WHOLE BLOOD ACTIVATION RESULTS IN ALTERED T CELL AND MONOCYTE CYTOKINE PRODUCTION PROFILES BY FLOW CYTOMETRY

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ABSTRACT

BACKGROUND: An excellent monitor of the immune balance of peripheral circulating cells is to determine their cytokine production patterns in response to stimuli. Using flow cytometry, a positive identification of cytokine producing cells in a mixed culture may be achieved. Recently, the ability to assess cytokine production following a whole-blood activation culture has been described. In this study, whole blood activation was compared to traditional PBMC activation and the individual cytokine secretion patterns for both T cells, T cell subsets and monocytes was determined by flow cytometry.

RESULTS: For T cell cytokine assessment (IFNg/IL-10 and IL-2/IL-4) following PMA +ionomycin activation: (1) a significantly greater percentages of T cells producing IFNγ and IL-2 were observed following whole-blood culture and (2) altered T cell cytokine production kinetics were observed by varying whole blood culture times. Four-color analysis was used to allow assessment of cytokine production by specific T cell subsets. It was found that IFNγ production was significantly elevated in the CD3+/CD8+ T cell population as compared to the CD3+/CD8- population following five hours of whole blood activation. Conversely, IL-2 and IL-10 production were significantly elevated in the CD3+/CD8- T cell population as compared to the CD3+/CD8+ population. Monocyte cytokine production was assessed in both culture systems following LPS activation for 24 hours. A three-color flow cytometric was used to assess two cytokines (IL-1a/IL-12 and TNFa/IL-10) in conjunction with CD14. Nearly all monocytes were stimulated to produce IL-1a, IL-12 and TNFα equally well in both culture systems, however monocyte production of IL-10 was significantly elevated in whole blood culture as compared to PBMC culture. IL-12 producing monocytes appeared to be a distinct subpopulation of the IL-1a producing set, whereas IL-10 and TNFa producing monocytes were largely mutually exclusive. IL-10 and TNFa producing monocytes may represent distinct monocyte subsets with unique functions.

CONCLUSIONS: Whole blood culture eliminates the need to purify cell populations prior to culture and may have significant utility for the routine monitoring of the cytokine balances of the peripheral blood T cell and monocyte populations. In addition, there are distinct advantages to performing whole-blood (WB) activation as compared to PBMC activation. These advantages would include retaining all various cell-cell interactions as well as any soluble factors present in serum that influence cell activation. In this study, alterations in cytokine production are demonstrated between whole blood and PBMC activation. It is likely that whole blood activation more accurately represents the in-vivo immune balance than PBMC activation.

BACKGROUND

The role of the immune system and cytokine regulation in the development and progression of a variety of disease states is becoming increasingly well defined. Correspondingly, improved techniques to evaluate cytokine production have been developed. The emergence of flow cytometric detection of intracellular cytokine production has occurred over the last decade and offers several unique advantages over most standard cytokine detection methods (1-6). Using multicolor staining techniques, the detection of several cytokines in conjunction with multiple surface markers now offers the ability to phenotype and positively identify cytokine producing cells. The clinical utility of cytokine detection by flow cytometry has been recently demonstrated for several disease states (7-9) and new developments were recently reviewed by Maino et al (10). Since this technique is relatively simple to perform with equipment already standard in many hospitals the routine clinical use of peripheral blood cytokine profiles to monitor the both the magnitude and direction of peripheral immune activation is likely to emerge. To date, the vast majority of cytokine flow cytometry assays included the activation in culture of purified peripheral mononuclear cells (PBMCs). There are potential drawbacks to PBMC activation. These include a relatively low sensitivity with respect to some cytokines and a limited ability to extrapolate to the in-vivo immune condition due to the 'artificial' purified nature of the PBMCs culture system. Recently, whole blood activation techniques have recently been introduced which are rapid to perform and may more accurately reflect in-vivo cytokine regulation (11-15).

Given the current wide spread use of PBMC culture for cellular activation, the vast cellular differences between the two culture situations and the likely emergence of whole blood culture, we have compared whole blood culture to standard PBMC culture techniques with regards to cytokine detection for both T cells and monocytes. A variety of cytokines were analyzed and varied culture times were investigated. Multicolor analysis was used to investigate cytokine producing cell sub-populations. The data demonstrate differences in cytokine production profiles and illustrates the advantages of whole blood culture for routine clinical monitoring of cytokine regulation by flow cytometry.

RESULTS

T cell cytokine expression is altered following whole blood activation. T cell cytokine production following whole-blood activation was compared to T cell cytokine production following PBMC activation. PBMCs were activated for 5 hours in medium containing 10 ng/ml PMA, 2 ug/ml ionomycin and $3\mu M$ monensin, whereas whole blood (1:20 in medium) was activated for 5 hours in medium containing 100 ng/ml PMA, 2 ug/ml ionomycin and $3\mu M$ monensin. It should be noted that 100ng/ml PMA is toxic to PBMCs in culture, wheras leukocytes in whole blood culture exposed to 10ng/ml PMA do not activate. The whole blood culture environment allows/requires higher PMA concentrations. For this comparison the readily detectable pro-inflammatory cytokines IFNg and IL-2 were assessed. The data showed that for each donor both cytokines were expressed on significantly greater percentages of T cells (CD3+) in the whole blood cultures as compared to the PBMC cultures. The mean percentages (from four separate experiments) of cytokine producing T cells for each culture condition are presented in **figure 1**.

