Introduction: Joint arthroplasty is a successful procedure for the treatment of the inflammatory or degenerative joint destruction that occurs in arthritis. One of the factors contributing to the failure of joint arthroplasty is the biologic response to wear debris and implanted biomaterials and the debris they generate (1-3). It would be beneficial to determine the sensitization levels to biomaterials in patients with failed joint arthroplasty, since this could influence the choice of illicit material if a significant immune response to a biomaterial is present. One current method to determine immunological reactivity is an in vitro assay of the patients blood cells cultured with suspected immune sensitizers (usually cobalt chrome alloy or PMMA cement)(4,5). This assay measures T cell activation, takes 6 days to complete, and does not always give a definitive answer. Complications due to biomaterial toxicity in culture, resulting in cell death instead of cell proliferation, can obscure the data interpretation. Recently, novel methods have evolved for evaluating cellular sensitization using human peripheral blood cells. With the discovery of CD69, it was evident that this is an early activation antigen. CD 69 expression is induced in vitro on cells of most hematopoietic lineage, including T and B lymphocytes and NK cells (6). It is rapidly induced after stimulation of the T cell receptor complex in T cells; after cross linking of surface immunoglobulins in B cells; and by IL-2, IL-6, Interferon alpha, CD16-antibodies, or NK targets on NK cells (7). In T cells, surface expression of CD69 can be detected as early as 30 minutes after the addition of a stimulat ing molecule, and after one hour 85-90 percent of T cells are positive, with increasing expression until 24 hours (8). It was our hypothesis that measuring early activation using CD69 expression markers would provide a quicker and more accurate means to determine cell sensitization levels. Metal and PMMA sensitivity might be determined faster and prior to toxicity in culture. 

Methods. Peripheral blood was obtained from 23 patients with total joint arthroplasties (either knee or hip), mononuclear cells (MNC) were separated from blood. A proliferation assay was undertaken and CD69 expression was evaluated after cell exposure to PMMA particles. 

Proliferation assay: 100ul of cell suspension as of 2.5x10^6 cells/ml in RPMI medium supplemented with 5% fetal calf serum (FCS) were dispensed into each wells of a 96-well tissue culture plate (Costar). 100ul aliquots of medium containing various concentrations of PMMA particle were added to appropriate wells. Wells with no particles (negative control) or with ConA (positive control) were also included on the plate. Plates were incubated at 37°C in a 5% CO2 atmosphere for 72 hours. 20ul of 3-(4,5 dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) solution (5mg/ml; Sigma, MO) was then added per well, 37°C for 6 hours. Medium was replaced by 10% SDS, 37°C overnight. The optical density (OD) of the solution was read at 590nm using a microplate spectrophotometer (Molecular Devices, Menlo Park, CA), and cell responses were expressed as OD values. Stimulation indexes (SI) for each response were then calculated by comparison with background proliferation (medium control).

Flow Cytometry for CD 69 expression: 200ul of cell suspension were mixed with 200ul of PMMA and incubated at 37°C for 1-3 days. Following culture, 10ul of the following antibodies were added: CD 69 (anti-human mononuclear T cell antibody), CD3 (anti-human T cell antibody), CD20 (anti-human B cell antibody). (Phar mingena, USA). Excess was removed by washing with phosphate buffered saline containing fetal calf serum. All samples were then evaluated by two color flow cytometry.

Results. Flow cytometric analysis revealed a high expression of CD69 in lymphocytes cultured with PMMA. CD69 peaked on the lymphocyte surface after 21 hours in culture, and remained at a constant level of expression through six days. For analysis purposes, patients were considered to exhibit a positive reaction to PMMA if CD69 expression increased over ten percent of the level observed on cells cultured in medium alone. Double labeling of the cells indicated that the predominant response occurred in the B-cell population (CD20) compared with the T-cell population (CD3). Twenty-five percent of the patient samples were positive for PMMA reactivity in the T cell population (CD3+/CD69+), while 85% of the patient samples were positive for PMMA reactivity in the B cell population (CD20+/CD69+). The mean percent of positive CD3+/CD69+ cells reacting to PMMA was 6.5% (+/- 1.36), while the mean percent of positive CD20+/CD69+ cells reacting to PMMA was 29.6% (+/- 5.6). There was a highly significant difference (p<0.001) in the CD69 antigen expression between T cells and B cells. This indicates that B-cell reactivity to PMMA was more potent than T-cell reactivity to PMMA in the total arthroplasty patient population, and the strength of the lymphocyte activation was higher in the patient B-cell component.

Comparison of CD69 expression to proliferation in response to PMMA in culture indicated that the activation marker was a more sensitive parameter of PMMA stimulation. Although there was no significant association between the proliferation stimulation index and the level of CD69 expression, the correlation coefficient indicated a higher association of cellular stimulation with B-cell CD69 expression compared with T-cell CD69 expression.

Discussion. Previous studies of lymphocyte activation by orthopaedic biomaterials have implied that T lymphocytes are the dominant cell in the response. A recent paper by Granchi et al (9) reports that CD69 T cell expression was increased in cells from arthroplasty patients stimulated with cobalt or chrome ions. However, the evidence for T cell reactivity to PMMA, beyond the activation of T cell cytokines (4), is rather scarce. The current data suggests that although T cell activation to PMMA does occur, the predominant reactive cell is usually a B cell. The high frequency of CD69+/CD20+ cells in cultures from total joint arthroplasty patients suggests that this response is common, although an association with clinical outcome remains to be determined. The high frequency of PMMA reactive B-cells in some patients is suggestive of polyclonal B cell activation, and we propose that the repetitive structural properties of the polymer may mimic the complex antigens (such as bacterial cell walls) that are recognized as T cell independent antigens (10).

References: