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

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13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information) We studied the effect of eosinophil 24hr. cultured supernatants and exogenous cytokines on growth and adhesion molecule expression on PC3, DU145 and LNCaP prostate cancer (Pca) cell lines. Hypo and hyperdense peripheral blood eosinophils (Eos) significantly inhibited LNCaP, PC3 and DU145 cell growth in vitro. This activity was enhanced by IL-5. DU145 and PC3 colony formation was inhibited by 50-75% by Eos cell lines, while peripheral blood Eos inhibited PC3 colony formation by 95%. 24hr. cultured supernatants significantly inhibited PC3 colony formation, causing 100% inhibition, and were more variable in their effects on DU145 (inhibition ranging from 9-100%). IL-4 and TNF-alpha inhibited PC3 and DU145 growth. ICAM-1, was upregulated on PC3 and DU145 by IL-1-alpha and TNF-alpha. IL-10 upregulated VCAM-1, ELAM, and E-Cadherin on PC3, while IL-12 upregulated ELAM and E-Cadherin. 24hr. supernatants had no measurable effect on adhesion molecule expression, however Eos:DU145 co-culture (E:T ratio 1:1) resulted in an upregulation of E-Cadherin on these cells. IDV density measurements of tumor cell cultures post Eos and conditioned supernatant treatment confirmed the inhibitory effects observed. The upregulation of E-Cadherin by Eos and IL-12 is intriguing. These observations may be useful for therapeutic strategies in prostate cancer.				
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Introduction

Prostate cancer is the most common cancer diagnosed in American men. It has been estimated that by the end of 2000, 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 tumors 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 and MCF-7 colony formation *in vitro*. The inhibition observed was 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 have previously established and are presently characterizing, on prostate cancer cell lines in eosinophil:tumor co-culture assays, and also the effect of cultured eosinophil supernatants on cell growth. Moreover this study investigates the potential regulation of cell adhesion molecule expression on prostate tumor cells as these molecules are involved in the migration of cells and eosinophils may either enhance or suppress prostate tumor cell migration or invasion, and hence metastasis.

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. 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 were maintained in the appropriate culture medium as recommended by the vendor; PC3 (7% F-12K medium supplemented with penicillin/streptomycin and gentamycin); DU145 and LNCAP in 10% RPMI 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 6-well plates (at 2.5×10^5 cells per well) or 12-well plates (at 1.5×10^5 cells per well). The plates were incubated overnight (16-24hr) at 37°C . Peripheral blood eosinophils were added at various effector to target (E:T) ratios and the plates incubated for an additional 24-48hr. Effector cells were then removed, the monolayers washed 3x with PBS and stained with H/E. LNCaP was extremely sensitive to both hypodense (22) eosinophils at 5:1 and 43:1 E:T ratios and hyperdense (24) eosinophils at 5:1 and 14:1 E:T ratios. PC3 was also sensitive to killing by eosinophil hypodense and hyperdense cell lines. Eosinophil cell lines were sterile sorted with a Becton Dickinson FACS SCAN Cell Sorter using the PE-labeled antibody to the eotaxin receptor. This chemokine receptor is found predominantly on eosinophils. These sublines were found also to be positive for 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. When 24hr. cultured eosinophil supernatants were added to subconfluent PC3 and DU145 monolayers, cell growth was dramatically inhibited.

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 H/E and counted manually. Both hypodense and hyperdense subpopulations of peripheral blood eosinophils inhibited PC3 colony formation in a dose-dependent manner, with the 50:1 E:T ratio resulting in 95% inhibition for the hypodense eosinophils and 91% inhibition 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. 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. 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 and at least three supernatant preparations (BLA 24, HMO 22 and HMO 24) completely prohibited colony formation. GRC.014.24 supernatant completely inhibited DU145 colony formation.

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 ($\text{TNF}\alpha$) were present in varying levels in all individuals tested (Table I). IL-4 concentrations ranged from 0 to >1000 pg/ml. $\text{TNF}\alpha$ concentrations were far less than IL-4, ranging from 10-224pg/ml.

E. Adhesion Molecule Expression. (E-selectin, ELAM, ICAM-1, VCAM-1, VLA-4). All cell lines were subcultured with their appropriate media (PC3 - F12K complete with 7% FBS; DU145 and LNCAP - RPMI complete with 10% FBS). Optimum incubation time and temperature was determined for those adhesion molecule antibodies that were not tested for flow cytometry use prior to purchase. Those antibodies purchased from Becton Dickinson, or comparable companies specializing in flow cytometry reagents, were used according to vendor specifications. The adhesion molecules tested were E-Selectin, ICAM-1, VCAM-1, and VLA-4. Moreover we tested E-Cadherin and N-Cadherin expression on PC3 and DU145. ELAM, ICAM-1 and VCAM-1 were examined by direct flow cytometric procedures and E-Cadherin, N-Cadherin and VLA-4 were analyzed by indirect flow cytometry, according to vendor's protocol.

F. Integrated Density Value Measurement of the monolayer assay. In order to quantitate the inhibitory activity observed in the monolayer assays, we utilized a Chemi-Imager 4000 (alpha Innotech Corp) to measure the density of the cells in the wells. The well area was selected and saved as the spot overlay. This spot overlay was used on each well of a plate in order to standardize the area of all wells. As wells were selected, their Integrated Density Value (IDV) was automatically calculated. The IDV is the sum of all pixel values after background correction. Auto background correction uses the Alpha Ease program which determines the average of the 10 lowest pixel values in each individual well and assigns that value as background. However, if no background is selected the background value is reported as zero. Because the average (AVG) is equal to IDV divided by the overlay value (which is constant), we used AVG as a comparative figure. We then compared the IDV of the control with that of the test samples. The percent inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{average IDV (Control)} - \text{average IDV (test)}}{\text{average IDV (control)}} \times 100$$

MRC-5 fibroblasts and tumor cells were seeded into the wells of either a 6-well plate (at 5×10^5 cells per well) or a 12-well plate (at 1.5×10^5 cells per well) as previously described. The plates were incubated overnight (18-24hr) at 37°C, 5% CO₂ at 95% humidified atmospheric conditions before treatment. Eosinophils were added at various effector to target (E:T) ratios and the plates were incubated for an additional 72hrs. The effector cells were then removed, the monolayers washed 3x with PBS and the wells stained with hematoxylin and eosin. At E:T ratio of 1:1, subline GRC.014.24S did not inhibit the growth of MRC-5 fibroblasts (fig. 24). As the E:T ratio increased the IDV increased giving a negative value of inhibition. Hypodense peripheral blood eosinophils (fig. 25) dose-dependently inhibited PC-3 tumor cell growth, while hyperdense eosinophils at 5:1 and 10:1 were comparable (75-78%) in their inhibition. Hypodense eosinophil cell line was less effective in inhibiting both DU145 and PC-3 cell growth however, this activity was upregulated by IL-5 (fig. 26 A&B). Similar results were observed with the hyperdense cell line (fig. 27). At the E:T ratio of 10:1 (fig. 27A) and 1:1 (fig. 27B) the IDV values increased, giving a negative value of inhibition. In these experiments, eosinophil:tumor cell clusters were observed. This thus created high density values which were readily detected by the Chemi-Imager. Killing thus seemed to have been masked if there were several of these eosinophil:tumor cell clusters present, even though the lawn of cells had been destroyed.

This was one of the draw backs to using the Chemi-Imager to quantitate cell destruction. We utilized an eosinophil subline, previously created by FACS sorting the hyperdense parent line using the CCR3 monoclonal antibody. Inhibition at 1:1 and 2:1 E:T ratios were marginally less than the parent line, but significantly higher (68% vs. 58%) at the higher E:T ratio, 5:1 (fig. 28). Having demonstrated growth inhibition (both monolayer cultures and colony formation) by peripheral blood eosinophils and eosinophil cell lines, we then examined conditioned supernatants. In figure 29, when DU145 and PC-3 tumor cells were cultured with 24hr conditioned supernatants from both the hypo and hyper-dense eosinophils. PC-3 tumor cells were inhibited far less than DU145 with all supernatants tested. Six of the eight preparations (hypo-hyperdense) inhibited DU145 growth by approximately 50%, while only two of the six samples tested inhibited PC-3 by 40%. When these supernatants were analyzed (prior study) for cytokine presence, both IL-4 and TNF- α were present. We then tested exogenous IL-4 and TNF- α on DU145 and PC-3 cell growth; IL-4 inhibited DU145 growth by 38-42% while TNF- α inhibited DU145 38-50% (fig. 30). While inhibition of PC-3 by IL-4 was comparable to that of DU145, TNF- α was more potent in its inhibition (fig. 30B). At 100ng/ml TNF- α completely destroyed PC-3 cells. These studies show that both IL-4 and TNF- α participate in the destruction and growth inhibition of prostate tumor cells in vitro.

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.
- ▶ Determined cytokine modulatory effects on cell adhesion molecules
- ▶ Upregulated tumor suppressor E-Cadherin on PC3 cells

Reportable Outcomes

Promotion from Assistant Professor to Associate Professor.

Activated eosinophils inhibit in vitro growth of prostate cancer cell lines, (Manuscript to submitted)
Late Abstract for AACR Spring 2000, (Eosinophil Cell Lines Inhibit Prostate Cancer Cell Growth In Vitro).

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.

Regulation of E-Cadherin Expression on Prostate Cancer Cells by Activated Eosinophils is Mediated by IL-12. **Furbert-Harris PM**¹, Laniyan I¹, Hunter KA², Vaughn T¹, Parish-Gause D¹, Forrest K¹, Brooks L¹, Albury R¹, Howland C¹, Tackey R¹, Okomo-Awich J¹, and Oredipe OA¹. Howard University Cancer Center, Howard University Hospital, Washington, DC 20060 and NIH, Bethesda, MD. Abstract submitted to the AACR Special Conference on Cytokines in Cancer, September 2000. (IL-12 Modulation of E-Cadherin expression on Prostate tumor cells).

In Vitro Inhibition of Prostate Cancer Cell Growth by Eosinophil Cell Lines. **Paulette M. Furbert-Harris**¹, Josephine Okomo-Awich¹, Debra Parish-Gause¹, Ibrahim Laniyan¹, Keith A. Hunter², Theresa R. Vaughn¹, Christina Howland¹, Kesha C. Forrest¹, and Oladipo Oredipe¹. Howard University, Washington, DC 20060, and NIH, Bethesda, MD. (In Preparation)

Modulation of Adhesion Molecule Expression on Prostate Tumor Cells by Conditioned Supernatants from Eosinophil Cell Lines. **Paulette M. Furbert-Harris**, Michelle Law, Debra Parish-Gause, Josephine Okomo-Awich,, Ibrahim Laniyan, Theresa R. Vaughn, Kesha C. Forrest, Christina Howland, and Oladipo Oredipe. (In Preparation)

EBV-Transformed Eosinophil Sublines Inhibit Prostate Tumor Cell Growth In Vitro. **Paulette M. Furbert-Harris**, Ibrahim Laniyan, Josephine Okomo-Awich, Debra Parish-Gause, Theresa R. Vaughn, Kesha C. Forrest, Christina Howland, and Oladipo Oredipe. (In Preparation)

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 for the 18 month period.

- 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.
- e. flow cytometric analysis of adhesion molecule expression post eosinophil:tumor cell co-culture.
- f. the modulation of adhesion molecules were studied by flow cytometry.
- g. effect of eosinophil supernatants with and without co-culture on adhesion molecule expression on prostate tumor cells.
- h. exogenous cytokine modulation of adhesion molecules on prostate tumor cells.

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 subpopulations 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 enhanced hypodense and hyperdense cell line 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 in these studies used to enhance eosinophil activity.

ICAM-1 was expressed on DU145 and PC3. This was upregulated by IL-1, TNF α and IL-10. ICAM-1 was induced on LNCaP by IL1 α and TNF α . ELAM-1 and VCAM-1 were absent on PC3 but ELAM-1 expression was induced by TNF α , IFN γ , IL-10 and IL-12, but not IL-4 and VCAM-1 was induced by IL-10 only. TNF α and IL-4, induced marginal ELAM-1 expression on DU145, not LNCaP. The most significant observation has been the induction of the suppressor adhesion molecule E-Cadherin by IL-12, and eosinophil enhancement of E-Cadherin on both DU145 and PC3. This has not been reported in prostate cancer and the only other report has been by Hiscox *et al* with human colon cancer cells, (Clin Exp Metastasis 13(5): 396-404 (1995). IL-12 is well known for its immunomodulatory activities. IL-12 is now being vigorously studied as an anti-cancer cytokine. The observation presented in this report reaffirms the potential significance of IL-12 as an anti-cancer therapeutic agent. Eosinophils produce IL-12, hence this cell gains continuing attention as an anti-cancer effector and the use of eosinophil cell lines which we have developed offer exquisite tools for clearly defining biologic activity of eosinophils and developing strategic manipulations in order to maximize their worth as anti-cancer effector agents.

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Personnel

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

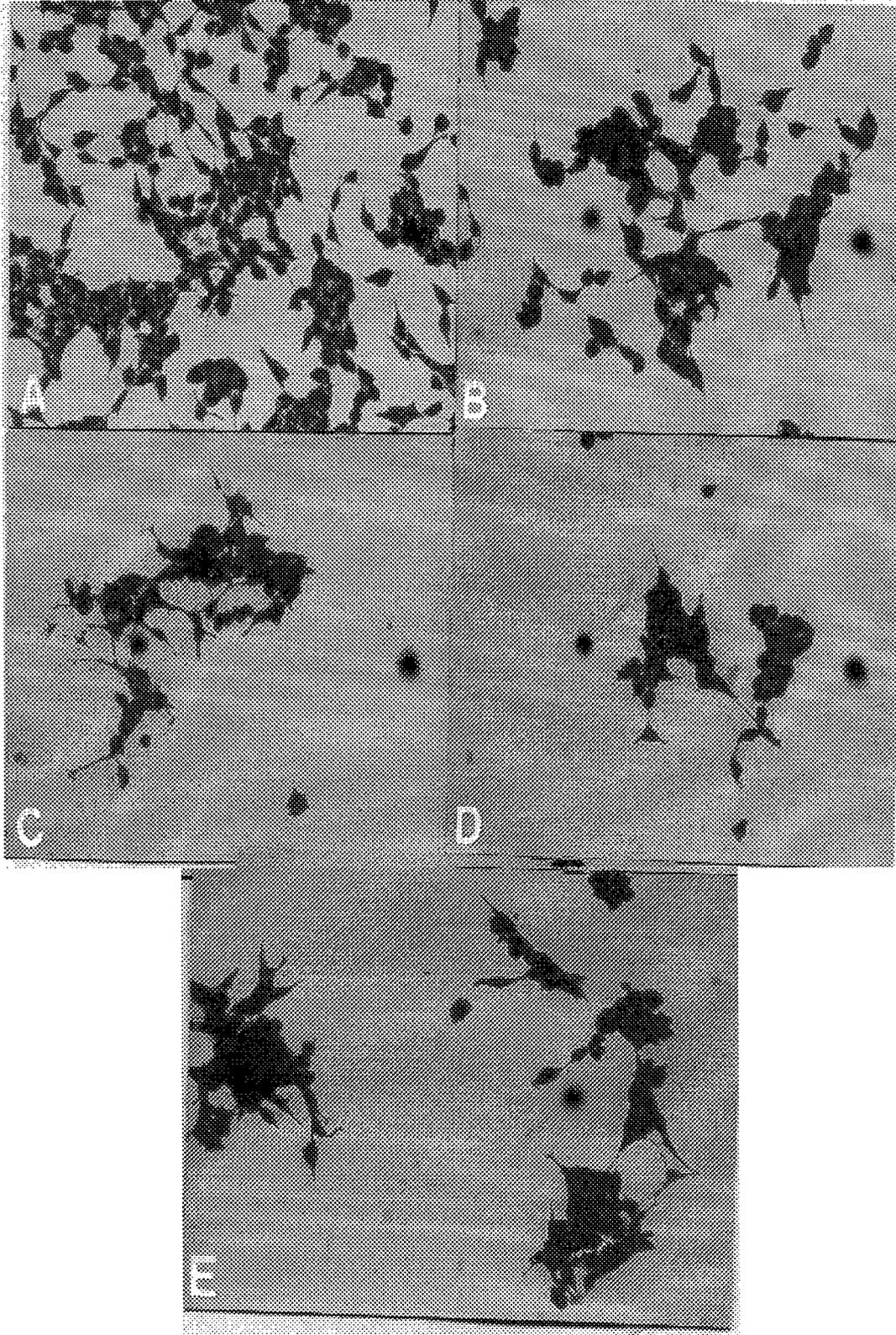


Fig. 1. LNCaP tumor cells were seeded into T25 flasks at 3×10^5 cells/flasks and allowed to grow to confluence (3-4 days) in media alone (A) or in the presence of hypodense eosinophilic cell line SD.031.22 at E:T ratios of 5:1 and 43:1 (B&C, respectively) and hyperdense cell line (SD.031.24) at E:T ratios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