Increased whole blood culture times alters relative expression of T cell cytokines. Considering that the kinetics of expression are not the same for all cytokines, it followed that optimal detection of a variety of cytokines might require modulating the length of the activation cultures. In particular, we speculated that Th2 cytokines (such as IL-10) that are known to counter the inflammatory response and are expressed later in a typical immune reaction might not be optimally expressed at an early timepoint. A panel of Th1/Th2 cytokines (IFNg, IL-2, IL-10 and IL-4) were assessed for both culture conditions by flow cytometry at both an early (5 hour) and a later (24 hour) timepoint. The whole blood activation protocol described above was employed for this assay. For the 5-hour timepoint, mitogens and monensin were added to the cells simultaneously and incubated for 5 hours to allow cytokine accumulation. For the 24-hour timepoint, mitogens were added to the cells immediately and incubated for 24 hours, with monensin added for the final 5 hours to allow cytokine accumulation. We found that the expression of IFNg and IL-2 was consistently lower in the whole blood cultures which were allowed to progress to 24 hours of activation, however the expression of IL-10 was unaltered (or trending towards higher) in the 24 hour activation cultures (**figure 2**). The expression of IL-4 did not achieve expression levels in most donors great enough to allow comparison of the two culture timepoints. Whole blood activation cultures allowed to progress past 24 hours contained substantial cellular debris and artifacts, and were judged unsuitable for intracellular cytokine analysis (**data not shown**).

Four color analysis allows complete phenotyping of cytokine producing T cells. A four-color analysis strategy was used to allow the assessment of two cytokines simultaneously in conjunction with two surface

markers. This technique allowed phenotyping of responding T cells for two cytokines. The antibody combinations used for cytokine and surface marker staining are presented in **table 1**. Using these antibody combinations, well-defined analysis regions could be drawn around the lymphocytes, CD3+ T cells, CD8+ T cells and CD8- T cells. The legitimacy of assuming the CD3+/CD8- cells are analogous to the CD3+/CD4+ T cell population is addressed in the discussion. Representative scatter plots demonstrating the gating and cytokine analysis strategy are shown in **figure 3**. To demonstrate the utility of this technique, an analysis of cytokine production for the CD3+ T cell population in general, as well as the CD8+ and CD8- subsets was performed using the whole blood activation protocol at both the 5 hour and 24 hour timepoints. It was found that the expression of IFNγ was significantly higher in the CD3+/CD8+ population as compared to the CD3+/CD8- population following 5 hours of activation. Conversely, the expression of both IL-2 and IL-10 was significantly higher in the CD3+/CD8- population as compared to the CD3+/CD8+ population (**figure 4**). The expression of IL-4 did not appear to be different between both T cell subsets, although in most of our healthy donors the expression was at such low levels that statistical comparisons were difficult. If increased numbers of IL-4 producing T cells were detected in Th2-based disease states (allergy, autoimmune disease, etc.) it would most likely have significant clinical utility. The cytokine expression patterns and statistical differences between the two T cell subsets were the same following 24 hours of whole blood activation as they were after 5 hours of whole blood activation (**data not shown**).

Distinct cytokine-secreting monocyte subpopulations may be resolved using flow cytometry. To assess monocyte cytokine production patterns, either activation of PBMCs or whole blood for 24 hours was performed in the presence of 10ug/ml LPS. Nearly all of the peripheral monocytes were stimulated to secrete IL-1α in both culture systems. A distinct subpopulation of those monocytes were stimulated to secrete IL-12. In addition, following LPS activation, monocytes secreting IL-10 and TNFα were resolved, however IL-10 and TNFα secreting monocytes were largely distinct and mutually exclusive subpopulations (**figure 5**).

Monocyte IL-10 production is altered in whole blood culture. Levels of monocytes stimulated to secrete IL-1α, IL-12, TNFα and IL-10 were averaged for all subjects to assess alterations in expression patterns for whole blood activation. It was found that mean monocyte expression of IL-1α, IL-12 and TNFα was unaltered between PBMC and whole blood culture conditions. Interestingly, mean monocyte expression of IL-10 was significantly elevated following whole blood culture as compared to PBMC culture (**Figure 6**).

DISCUSSION

The use of whole blood culture to perform cytokine flow cytometry was first described in 1997 (**11-13**) and again reported more recently by Tayebi et al. (**14**) and Farrell et al. (**15**). In these articles a protocol using diluted whole blood in culture to retain unfractionated cell populations and soluble plasma proteins was described and he ability to detect of cytokines by flow cytometry was demonstrated. We have compared whole blood activation to traditional PBMC activation and assessed differences between the two systems. Flow cytometric analysis of T cell and monocyte cytokine production profiles were performed for both culture systems using varied culture times and multicolor flow cytometry analysis.

Whole blood activation for 5 hours was found to result in significantly higher percentages of both IFNγ and IL-2 positive T cells as compared to PBMC activation. The distribution of cytokine secretion was found to vary over time during whole blood culture, with IFNγ and IL-2 significantly decreased at 24 hours (as compared to 5 hours) and IL-10 slightly increased. Four-color analysis was used to simultaneously assess Th1 and Th2 cytokine production (IFN/IL-10 or IL-2/IL-4) in CD3+/CD8+ and CD3+/CD8- T cell subsets. Although controversial, it is generally accepted that in healthy individuals the vast majority of the CD3+/CD8- T cells are comprised of the CD4+ T cell subset. Differential expression of cytokine production by the T cell subsets was observed. The CD8+ T cell subset was found to be composed primarily of producers of IFNγ, while the CD8- (CD4+) T cell subset was composed primarily of IL-2 producers. IL-10 was produced almost exclusively by the CD8-(CD4+) subset.

Here, we have analyzed T cell cytokine production for the CD3+/CD8+ (suppressor/cytotoxic) and CD3+/CD8- (helper/inducer) phenotypes. The assumption made was that the CD3+/CD8- T cell subset is largely analogous to the CD4+ T cell subset. The legitimacy of assuming the CD3+/CD8- population to be analogous to the CD4+ T cell population is controversial and has generated significant discussion. For our protocol this assumption was necessary for two reasons: (1) the CD4 molecule is internalized on T cells rapidly after PMA/ionomycin activation to nearly nondetectable surface levels and (2) to facilitate single-tube phenotyping and double-

cytokine analysis of both the CD4 and CD8 T cell subsets. A necessary assumption to utilize this method is that the CD3+/CD4+/CD8+ and the CD3+/CD4-/CD8- populations are negligible. Of course, in many disease states (such as HIV infection) this would *not* be the case and therefore this assumption would be invalid. Although the 'normal ranges' for these phenotypes would have to be established for individual laboratories, it is the opinion of the authors that in *healthy* individuals these populations are in fact a negligible proportion of the T cell compartment. It is noteworthy that for mitogenic stimuli other than PMA/ionomycin the internalization of CD4 is typically not a problem, however levels of cytokine producing cells induced by other stimuli are also typically not as great as those induced by PMA/ionomycin. It is therefore highly desirable to stimulate cells with PMA/ionomycin in most cases to determine cytokine production profiles.

Several alternatives to allow positive identification of CD4+ T cells following PMA+ionomycin activation have recently been suggested. These include prestaining CD4 prior to activation **(16)** and the staining (after activation) of intracellular CD4. For prestaining of CD4, it has been reported that the addition of monensin abrogates the protolytic effects of internalization and allows stable detection of CD4. The effects of prestaining CD4 on cellular activation would have to be determined for the experimental conditions. In disease states where positive identification of CD4 would be desired it is likely that staining intracellular CD4 with a bright fluorochrome would be the most successful option. Staining CD4 in addition to CD8 would require a separate analysis tube (when used with our two-cytokine four-color analysis system). If the assumption to assess CD3+/CD8- cells as the helper inducer subset is appropriate for the patient/donor population, the single tube dual cytokine/dual surface marker assay system described here would then have significant advantages.

Monocytes were identified by expression of CD14. The expression of IL-1α, IL-12, TNFα and IL-10 were assessed in monocytes for both culture systems. In both systems, IL-12 producing monocytes were found to be a distinct subpopulation of the whole, with the majority of the monocytes producing IL-1α. Interestingly, TNFα and IL-10 secreting monocytes were resolved and found to be largely mutually exclusive. TNFα and IL-10 secreting monocytes may represent exclusive functional monocyte subsets that may be distinguished based on cytokine secretion profiles. Percentages of monocytes expressing each cytokine were largely unchanged between whole blood and PBMC culture, with the notable exception of IL-10. Monocytes secreting IL-10 were significantly elevated in following whole blood activation. It is of note that the level of LPS was the same for both culture conditions.

The reasons for the altered cytokine production patterns between the two culture systems are unknown, but are most probably due to one (or some combination) of the following factors:

- Loss of specific cell populations through purification in the PBMC culture.
- Soluble plasma molecules that are retained in the whole blood culture system.
- Increased levels of PMA are tolerated in whole blood culture (T cell).
- Increased survival of cytokine-secreting cells in whole blood culture.
- Altered adherence and activation of leukocytes in PBMC cultures.

It is important to note that for whole blood culture there is an ability (and necessity) to use a much higher concentration of PMA than in PBMC culture. In this study 100ng/ml PMA was used in the whole blood culture as compared to 10ng/ml in the PBMC culture. In our preliminary work it was found that if the whole blood PMA level (100 ng/ml) were used in PBMC cultures, cell death resulted (data not shown). If the PMA level suitable · for PBMC culture (10 ng/ml) was used in whole blood culture little to no cytokine production occurred. The reason for this difference is unknown, but it remains clear that the whole blood culture (probably due to the presence of large levels of RBCs) allows the use of the much higher PMA concentrations. It is certainly possible that the higher cytokine production observed in the whole blood cultures is attributable to the elevated PMA concentration, but it is also likely that the other variable mentioned above play an important role as well. The ultimate goal of using polyclonal activators for cytokine flow cytometry is to drive as many leukocytes as possible to their natural cytokine producing activation endpoint, allowing a picture of the cellular cytokine regulation and production profiles to be achieved. Even if the elevated PMA concentration is responsible by allowing many more T cells to be activated, it is beneficial since clearer window to the cytokine bias of the peripheral immune system is the result. The monocyte data, where an equal level of LPS (10 μg/ml) was acceptable for use in both culture systems, argues in favor of either natural cell interactions or plasma factors influencing the whole blood cytokine profile outcome.

CONCLUSION

Assessment of cytokine production profiles by flow cytometry may be performed following either whole blood activation or PBMC activation. Whole blood activation, which eliminates cellular purification steps, is less labor intensive to perform and may represent a substantial improvement over PBMC culture. In this study, altered cytokine levels and altered cytokine production profiles are observed between whole blood and PBMC culture activation for both T cell subsets and monocytes. It is highly likely that whole blood culture more accurately reflects the in-vivo cellular cytokine production patterns than does PBMC culture.

Considering that the goal of cytokine production assays using polyclonal activators is to reveal the cytokine production potential of cells upon full immunologic activation, these data show that the use of whole blood culture to assess the cytokine balances by flow cytometry appears to have several practical advantages. They include: (1) the detection of larger percentages of IL-2 and IFNγ producing T cells; (2) potential increase in ability to detect T cell IL-10 production by increasing culture times; (3) the ability to determine the phenotype the T cell subsets responsible for dual cytokine production using multicolor analysis; (4) the ability to detect altered monocyte IL-10/TNFα production.

Considering that whole blood culture probably more accurately reflecting the in vivo situation, and that the use of whole blood activation is a rapid non-labor intensive protocol, whole blood culture may be preferred for routine monitoring of cytokine secretion patterns in clinical laboratories for diagnostic and prognostic purposes.

MATERIALS AND METHODS

Blood donors. Whole blood samples were obtained from adult donors into ACD or sodium heparin anticoagulant vacutainers. The subjects had been screened by the NASA-JSC Test Subject Facility for most major infectious diseases and were found to be in good health. Institutional Review Board (NASA-JSC) approval was obtained for this study and written informed consent was obtained from all subjects.

Peripheral mononuclear cell isolation. For PBMC isolation, 4.0 ml of whole ACD anticoagulated blood was diluted 1:1 with sterile phosphate buffered saline (PBS) and subjected to centrifugation over a Ficoll-Hypaque density gradient (Pharmacia, Upsala Sweden). The isolated cells were washed 3 times in sterile PBS and resuspended in RPMI medium 1640 (Gibco BRL, Grand Island, NY) containing 10% fetal bovine serum (GIBCO) and 1×10^5 mg/ml penicillin and streptomycin (GIBCO). The final concentration of cells was determined using a hemacytometer. A final concentration of approximately 1.0×10^6 cells/ml was used in all PBMC activation experiments.

PBMC culture conditions for activation. For PBMC T cell activation, isolated cells were cultured in RPMI medium 1640 (Gibco BRL) containing 10 ng/ml phorbol myristate acetate (PMA- Sigma, St. Louis, MO) and 2 μg/ml ionomycin (Sigma). The cells were cultured for the indicated length of time (5 or 24 hours). In all cases 3 μM monensin (Sigma) was added to the medium for the final five hours to abrogate extracellular transport of cytokines and allow intracellular accumulation (17). The cells were fixed in 4.0% paraformaldehyde and then washed 3 times in sterile PBS prior to staining. For PBMC monocyte activation the cells were cultured in RPMI medium 1640 (Gibco BRL) containing 10 mg/ml LPS (Sigma, St. Louis, MO) and 3 mM monensin (Sigma) for 24 hours. The cells were then fixed in 4.0% paraformaldehyde and then washed 3 times in sterile PBS prior to staining.

Whole blood culture conditions for activation. For whole blood T cell activation, 50μl of heparinized whole blood was added to 1.0 ml of RPMI medium 1640 (Gibco BRL) containing 100 ng/ml PMA (Sigma, St. Louis, MO) and 2 μg/ml ionomycin (Sigma) to create a 1:20 dilution of whole blood in medium. Cells were cultured for the indicated length of time (5 or 24 hours). In all cases 3 μM monensin (Sigma) was added to the medium for the final five hours to allow cytokine accumulation. Following activation the RBCs were lysed using FACSlyse (Becton-Dickinson, Mountain View, CA) according to the manufacturers instructions. The cells were fixed in 4.0% paraformaldehyde and then washed 3 times in sterile PBS prior to staining. For whole blood monocyte activation 300ml of heparinized whole blood was added to 2.0 ml of RPMI medium 1640 (Gibco BRL) containing 10 mg/ml LPS (Sigma, St. Louis, MO) and 3 mM monensin (Sigma) for 24 hours. Following activation the RBCs were lysed using FACSlyse (Becton-Dickinson, Mountain View, CA) according to the manufacturers instructions. The cells were fixed in 4.0% paraformaldehyde and then washed 3 times in sterile PBS prior to staining.

Fluorescent antibody staining. Following fixation, the cells were then washed 3 times in PBS. To detect intracellular production cytokine in conjunction with a surface marker (both culture conditions), the fixed cells were resuspended in 200 μl of permeabilization buffer, consisting of 5.0% non-fat dry milk and 0.5% saponin in PBS containing 0.5 μg of each labeled antibody. The antibody combination for each tube (with manufacturers) is presented in **table 1.** The cells were incubated at room temperature for 60 minutes and then washed in PBS containing 0.5% saponin. The cells were then resuspended in 1.0% paraformaldehyde for analysis.

Flow cytometry analysis. For analysis, a Coulter XL flow cytometer was configured for four color analysis with appropriate electronic compensation for spectral overlap of the light emitted by the different fluorochromes. The target cells (CD3+ T cells or CD14+ monocytes) were then 'gated' for immunofluorescence analysis, including only the target cells in the data analysis. Thresholds for positive were set using isotype matched control antibodies or internal cells distinctly negative for the target antigens.

ABBREVIATIONS

PBMC	Perhpheral blood mononuclear cell
PMA	Phorbol-myristal acetate
IFN	Interferon-gamma
IL-10	Interleukin-10
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-1	Interleukin-1
IL-12	Interleukin-12
TNF	Tumor necrosis factor
RBC	Red blood cell
ACD	Acid-citrate-dextrose
NASA	National Aeronautics and Space Administration
JSC	Johnson Space Center
PBS	Phosphate buffered saline

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Figure Legends

Figure 1. T cell IFN and IL-2 production is altered in whole blood culture as compared to PBMC culture. PBMC stimulation was performed for 5 hours using 10 ng/ml PMA and 2 ug/ml ionomycin. Whole blood activation was performed using a 1:20 dilution of whole blood in media containing 100 ng/ml PMA and 2 ug/ml ionomycin. 3 uM mononsin was added to all cultures to abrogate extracellular transport and allow intracellular accumulation of cytokines. Error bars represent the standard error, mean of 7 experiments. Significant differences are indicated (*).

Figure 2. Optimal T cell cytokine production in whole blood culture is time dependant. For all cytokines measured (with IL-10 an exception) 5 hour whole blood activation is sufficient for optimal production. IL-10 expression is slightly elevated at 24 hours as compared to 5 hours. This result is consistently seen and fits with the role of IL-10 as a down regulator of the inflammatory response whose expression occurs late in the kinetics of an immune reaction. Error bars represent the standard error, mean of 6 experiments. Significant differences are indicated (*).

Figure 3. Representative scatter plots demonstrating the gating and analysis strategy for four-color dual-cytokine and phenotype T cell analysis by flow cytometry. (A) lymphocyte gate; (B) pan-T cell gate; (C) CD8+ and CD8- T cell gate; (D) T cell isotype control analysis; (E) and (F) IFNg/IL-10 analysis for T cell subsets; (G) and (H) IL-2/IL-4 analysis for T cell subsets. NOTE: The legitimacy of assuming the CD3+/CD8- population to be analogous to the T helper subset is discussed in the text.

