CD4+Leu8- and CD4+CD45RA+ cells. All activated T cell subsets in stage I-III were higher than stage IV. This study demonstrates significant differences in the PBL profile between stages of breast cancer patients. These differences may be an indication of patient response to their tumor and their ability to combat disease progression.

452C

THE EFFECT OF TPA AND OTHER DIFFERENTIATION REAGENTS ON I29 LYMPHOMA CELL LINES. I.ZAN-BAR*, D.ROKER. Dept of Human-Microbiol, Faculty of Medicine, Tel-Aviv University, Tel-Aviv, ISRAEL.

Murine B cell lymphoma I29 cell lines can be stimulated by LPS, IL-4, IL-5, IL-6, and other B cells "pushers" to secrete immunoglobulins (Ig) and to differentiate from IgM to IgG1, IgG2, IgE, or IgA bearing cells. In the present work the effects of TPA, Retinoic Acid (RA), Vitamin D (VitD), and DMSO were examined on I29 cells. The changes in cell proliferation, cell differentiation and Ig secretion were measured. No direct or indirect effect of DMSO, RA, and VitD on Ig secretion of I29 cells could be detected. TPA induces Ig secretion and has direct effect on I29 cell differentiation in isotype switch and IgA secretion. In spite of the isotype switch, no changes in DNA quantities (and thus in cell aneuploidity), could be detected. No direct or indirect effect of DMSO, RA and VitD on I29 cell differentiation could be detected. VitD has no effect on I29 lymphoma cell division, while DMSO, RA, and TPA have dramatic effect in reduction and in temporal blockage (4-5 days) of cell proliferation. Thus while TPA induces both differentiation and reduction in the rate of cell proliferation, RA and DMSO reduce the rate of cell division with no effect on I29 cell differentiation.

453A

A DEFECT OF IMMUNOREGULATORY T CELL SUBSETS IN PATIENTS WITH HISTIOCYTOSIS X (H-X) DEMONSTRATED WITH ANTI-2H4 ANTIBODY. B.T. Shannon* and W.A. Newton, Ohio State University, Columbus, OH, USA.

The CD4 lymphocyte population includes a subset that induces suppressor T lymphocytes (CD8) and can be distinguished by dual-color fluorescence analysis with anti-2H4 and anti-CD4 monoclonal antibodies. Previous studies from our laboratory have demonstrated poor suppressor cell function in conjunction with markedly reduced absolute numbers of CD8 lymphocytes in H-X patients with active disease (J. Clin. Immunol. 6:510, 1986). In this study, we examined the CD4+2H4+ population of H-X patients with active disease in order to determine whether the reduction of the CD8 subpopulation might be explained in part by a reduction of the CD4+2H4+ subpopulation. Patients with active disease had a markedly decreased percentage of CD4+2H4+ cells (9±2%) in their peripheral blood compared with patients with inactive disease (18±5%) or age matched controls (19±4%) ($P < 0.001$). Furthermore, the CD4+2H4+ subpopulation in patients with active disease could not be expanded utilizing certain biologic response modifiers thought to modify the progression of the disease. These results may explain in part certain clinical manifestations of the disease.

454B

LIGHT SCATTERING OF MICRO-PARTICLE LABELED LYMPHOCYTES STUDIED BY FCM. H.S.P. Garritsen*, B.G. de Grooth, J. Greve. University Twente, Dept. of Applied Physics, Cell Characterization Group.

Biotinylated magnetic microparticles (MACS ,Becton Dickinson) with a diameter of 50-100nm can be used to label cells. They are used to separate cells on the basis of their surface determinants by coupling the microparticles to primary monoclonal antibodies. We have investigated wether the microparticle labeled cells can be identified on the basis of their scatter profile in FCM. In agreement with the notion of the manufacturer the FLS and SSC of the labeled lymphocytes was not influenced by the MACS microparticles with. laser excitation wavelengths of 488 and 632nm.
Also no change in the depolarized orthogonal light scatter signal was found. However when this labeling was combined with a silver enhancement procedure (1), both orthogonal light scattering and the depolarized orthogonal light scattering showed a large increase. Similar results were obtained with monoclonal antibody labeled 5nm and 20nm colloidal gold particles instead of MACS particles. The influence of this procedure on other intrinsic scatter parameters such as 10 degrees scattering and axial light loss will be presented.
(1) De Waele M. et.al. J. Histochem. Cytochem. **34** 935 (1986)

455C

DIFFERENTIAL SUPPRESSIVE EFFECT OF THC ON MURINE T CELL SUBSETS. S.H. Pross, R.H. Widen*, J.H. Smith Univ. of South Florida, Tampa, FL

Tetrahydrocannabinol (THC) is the major psychoactive component of marijuana and has been shown to suppress the proliferative response of human and murine T cells to the mitogens PHA and con A. The effect of THC on the different subpopulations of T cells has not been characterized. We examined the effect of THC on the percentage and number of CD4 and CD8 positive cells after incubation of murine splenocytes with PHA or con A. Normal mouse splenocytes are 20-30% CD4+ and 5-15% CD8+. After 72 hr incubation with PHA, the percentages change to 35% CD4+ and 60% CD8+. THC caused a dose dependent suppression of proliferation, with the predominant effect on the CD8+ cells. Similar dose dependent reductions in mainly CD8+ cells with con A as the stimulus. The absolute numbers of CD4 and CD8 positive cells also showed similar dose dependent rdeuctions in the presence of THC, with the CD8+ cells demonstrating the greatest sensitivity, although suppression of both populations was demonstrable at high concentrations of THC.

456A

ROUTINE THREE COLOR IMMUNOPHENOTYPING. C. C. Stewart and K. Foon Roswell Park Memorial Institute, Buffalo, N.Y.

Immunophenotyping by flow cytometry has become the preferred method for identification of hematopoietic cells. Most immunophenotyping laboratories develop a profile of the patients blood or bone marrow cells by analysing several samples each containing a single antibody. Some laboratories routinely use two antibodies per sample, one labeled with fluorescein, the other with phycoerythrin. We have developed the methodology to use three antibodies per sample of bone marrow or lysed whole blood. The third antibody is biotinylated and the phycoerythrin-Texas Red avidin complex, DuoChrome, is used as a second step reagent for the third color. Panels containing three antibodies can be developed to determine specific subsets. For example, using FL-CD3, PE-CD4 and DC-CD8, ten separate subpopulations of $CD3^+$ T cells can be routinely resolved. By backgating on $CD3^+$ cells first, both small and large T-cells are resolved. Large cells have been ignored by the flow cytometry community because the "lymphogate" excludes them. In patients with disease, the large T-cell population can be as high as 25% of the T-cells. The subsets of $CD56^+$ cells can be resolved using FL-CD3, PE-CD56 and DC-CD8. We have found up to 40% of $CD56^+$ cells from normal individuals are large granular cells that would not have been included in the "lymphogate." The routine use of three antibodies not only reduces the number of samples that have to be analysed but provides new insights into the subsets of normal and neoplastic leukocytes. The biological and clinical significance of these newly resolved populations has yet to be fully understood.