

AD _____

Award Number: DAMD17-98-1-8485

TITLE: Modulation of Adhesion Molecule Expression on Prostate Tumor Cells After Co-Culture with Eosinophilic Cell Lines

PRINCIPAL INVESTIGATOR: Paulette Furbert-Harris, Ph.D.

CONTRACTING ORGANIZATION: Howard University
Washington, DC 20059

REPORT DATE: October 1999

C
TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE October 1999	3. REPORT TYPE AND DATES COVERED Annual (01 Oct 98 - 30 Sep 99)	
4. TITLE AND SUBTITLE Modulation of Adhesion Molecule Expression on Prostate Tumor Cells After Co-Culture with Eosinophilic Cell Lines			5. FUNDING NUMBERS DAMD17-98-1-8485	
6. AUTHOR(S) Paulette Furbert-Harris, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Howard University Washington, DC 20059 e-mail: pfurbert-harris@fac.howard.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) We have demonstrated that cell lines developed from metrizamide density fractions of peripheral blood hypodense (.22) and hyperdense (.24) eosinophils significantly inhibited LNCaP, PC3, DU145 and HPC1 monolayer growth cultures <i>in vitro</i> . This activity was enhanced when the eosinophils were pretreated with interleukin-5. DU145 and PC3 colony formation was inhibited 50-75% by eosinophil cell lines, while peripheral blood eosinophils inhibited PC3 colony formation by as much as 95%. 24hr. cultured eosinophil supernatants significantly inhibited PC3 colony formation with 3/7 supernatants causing 100% inhibition. Inhibitory activity of the 24hr. supernatants towards DU145 was more variable, ranging from 9-90% inhibition of colony formation. There was slight enhancement of growth with one preparation. IL-4 and TNF α , which were found present in the supernatants also inhibited growth of monolayers cultures of PC3, DU145 and HPC1. These data strongly support the hypothesis that eosinophils can destroy prostate tumor cells <i>in vitro</i> . The eosinophil cell lines and cytokine modulation of their activity offer an exquisite tool for more detailed study (both cellular and molecular) of a role for eosinophils as anti-prostate cancer effector cells.				
14. SUBJECT TERMS Prostate Cancer			15. NUMBER OF PAGES 39	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

_____ Where copyrighted material is quoted, permission has been obtained to use such material.

_____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

_____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

NA In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

NA In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

NA In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

NA In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Paulette Tubert-Harris 1/5/00
PI - Signature Date

Table of Contents

	Page Number
Front Cover	1
Standard Form 298	2
Foreword	3
Table of Contents	4
Introduction	5
Body	5
Key Research Accomplishments	7
Reportable Outcomes	7
Conclusion	7
References	8
Appendices	9

5. Introduction

Prostate cancer is the most common cancer diagnosed in American men. It has been estimated that by the end of 1999, 179,000 men will have been diagnosed with prostate cancer and that 37,000 deaths will have resulted(1). Prostate cancer incidence and mortality rates for African American males are the highest of any racial or ethnic group in the world (2). Prostate cancer incidence in this group is 180.6/100,000, compared to 143.7/100,000 for Caucasians and 24.2/100,000 for Koreans (2). The mortality rate for African American is 53.7 compared to 24.1 for Caucasians and 6.6 for Chinese.

Several new treatment approaches towards the eradication of prostate cancer have focused on regulating the immune response system to antigens expressed on prostate cancer cells (3-7). Moreover the strategy of utilizing cytokine gene therapy in order to amplify the host response to tumor is quickly gaining momentum. Many of the cytokines which have been used (e.g. IL-2, IL-4, IL-5 and GM-CSF) are known to either attract and/or regulate eosinophil activity(8).

Eosinophils have been traditionally known as anti-helminthic effector cells and inflammatory agents in hypersensitivity reactions, particularly allergic asthma(9). Evidence exists, however, for a potential role for eosinophils in cancer. We have recently shown that activated eosinophils destroy MCF-7 and MDA-231 breast cancer cell monolayer formation *in vitro* and inhibit MCF-7 colony formation (manuscript in preparation). The inhibition observed is partially mediated by cytokines IL-4 and TNF α which were secreted into 24-hr conditioned supernatants. In this study, we have examined the inhibitory activity of activated eosinophils and eosinophilic cell lines which we had previously established and are presently characterizing (manuscript in preparation) on prostate cancer cell lines in eosinophil:tumor co-culture assays, and also the effect of cultured eosinophil supernatants on cell growth.

6. Body

Propagation of Cell Lines: To date all six eosinophilic cell lines have been retrieved from storage at -160°C, cultured in RPMI medium supplemented with penicillin/streptomycin (50 units/50 ug/ml, respectively), gentamycin (50ug/ml) and 10% fetal bovine serum. At present we have data with 3 of the cell lines and 2 sublines. **Tumor:** PC-3, DU145 and LNCaP cells were obtained from ATCC and established in culture, frozen and retrieved prior to use. They are being maintained in the appropriate culture medium as recommended by the vendor, PC3 (7% F-12K medium supplemented with penicillin/streptomycin and gentamycin). In a collaborative study, we at Howard University have very recently immortalized a primary prostate culture HPC1 from an African American which is presently being characterized. These cells are also cultured in 10% RPMI medium containing penicillin/streptomycin and gentamycin and were used preliminarily in this study.

Growth Inhibition of PC3, LNCaP, DU145 and HPC1 Tumor Cells by Activated Eosinophils and Eosinophilic Cell Lines.

A. Monolayer. Tumor Cells (PC3, LNCaP, DU145 and HPC1) were seeded into the wells of either a 6-well plate (at 2.5×10^5 cells per well) or a 12-well plate (at 1.5×10^5 cells per well). The plates were incubated overnight (16-24hr) at 37°C , 5% CO_2 . Eosinophils were added at various effector to target (E:T) ratios and the plates were incubated for an additional 24-48hr. The effector cells were then removed, the monolayers washed 3x with PBS and stained with hematoxylin and eosin before being photographed. As another form of documentation, the entire plate was scanned into power point and is represented.

LNCaP was extremely sensitive to both hypodense eosinophils at 5:1 and 43:1 E:T ratios (Fig. 1B and 1C, respectively) and hyperdense eosinophils at 5:1 and 14:1 E:T ratios (Fig. 1D and 1E, respectively). At the same E:T ratios, PC3 was also sensitive to killing by eosinophil hypodense and hyperdense cell lines (Fig. 2). Moreover, eosinophil cell lines established from both hypodense and hyperdense eosinophil fractions from another donor also inhibited PC3 cell growth at the E:T ratio of 10:1 (Fig. 3B- hypodense cell line; 3E-hyperdense cell line), and HPC1 cell growth also at 10:1 E:T ratio (Fig. 11B). IL-5 pretreatment of the eosinophils resulted in even greater killing of both PC3 (Fig. 3C and 3F) and HPC1 (Fig. 11C). The entire 12-well plates were scanned in an effort to get a more comprehensive evaluation of tumor cell damage (Fig. 4), (Fig. 12).

Eosinophil cell lines were sterile sorted with a Becton Dickinson FACS SCAN Cell Sorter using the PE-labelled antibody to the eotaxin receptor. This chemokine receptor is found predominantly on eosinophils. These sublines were found also to be positive for the eosinophil markers CD15 and CD49d. Both the parent eosinophil cell line GRC.014.24 and the two sublines GRC014.24.S1 and GRC.014.24.S2 markedly inhibited PC3 cell growth (Fig. 5).

When 24hr. cultured eosinophil supernatants were added to subconfluent PC3 (Fig. 6), DU145 (Fig. 9) and HPC1 (Fig. 13) monolayer cell growth was dramatically inhibited except for donor BLA who's eosinophil supernatants showed little damage to the PC3 monolayer, but completely destroyed both DU145 and HPC1 monolayers.

B. Colony Formation. PC3 and DU145 cells were seeded into the wells of 6-well tissue culture plates at 100 cells per well. The plates were incubated overnight at 37°C , 5% CO_2 . At this time effector cells were added at various E:T ratios and the plates were incubated for ten days. The plates were harvested, washed with PBS 3x, then stained with hematoxylin and eosin and counted manually. Both hypodense and hyperdense subpopulations of peripheral blood eosinophils inhibited PC3 colony formation in a dose-dependent manner, (Fig. 7A), with the 50:1 E:T ratio resulting in 95% inhibition for the hypodense eosinophils and 91% inhibition for both 10:1 and 50:1 E:T ratios for the hyperdense eosinophils. The cell line GRC.014.24 inhibited colony formation by 71 and 75% at E:T ratios 1:2 and 2:1, respectively, while the subline GRC.014.24.S1 had minimal effect at both E:T ratios (7% and 18%, respectively). At the E:T ratio of 2:1, GRC.014.24 inhibited DU145 by 88% and the sublines S1 and S2 inhibited colony formation by 81 and 54%, respectively (Fig. 10A). The hypodense cell line BJA.060.22 inhibited colony formation by 50%.