Figure 4. Differential expression of cytokines by T cell subsets. Whole blood cultures were activated for 5 hours in media containing PMA+ionomycin+monensin and stained for surface phenotype and intracellular cytokines using the four-color analysis protocol described. Error bars represent the standard error (n=5) and significant differences are indicated (*).

Figure 5. Representative scatter plots demonstrating the gating and analysis strategy for three-color dual-cytokine monocyte analysis by flow cytometry. A typical (A) whole blood vs. (B) PBMC result for a healthy subject is presented. Note the presence of distinct granulocytes in the whole blood analysis, and their absence in the PBMC analysis.

Figure 6. Mean percentages of monocytes expressing intracellular IL-1α, IL-12, TNFα and IL-10 demonstrate that cytokine production in whole blood culture is altered with respect to PBMC culture. PBMC stimulation was performed for 24 hours using 10 ug/ml LPS. Whole blood activation was performed for 24 hours using a 300 μl/2.0 ml dilution of whole blood in media containing 10 ug/ml LPS. 3 uM mononsin was added to all cultures for the final 5 hours to abrogate extracellular transport and allow intracellular accumulation of cytokines. Error bars represent the standard error, mean of 7 experiments. Significant differences are indicated (*).

Table 1: Four color antibody panel used for cytokine and surface marker analysis

Tube	FL1	FL2	FL3	FL4
1.	control*	control*	CD3[@]	CD8[@]
2.	IFNg*	IL-10[#]	CD3[@]	CD8[@]
3.	IL-2*	IL-4*	CD3[@]	CD8[@]
4.	control*	control*		CD14*
5.	IL-1α[#]	IL-12[#]		CD14*
6.	TNFα[#]	IL-10[#]		CD14*

FL1: FITC; FL2: phycoerytherin; FL3: ECD; FL4: phycoerytherin/cyanin5. *Becton-Dickinson, Mountain View, CA; [#]Pharmingin, San Diego, CA; [@]Beckman-Coulter Corporation, Miami, FL.

