

# Lack of immune response to methyl methacrylate in lymphocyte cultures

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To analyze whether or not methyl methacrylate is immunologically inert, peripheral blood mononuclear cells were cultured with finely pulverized methyl methacrylate. Phytohemagglutinin (PHA) lectin, purified protein derivative of tuberculin (PPD) antigen, and culture medium alone were used as positive and negative controls. Lymphocyte kinetics on culture Days 0, 1, 3, and 5 were studied. Major histocompatibility complex locus II antigen (MHC locus II antigen; Ia) and interleukin-2 receptor (IL-2R; Tac) expression were analyzed using the avidin-biotin-peroxidase complex (ABC) method and lymphocyte DNA synthesis using <sup>3</sup>H-thymidine incorporation and beta-scintillation counting. On culture Days 1 and 3, lymphocytes and monocytes

were seen under the light microscope to be attached to methyl methacrylate particles. However, the results disclosed no methyl methacrylate-induced DNA synthesis, although methyl methacrylate-induced MHC locus II antigen and IL-2R activation marker expression were recorded; notably, this expression was less pronounced than that seen in PHA or PPD antigen driven lymphocyte response. The results suggest that methyl methacrylate is essentially an immunologically inert implant material. However, it seems to induce inflammatory mononuclear cell migration and adhesions leading to slightly nonspecific lymphocyte reaction.

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The immunologic response to methyl methacrylate cement in replaced joints has been debated since the early period of arthroplastic surgery. Charnley (1975, 1979) studied the tissue reaction around cemented prostheses using conventional histologic methods and concluded that methyl methacrylic cement was relatively inert, causing little tissue response as long as there was good fixation. Our recent research (Tallroth et al. 1989, Santavirta et al. 1989, Eskola et al. 1990, Santavirta et al. 1990c) and observations made by others (Harris et al. 1976, Maguire et al. 1987, Jones and Hungerford 1987) indicate that some patients develop a foreign-body reaction to cemented prostheses. In fact, 5 percent of our revision arthroplasties have shown an aggressive granulomatous reaction (Tallroth et al. 1989). We have previously demonstrated an immunologic response around loose total hip replacements; the reaction is different in aggressive granulomatous cases when compared with those with common prosthetic loosening (Santavirta et al. 1990c).

We analyzed the human immunologic response to methyl methacrylate in human lymphocyte cultures.

## Material and methods

### Human lymphocyte cultures

From 4 healthy volunteers, venous blood was collected in syringes containing preservative-free heparin. After an RPMI-1640 dilution (1:1), a Lymphoprep<sup>®</sup> (specific gravity 1.078 g/mL, Nyegaard, Oslo, Norway) density gradient isolation of peripheral blood mononuclear cells was performed (400 g, 40 min, +22 °C). Next,  $2 \times 10^5$  isolated and washed cells were placed in 0.2 mL (Falcon 3072, Becton Dickinson, Oxnard, CA) or 2 mL (Nuclon Delta SI, Roskilde, Denmark) flat-bottomed, cell-culture wells. In a preliminary study, 10, 100, 1,000, and 10,000 µg of finely pulverized methyl methacrylate (particle diameters 30–50 µm) were tested. Totally, 10 mg per well was used in subsequent experiments, because methyl methacrylate is nontoxic, and 10 mg could easily be visualized as microscopic particles in the cytocentrifuged specimens. As negative controls, we used plain 10 percent FCS-RPMI-1640 medium without methyl methacrylate; and as positive controls, we used either phytohemagglutinin (PHA, 1.25 µg per well; Wellcome Diagnostics, Dartford, U.K.) or

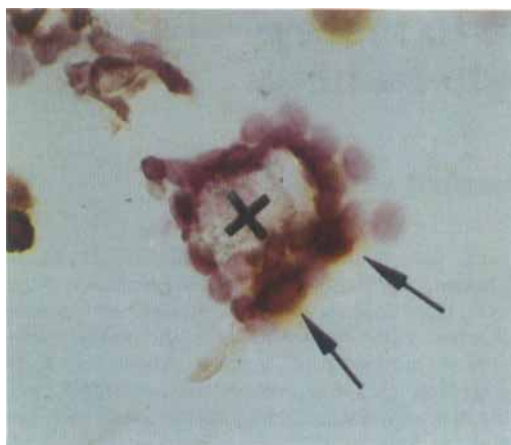


Figure 1. Human peripheral blood mononuclear cells cultured with finely pulverized methyl methacrylate (particle size 30–50  $\mu\text{m}$ ) are stained with anti-MHC locus II antibodies using immunoperoxidase technique. Methyl-methacrylate particle (marked with X) is surrounded by some lymphocytes and Ia-positive monocytes (arrows),  $\times 400$ .