C. 24hr. Cultured Supernatants Inhibit Prostate Tumor Cell Growth In Vitro. Subconfluent monolayers of PC3, DU145 and HPC1 prostate cells were incubated overnight with 24-hr. cultured supernatants in 12-well tissue culture plates. Both hypodense and hyperdense eosinophil cultured supernatants markedly inhibited PC3 colony formation (Fig. 7C) and at least three supernatant

preparations (BLA 24, HMO 22 and HMO 24) completely prohibited colony formation. On the otherhand DU145 was less sensitive to many of the supernatant preparations, particularly BLA 24, HMO 22, YDA 22 and WCH 22, (Fig. 10B). GRC.014.24 supernatant completely inhibited DU145 colony formation (Fig. 10C).

D. Cytokine Presence in 24hr. Eosinophil Supernatants. 24hr. cultured supernatants from peripheral blood eosinophil hypodense and hyperdense subpopulations (M22 and M24, respectively) were evaluated by enzyme-linked immunoassay (ELISA) analysis using commercial kits. Interleukin-4 (IL-4) and Tumor Necrosis Factor Alpha (TNF α) were present in varying levels in all individuals tested (Table I). IL-4 concentrations ranged from 0 to >1000 pg/ml. TNF α concentrations were far less than IL-4, ranging from 10-224pg/ml. GM-CSF was only found in donor 6 supernatants at 450pg/ml, and IL-5 was absent in all 6 samples.

7. Key Research Accomplishments

- ▶ Retrieval of all eosinophilic cell lines
- ▶ Demonstration of functional cytotoxic/cytostatic activity with 3 of the lines and 2 sublines
- ▶ Establishment of new prostate cell line in collaboration with clinical investigators at Howard University Hospital
- ▶ Use of new prostate cell line in eosinophil co-culture assays.

8. Reportable Outcomes

Activated eosinophils inhibit cell in vitro growth of prostate cancer cell lines, (Manuscript in preparation)

Late Abstract in preparation for AACR Spring 2000

Ahaghotu C, Marshalleck J, Dennery M, Vaughn T, Laniyan I, Jackson A,
Furbert-Harris P. A Novel Primary Prostate Cancer Cell Line Derived from an African American Patient. The American Urological Association, Inc. 95th Annual Meeting, 1999.

9. Discussion/Conclusions

We hypothesized that activated eosinophils which may be found in tumor infiltrates produce cytokines which are both tumor inhibitory and enhancing. Moreover these cytokines may modify adhesion molecule expression on tumor cells thereby modifying their mortality and metastatic capabilities. The tasks of the first 12-months entailed:

- a. culturing and propagation of both prostate cells and eosinophilic cell lines.
- b. growth inhibition assays (monolayer/colony).
- c. cytokine enhancement of eosinophil activity.
- d. exogenous cytokine activity against prostate tumor cell growth.

The data presented have clearly shown that subpopulations of activated eosinophils, (hypodense and hyperdense) from individuals with mild to moderate eosinophilia inhibit the growth of PC3, tumor cells (both monolayer and colony formation). Furthermore eosinophil cell lines established from these subpopulation inhibited LNCaP, PC3, DU145 and the newly established prostate cell line HPC1. In the colony assay PC3 was more sensitive than DU145, to eosinophil killing. IL-5 which is known to activate eosinophils, when used to pretreat both hypodense and hyperdense cell lines, enhanced their killing of both PC3 and HPC1 tumor cells.

Both LNCaP and HPC1 failed to form colonies and hence we simply used monolayers to test eosinophil activity. IL-5 has been the only cytokine used thus far to enhance eosinophil activity. We will continue to study this with other cytokines known to stimulate eosinophils (e.g. IL-3, GM-CSF, IL-4). ELISA analysis of eosinophil supernatants revealed the presence of IL-4, TNF α and GM-CSF.

We will continue to characterize the supernatants for other cytokines. In addition to completing the tasks for year one, we have already begun to assess the baseline presence of adhesion molecules on the tumor cells and will begin with the cytokine modulation of these in the very near future.

10. References

1. American Cancer Society (1998): Facts and figures.
2. Brawley OW and Kramer BS. Comprehensive textbook of Genitourinary Oncology. NJ Vogesang, PT Scardino, WU Shiplay, OS Coffey Eds. (Williams and Wilkins, Baltimore, 1996.
3. Tjoa BA, Erickson SJ, Bowes VA, Ragde H, Kenny GM, Cobb OE, Ireton RC, Troychak MJ, Boynton AL and Murphy GP. Follow-up evaluation of prostate cancer patients infused with autologous dendritic cells pulsed with PSMA peptides. *Prostate* 32:272-278, 1997.
4. Tjoa BA, Simmons SJ, Bowes VA, Radge H, Rogers M, Elgamal A, Kenny GM, Cobb OE, Ireton RC, Troychak MJ, Salgaller ML, Boynton AL and Murphy GP. Evaluation of Phase I/II clinical trials in prostate cancer with dendritic cells and PSMA peptides. *Prostate* 36: 39-44, 1998.
5. Zhang S, Zang HS, Reuter VE, Slovin SF, Scher HI and Livingston PO. Expression of potential target antigens for immunotherapy on primary and metastatic cancers. *Clin Cancer Res* 4: 295-302, 1998.
6. Correale P, Walmsley K, Zaremba S, Zhu M, Schlom J and Tsang KY. Generation of human cytolytic T lymphocyte lines directed against prostate-specific antigen (PSA) employing a PSA oligopeptide. *J Immunol* 161:3186-3194, 1998.
7. Simons JW and Mikhak B. Ex vivo gene therapy using cytokine-transduced tumor vaccines: molecular and clinical pharmacology. *Semin Oncol* 25: 661-676, 1998.
8. Silberstein DS. Eosinophil function in health and disease. *Critical Reviews in Oncology/Hematology* 19(1995): 47-77, 1994.
9. Gaga M, Frew AJ, Veronica AV and Kay AB. Eosinophil activation and T lymphocyte infiltration in allergen-induced late phase skin reactions and classical delayed-type hypersensitivity. *J Immunol* 147: 816-822, 1991.

11. Apendices.

- Figure 1. Inhibition of LNCaP Tumor Cell Growth by Eosinophil Cell Lines
- Figure 1. Legend
- Figure 2. Inhibition of PC3 Tumor Cell Growth by Eosinophil Cell Lines
- Figure 2. Legend
- Figure 3. IL-5 Pretreatment of Eosinophil Cell Lines Enhances Growth Inhibition of PC3 Tumor Cells In Vitro
- Figure 3. Legend
- Figure 4. IL-5 Pretreatment of Eosinophil Cell Lines Enhances Growth Inhibition of PC3 Tumor Cell In Vitro
- Figure 4. Legend
- Figure 5. CD15 and CD49d Positive Eosinophil Sublines Inhibit PC3 Cell Growth In Vitro
- Figure 5. Legend
- Figure 6. 24-hr. Cultured Eosinophil Supernatants Inhibit PC3 Cell Growth In Vitro
- Figure 6. Legend
- Figure 7. Eosinophils Inhibit PC3 Colony Formation In Vitro
- Figure 7. Legend
- Figure 8. Eosinophil Cell Lines Inhibit DU145 Cell Growth In Vitro
- Figure 8. Legend
- Figure 9. 24-hr. Cultured Supernatants Inhibit DU145 Cell Growth In Vitro
- Figure 9. Legend
- Table 1. Cytokine Concentrations in 24hr Eosinophil Culture Supernatants (pg/ml)

Table 1.	Legend
Figure 10.	DU145 Colony Inhibition by Eosinophil Cell Lines and Cultured Supernatants
Figure 10.	Legend
Figure 11.	HPC1 Prostate Cell Growth Inhibition by Eosinophil Hypodense and Hyperdense Cell Lines
Figure 11.	Legend
Figure 12.	HPC1 Prostate Cell Growth Inhibition by Eosinophil Hypodense and Hyperdense Cell Lines
Figure 12.	Legend
Figure 13.	24-hr. Cultured Supernatants Inhibit HPC1 Cell Growth In Vitro
Figure 13.	Legend