T CELL CYTOKINE EXPRESSION IS ALTERED IN WHOLE BLOOD CULTURE
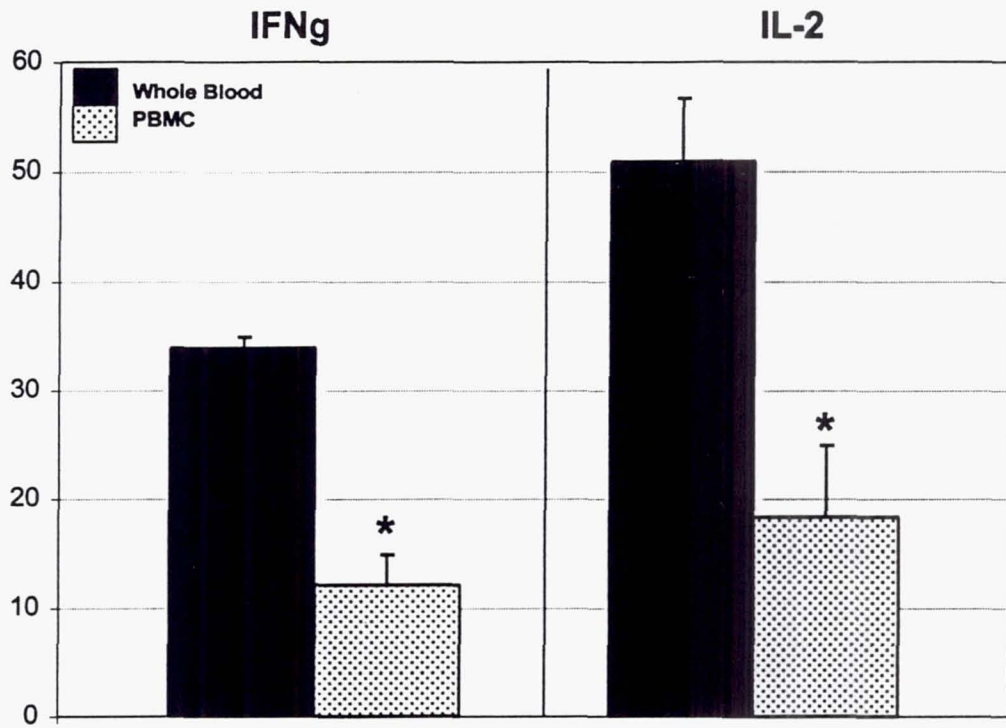
Figure 1

TIME DEPENDENCE OF OPTIMAL T CELL CYTOKINE EXPRESSION IN WHOLE BLOOD CULTURE
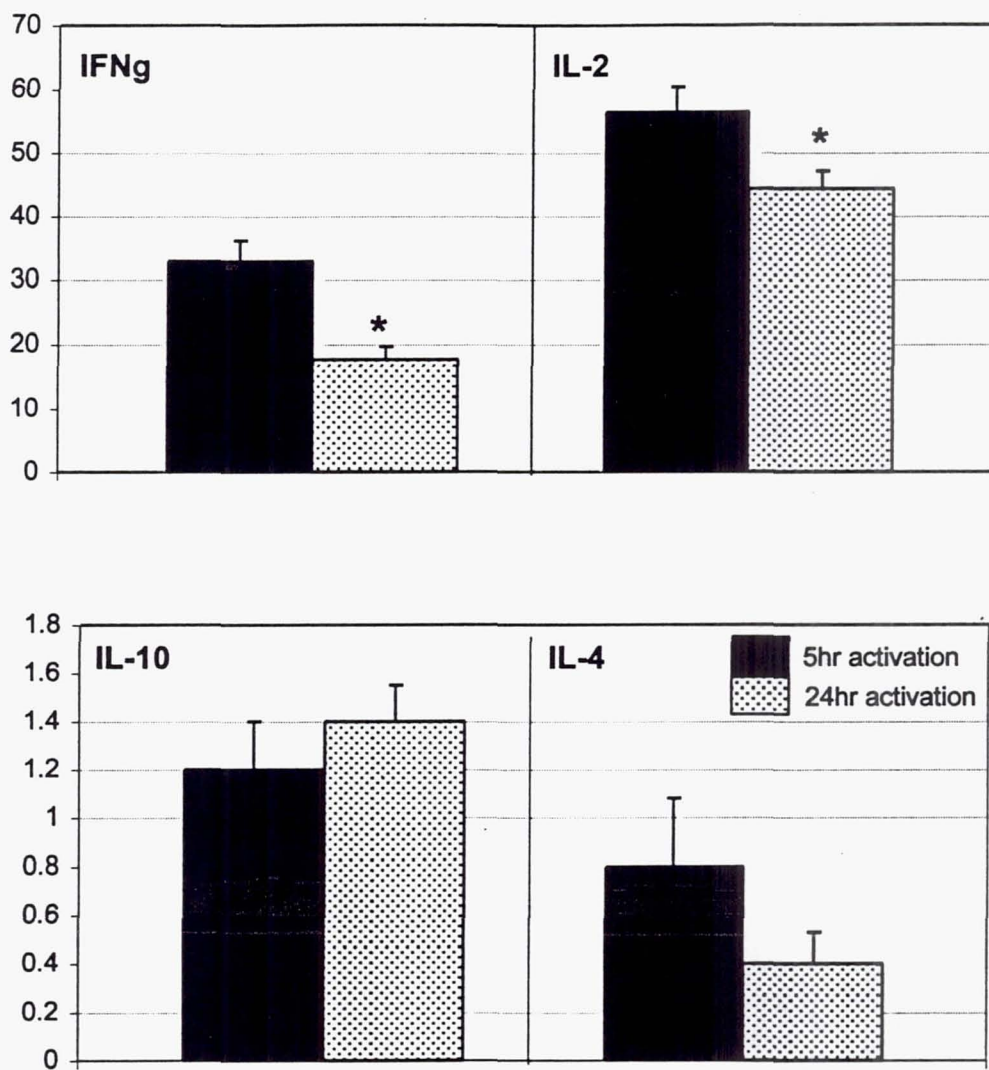
Figure 2

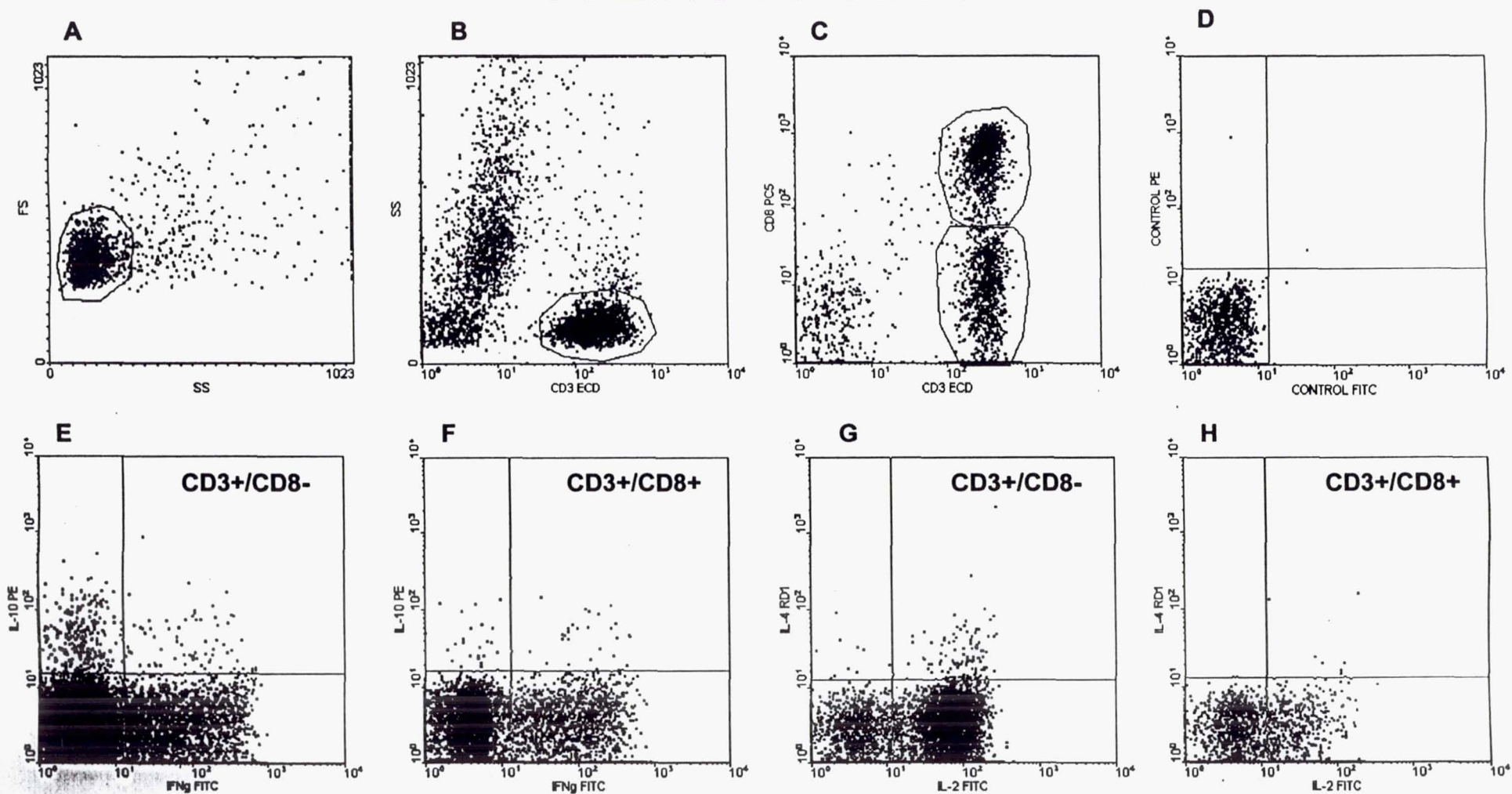# FOUR COLOR ANALYSIS OF CYTOKINE PRODUCTION BY
T CELL SUBPOPULATIONS

FIGURE 3

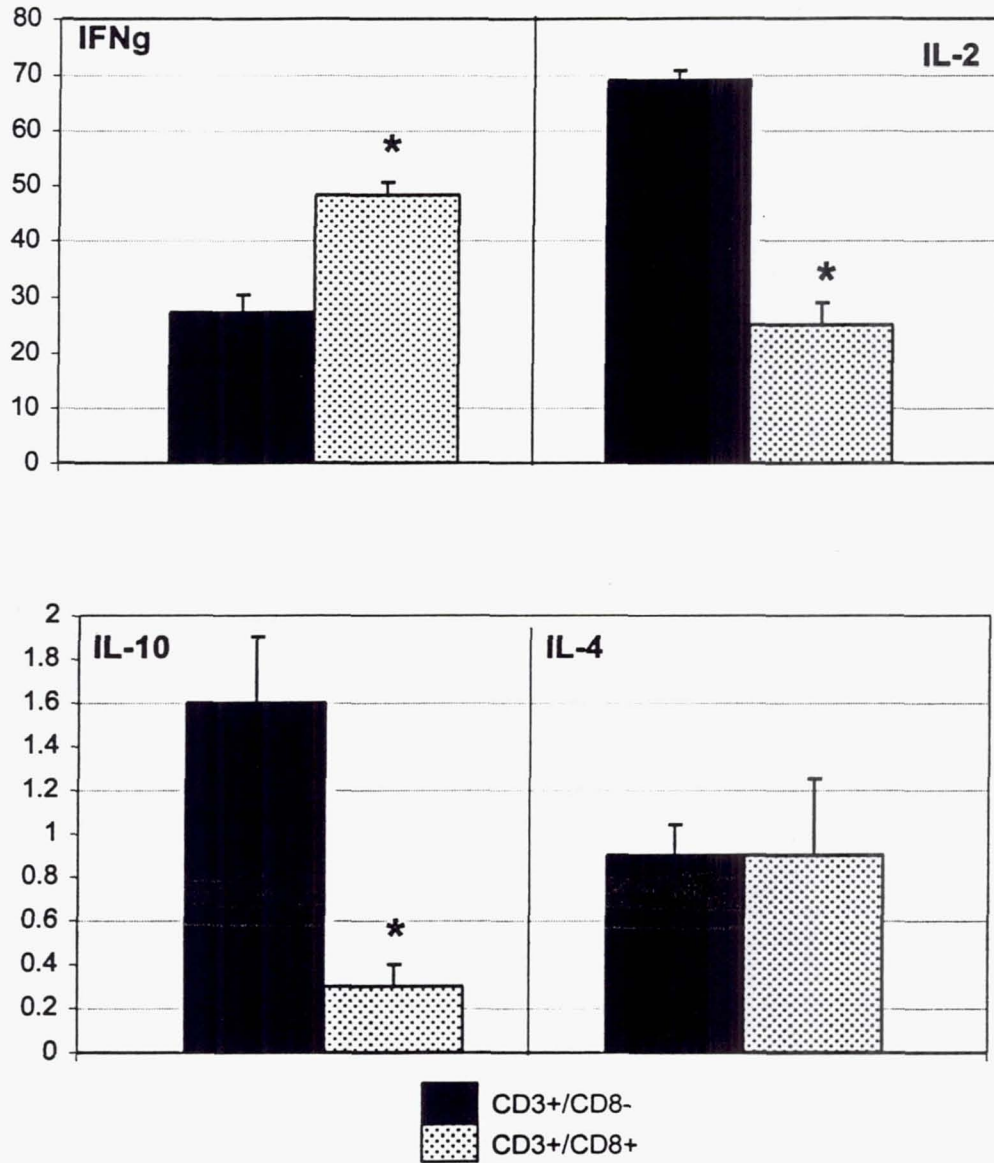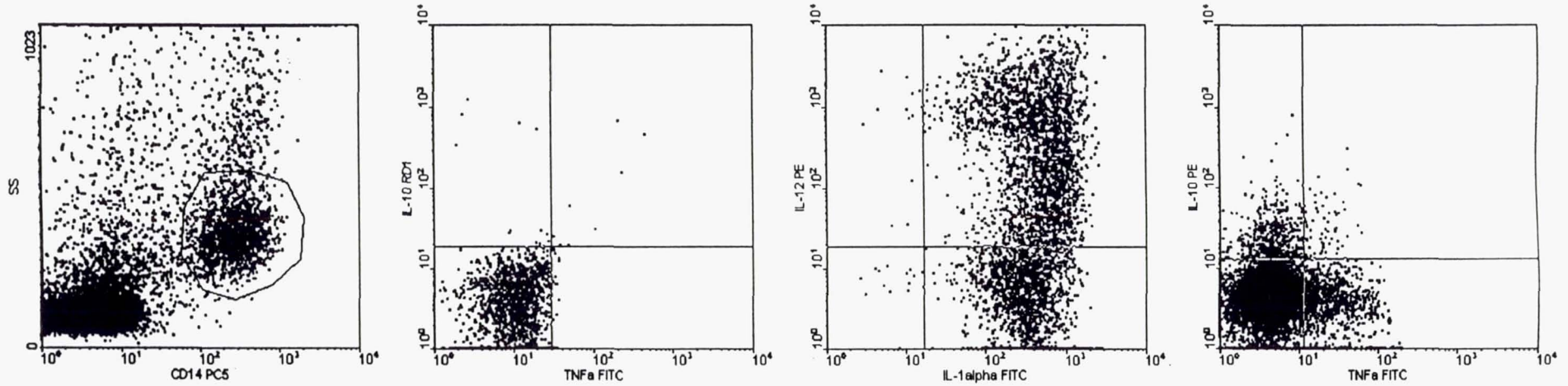# T CELL WHOLE BLOOD CYTOKINE EXPRESSION IS ALTERED BETWEEN CD8- vs. CD8+ SUBSETS
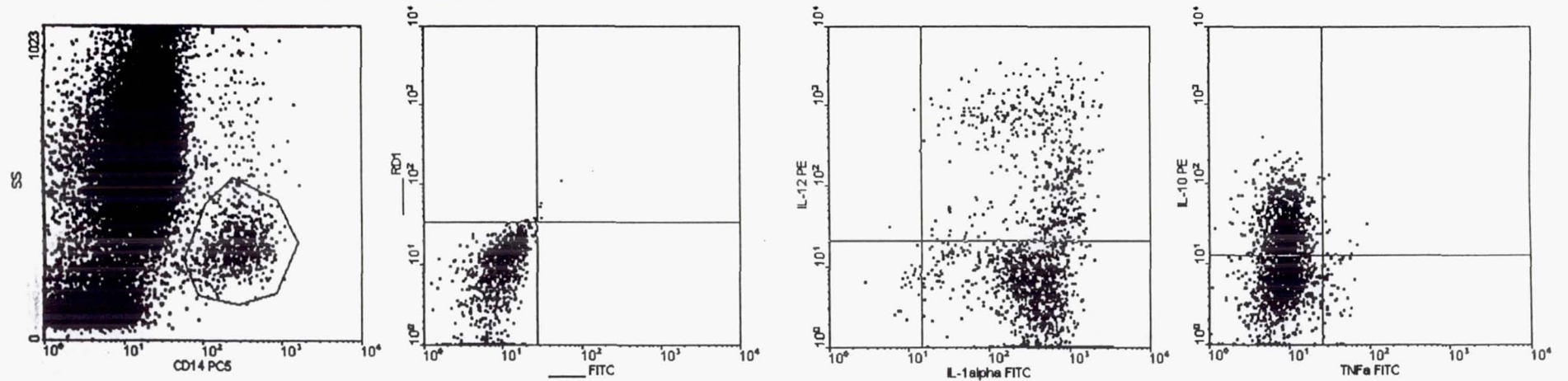