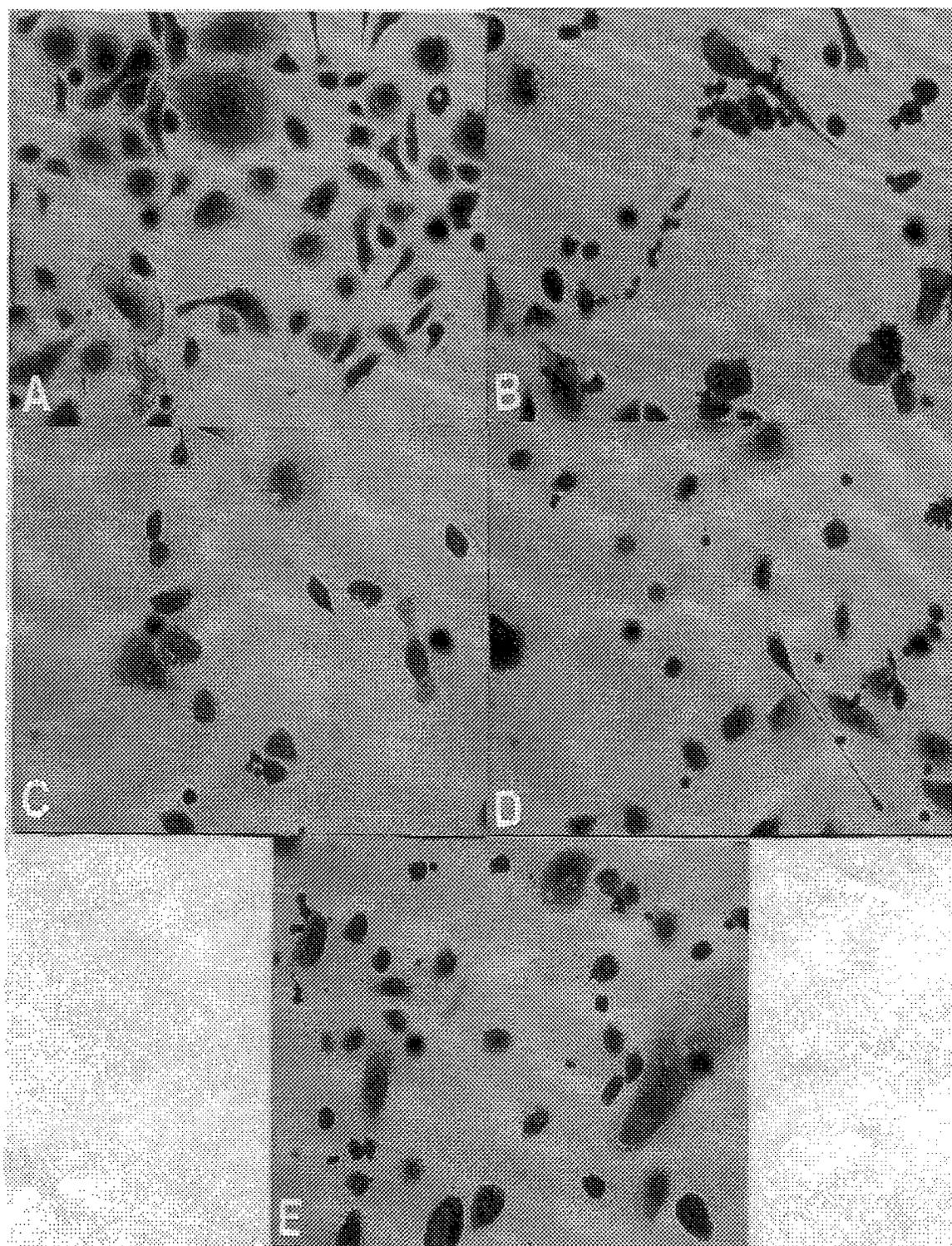


Fig. 2 PC-3 tumor cells were seeded into duplicate T25 flasks at 3×10^5 cells/flask and allowed to grow to confluence (3-4 days) in media alone, and in co-culture with hypodense eosinophilic cell line SD.031.22 at E:T ratios of 5:1 and 43:1 (B, C, respectively) and hyperdense cell line SD.031.24 at E:T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

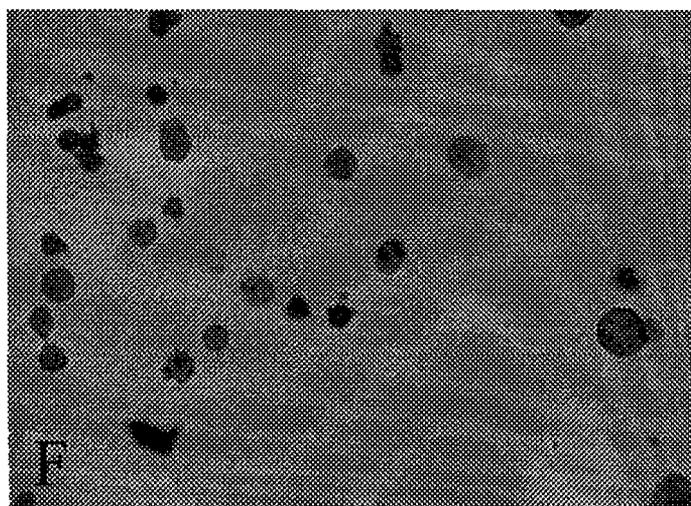
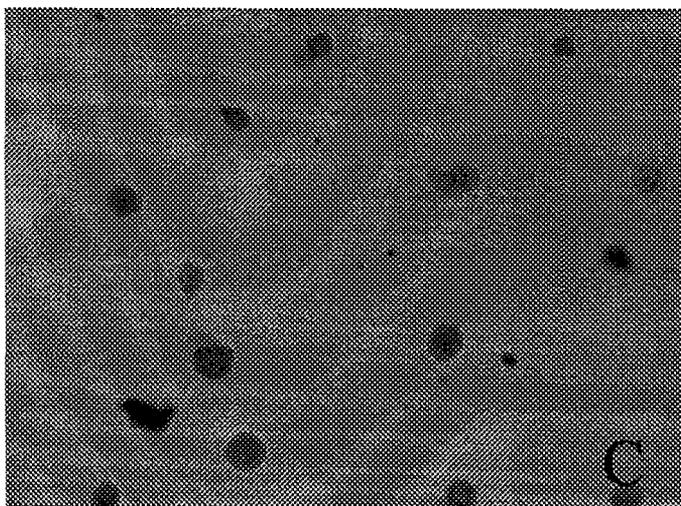
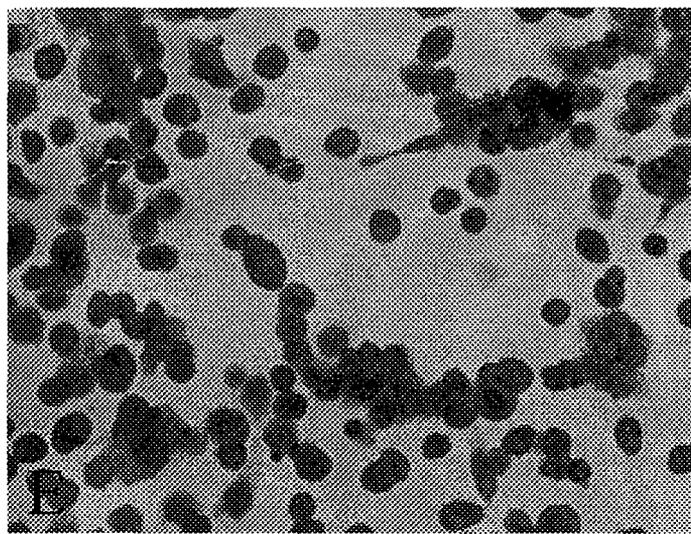
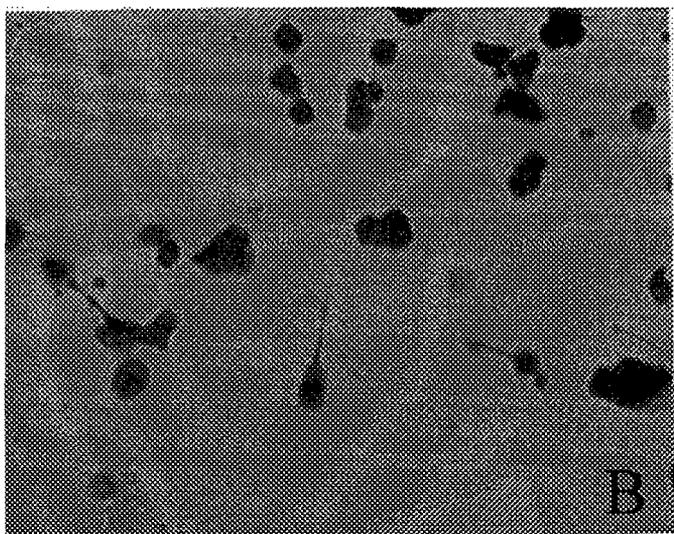
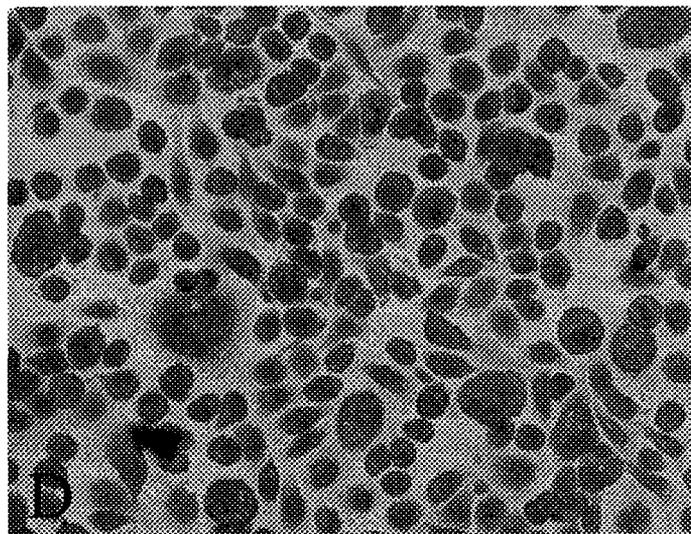
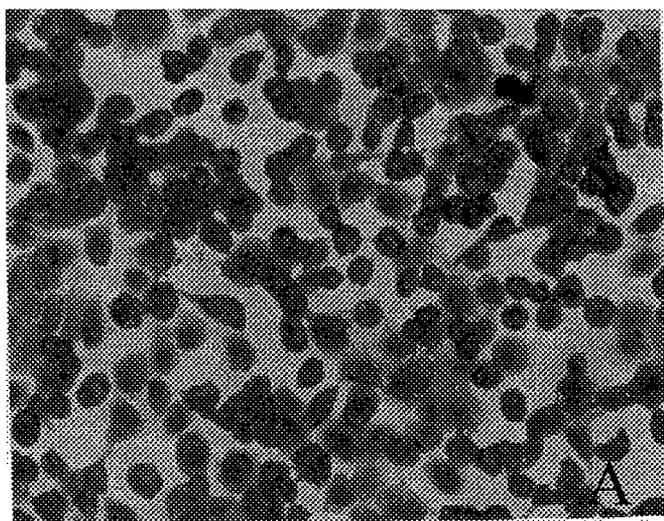
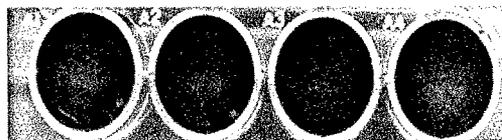


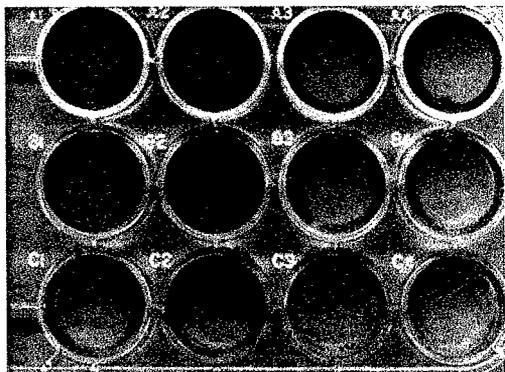
Photo Micrograph

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.

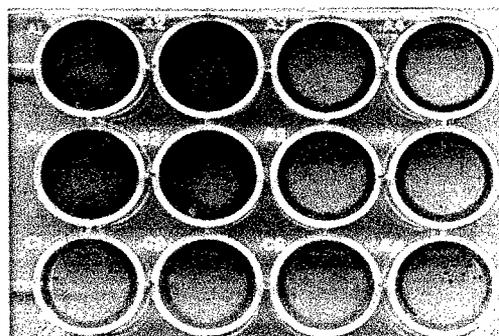
Fig. 4. IL-5 Treatment of Eosinophil Cell Lines Enhances Growth Inhibition of PC3 Tumor Cells In Vitro



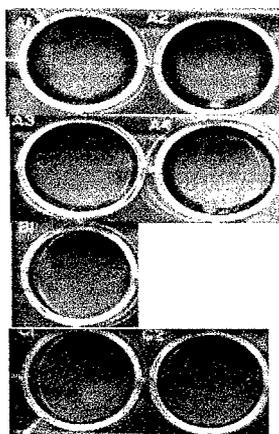
Control



A1-2:GRC.014.24:PC-3, 1:1
 B1-2:GRC.014.24:PC-3, 10:1
 C1-2:GRC.014.24:PC-3, 25:1
 A3-4:GRC.014.24+IL-5:PC-3, 1:1
 B3-4:GRC.014.24+IL-5:PC-3, 10:1
 C3-4:GRC.014.24+IL-5:PC-3, 25:1



A1-2:BJA.060.22:PC-3, 1:1
 B1-2:BJA.060.22:PC-3, 10:1
 C1-2:BJA.060.22:PC-3, 25:1
 A3-4:BJA.060.22+IL-5:PC-3, 1:1
 B3-4:BJA.060.22+IL-5:PC-3, 10:1
 C3-4:BJA.060.22+IL-5:PC-3, 25:1



WCH22, 5:1 (Peripheral Blood Eosinophils)
 WCH22, 10:1 (Peripheral Blood Eosinophils)
 WCH22, 25:1 (Peripheral Blood Eosinophils)
 WCH24,5:1 (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

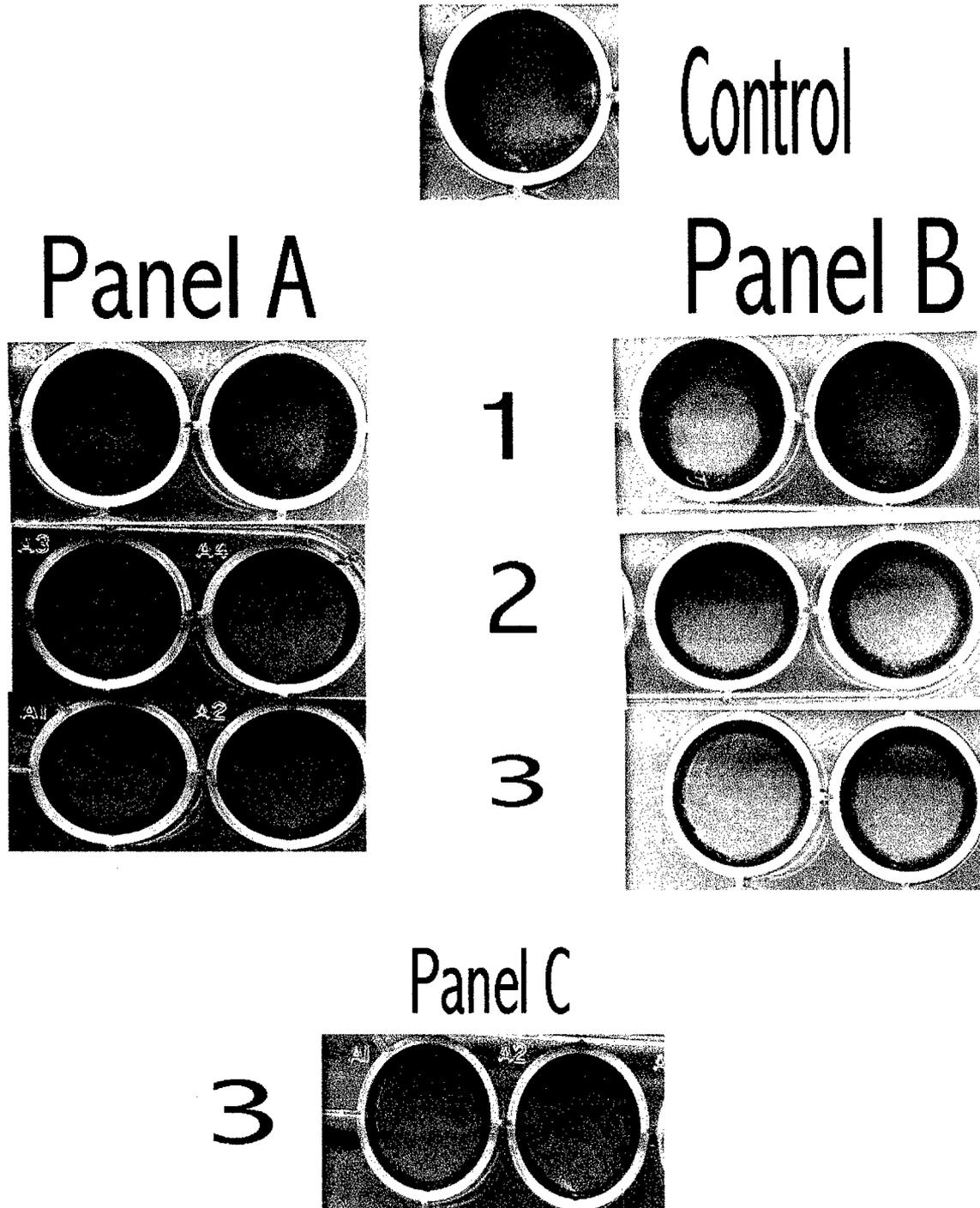
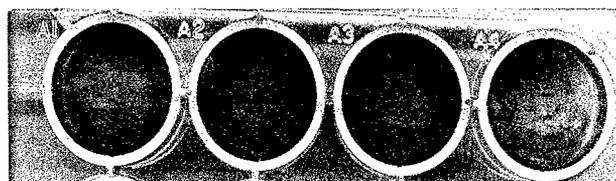
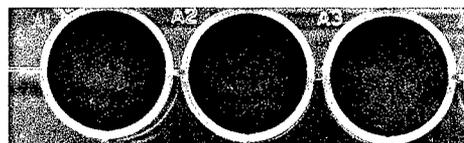