Fig. 1. Inhibition of LNCaP Tumor Cell Growth by Eosinophil Cell Lines

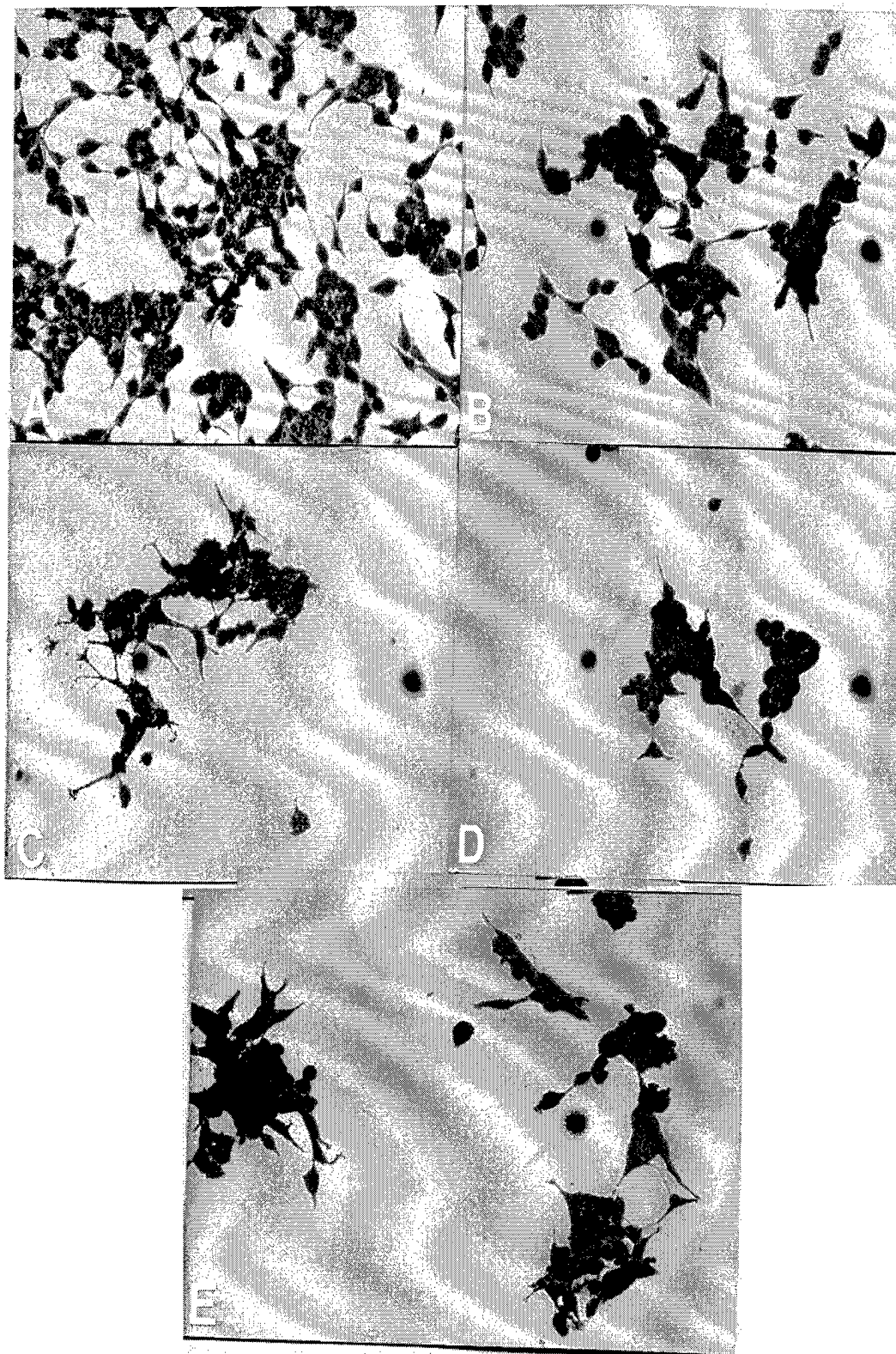


Fig. 1. LNCaP tumor cells were seeded into T25 flasks at 3×10^5 cells/flasks and allowed to grow to confluence (3-4 days) in media alone (A) or in the presence of hypodense eosinophilic cell line SD.031.22 at E:T ratios of 5:1 and 43:1 (B&C, respectively) and hyperdense cell line (SD.031.24) at E:T ratios of 5:1 and 14:1 (D and E, respectively). All experiments were performed in duplicate.

Fig. 2. Inhibition of PC3 Tumor Cell Growth by Eosinophil Cell Lines

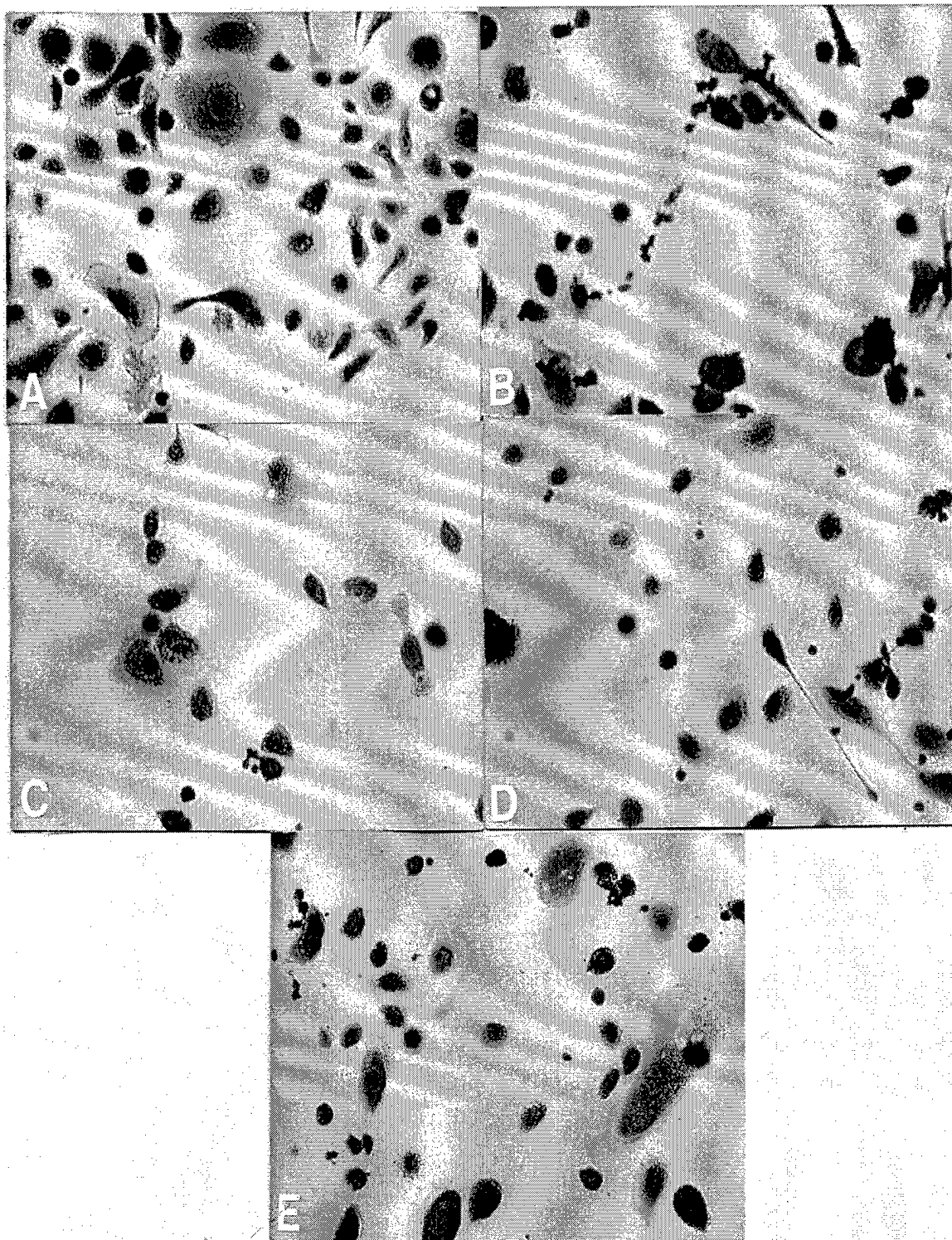
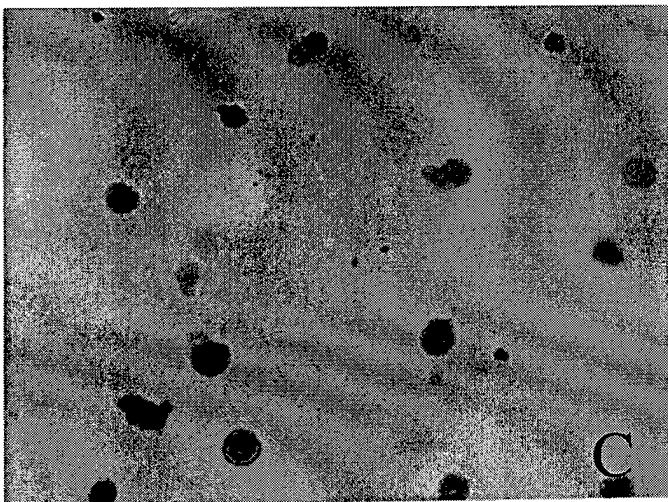
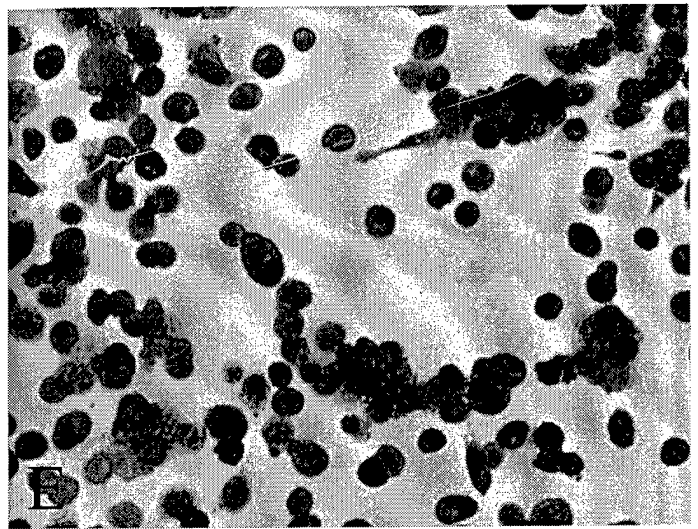
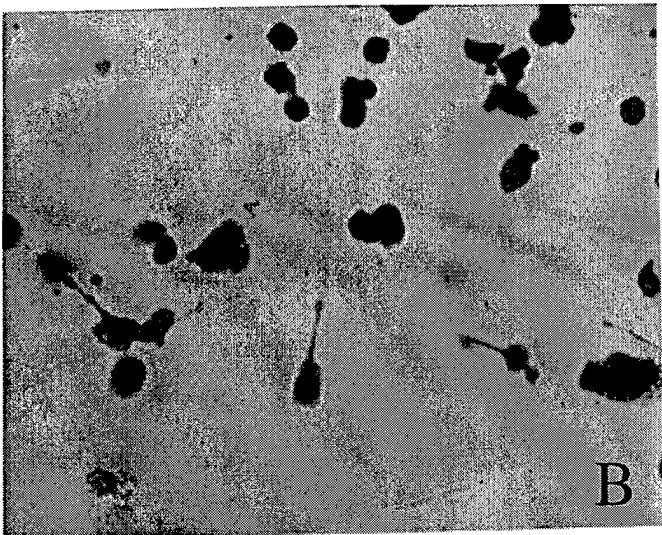
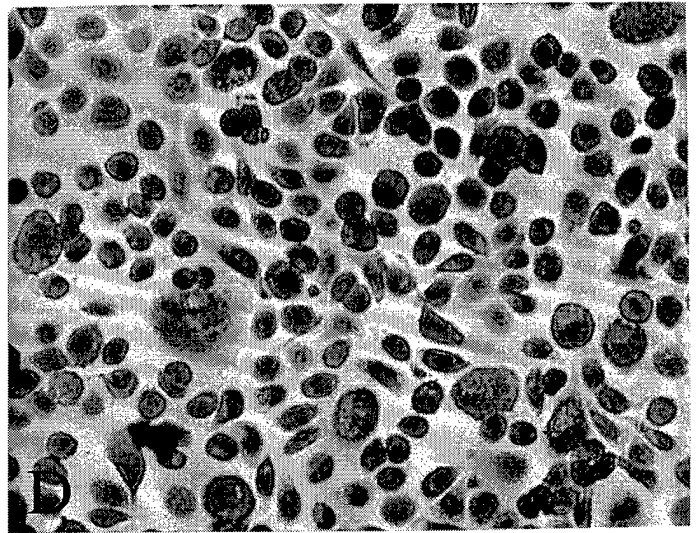
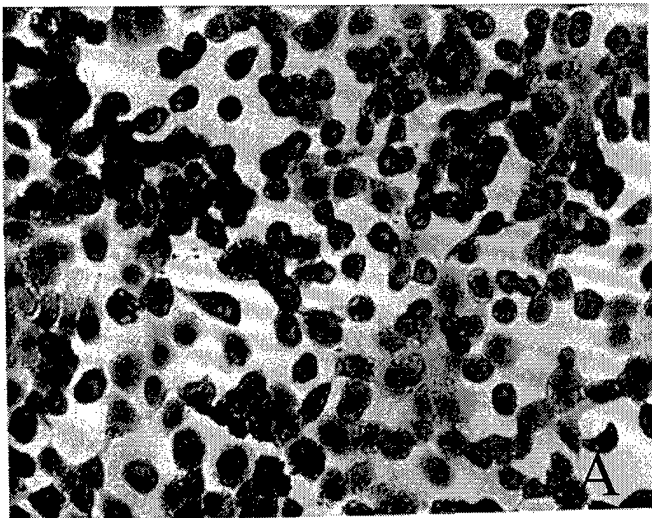