Table 1. Methyl methacrylate cement (10 mg/mL) induced  $^3\text{H}$ -thymidine incorporation in peripheral blood mononuclear cell cultures as compared with negative (culture medium alone) and positive mitogen (PHA) and antigen (PPD) controls. Mean SEM (cpm  $10^2/0.2$  mL well)

|                | Culture day |     |      |      |     |      |     |    |
|----------------|-------------|-----|------|------|-----|------|-----|----|
|                | 0           |     | 1    |      | 3   |      | 5   |    |
| Culture medium | 3.1         | 0.6 | 3.5  | 0.5  | 11  | 2.8  | 60  | 21 |
| Cement         | 3.5         | 0.8 | 3.0  | 0.3  | 12  | 4.8  | 49  | 14 |
| PPD            | 2.7         | 0.3 | 4.7  | 1.5  | 38  | 14.8 | 147 | 42 |
| PHA            | 3.8         | 0.5 | 35.0 | 27.0 | 286 | 55.3 | 106 | 30 |

purified protein derivate of tuberculin (PPD, 10  $\mu\text{g}$  per well; Statens Serum Institute, Copenhagen, Denmark). The lymphocyte culture was kept in a 5 percent  $\text{CO}_2$ -air mixture at  $+37^\circ\text{C}$ . End-point analysis was performed 0, 1, 3, and 5 days later to analyze the activation kinetics (Konttinen et al. 1988).

### $^3\text{H}$ -thymidine incorporation

We added 1  $\mu\text{Ci}$  ( $6\text{-}^3\text{H}$ )-thymidine (specific activity 5 Ci/mmol, Amersham, U.K.) to each well 18–24 h before the cultures were harvested with a semiautomatic multi-sample harvester (MHCI, Skatron, Lierbyen, Norway) on glass-fiber filters in triplicate. The filters were suspended in a scintillation solution before beta-scintillation counting in a CK B Wallac 81000 counter (Wallac, Turku, Finland).

### ABC staining

The cultured cells were plated, and cytocentrifuged preparations were made. Avidin-biotin-peroxidase complex (ABC; Hsu et al. 1981) staining consisted of the following steps:

1. Fixation of the samples (5 min in  $+4^\circ\text{C}$  acetone).
2. Inhibition of endogenous peroxidase (5 min in 0.3 percent  $\text{H}_2\text{O}_2$ ).
3. Blocking of nonspecific binding sites (20 min in 1:5 diluted normal horse serum).
4. Incubation with mononuclear antibodies (30 min MoAB anti-CD2 = T lymphocyte marker, anti-C3bi = monocyte marker, anti-IL-2R = interleukin-2 receptor or activated T cell marker, anti-MHC locus II antigen or activated T cell marker).
5. Incubation with biotinylated horse antimouse antibodies (30 min).
6. Incubation with ABC complex (30 min).
7. Development of color reaction (3,3'-diaminobenzidine 50 mg/150 mL PBS in 0.003 percent  $\text{H}_2\text{O}_2$ ).
8. Counterstaining with hematoxylin.
9. Dehydration, clearing, and mounting; between each step, the cytospin specimens were washed twice for 5 min in PBS (0.1 mol/L, pH 7.4).

The methods have been reported in detail (Konttinen et al. 1988, Santavirta et al. 1990a).

### Results

Methyl methacrylate did not cause an increase in lymphocyte DNA synthesis as assessed by  $^3\text{H}$ -thymidine incorporation. The lymphocyte proliferation caused by PHA showed that the indicator cells had a full-action capacity. The proliferation recorded for cell-culture medium alone, cement, PPD, and PHA on culture Days 1, 3, and 5 indicated that the stimulation induced by methyl methacrylate was not different from that induced by culture medium alone, and was less than the stimulation induced by the antigen PPD or the mitogen PHA (Table 1).

The more sensitive analysis of lymphocyte activation showed that methyl methacrylate induced an expression of MHC locus II antigen on the surface of monocytes. This monocyte activation was seen as early as culture Day 1. The number of Ia-positive cells only slightly increased during methyl-methacrylate stimulation, while the intensity of Ia expression was clearly increased. Methyl methacrylate induced only a slight IL-2R expression on the surface of lymphocytes on culture Day 5 (Table 2).