Fig. 5. Sublines from the eosinophil parent line GRC.014.24 were sterile-sorted with a FACS SCAN cell sorter using antibody to the eosinophil specific eotaxin receptor. The sublines S1 and S2 were found to be positive for both CD15 and CD49d markers. The co-culture was set up similarly to that described 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

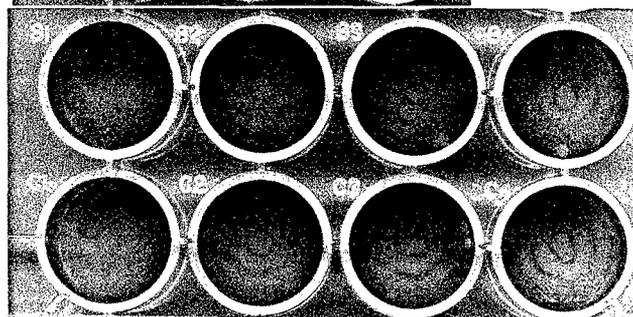


Control



A1-2: BLA22

A3: BLA24

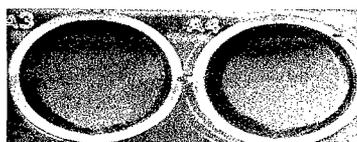


B1-2: HMO22

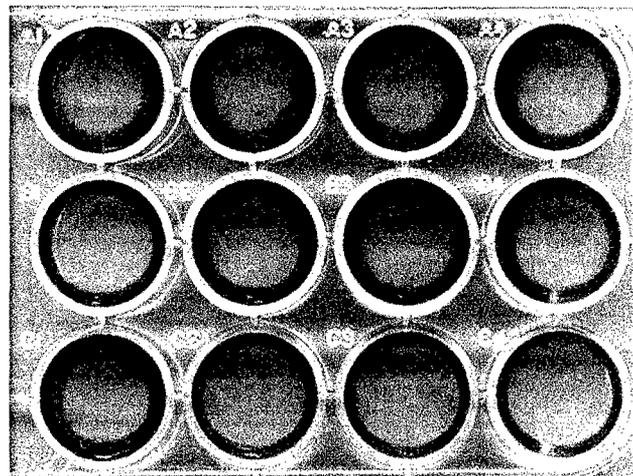
B3-4: YDA22

C1-2: YDA24

C3-4: WCH22



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 cells 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

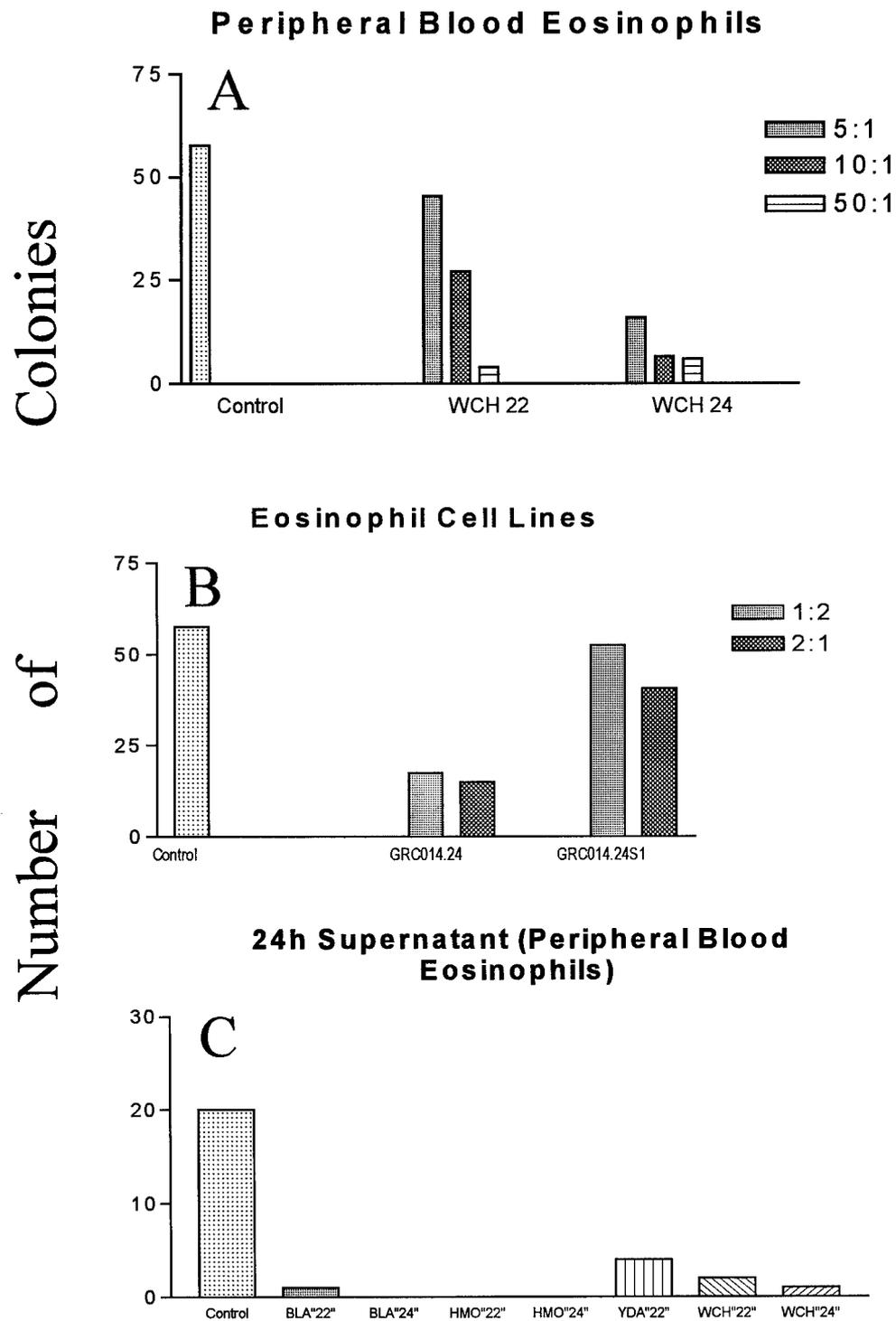
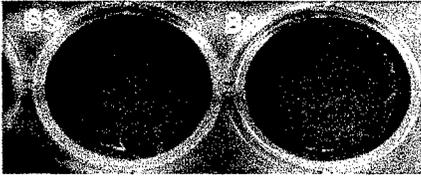
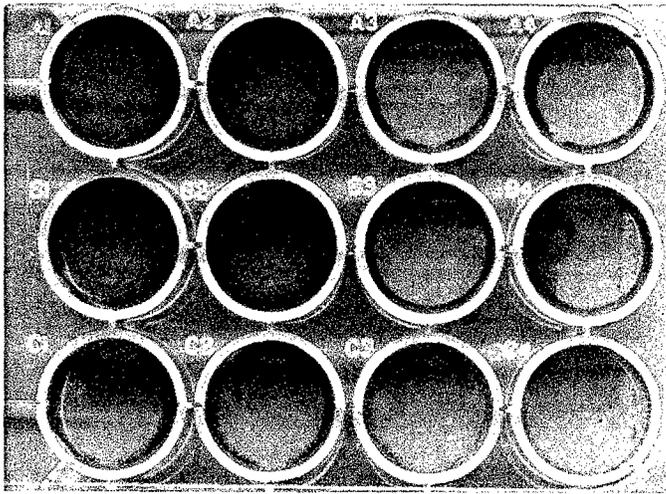


Fig. 7. PC-3 cells were seeded into duplicate and sometimes triplicate wells of a 6-well plate at 100 cells per well. After 24hr incubation eosinophils [fresh peripheral blood eosinophils (panel A); eosinophil cell lines (panel B)], and cultured supernatants (panel C) from both peripheral blood eosinophils (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.

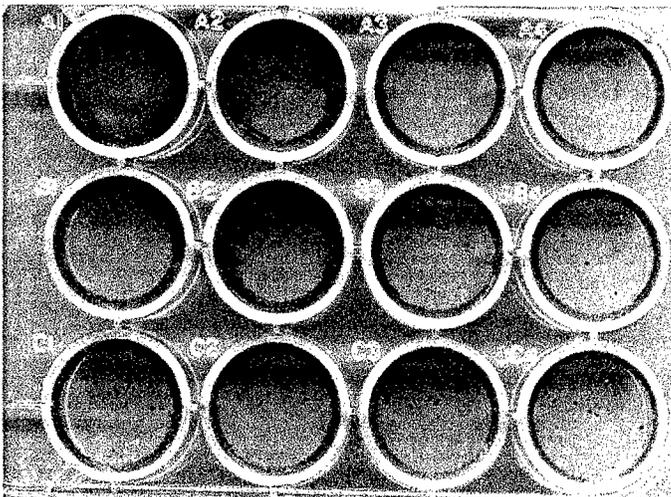
Fig. 8 Eosinophil Cell Lines Inhibit DU145 Cell Growth In Vitro



Control



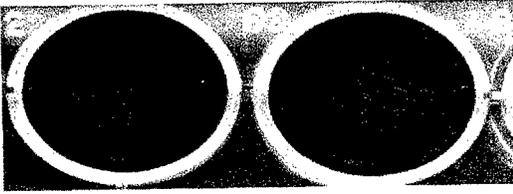
A1-2: BJA.060.22:DU145, 1:1
B1-2: BJA.060.22:DU145, 10:1
C1-2: BJA.060.22:DU145, 25:1
A3-4: BJA.060.22+IL-5:DU145, 1:1
B3-4: BJA.060.22+IL-5:DU145, 10:1
C3-4: BJA.060.22+IL-5:DU145, 25:1



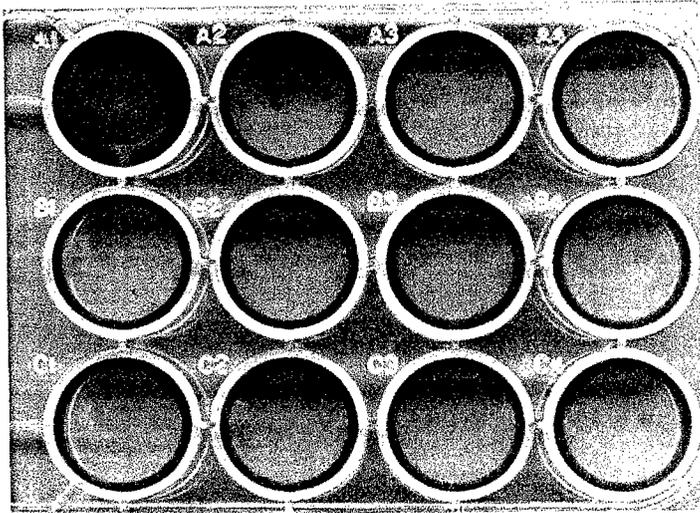
A1-2: GRC.014.24:DU145, 1:1
B1-2: GRC.014.24:DU145, 10:1
C1-2: GRC.014.24:DU145, 25:1
A3-4: GRC.014.24+IL-5:DU145, 1:1
B3-4: GRC.014.24+IL-5:DU145, 10:1
C3-4: GRC.014.24+IL-5:DU145, 25:1

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.

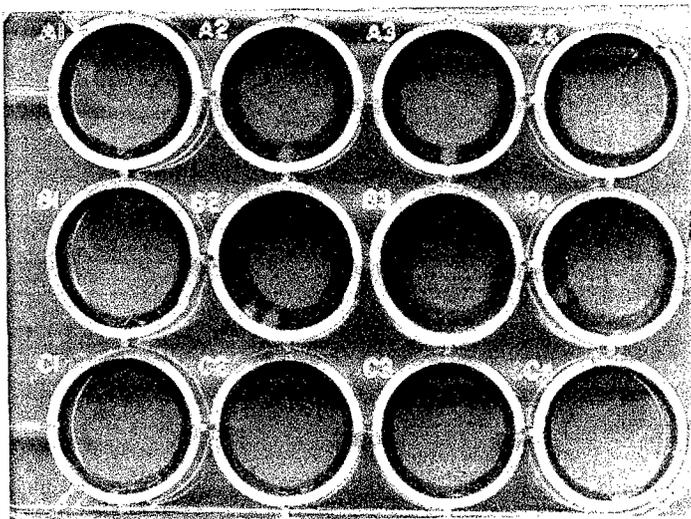
Fig. 9. 24hr Cultured Supernatants Inhibit DU145 Cell Growth In Vitro



Control



A1-2: BLA22
A3: BLA24
A4: HMO24
B1-2: HMO22
B3-4: YDA22
C1-2: YDA24
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

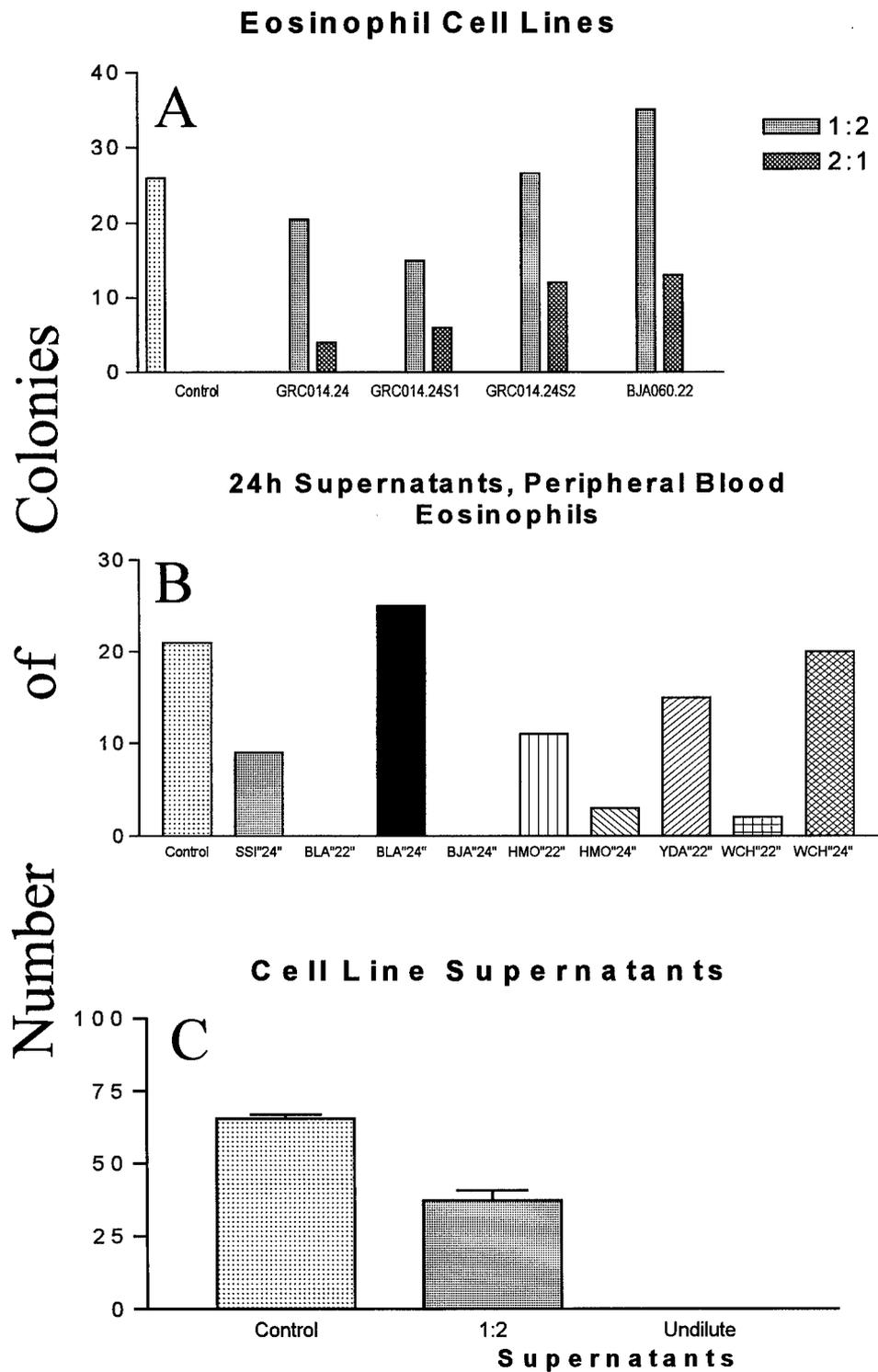
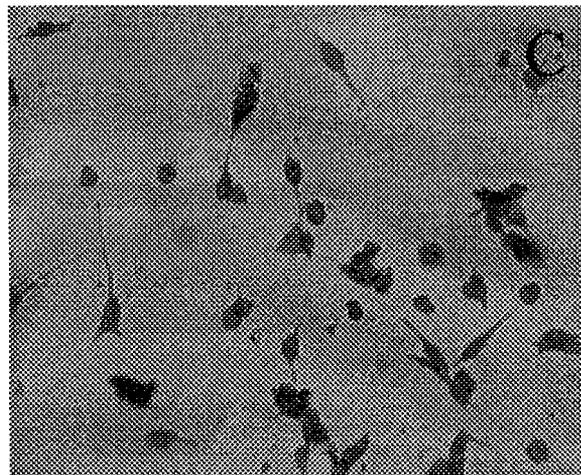
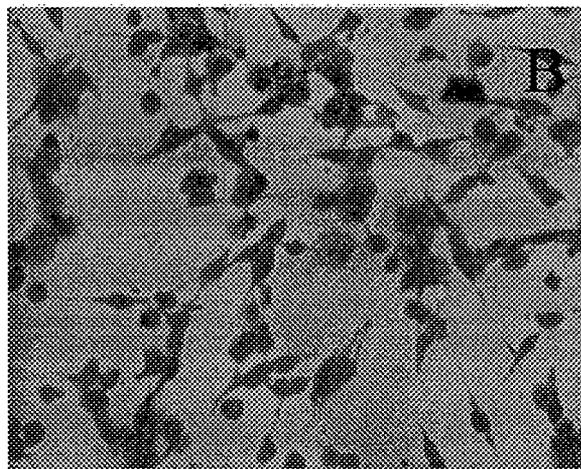
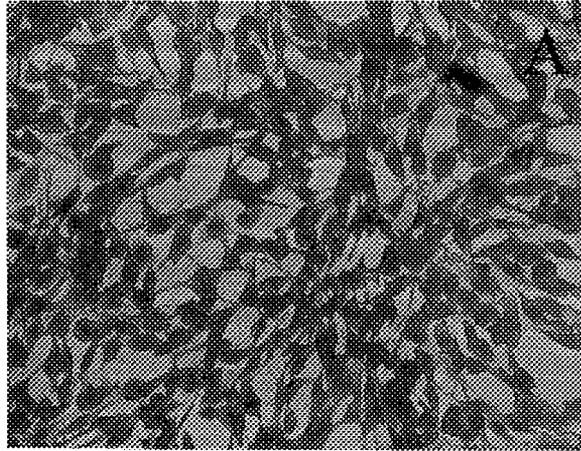