Fig. 2 PC-3 tumor cells were seeded into duplicate T25 flasks at 3×10^5 cells/flask and allowed to grow to confluence (3-4 days) in media alone, and in co-culture with hypodense eosinophilic cell line SD.031.22 at E:T ratios of 5:1 and 43:1 (B, C, respectively) and hyperdense cell line SD.031.24 at E:T ratios of 5:1 and 14:1 (D & E, respectively).

Fig. 3. Interleukin-5 Pretreatment of Eosinophil Cell Lines Enhances Growth Inhibition of PC3 Tumor Cells In Vitro



15

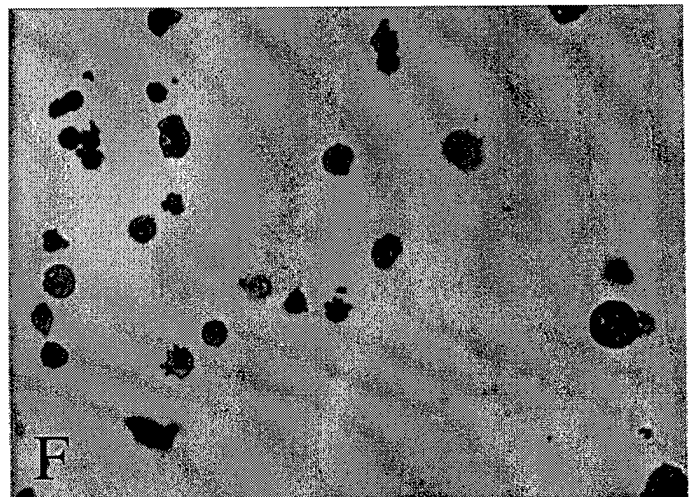
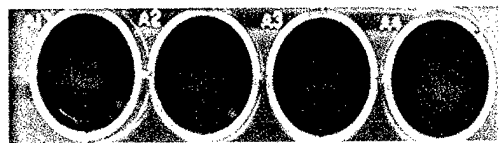


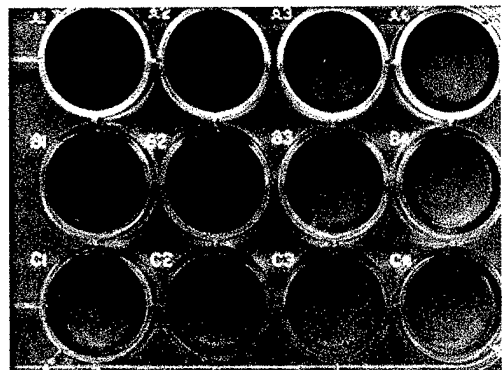
Photo Micrograph

Fig. 3. PC-3 tumor cells were seeded into the wells of a 12 well culture plate at 1.5×10^5 cells/well. Prior to this effector eosinophil cell lines were pretreated with IL-5 (1 ng/ml) for 3 days. On day 4, eosinophils were added and the plate incubated for 24hr or until the control wells were confluent (24-48hrs.). Effector cells were removed and the wells washed 3X with PBS, then fixed and stained with H&E. Photomicrographs were taken as well as a scan of the entire well or plate.

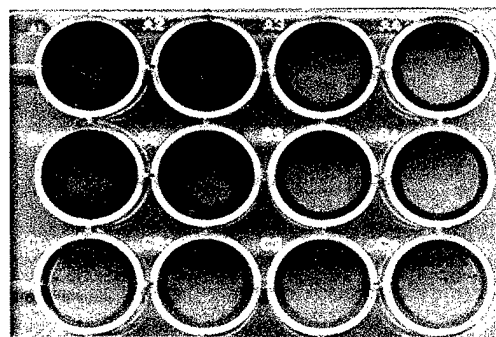
Fig. 4. IL-5 Treatment of Eosinophil Cell Lines Enhances Growth
Inhibition of PC3 Tumor Cells In Vitro



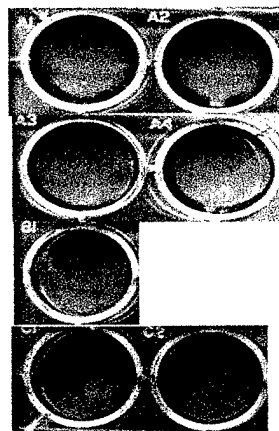
Control



- A1-2: GRC014"24":PC-3, 1:1
- B1-2:GRC014"24":PC-3, 10:1
- C1-2:GRC014"24":PC-3, 25:1
- A3-4: GRC014"24"+IL-5:PC-3, 1:1
- B3-4: GRC014"24"+IL-5:PC-3, 10:1
- C3-4: GRC014"24"+IL-5:PC-3, 25:1



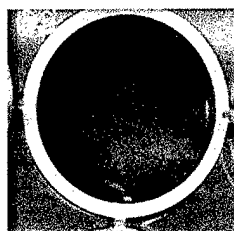
- A1-2: BJA060"22":PC-3, 1:1
- B1-2:BJA060"22":PC-3, 10:1
- C1-2:BJA060"22":PC-3, 25:1
- A3-4: BJA060"22"+IL-5:PC-3, 1:1
- B3-4: BJA060"22"+IL-5:PC-3, 10:1
- C3-4: BJA060"22"+IL-5:PC-3, 25:1