Table 2. Lymphocyte activation in peripheral blood mononuclear cell cultures exposed to methyl methacrylate cement compared with negative (culture medium alone) and positive (PPD and PHA) controls. Mean SEM

| Day            | IL-2R |     |     |     |     |     |      |      | MHC locus II Ag |     |     |     |     |      |     |     |
|----------------|-------|-----|-----|-----|-----|-----|------|------|-----------------|-----|-----|-----|-----|------|-----|-----|
|                | 0     |     | 1   |     | 3   |     | 5    |      | 0               |     | 1   |     | 3   |      | 5   |     |
| Culture medium | 0.5   | 0.3 | 0.6 | 0.1 | 0.5 | 0.3 | 0.4  | 0.3  | 2.6             | 1.1 | 5.0 | 1.5 | 5.9 | 1.8  | 6.9 | 1.6 |
| Cement         |       |     | 0.6 | 0.3 | 0.9 | 0.5 | 7.3  | 2.2  |                 |     | 7.5 | 3.1 | 20  | 4.2  | 17  | 3.3 |
| PPD            |       |     | 0.5 | 0.1 | 1.2 | 0.7 | 13.8 | 8.1  |                 |     | 4.5 | 1.3 | 28  | 6.5  | 43  | 6.5 |
| PHA            |       |     | 0.6 | 0.2 | 6.4 | 2.7 | 27.8 | 11.1 |                 |     | 9.0 | 4.8 | 44  | 11.8 | 72  | 9.2 |

CD2 positive lymphocytes, as well as some C3b-positive monocytes, were seen attached to methylmethacrylate particles, in particular during culture Days 1 and 3. Subsequently, some activated lymphocytes were seen on culture Day 5 in cultures containing methyl methacrylate.

## Discussion

Methyl methacrylate is one of the most common materials implanted in man. It has been considered totally or at least relatively inert (Charnley 1975 and 1979, Mears 1979). Histologic studies in man (Charnley 1979, Linder and Hansson 1983) and in animals (Draenert 1981) have demonstrated that lasting close contact between cement and bone may be achieved without signs of tissue irritation. It is generally known that in some cases a pseudo-synovium-like membrane develops with time at the interface between bone and cement (Charnley 1975). Even in well-fixed total hip replacements, this membrane contains macrophages and osteoclasts. Macrophage and osteoclast activity have been suggested to be related to micromovement of the prosthesis/cement complex (Charnley 1979). Further, the so-called pseudosynovium is known to contain PG-E2, collagenase, and phosphatase (Maguire et al. 1987).

Previously, we have shown in common loosening of a cemented prosthesis stem that the cellular response is characterized by a mesenchymoid reaction that typically involves numerous activated fibroblasts containing carboxy terminal propeptide of interstitial Type I collagen, some C3bi-R and, nonspecific, esterase-positive macrophages, and the virtual absence of the lymphoid series (Santavirta et al. 1990c). In aggressive granulomatosis around well-fixed cemented femoral stems, most of the cells in the reactive zones were C3bi-R monocytes. In aggressive granulomatous reaction against the cement/prosthesis complex, there is an imbalance

between monocyte/macrophage response and the fibroblast response. The reason for this phenomenon is unknown. There appears to be an immunologic response against the cemented total joint prosthesis in vivo that is accentuated in infections (Santavirta et al. 1989). The most aggressive form of this reaction has been defined as aggressive granulomatosis (Harris et al. 1976, Tallroth et al. 1989) or cement disease (Jones and Hungerford 1987). Charnley (1979) postulated that this reaction is due to poor fixation and micromovement. Quite recently, aggressive granulomatosis has also been found in cementless total hip replacements; the adverse reaction is caused by polyethylene debris originating from the acetabular component (Santavirta et al. 1990b).

Previous research on immunologic response to methyl methacrylate cement has been based on indirect evidence. The human lymphocyte culture method used here is a reliable direct method to investigate the immunologic response to biomaterials (Santavirta et al. 1990a). In human lymphocyte cultures, the  $^3\text{H}$ -thymidine incorporation method appears to be relatively insensitive for tracing slight changes in lymphocyte activation caused by methyl methacrylate. With the more sensitive activation-marker method, methyl methacrylate was found to cause MHC locus II (Ia) antigen expression on the surface of monocytes as early as Day 1. This reaction resembles that induced by PPD or PHA. In methyl-methacrylate stimulation, the activated monocytes did not appear to induce subsequent lymphocyte activation; the number of Ia-positive cells was much lower on Days 3 and 5 of methyl-methacrylate stimulation. In addition, only a slight Tac expression on the lymphocytes was seen on Day 5 in methyl-methacrylate stimulation. The increase seen in background, as well as using cement on Day 5, is due to spontaneous proliferation. The stimulation by PHA on Day 3 corresponds with our laboratory standards, and is about 30-fold; after this, the stimulatory effect gradually subsides.

The most probable explanation of our findings is that the monocyte activation is nonspecific and is due to the presence of phagocytizable particles in the culture. This type of nonimmunologic monocyte activation can also be responsible for the cellular profile observed in histologic samples in prosthetic loosening. We have previously shown that histiocytes and activated macrophages are abundant, and that lymphocytes are absent in the reactive-tissue reaction around the cement/prosthesis complex (Santavirta et al. 1990c).

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