Fig. 10. DU145 cells were seeded into 6-well plates at 100 cells/well and incubated overnight at 37°C. At 24hrs. eosinophil cell lines were added at E:T ratios of 1:2 and 2:1. The plates were then cultured for 10 days at 37°C, after which the plates were stained with H & E and the colonies counted (Panel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

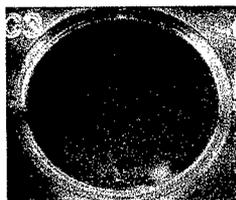
Fig. 11. HPCI Prostate Cell Growth Inhibition by Eosinophil Hypodense and Hyperdense Cell Lines



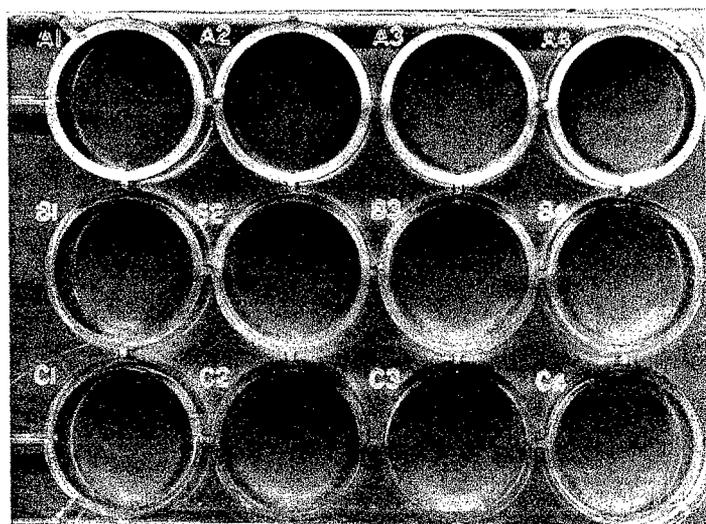
Photomicrograph

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.

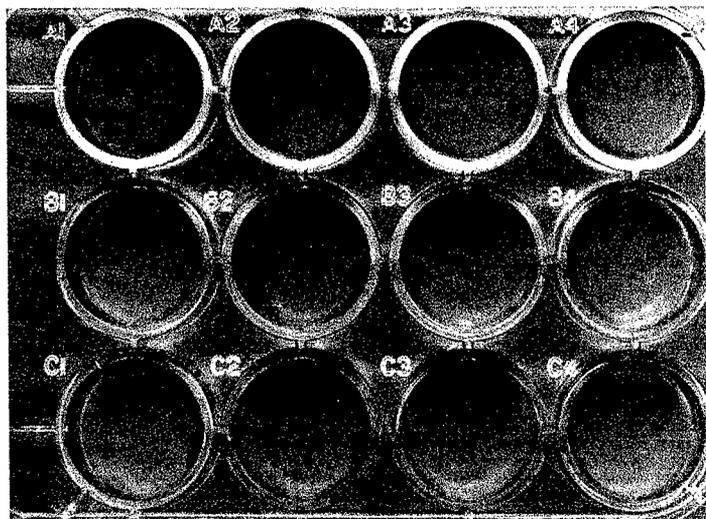
Fig. 12. HPCI Prostate Cell Growth Inhibition by Eosinophil Hypodense and Hyperdense Cell Lines



Control



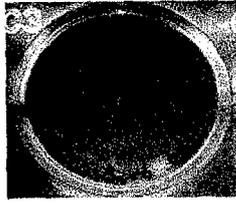
A1-2:GRC.014.24:HPCI, 1:1
B1-2:GRC.014.24:HPCI, 10:1
C1-2:GRC.014.24:HPCI, 25:1
A3-4:GRC.014.24+IL-5:HPCI, 1:1
B3-4:GRC.014.24+IL-5:HPCI, 10:1
C3-4:GRC.014.24:+IL-5HPCI, 25:1



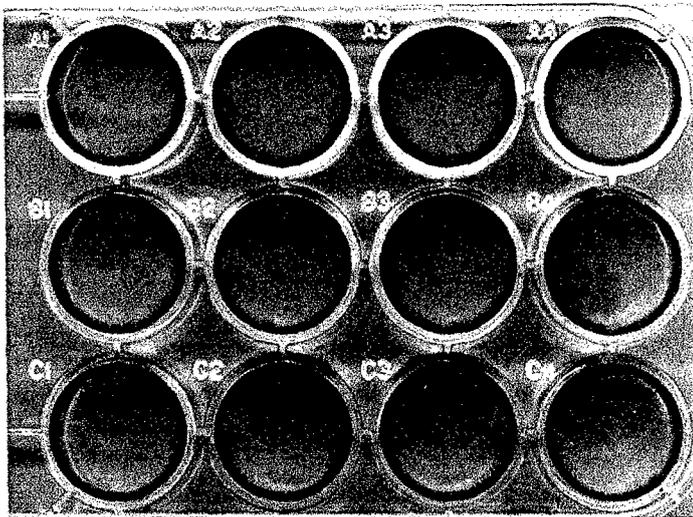
A1-2:BJA.060.22:HPCI, 1:1
B1-2:BJA.060.22:HPCI, 10:1
C1-2:BJA.060.22:HPCI, 25:1
A3-4:BJA.060.22+IL-5:HPCI, 1:1
B3-4:BJA.060.22+IL-5:HPCI, 10:1
C3-4:BJA.060.22+IL-5:HPCI, 25:1

Fig. 12. HPC1 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.

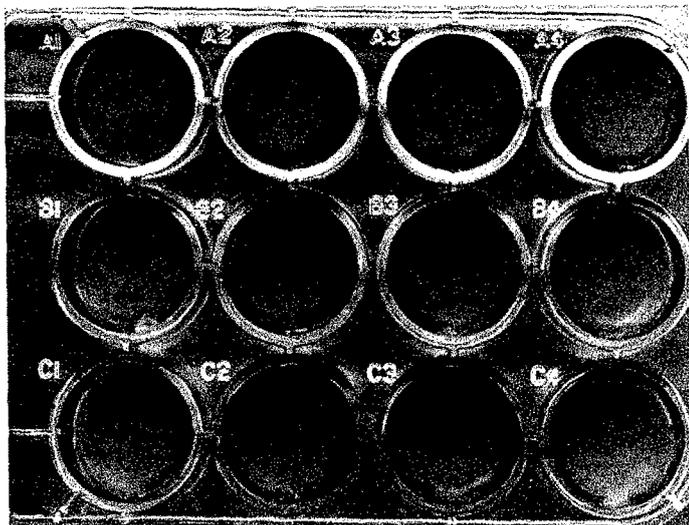
Fig 13. 24hr Eosinophil Cultured Supernatants Inhibit HPCI Cell Growth In Vitro



Control

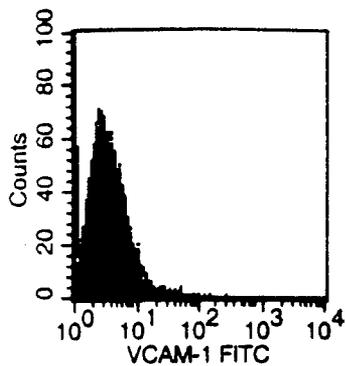


A1-2: BLA22
A3: BLA24
A4: HMO24
B1-2: HMO22
B3-4: YDA22
C1-2: YDA24
C3-4: WCH"22"

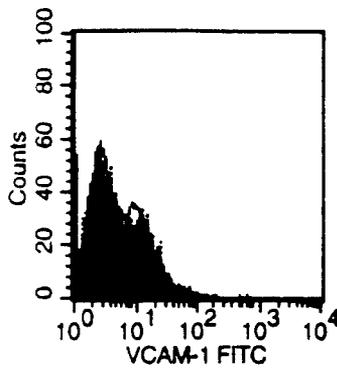


A1-2: +IL-4 @ 10ng/ml
A3-4: +IL-4 @ 50ng/ml
B1-2: +IL-4 @ 100ng/ml
B3-4: +TNF-alpha @ 10ng/ml
C1-2: +TNF-alpha @ 50ng/ml
C3-4: +TNF-alpha @ 100ng/ml

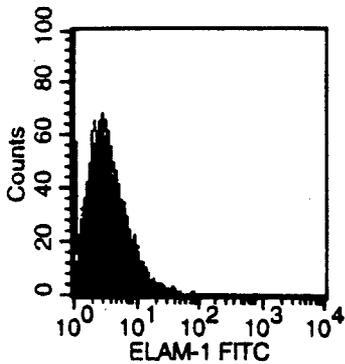
Fig. 13. HPC1 cells (1.5×10^5 /well) were cultured for 24hrs. were incubated for an additional 24-48hrs with cultured eosinophil supernatants (Panel A) and with IL-4 and TNF α (Panel B). The plates were harvested, stained with H & E and scanned into power point.



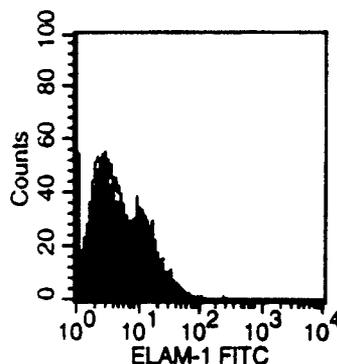
DU145 Untreated



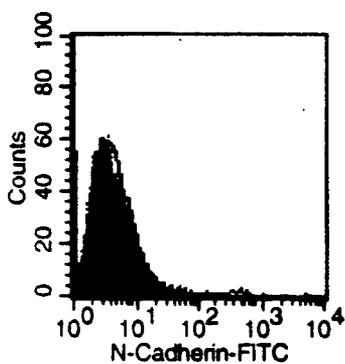
DU145 + Eos.



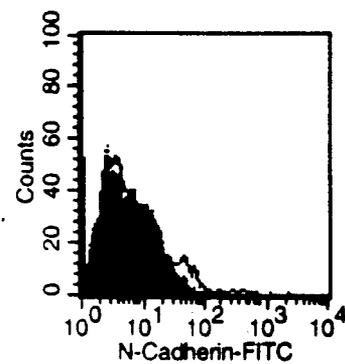
DU145 Untreated



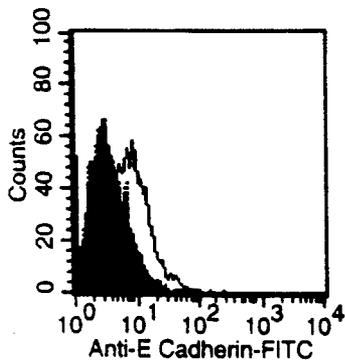
DU145 + Eos.



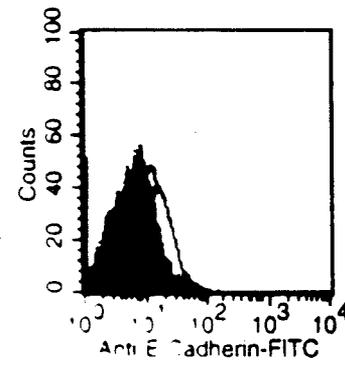
DU145 Untreated



DU145 + Eos.



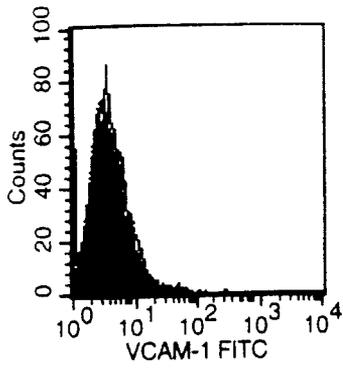
DU145 Untreated



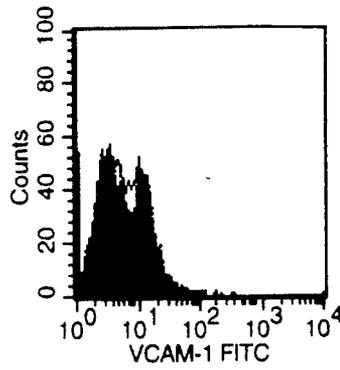
DU145 + Eos.

Fig. 14. Comparison between untreated DU145 and treated DU145 with eosinophils for 24hrs. at 1:1 ratio. The bottom row shows up-regulation of E-Cadherin expression in the presence of eosinophils.

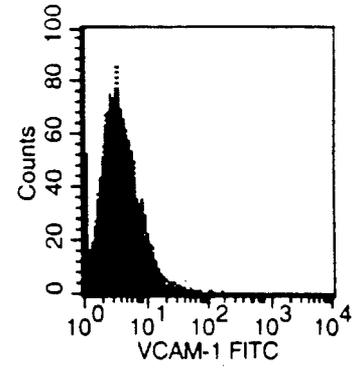
Test's control  Test 



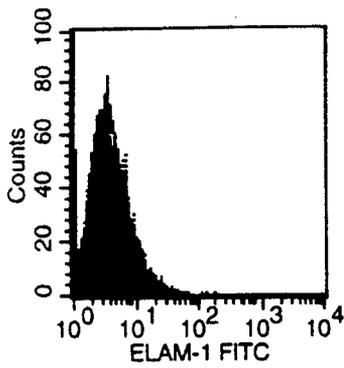
PC-3 Untreated



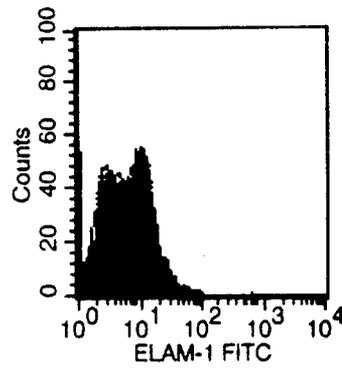
PC-3 + Eos.



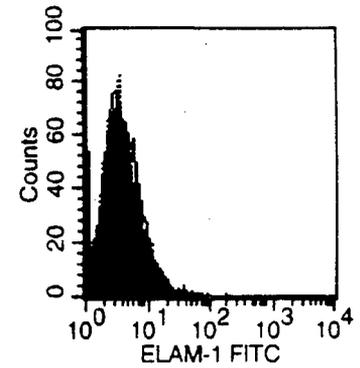
PC-3 + 24hrs Sup.



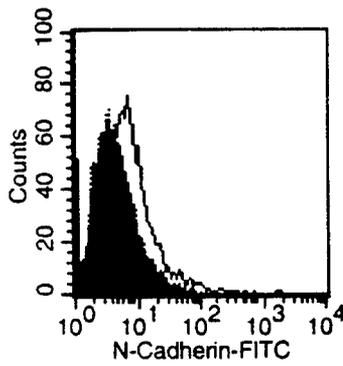
PC-3 Untreated



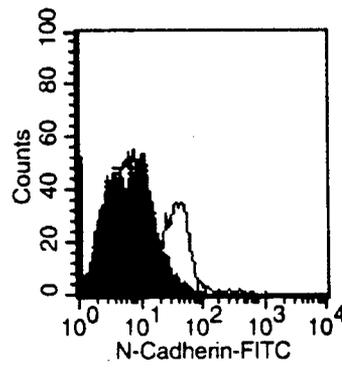
PC-3 + Eos.



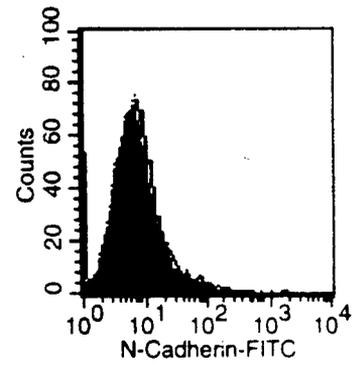
PC-3 + 24hrs Sup.



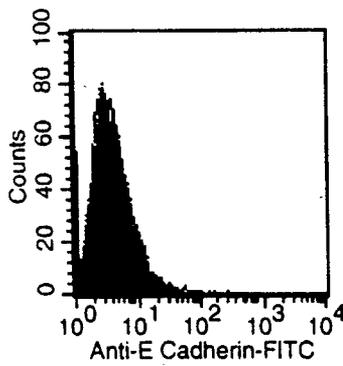
PC-3 Untreated



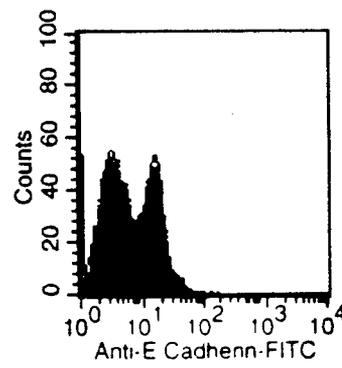
PC-3 + Eos.