- WCH"22", 5:1 (Peripheral Blood Eosinophils)
- WCH"22", 10:1 (Peripheral Blood Eosinophils)
- WCH"22", 25:1 (Peripheral Blood Eosinophils)
- WCH"24", 5:1 (Peripheral Blood Eosinophils)

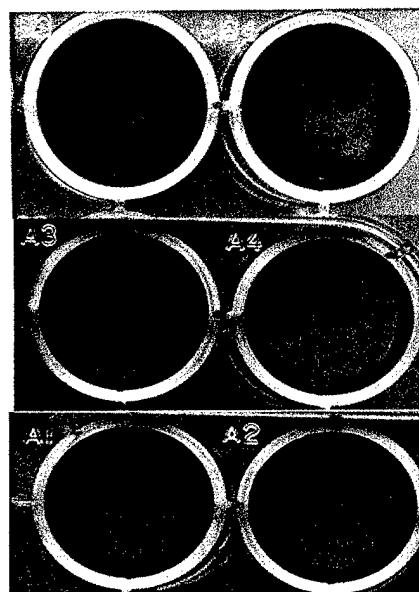
Fig. 4. Eosinophils, both peripheral blood and eosinophil cell lines were co-cultured in duplicate wells of a 12-well tissue culture plate with PC3 tumor cells at E:T ratios 1:1, 10:1 and 25:1 as described in fig. 3. The plates were harvested and stained with H & E then scanned into power point for presentation. The alpha numeric represents the donor and the numbers in quotations represent the eosinophil subpopulations (22-hypodense and 24-hyperdense).

Fig. 5. CD15 and CD49d Positive Eosinophil Sublines Inhibit PC3 Cell Growth In Vitro

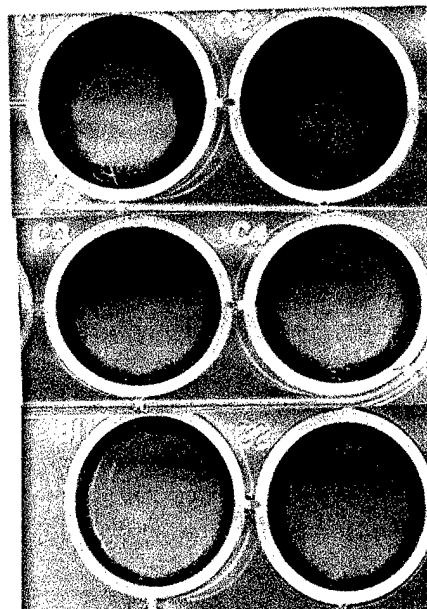


Control

Panel A



Panel B

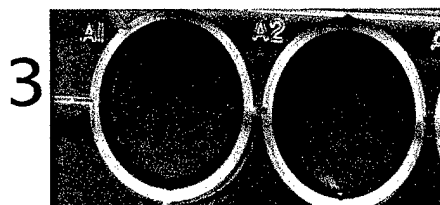


1

2

3

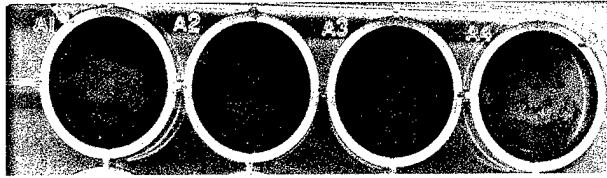
Panel C



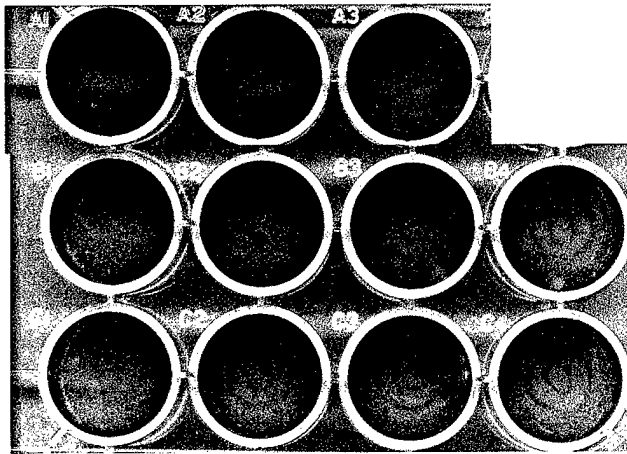
3

Fig. 5. Sublines from the eosinophil parent line GRC.014.24 were sterile-sorted with a FACS SCAN cell sorter using antibody to the eosinophil specific eotaxin receptor. The sublines S1 and S2 were found to be positive for both CD15 and CD49d markers. The co-culture was set up similarly to that described in figures 3 and 4. Numbers 1, 2 and 3 represent E:T ratios 1:2, 2:1 and 5:1, respectively.

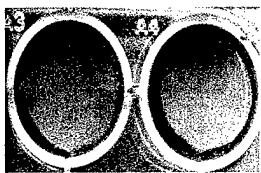
Fig. 6 24-hr Cultured Eosinophil Supernatants Inhibits PC3 Cell Growth In Vitro



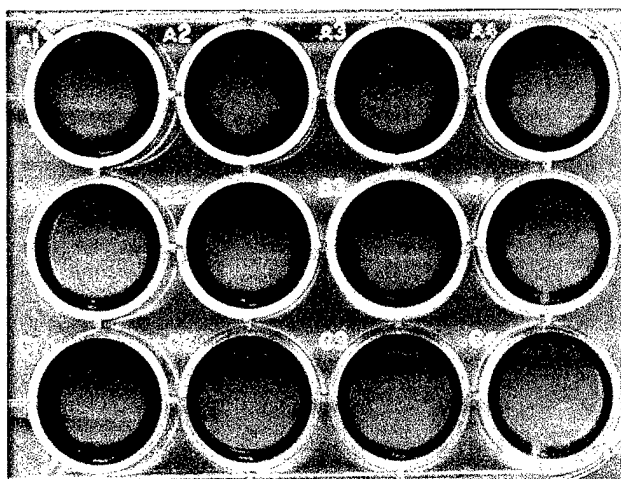
Control



A1-2: BLA"22"
A3: BLA"24"
B1-2: HMO"22"
B3-4: YDA"22"
C1-2: YDA"24"
C3-4: WCH"22"



GRC.014.24



A1-2: +IL-4 @ 10ng/ml
A3-4: +IL-4 @ 50ng/ml
B1-2: +IL-4 @ 100ng/ml
B3-4: +TNF-alpha @ 10ng/ml
C1-2: +TNF-alpha @ 50ng/ml
C3-4: +TNF-alpha @ 100ng/ml

Fig. 6. PC-3 tumor cells were incubated (1.5×10^5 cells/well) overnight at 37°C . Duplicate wells were then treated with 24hr. cultured supernatants from peripheral blood eosinophil hypodense (22) and hyperdense (24) subpopulations, from donors BLA, HMO, YDA and WCH. Tumor 1cells were also treated with IL-4 and $\text{TNF}\alpha$ at 10, 50 and 100ng/ml 24-48hr later.

