mechanisms. There is compelling evidence that both mast cells and basophils are derived from hematopoietic stem cell precursors (5, 6, 10, 11), and recent evidence indicates that they may develop from the same stem cell (12).

Basophils and mast cells are distinguished from each other mainly on the basis of morphologic differences. According to morphologic criteria, the cells derived from human fetal liver described in this report appear to be basophilic rather than mast cells. The granules have the particulate appearance characteristic of basophils rather than the crystalline appearance regularly seen in mast cell granules. The nuclei are segmented and have a chromatin pattern like that of other granulocytes, whereas the nuclei of mast cells are round or oval.

The cells cultured from human fetal liver were identified as basophils by their morphologic characteristics, as described above, by their histamine content, and by the affinity of their surface for IgE. Basophils and mast cells are the only cell types known to store histamine. Histamine was clearly detectable in the cells cultured from human fetal liver. It is of interest, however, that the amount of histamine per 10^6 cells varied greatly in different cultures, ranging between 50 and 450 ng per 10^6 cells. These differences could not be recognized by simple toluidine blue staining. The affinity of the cells for IgE was the major feature characterizing them as basophils. Thus, nearly all of the cells bound IgE specifically after short periods of incubation. The high affinity of the cell surface receptors for IgE was demonstrated by the persistence of binding of the IgE after washing.

During 5–7 days in culture, the percentage of cells stained with toluidine blue increased about 200-fold, and IgE receptor sites appeared. These observations suggest that during culture a population of precursor cells underwent selective growth and differentiation into basophils. In contrast to mouse cultured mast cells, which could be generated from bone marrow precursors only in the presence of conditioned medium derived from concanavalin A-treated spleen cells (5, 6), the human basophils were generated without the addition of such special conditioning medium. It is possible that the selective growth of basophils from a heterogeneous population of human liver cells is stimulated by the release of a growth factor(s) from certain cells present in the culture of fetal liver. It seems likely that this culture system can be further developed to permit longer culture periods and greater expansion of the basophil population. It also seems possible that it is the loss of such a growth factor that accounts for the sudden failure of the culture after 7 days.

The present report indicates that basophils that have morphologic and biochemical characteristics similar to those of basophils isolated from marrow or blood can be obtained from human fetal liver during *in vitro* culture. The cell culture system described here should facilitate the further study of basophil function. It should also prove useful for studying the process of basophil differentiation and the development of cytoplasmic granules and IgE receptors during cell maturation.

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