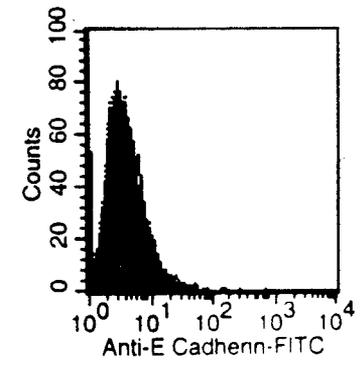
PC-3 + 24hrs Sup.



PC-3 Untreated



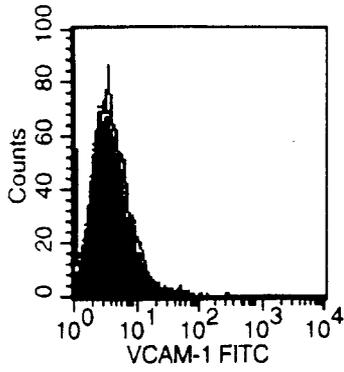
PC-3 + Eos.



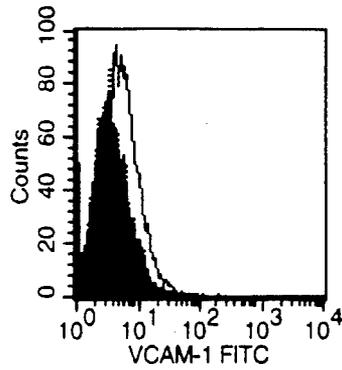
PC-3 + 24hrs Sup.

Fig. 15. Comparison among PC-3 untreated, PC-3 treated 24hrs. with eosinophils (1:1 ratio) and PC-3 treated 24hrs. with 24hrs. eosinophils' supernatant. No significant change was detected.

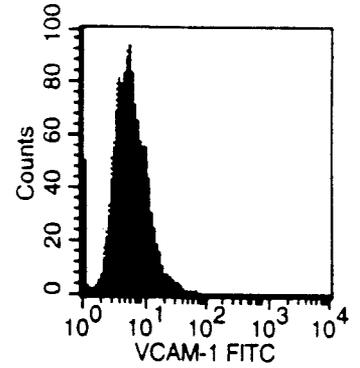
Test's control  Test 



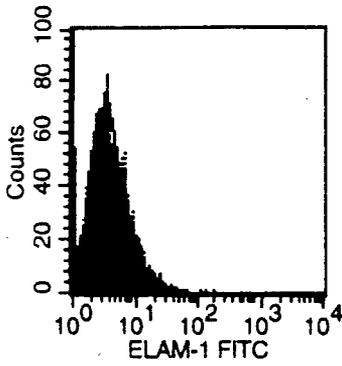
PC-3 Untreated



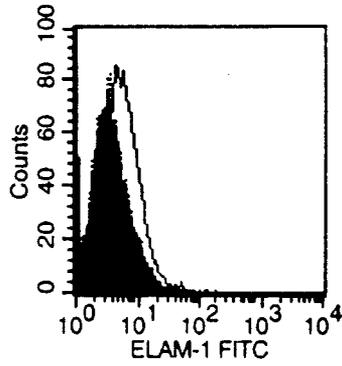
PC-3 + IL-10



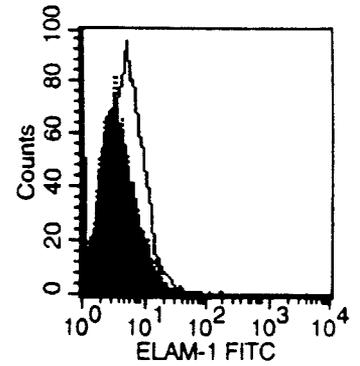
PC-3 + IL-12



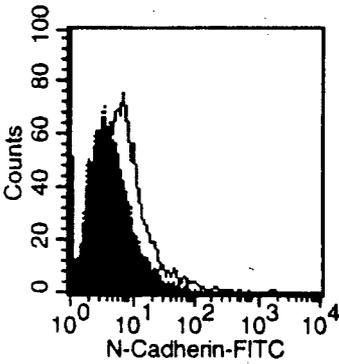
PC-3 Untreated



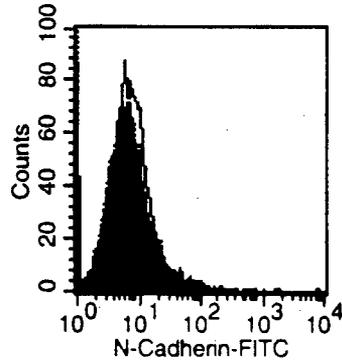
PC-3 + IL-10



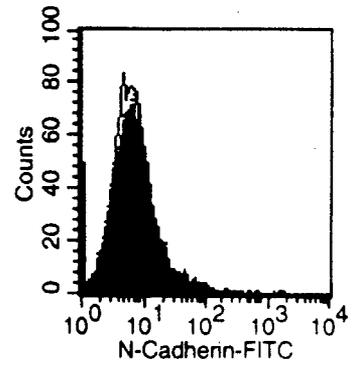
PC-3 + IL-12



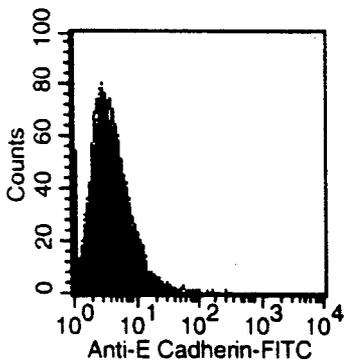
PC-3 Untreated



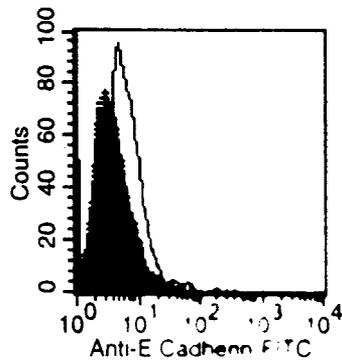
PC-3 + IL-10



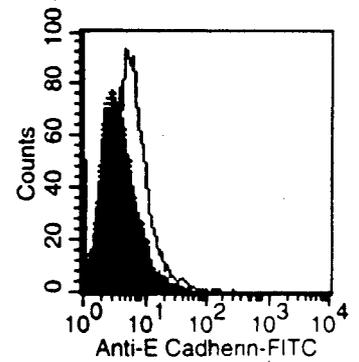
PC-3 + IL-12



PC-3 Untreated



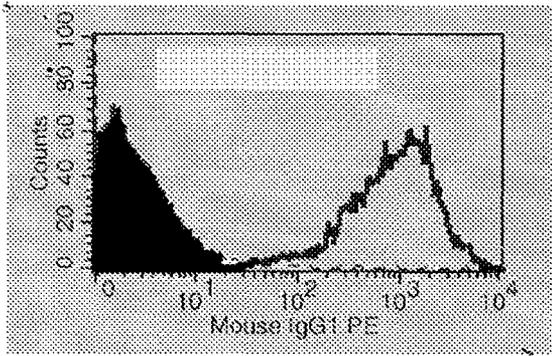
PC-3 + IL-10



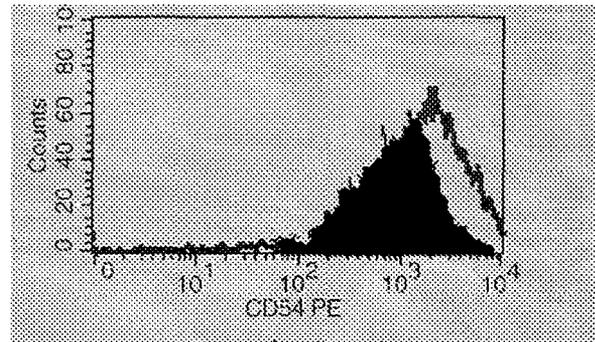
PC-3 + IL-12

Fig. 16. Comparison among PC-3 untreated, PC-3 treated 24hrs. with 10ng/ml of IL-10 and PC-3 treated 24hrs. with 10ng/ml of IL-12. IL-10 up-regulated VCAM, ELAM and E-Cadherin whereas IL-12 up-regulated ELAM and E-Cadherin only.

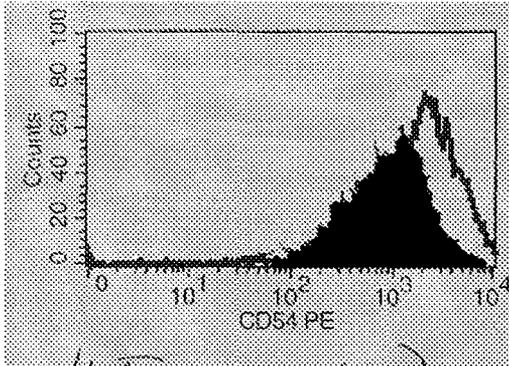
Test's control ■ Test □



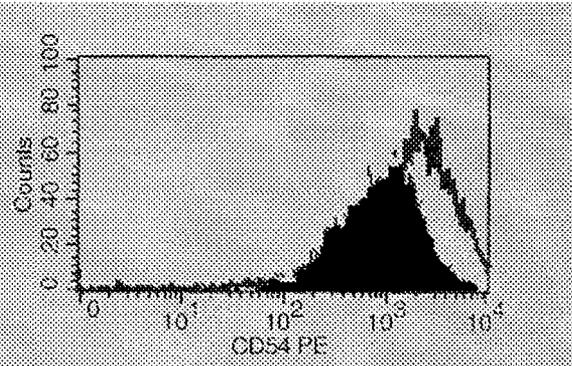
Isotype Control (■) vs. ICAM-1



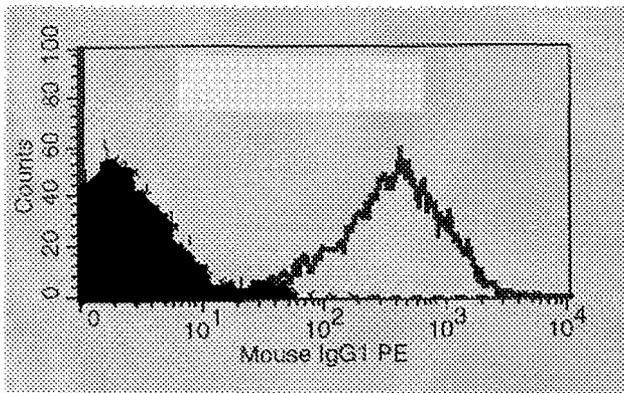
Untreated (■) vs. +TNF-a @ 1ng/ml



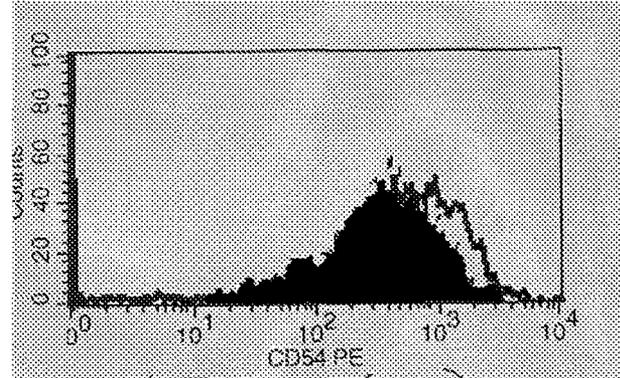
Untreated (■) vs. +TNF-a @ 10ng/ml



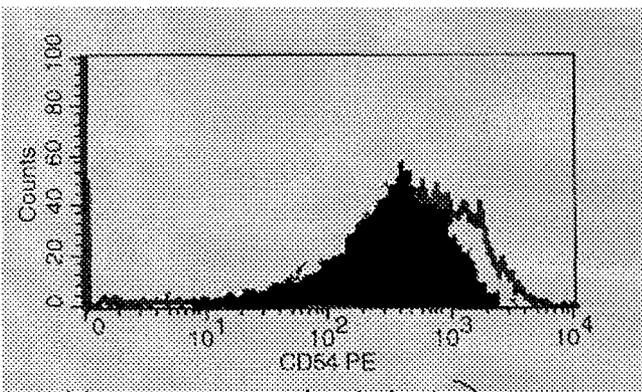
Untreated (■) vs. +TNF-a @ 100ng/ml



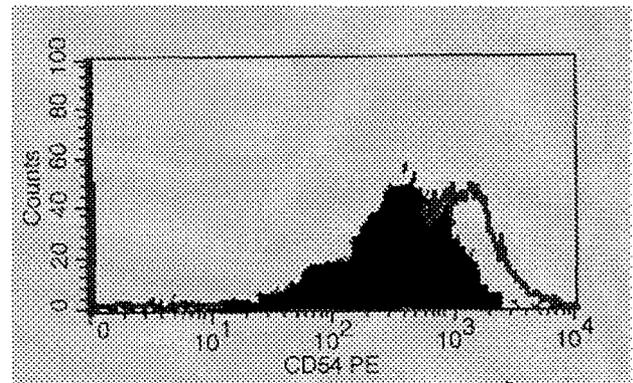
Isotype Control (■) vs. ICAM-1



Untreated (■) vs. +IL-10 @ 1ng/ml

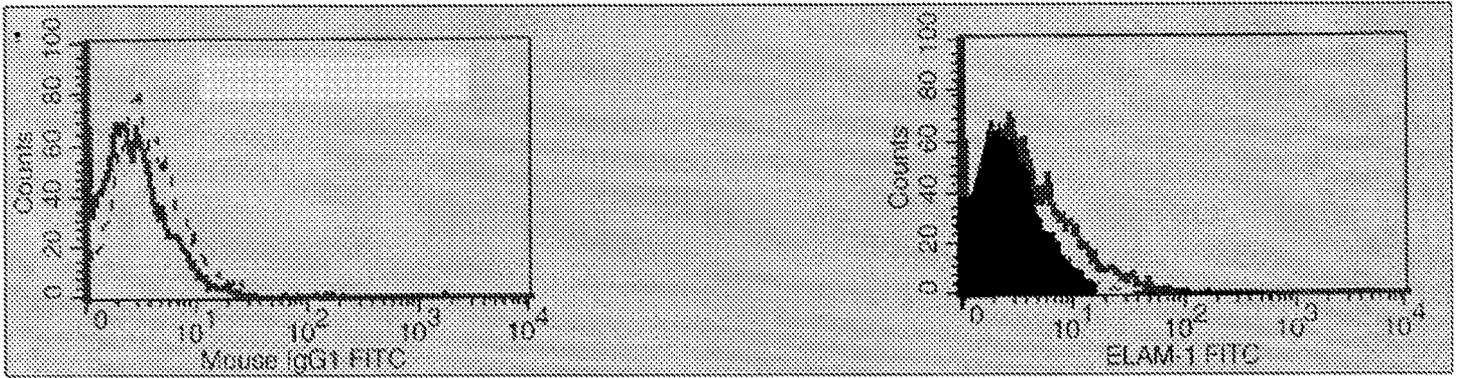


Untreated (■) vs. +IL-10 @ 10ng/ml



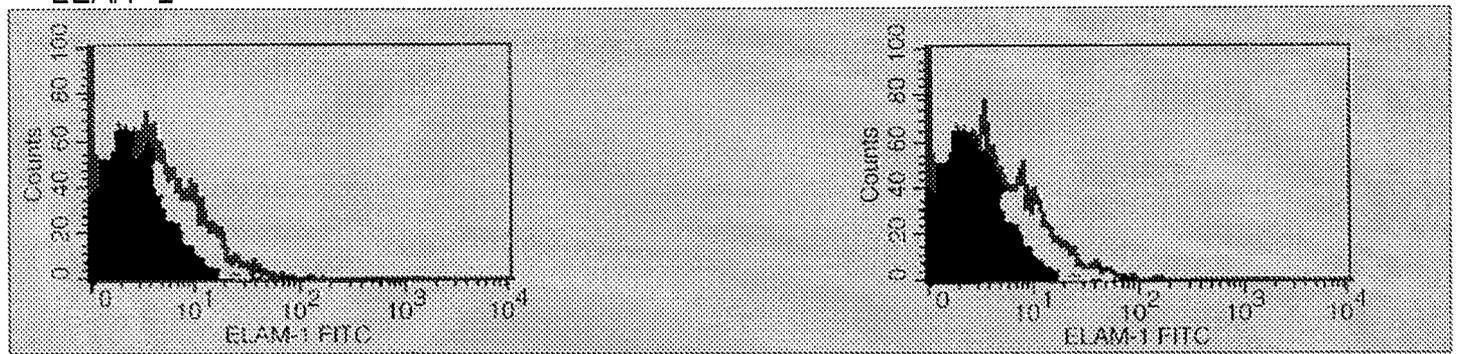
Untreated (■) vs. +IL-10 @ 100ng/ml

Fig. 17. Effect of TNF- α and IL-10 at 1ng/ml, 10ng/ml, and 100ng/ml on DU145 prostate cancer line expression of ICAM-1.