Fig. 7. Eosinophil Inhibit PC-3 Colony Formation In Vitro

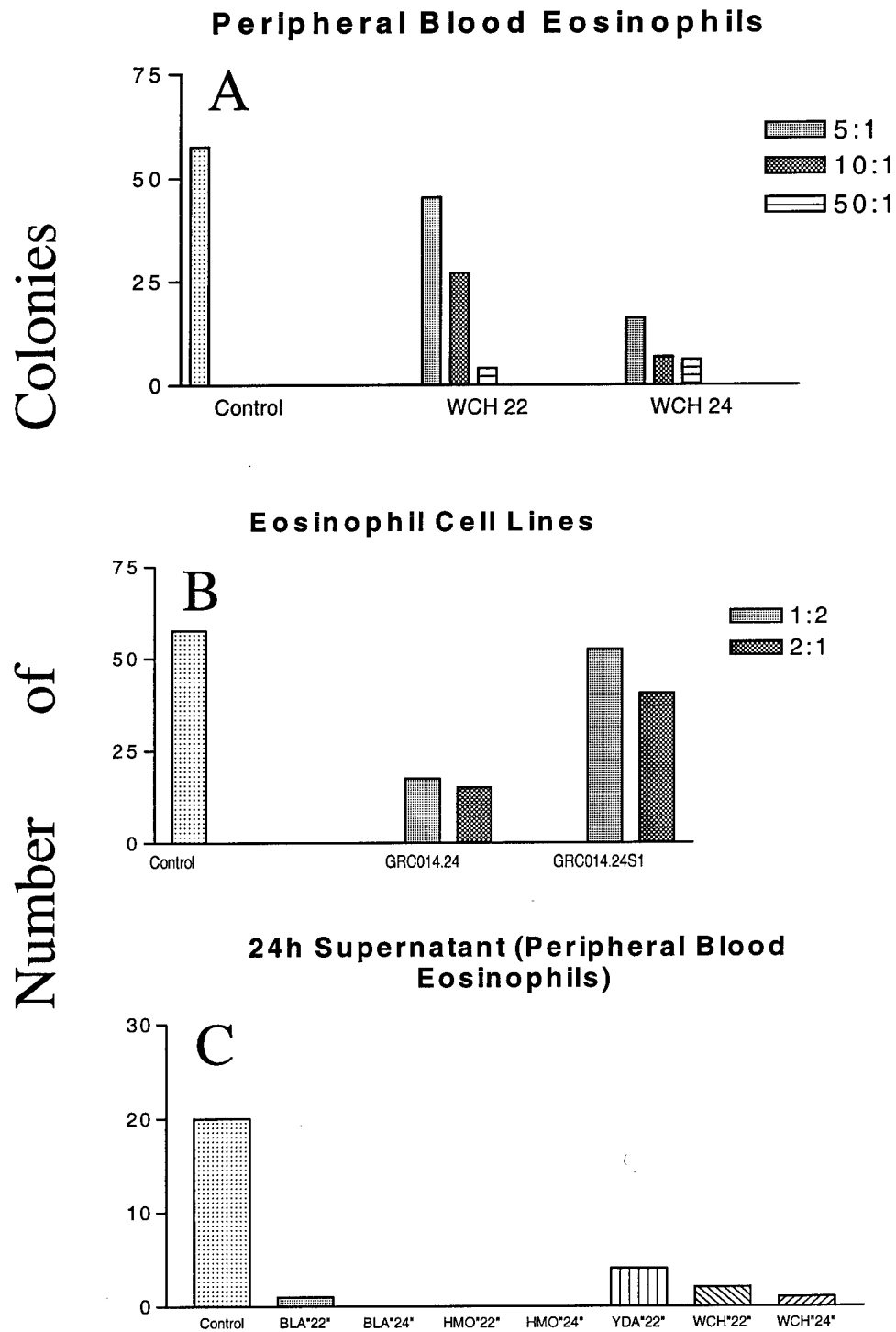
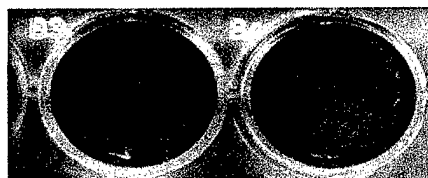
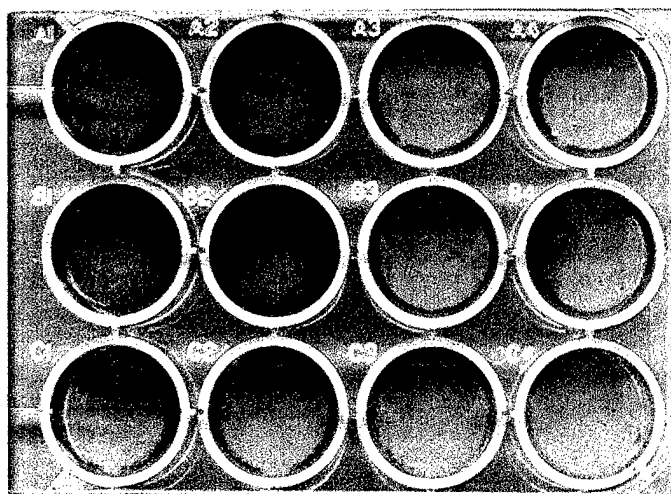


Fig. 7. PC-3 cells were seeded into duplicate and sometimes triplicate wells of a 6-well plate at 100 cells per well. After 24hr incubation eosinophils [fresh peripheral blood eosinophils (panel A); eosinophil cell lines (panel B)], and cultured supernatants (panel C) from both peripheral blood eosinophils (WCH 22 and WCH 24) and eosinophil cell line (GRC.014.24.2) and the plates were further incubated for 10 days. The plates were then harvested, stained with H & E and the colonies counted manually.

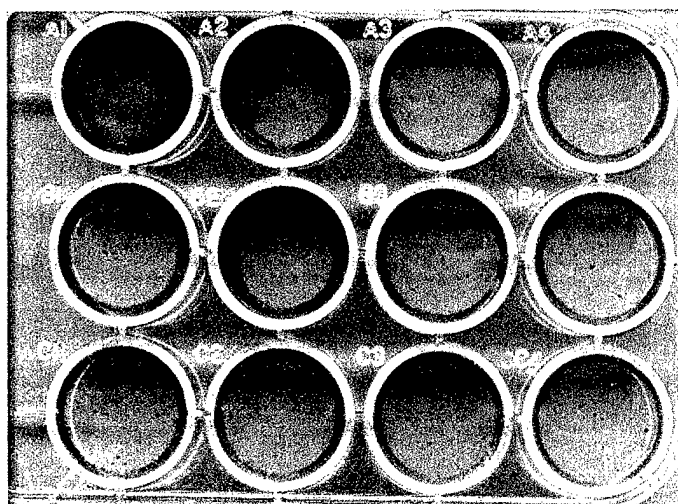
Fig. 8 Eosinophil Cell Lines Inhibit DU145 Cell Growth In Vitro



Control



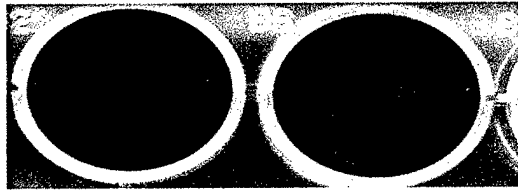
- A1-2: BJA060"22":DU145, 1:1
- B1-2: BJA060"22":DU145, 10:1
- C1-2: BJA060"22":DU145, 25:1
- A3-4: BJA060"22"+IL-5:DU145, 1:1
- B3-4: BJA060"22"+IL-5:DU145, 10:1
- C3-4: BJA060"22"+IL-5:DU145, 25:1



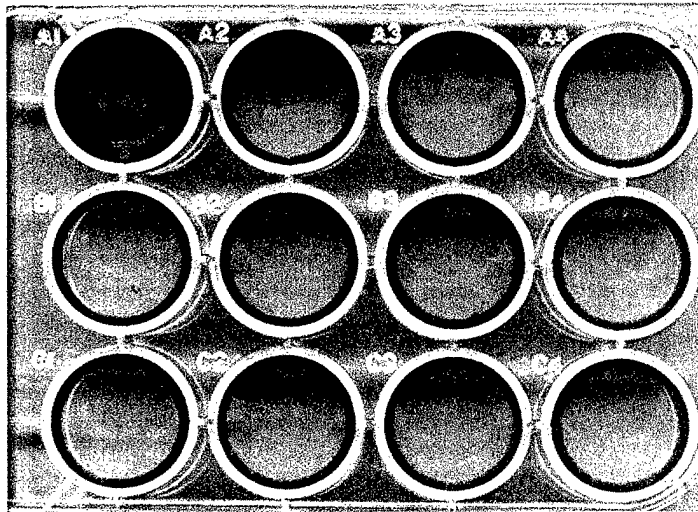
- A1-2: GRC014"24":DU145, 1:1
- B1-2: GRC014"24":DU145, 10:1
- C1-2: GRC014"24":DU145, 25:1
- A3-4: GRC014"24"+IL-5:DU145, 1:1
- B3-4: GRC014"24"+IL-5:DU145, 10:1
- C3-4: GRC014"24"+IL-5:DU145, 25:1

Fig. 8. DU145 prostate cells were seeded into duplicate wells of a 6-well plate at 1.5×10^5 cells/well and incubated overnight at 37°C . IL-5 treated and untreated eosinophil cell lines were added at E:T ratios 1:1, 10:1, and 25:1. The plates were incubated for an additional 24-48hr. Effector cells were removed, the plates were washed 3x with PBS then fixed and stained with hematoxylin and eosin. The entire plate or individual wells were then scanned into power point for presentation.

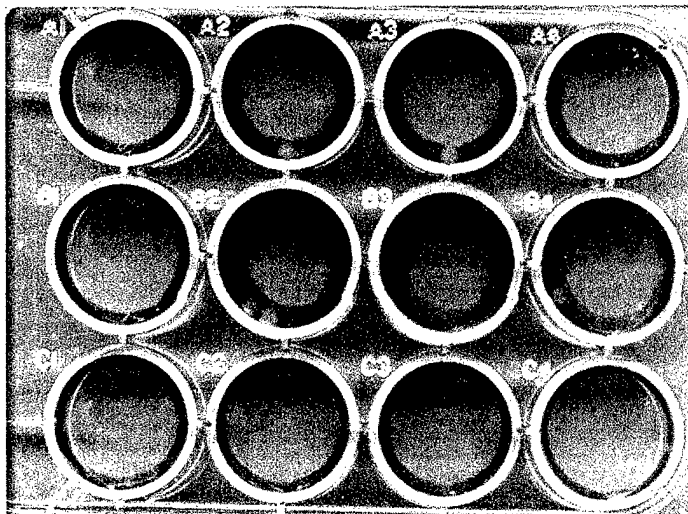
Fig. 9. 24hr Cultured Supernatants Inhibit DU145 Cell Growth In Vitro



Control



A1-2: BLA"22"
A3: BLA"24"
A4: HMO"24"
B1-2: HMO"22"
B3-4: YDA"22"
C1-2: YDA"24"
C3-4: WCH"22"



A1-2: +IL-4 @ 10ng/ml
A3-4: +IL-4 @ 50ng/ml
B1-2: +IL-4 @ 100ng/ml
B3-4: +TNF-alpha @ 10ng/ml
C1-2: +TNF-alpha @ 50ng/ml
C3-4: +TNF-alpha @ 100ng/ml

Fig. 9. DU145 cells (1.5×10^5) were treated with 24hr. cultured supernatants from various donor peripheral blood eosinophil subpopulations. Cells were also treated with IL-4 and TNF α at 10, 50 and 100ng/ml. The plates were stained with H & E and scanned into power point for presentation.