Figure 4

MONOCYTE CYTOKINE-PRODUCING SUBPOPULATIONS

LPS ACTIVATION – 24 HOURS

SUBJECT A: PBMC CULTURE

SUBJECT A: WHOLE BLOOD CULTURE

GATING STRATEGY   **ISOTYPE CONTROLS**   *IL-1a vs. IL-12*   *TNFa vs. IL-10*

FIGURE 5

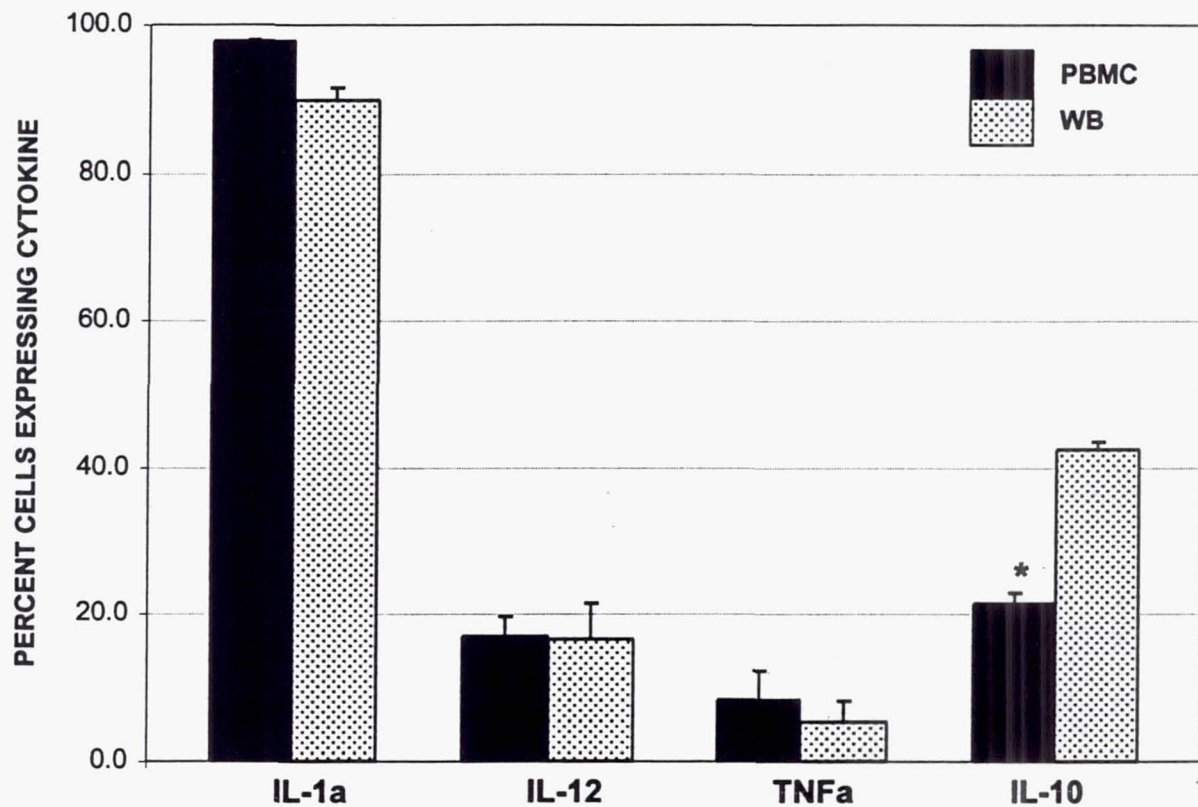# MONOCYTE IL-10 EXPRESSION IS ALTERED FOLLOWING WHOLE BLOOD CULTURE

FIGURE 6