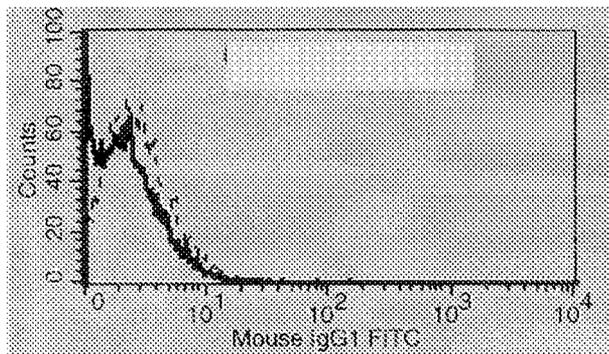
Isotype Control (--) vs. ELAM-1

Untreated (■) vs. +TNF-a @ 1ng/ml

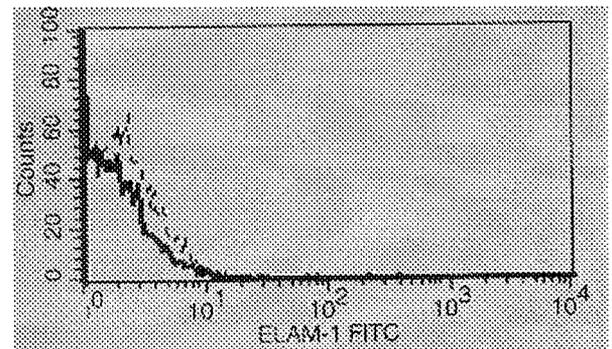


Untreated (■) vs. +TNF-a @ 10ng/ml

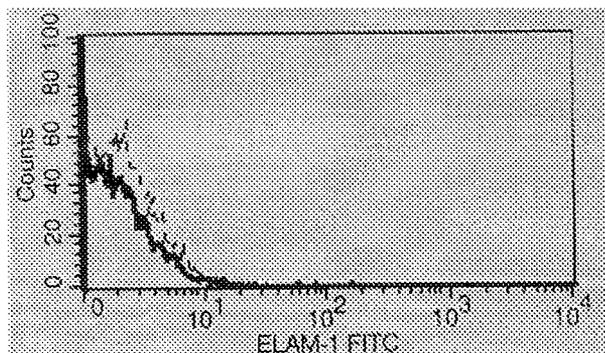
Untreated (■) vs. +TNF-a @ 100ng/ml



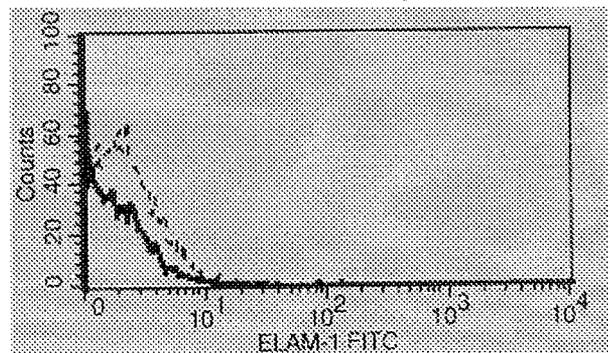
Isotype Control (--) vs. ELAM-1



Untreated (--) vs. +IL-10 @ 1ng/ml

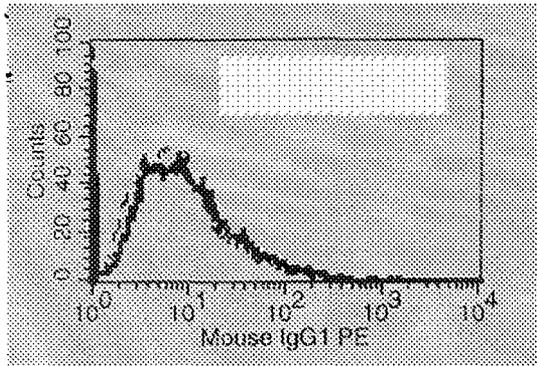


Untreated (--) vs. +IL-10 @ 10ng/ml

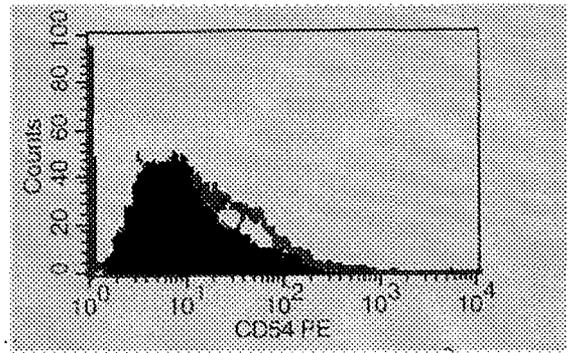


Untreated (--) vs. +IL-10 @ 100ng/ml

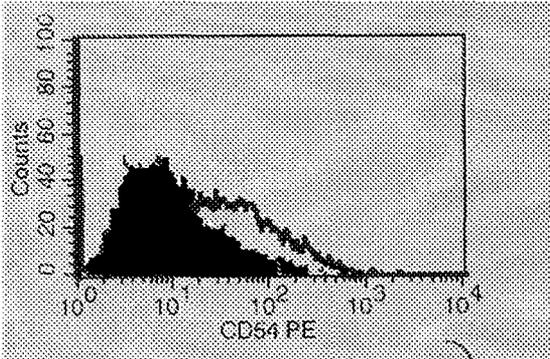
Fig. 18. Effect of TNF- α and IL-10 at 1ng/ml, 10ng/ml, and 100ng/ml on DU145 prostate cancer cell line expression of ELAM-1.



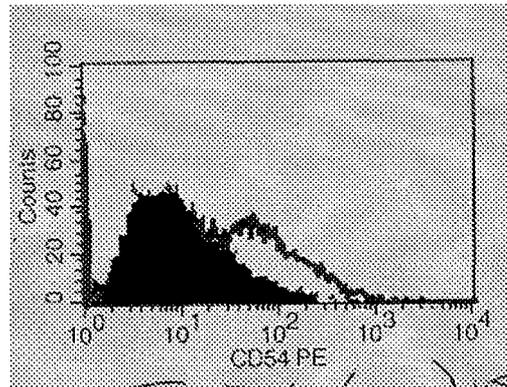
Isotype Control (-) vs. ICAM-1



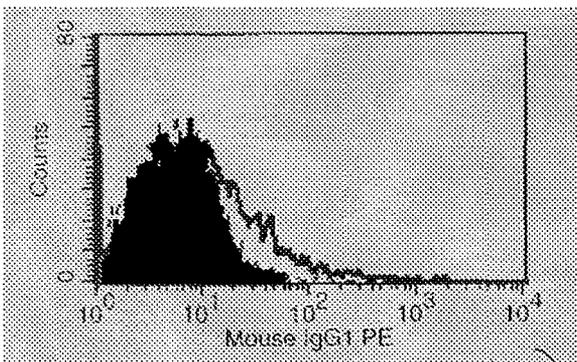
Untreated (■) vs. +TNF-a @ 1ng/ml



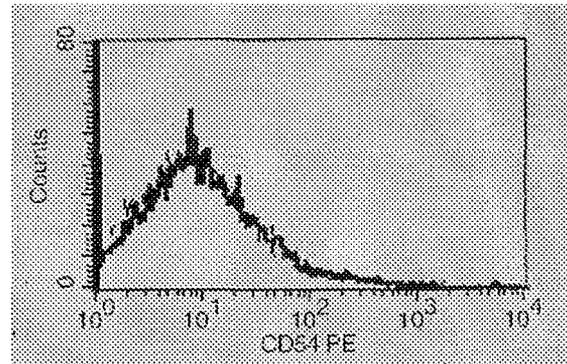
Untreated (■) vs. +TNF-a @ 10ng/ml



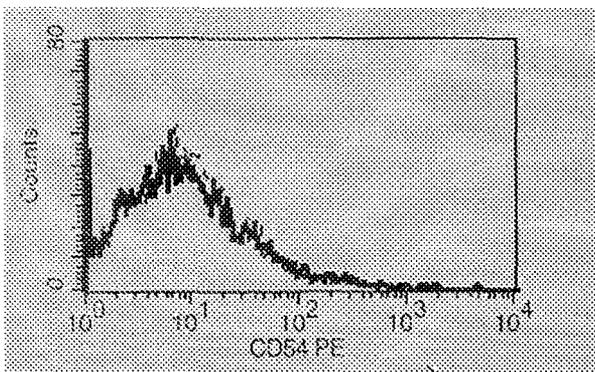
Untreated (■) vs. +TNF-a @ 100ng/ml



Isotype Control (■) vs. ICAM-1



Untreated (-) vs. +IL-10 @ 1ng/ml

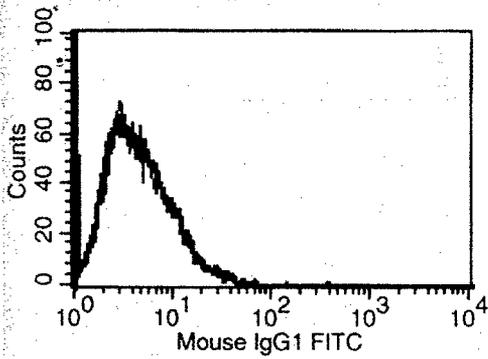


Untreated (-) vs. +IL-10 @ 10ng/ml

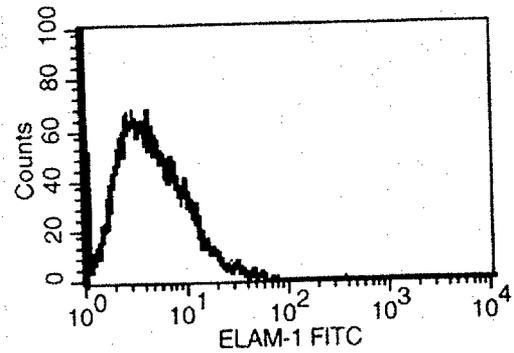
Fig. 19. Effect of TNF- α and IL-10 at 1ng/ml, 10ng/ml, and 100ng/ml on LNCaP prostate cancer cell line expression of ICAM-1.

LNCaP

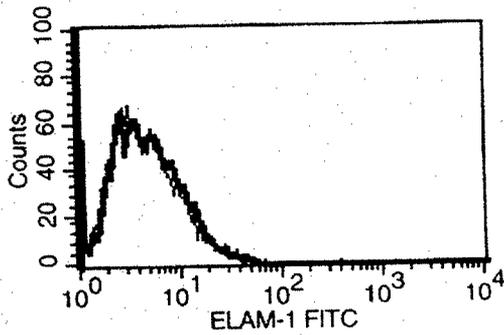
Fig.20



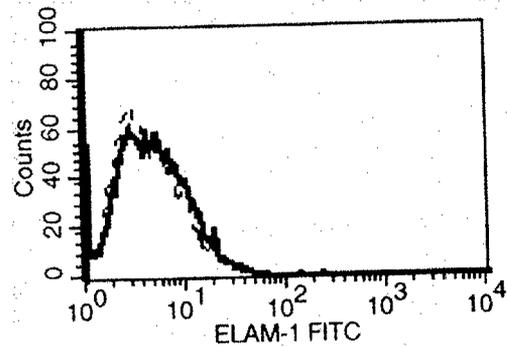
Isotype Control (--) vs.
ELAM-1



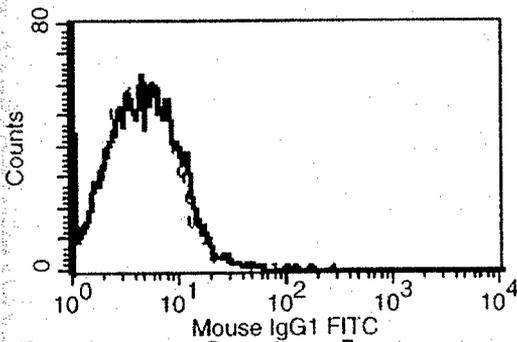
Untreated (--) vs.
+TNF-a @ 1ng/ml



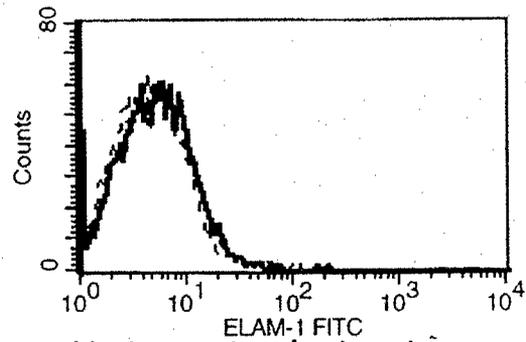
Untreated (--) vs.
+TNF-a @ 10ng/ml



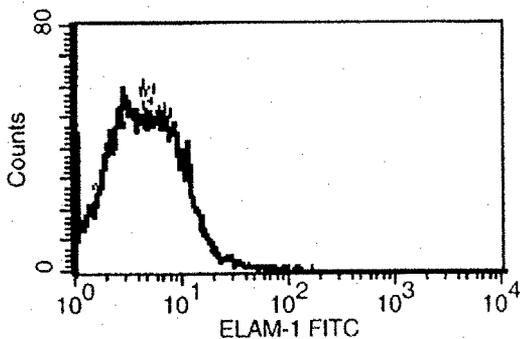
Untreated (--) vs.
+TNF-a @ 100ng/ml



Isotype Control (--) vs.
ELAM-1



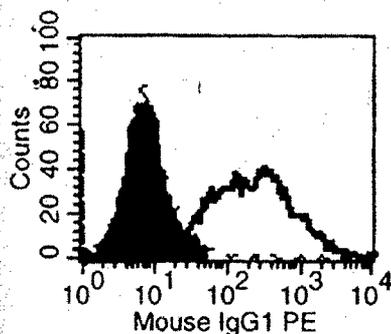
Untreated (--) vs.
+IL-10 @ 1ng/ml



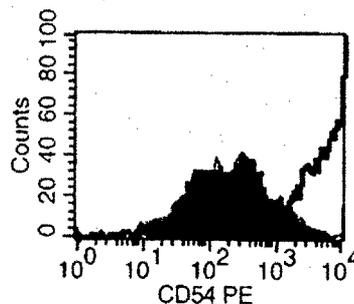
Untreated (--) vs.
+ IL-10 @ 10ng/ml

Fig. 20. Effect of TNF- α and IL-10 at 1ng/ml, 10ng/ml, and 100ng/ml on LNCaP prostate cancer cell line expression.

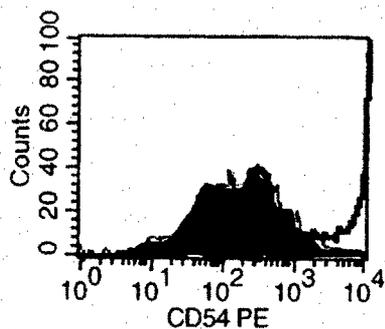
PC-3



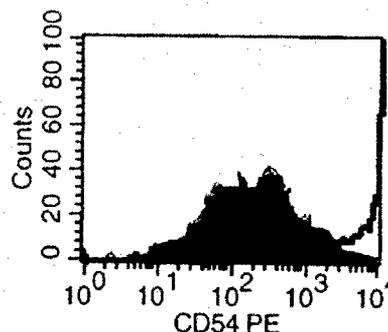
Isotype Control (■) vs. ICAM-1



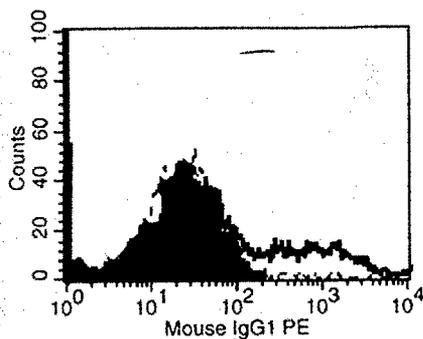
Untreated (■) vs. +TNF-a @ 1ng/ml



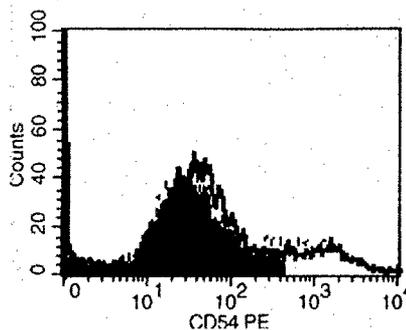
Untreated (■) vs. +TNF-a @ 10ng/ml



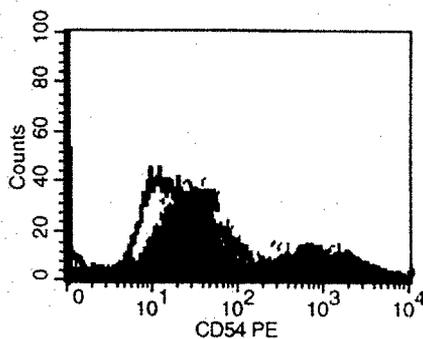
Untreated (■) vs. +TNF-a @ 100ng/ml



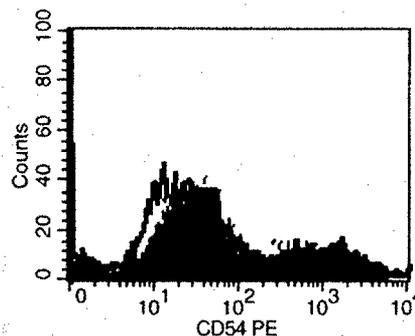
Isotype Control (■) vs. ICAM-1



Untreated (■) vs. +IL-10 @ 1ng/ml



Untreated (■) vs. +IL-10 @ 10ng/ml

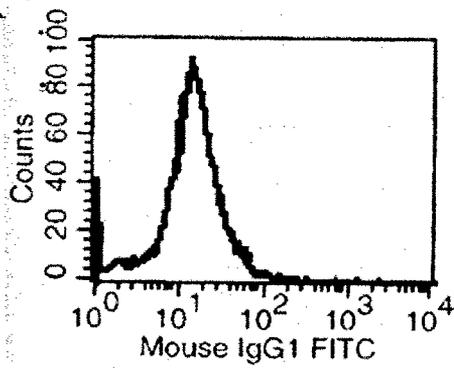


Untreated (■) vs. +IL-10 @ 100ng/ml

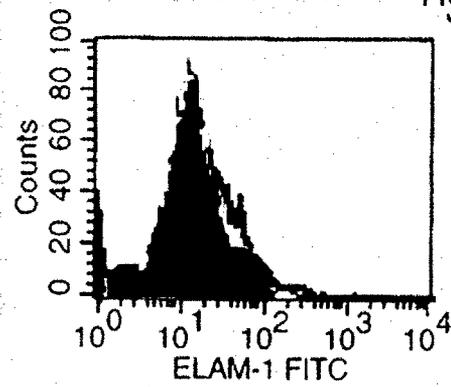
Fig. 21. Effect of TNF- α and IL-10 at 1ng/ml, 10ng/ml, and 100ng/ml on PC-3 prostate cancer cell line expression of ICAM-1.