Fig. 10. DU145 Colony Inhibition by Eosinophil Cell Lines and Cultured Supernatants

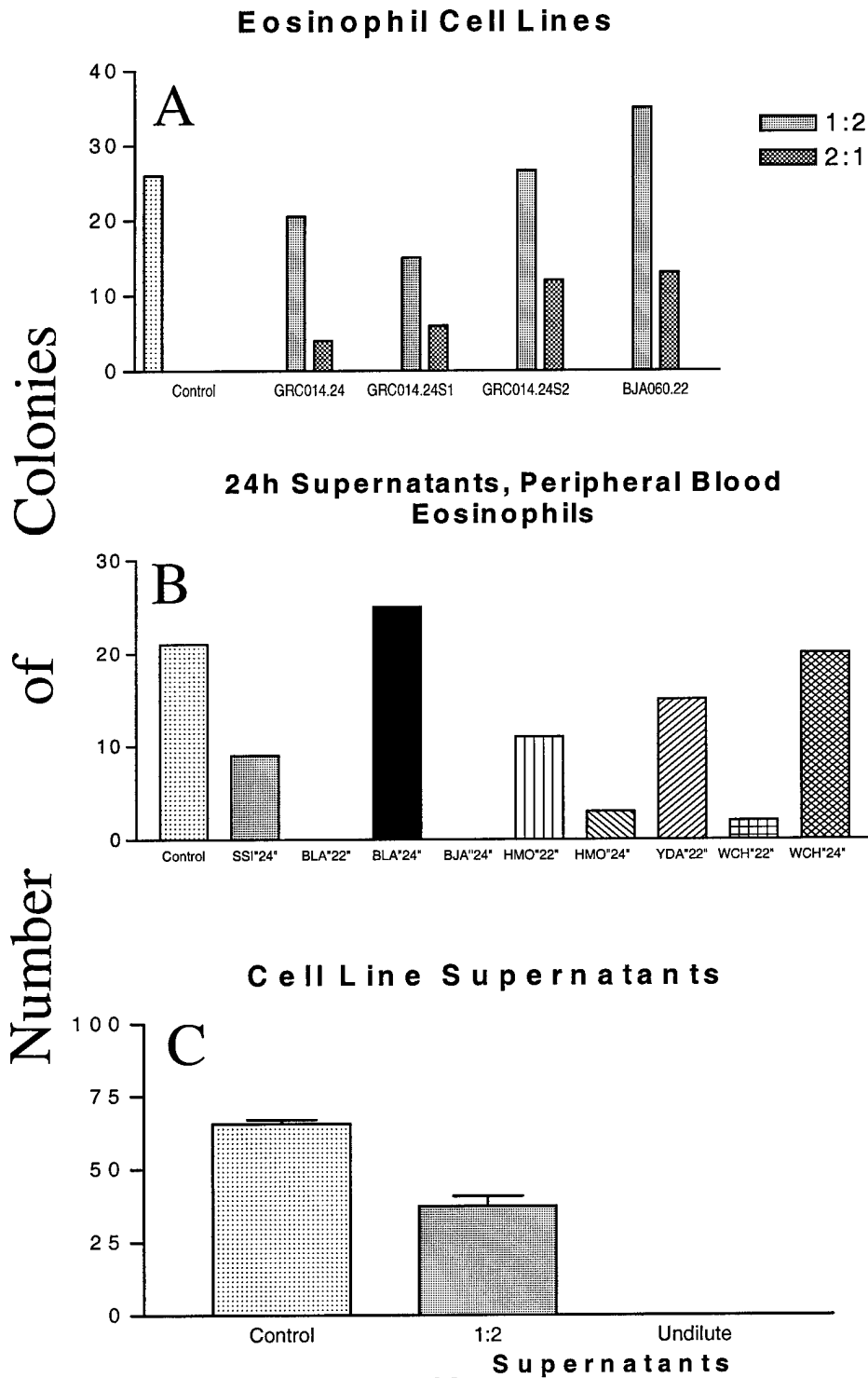


Fig. 10. DU145 cells were seeded into 6-well plates at 100 cells/well and incubated overnight at 37°C. At 24hrs. eosinophil cell lines were added at E:T ratios of 1:2 and 2:1. The plates were then cultured for 10 days at 37°C, after which the plates were stained with H & E and the colonies counted (Panel A). Parent Tumor cells were also incubated with supernatants (Panel B) from cultured eosinophils from various donors and also from a cultured eosinophil cell line (Panel C).

Fig. 11. HPCI Prostate Cell Growth Inhibition by Eosinophil Hypodense and Hyperdense Cell Lines

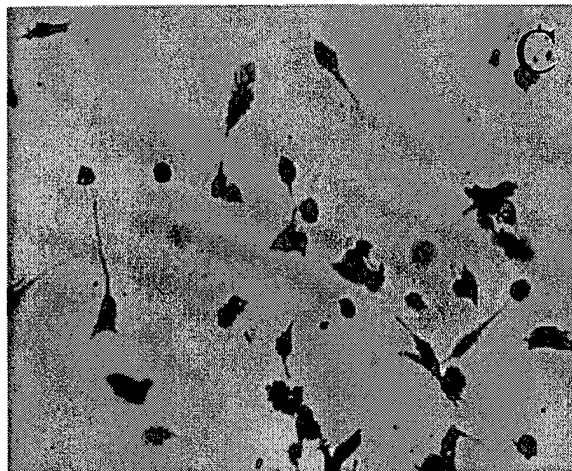
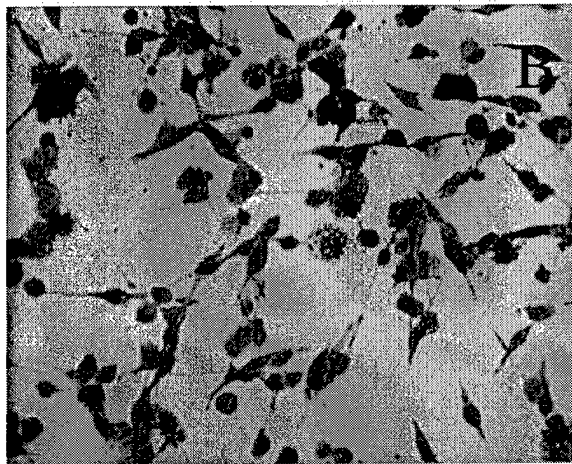
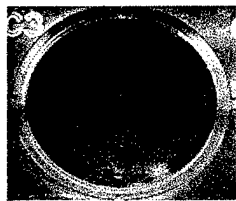
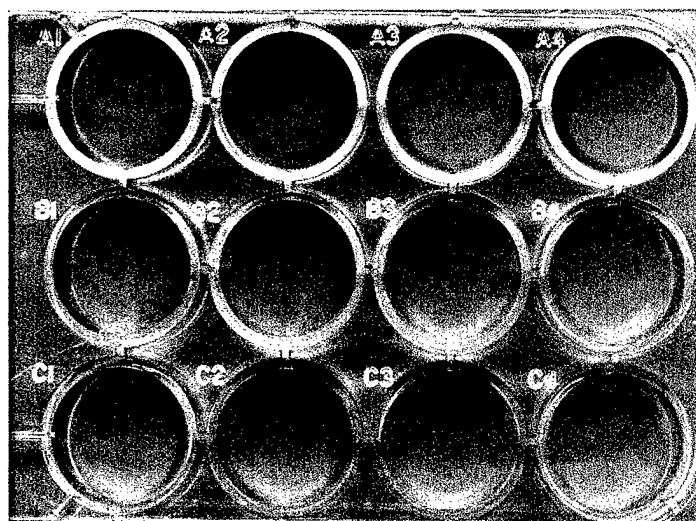


Fig. 11. HPC1 cells were seeded into the wells of a 12-well tissue cluster plate at 1.5×10^5 cells/well. Eosinophil cell lines (IL-5 treated and untreated) were added 24hrs. later the E:T ratio of 10:1. The plates were further incubated for 24-48hr., then harvested and photomicrographs taken.

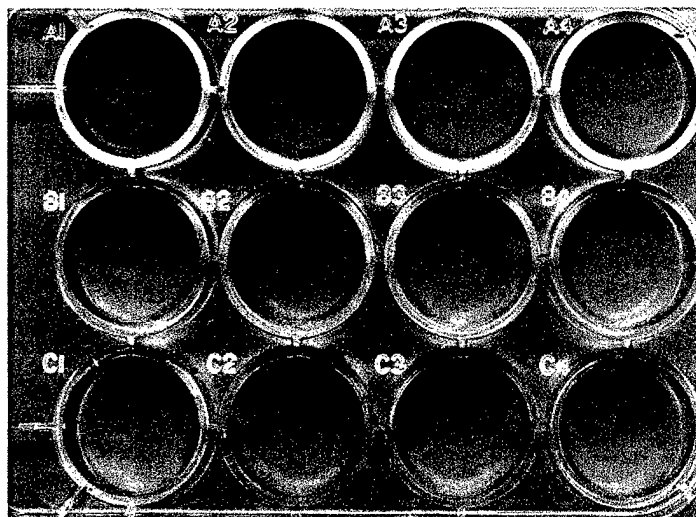
Fig. 12. HPCI Prostate Cell Growth Inhibition by Eosinophil Hypodense and Hyperdense Cell Lines



Control



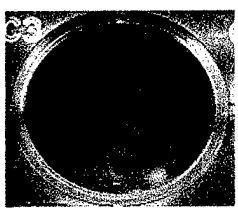
A1-2: GRC014"24":HPCI, 1:1
B1-2: GRC014"24":HPCI, 10:1
C1-2: GRC014"24":HPCI, 25:1
A3-4:GRC014"24"+IL-5:HPCI, 1:1
B3-4:GRC014"24"+IL-5:HPCI, 10:1
C3-4:GRC014"24":+IL-5HPCI, 25:1



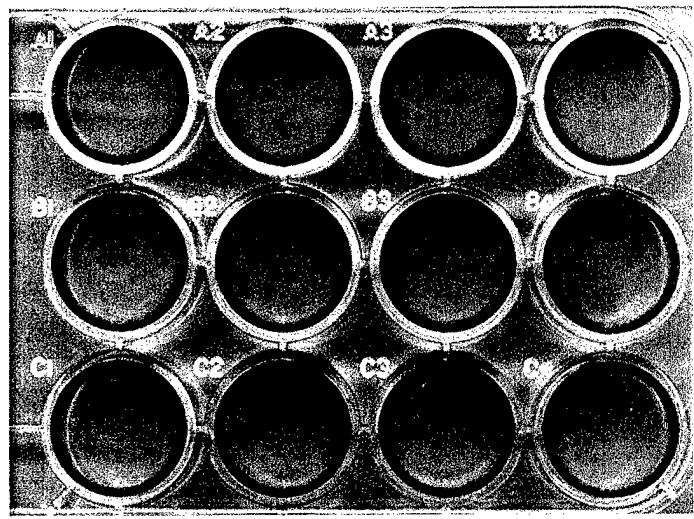
A1-2: BJA060"22":HPCI, 1:1
B1-2: BJA060"22":HPCI, 10:1
C1-2: BJA060"22":HPCI, 25:1
A3-4:BJA060"22"+IL-5:HPCI, 1:1
B3-4:BJA060"22"+IL-5:HPCI, 10:1
C3-4:BJA060"22"+IL-5:HPCI, 25:1

Fig. 12. HPCI cells were seeded into duplicate wells of a 12-well tissue culture plate similarly to that described in fig. 11. Effector cells (IL-5 treated and untreated) were added at E:T ratios of 1:1, 10:1 and 25:1. The plates were stained and scanned into power point.

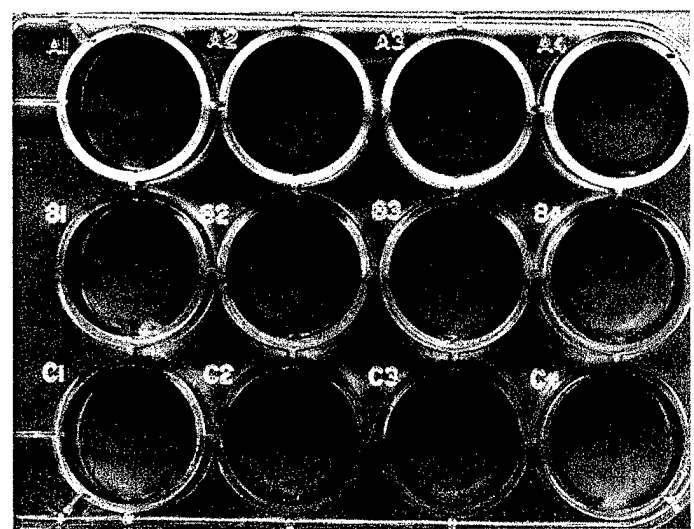
Fig 13. 24hr Eosinophil Cultured Supernatants Inhibit HPCI Cell Growth In Vitro



Control



A1-2: BLA"22"
 A3: BLA"24"
 A4: HMO"24"
 B1-2: HMO"22"
 B3-4: YDA"22"
 C1-2: YDA"24"
 C3-4: WCH"22"



A1-2: +IL-4 @ 10ng/ml
 A3-4: +IL-4 @ 50ng/ml
 B1-2: +IL-4 @ 100ng/ml
 B3-4: +TNF-alpha @ 10ng/ml
 C1-2: +TNF-alpha @ 50ng/ml
 C3-4: +TNF-alpha @ 100ng/ml

Fig. 13. HPC1 cells (1.5×10^5 /well) were cultured for 24hrs. were incubated for an additional 24-48hrs with cultured eosinophil supernatants (Panel A) and with IL-4 and TNF α (Panel B). The plates were harvested, stained with H & E and scanned into power point.

Table 1. CYTOKINE CONCENTRATIONS IN 24HR EOSINOPHIL CULTURE SUPERNATANTS (pg/ml)

Donor	IL-4		IL-5		TNF α		GM-CSF	
	22	24	22	24	22	24	22	24
1	>1000	>1000	440	435	50	63	0	0
2	316	3	0	0	100	56	0	0
3	>1000	631	0	0	50	16	0	0
4	>1000	0	nt	nt	129	200	nt	nt
5	200	20	0	0	100	224	nt	nt
6	8	>1000	0	186	10	7.9	450	450

Table 1. 24hr conditioned supernatants were tested for cytokines IL-4, IL-5, TNF α and GM-CSF using commercial enzyme linked immunoassay kits.

The American Urological Association, Inc.® 95th Annual Meeting

Presenting Author: Morice Dennerly

Presentation Type: Paper

Category: 44 Basic Research

Keywords: African American, Cell culture, Prostate cancer

A NOVEL PRIMARY PROSTATE CANCER CELL LINE DERIVED FROM AN AFRICAN AMERICAN PATIENT.

Chiledum Ahaghotu, Washington, D.C.; Josephine Marshalleck, Washington, D.C.; Morice Dennerly, Washington, D.C.; Theresa Vaughn, Washington, D.C.; Ibouraima Laniyan, Washington, D.C.; Aaron Jackson, Washington, D.C.; and Paulette Furbert-Harris, Washington, D.C..

Introduction and Objectives. Current epidemiological data suggests that African American prostate cancer patients tend to present with relatively aggressive phenotype compared to other ethnic groups in the U.S. The disparity probably represents genetic and environmental factors, both of which warrant in vitro studies. We present preliminary data on a primary prostate cancer cell culture system derived from an African American patient.

Methods. Malignant prostate tissue was obtained transurethrally from an African American patient presenting with hormone-refractory local tumor progression following radical retropubic prostatectomy. Tissue samples were submitted for H&E and immunohistochemical staining with PSA, PAP, CAM 5.2/AE-1, CK 7, 20, 903 and Lewis X antibodies. Remaining tissue was transported in Hank's balanced salt solution and processed by standard isolation and primary culture techniques. Briefly, collagenase-digested single-cell suspensions were dispensed into six-well plates coated with type I rat tail collagen. The plates underwent undisturbed CO₂ incubation at 37° C in prostate-specific serum-free culture media for 96 hours. Refeeding was done every 3 to 4 days. Actively proliferating primary cultures (1:2 split) were immortalized by Human Papilloma viral transfection using the LXSNI6E6E7 retroviral construct. Confluent flasks were harvested and cell pellets were paraffin-embedded and submitted for immunohistochemical staining.

Results. Standard H&E staining of original prostate tissue samples demonstrated sheets of anaplastic cells with no evidence of residual stromal or epithelial elements. Immunohistochemical staining was positive for PSA, PAP, and CAM 5.2/AE-1 (cytokeratins 39, 40, 43, 48, 50, 50.6 kd) and negative for Lewis X, CK 7, 20, and 903 (cytokeratins 46, 54, 56, 56.5, 58, 68 kd) antibodies. Despite marked similarities in histologic appearance between original tissue samples and primary cell culture, immunohistochemical staining of primary cells with previously described antibodies were negative.

Conclusions. Our laboratory presents preliminary findings on the first reported primary prostate cancer cell lined derived from an African American patient with hormone refractory disease. Further characterization is in progress.

Source of funding: Department of Defense:DAMD17-98-1-8485, Howard University Cancer Center.

Although you are not required to fax a copy of your abstract, to ensure the integrity of your abstract as submitted, we encourage you to fax a signed copy to Marathon Multimedia at 507-645-8105.