PC-3

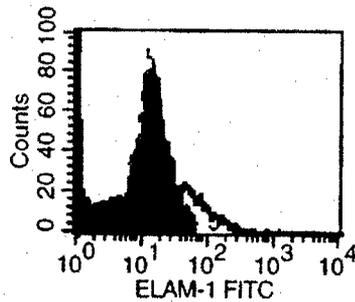
Fig.22



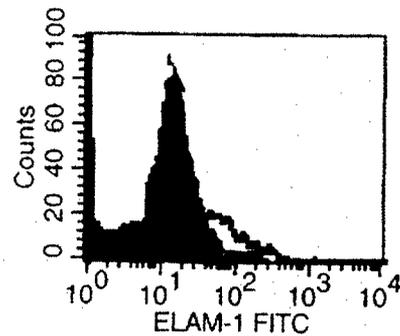
Isotype Control (--) vs. ELAM-1



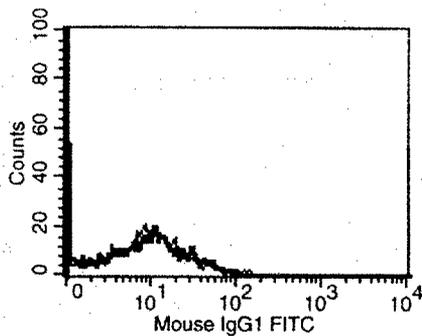
Untreated (■) vs. +TNF-α @ 1ng/ml



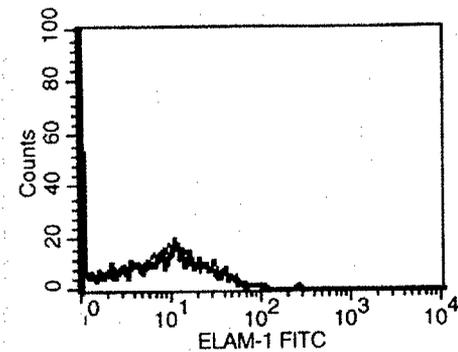
Untreated (■) vs. +TNF-α @ 10ng/ml



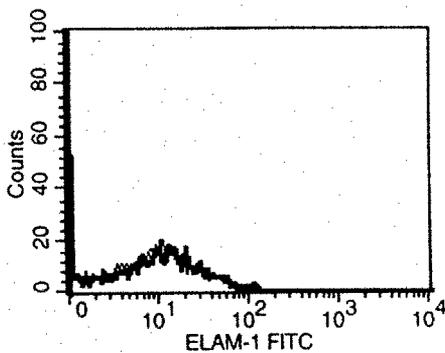
Untreated (■) vs. +TNF-α @ 100ng/ml



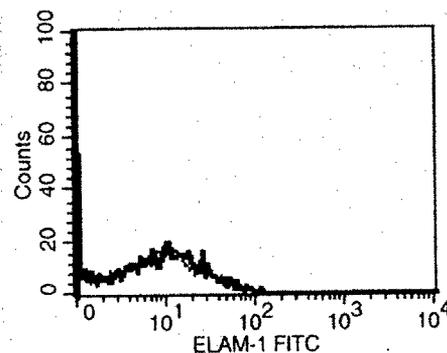
Isotype Control (--) vs. ELAM-1



Untreated (--) vs. +IL-10 @ 1ng/ml



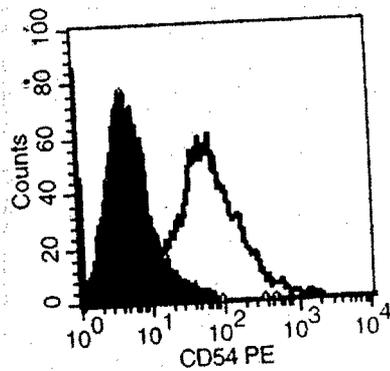
Untreated (--) vs. +IL-10 @ 10ng/ml



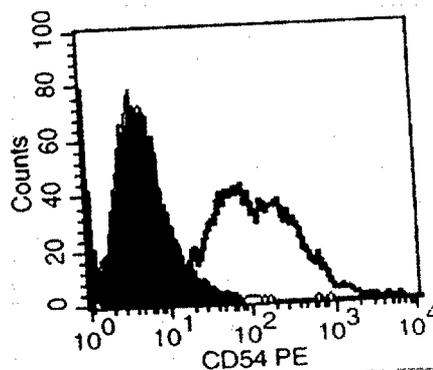
Untreated (--) vs. +IL-10 @ 100ng/ml

Fig. 22. Effect of TNF- α and IL-10 at 1ng/ml, 10ng/ml, and 100ng/ml on PC-3 prostate cancer cell line expression of ELAM-1.

PC-3

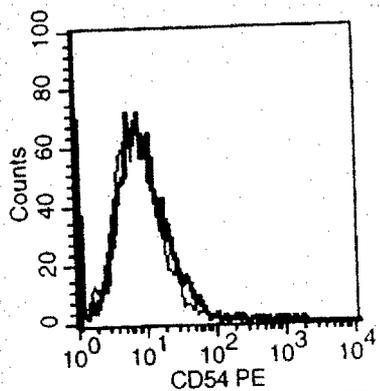


Isotype Control (■) vs. ICAM-1

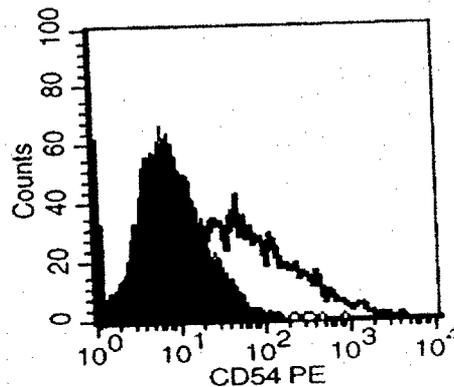


Isotype Control (■) vs. +IL-1

LNCaP

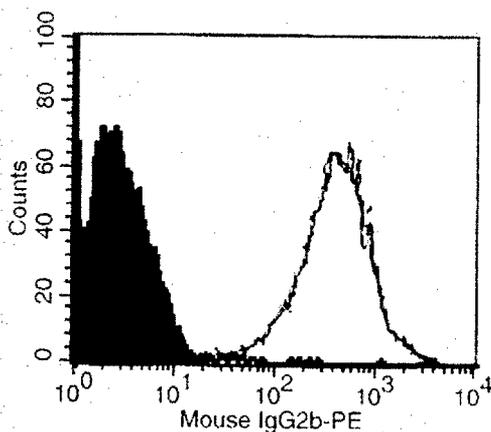


Isotype Control (--) vs. ICAM-1

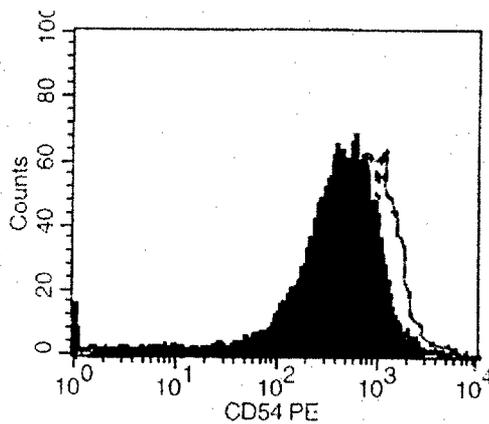


Isotype Control (■) vs. +IL-1

DU145



Isotype Control (■) vs. ICAM-1



Untreated (■) vs. +IL-1

Fig. 23. Effect of IL-1 α on PC-3, LNCaP, and DU145 prostate cancer cell lines expression of ICAM-1.

Fig.24 Inhibitory Effect of Eosinophil Cell Line on MRC-5 Fibroblast Cell Growth

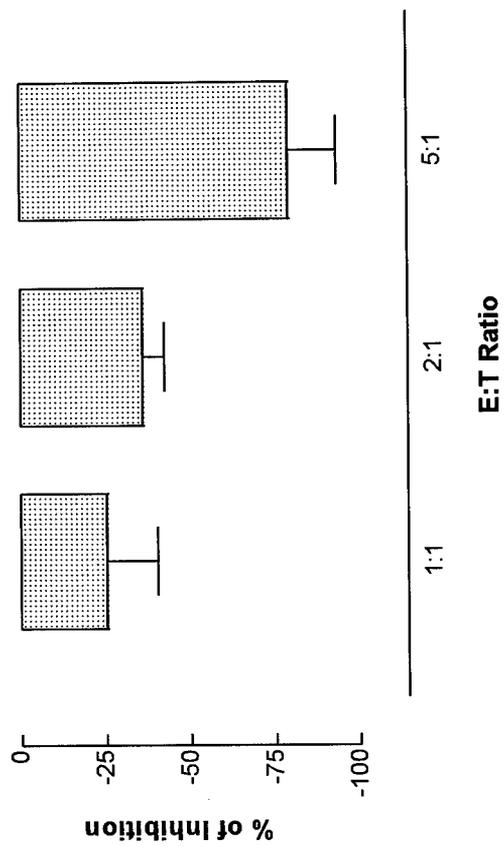


Fig. 24. MRC-5 fibroblast cells were seeded into the wells of 6-well cluster plates at 1.5×10^5 cells/well and incubated overnight. Sorted eosinophil cell lines were added at various E:T ratios in duplicates. The plates were incubated for an additional 72hrs, rinsed 3x with PBS, stained with H & E and the IDV's were measured. The percentage of inhibition was determined against the assay control.

**Peripheral Blood Eosinophils Inhibit PC-3
Tumor Cell Growth**

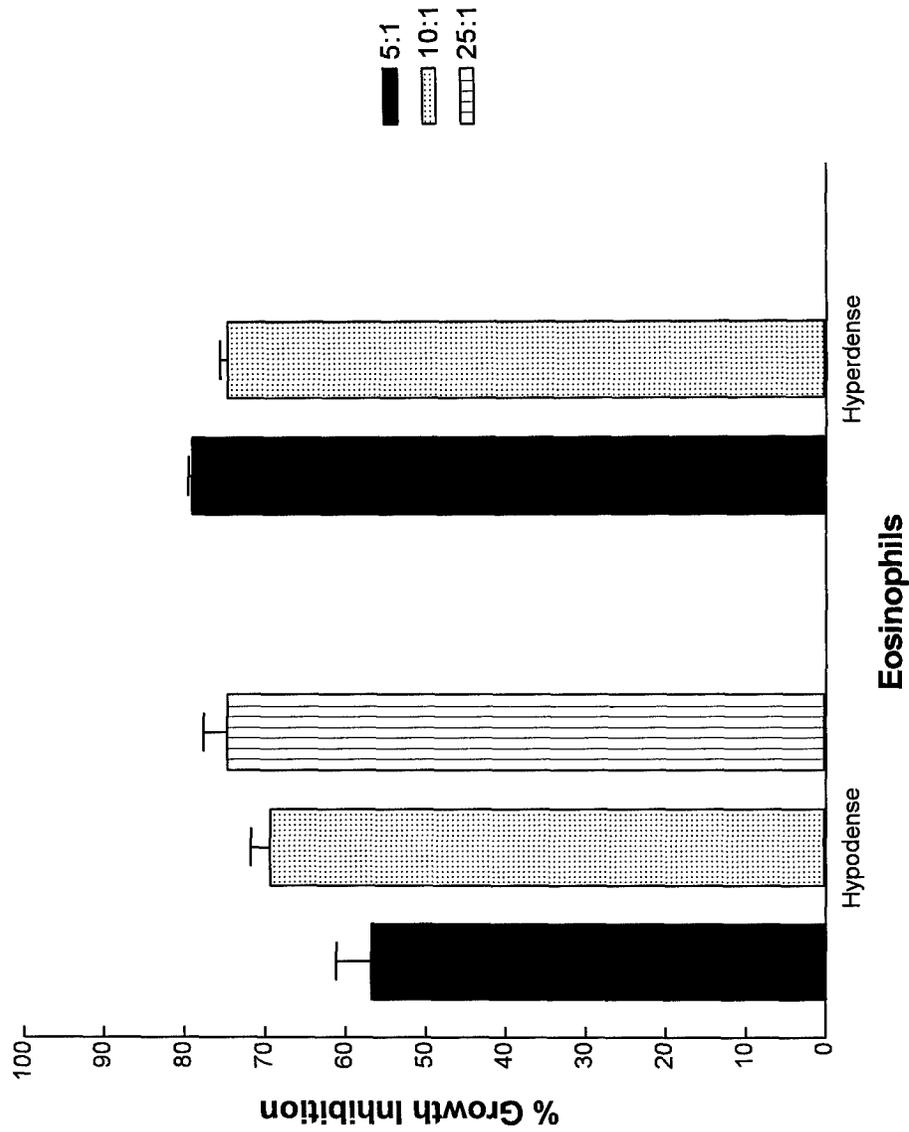


Fig. 25

Fig. 25. PC-3 tumor cells were seeded into the wells of a 12-well culture plate at 1.5×10^5 cells/well and incubated overnight. Peripheral blood eosinophils metrizamide fractions were added in duplicates at various E:T ratios. The plates were incubated for an additional 72hrs, rinsed 3x with PBS, stained with H & E and the IDV's were measured. The percentage of growth inhibition was determined against the assay control.

Hypodense Eosinophil Cell Line Inhibits Tumor Cell Growth

Fig. 26

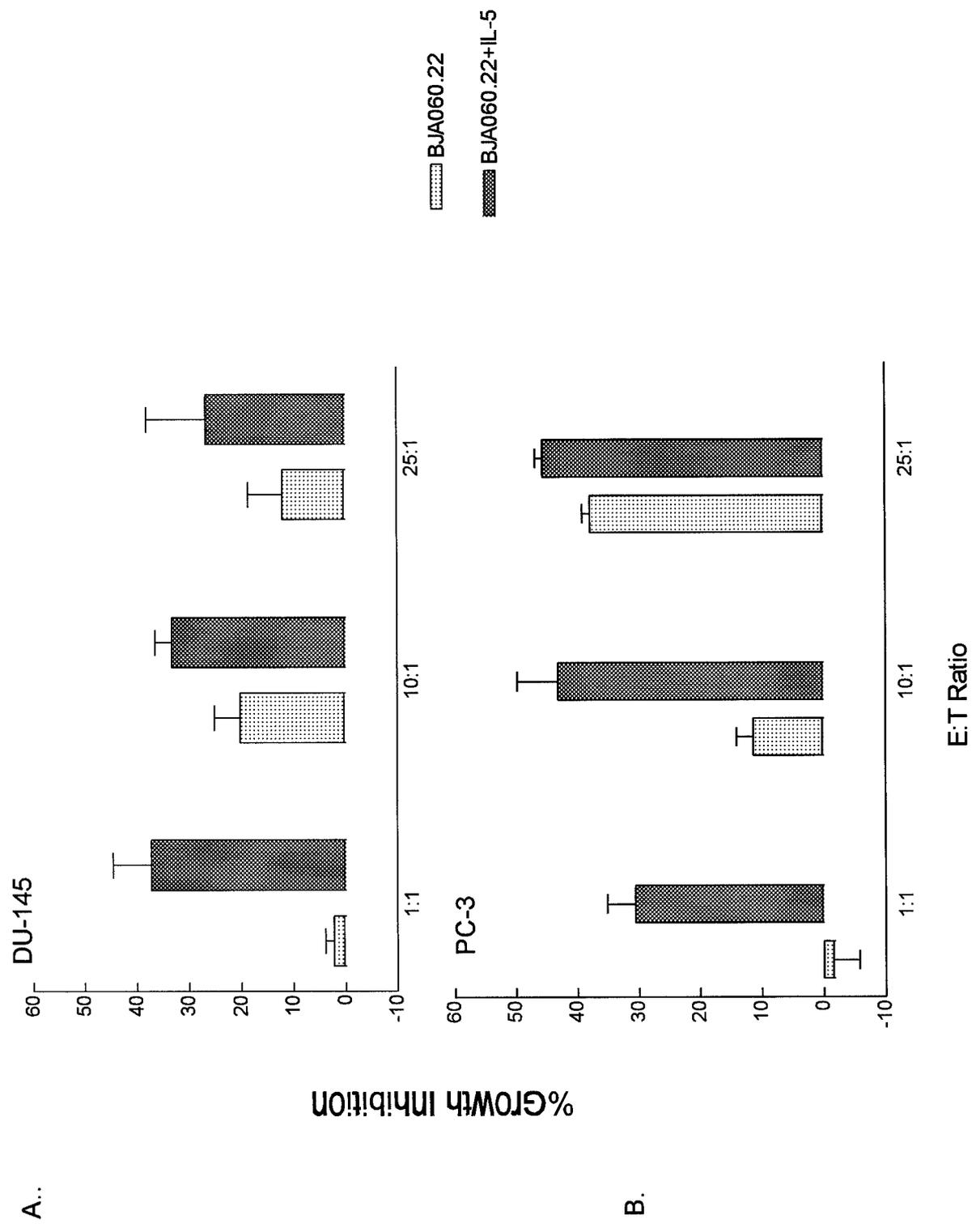


Fig. 26. PC-3 and DU145 tumor cells were seeded into the wells of a 12-well culture plate at 1.5×10^5 cells/well and incubated overnight. Prior to this, effector eosinophils cell lines were pretreated with IL-5 (1 ng/ml) for 3 days. On day 4, pretreated and non-treated eosinophils were added in duplicates at various E:T ratios. The plates were incubated for an additional 72hrs, rinsed 3x with PBS, stained with H & E and the IDV's recorded. The percentage of growth inhibition was determined against the control average value.

Hyperdense Eosinophil Cell Lines Inhibits Tumor Cell Growth

Fig. 27

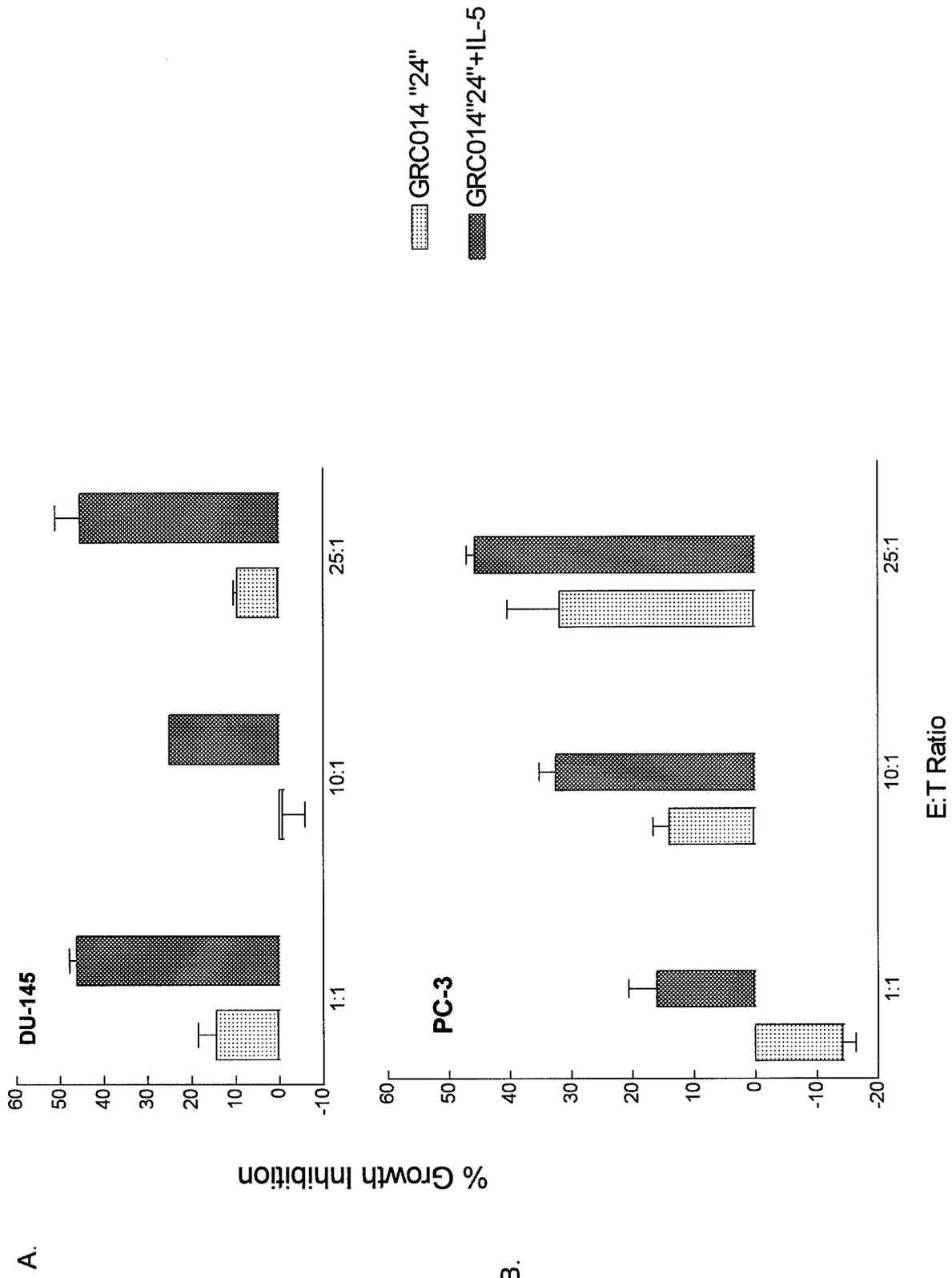


Fig. 27. PC-3 and DU145 tumor cells were seeded into the wells of a 12-well culture plate at 1.5×10^5 cells/well and incubated overnight. Prior to this, effector eosinophil cell lines were pretreated with IL-5 (1 ng/ml) for 3 days. One day 4, pretreated and non-treated eosinophils were added in duplicates at different E:T ratios. The plates were incubated for an additional 72hrs, rinsed 3x with PBS, stained with H & E and the IDV's recorded. The percentage of growth inhibition was determined against the control average value.

**Hyperdense Eosinophil Sub Line Inhibits PC-3
Tumor Cell Growth**

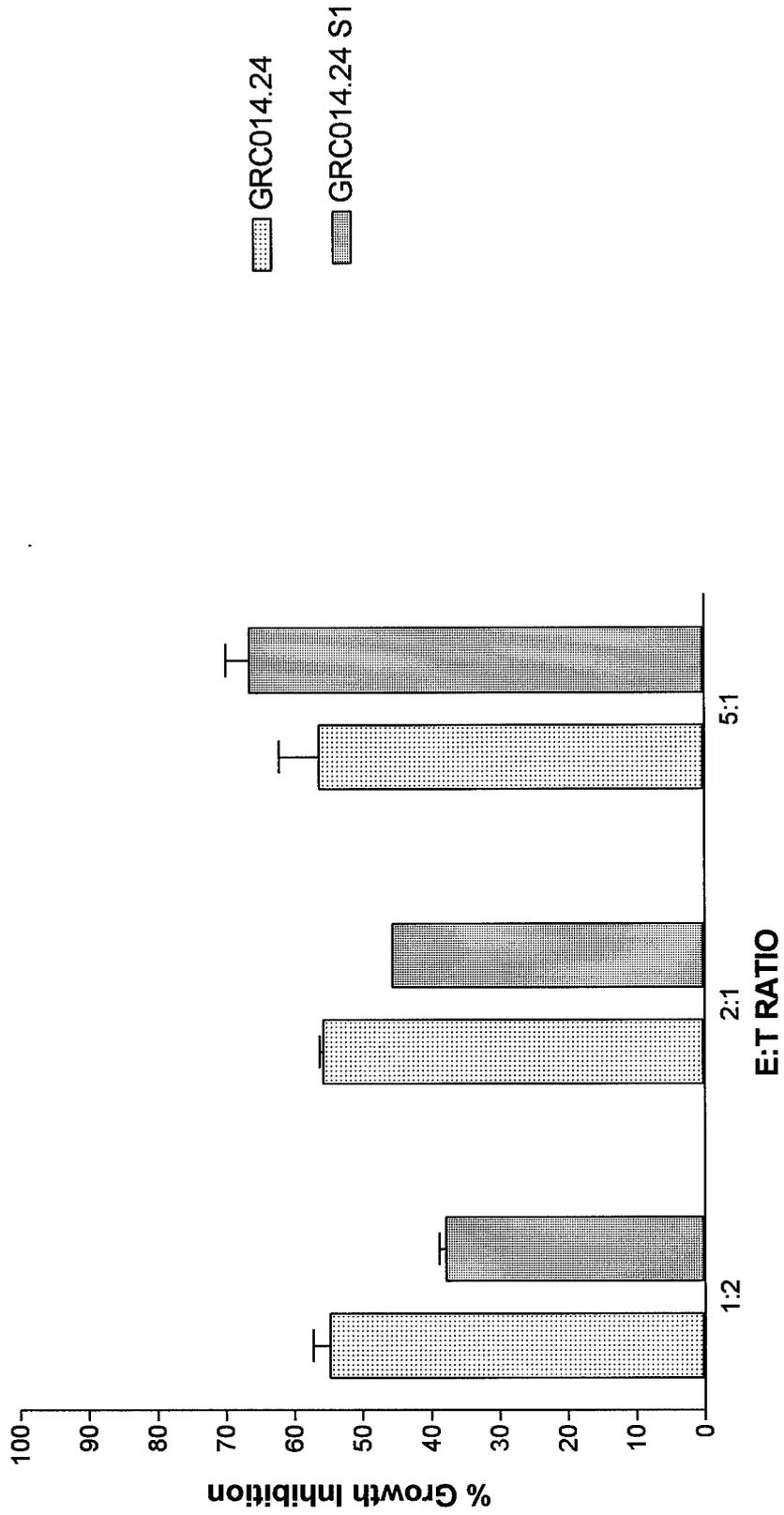


Fig. 28

Fig. 28. PC-3 tumor cells were seeded into the wells of a 12-well culture plate at 1.5×10^5 cells/well and incubated overnight. Hyperdense metrizamide gradient eosinophil cell lines and CCR3⁺ subline were added at various E:T ratios in duplicates. The plates were incubated for an additional 72hrs, rinsed 3x with PBS, stained with H & E and IDV's were measured. The percentage of growth inhibition was determined against the assay control.

**Inhibition of Prostate Tumor Cells Growth by
24 Hour Peripheral Blood Eosinophil
Conditioned Media**

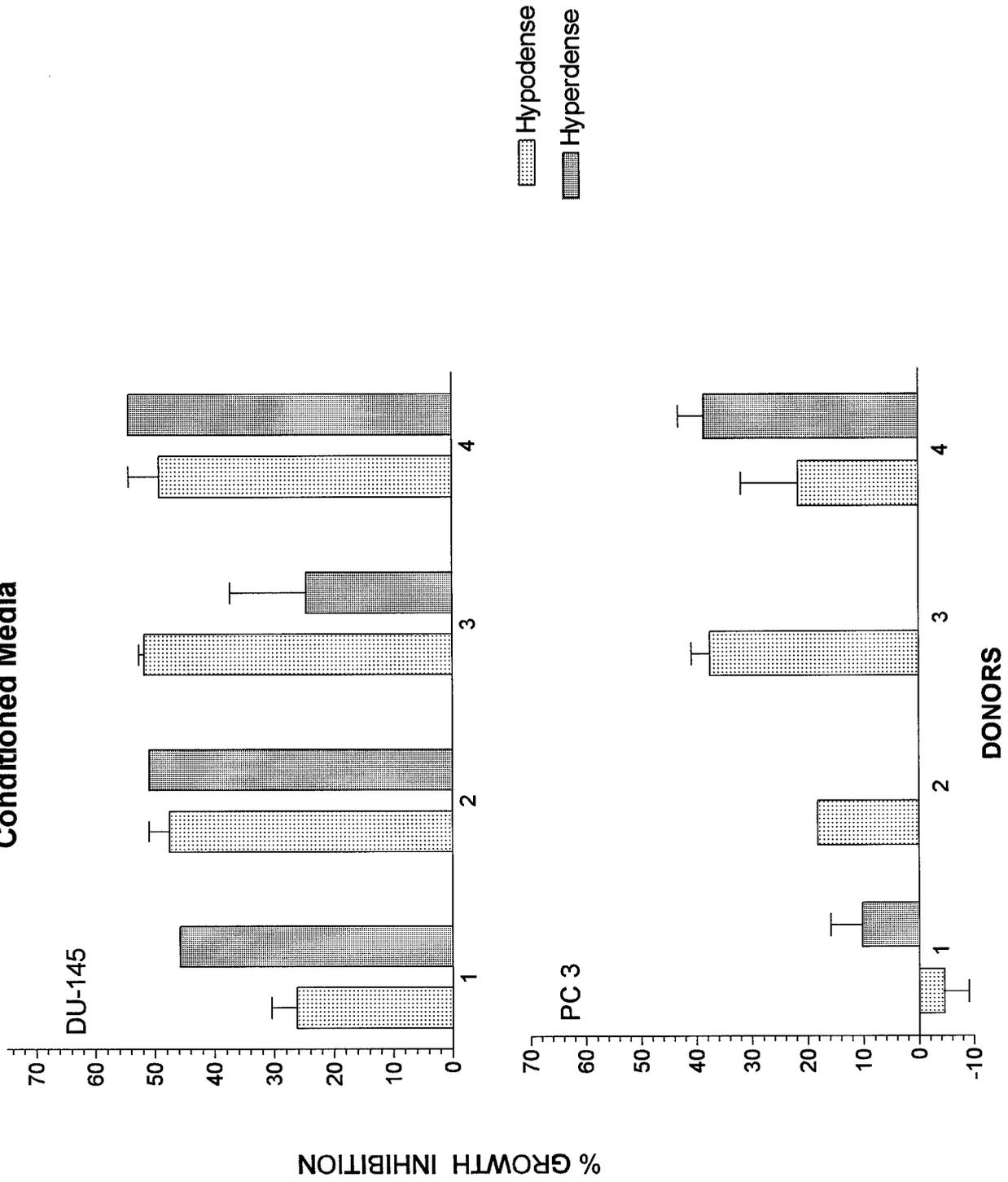


Fig. 29

Fig. 29. PC-3 and DU145 tumor cells were seeded into the wells of a 12-well culture plate at 1.5×10^5 cells/well and incubated overnight. Supernatants were replaced with 24hr peripheral blood eosinophil conditioned media. Duplicates of each metrizamide fraction (hyperdense and hypodense) from different donors were incubated for an additional 72hrs. Wells were rinsed 3x with PBS, stained with H & E and the IDV's were recorded. Percentage of growth inhibition was determined against the control average value.

Fig. 30 Inhibition of Prostate Tumor Cell Growth by IL-4 and TNF-Alpha

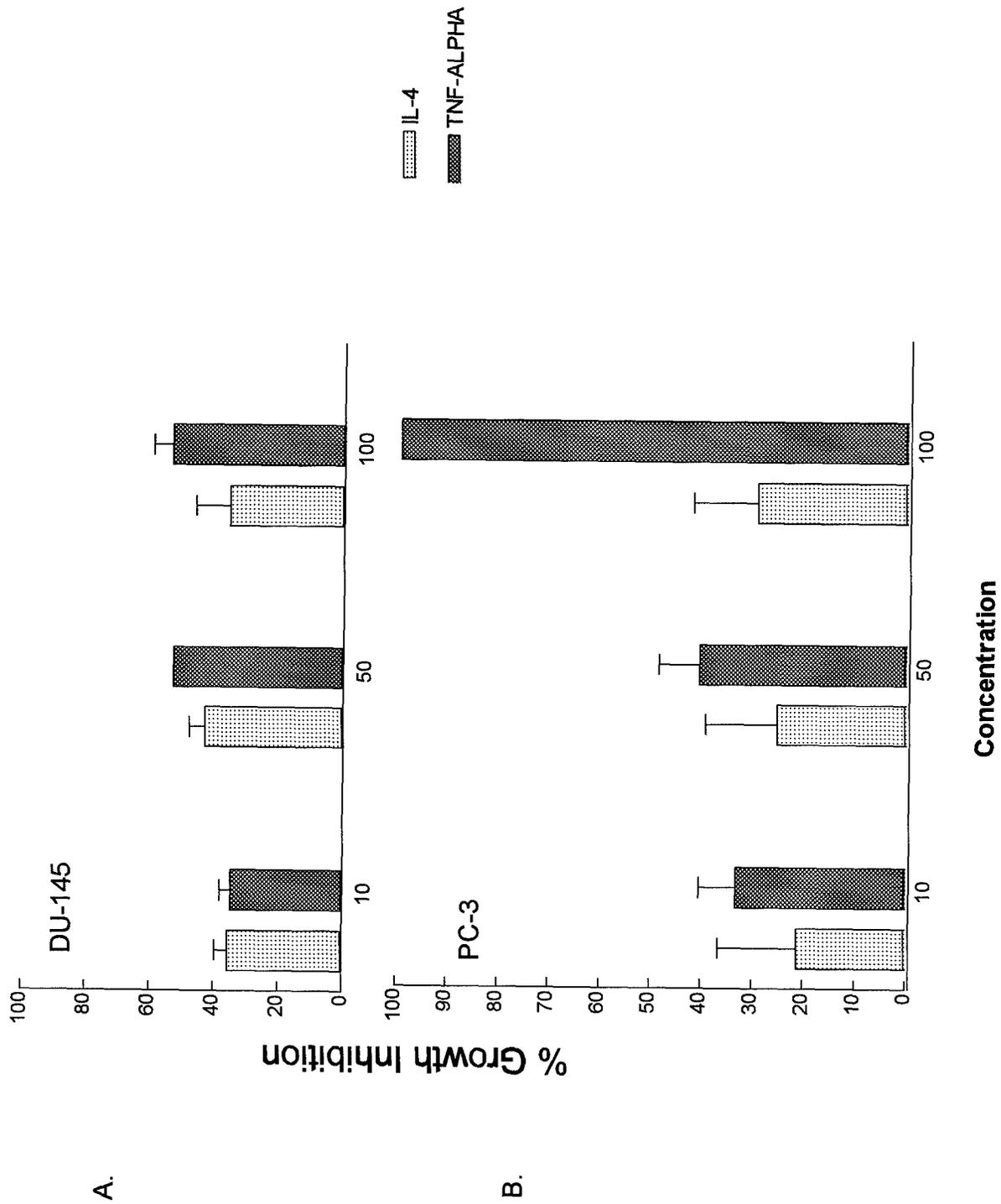


Fig. 30. PC-3 and DU145 tumor cells were seeded into the wells of a 12-well culture plate at 1.5×10^5 cells/well and incubated overnight. The cells were treated with cytokines in duplicate at various concentrations for an additional 72hrs. The wells were washed 3x with PBS, stained with H& E and the IDV's recorded. Percentage of growth inhibition was determined against the assay control.



DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y)

21 Feb 03

MEMORANDUM FOR Administrator, Defense Technical Information
Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir,
VA 22060-6218

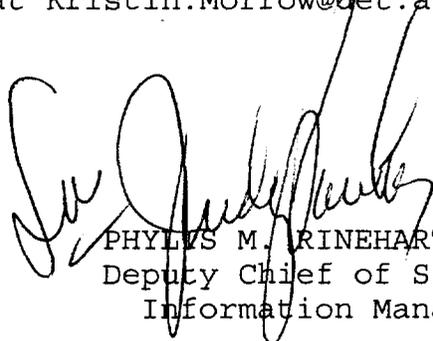
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PHYLLIS M. RINEHART
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