LYMPHOCYTE RESPONSE TO POLYMETHYL METHACRYLATE IN LOOSE TOTAL HIP PROSTHESSES

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We investigated the lymphocyte-mediated immune response to polymethylmethacrylate bone cement in 26 patients who had revision surgery for aseptic loosening of cemented total hip arthroplasties, at a mean time of seven years after the first replacement. We studied eight patients with cemented total hip arthroplasties which were not loose as controls.

Patch tests to polymethylmethacrylate bone cement were positive in 13 patients with loosening, and these patients had higher lymphoblast transformation values against polymethylmethacrylate bone cement patients with a negative skin reaction (p < 0.01) or those in the control group (p < 0.001).

Specific monoclonal antibodies were used to assess the percentage of certain cells of the immune system according to their cluster of differentiation (CD). There was a higher number of total T and B lymphocytes (CD2 and CD22) and interleukin-2 receptor-positive lymphocytes (activated cells, CD25) in patients with loose prostheses. More CD25 lymphocytes were found in patients with positive patch tests. The activation of the lymphocyte-mediated immune response was not related to the presence or absence of aggressive granulomatous lesions at the cement–bone interface.

Both mechanical and biological factors have been implicated in aseptic loosening of cemented total hip arthroplasty (Cavelier et al 1981; Radin et al 1982; Dubois 1984). The interface membrane between bone and cement, which resembles synovium and is rich in macrophages, may have a causative role in such loosening (Freeman, Bradley and Revell 1982; Goldring et al 1983). Macrophages have been shown to produce alkaline phosphatase and prostaglandins, both of which cause local osteolysis (Levack, Revell and Freeman 1987). It is uncertain whether the membrane represents an immunological response of bone to the toxic effect of cement.

We have studied the immunological response to polymethylmethacrylate bone cement (PMMA) in patients with aseptic loosening of hip replacements. We investigated different lymphocyte clusters in blood samples, using monoclonal antibodies.

PATIENTS AND METHODS

We performed the immunological studies on 26 patients who had undergone revision surgery of a previous cemented total hip prosthesis for painful aseptic loosening. The average time between the first hip arthroplasty and the revision was seven years (1 to 17). There were 15 women and 11 men with a mean age of 60 years (17 to 73) at the time of the first arthroplasty. The reason for the hip replacement was primary osteoarthritis in 21 patients, secondary osteoarthritis in three, traumatic hip dislocation in one and epiphyseolysis of the femoral head in one. Patients with rheumatoid arthritis were excluded because they frequently have abnormal immune responses. Charnley–Müller prostheses had been used in ten patients, Müller type in seven (both Protek AG, Berne, Switzerland), McKee–Farrar in seven (Zimmer, Bridgend, Wales) and Charnley low friction in two (Thackray, Leeds, England). At the time of revision, eight patients had bilateral hip replacements.

Patients were routinely assessed by radiography during follow-up. At the time of revision, the interface membrane between bone and cement was removed and examined by a pathologist. Although the patients had no clinical or laboratory evidence of infection, a sample of the removed tissue was subjected to bacterial screening. Cultures were sterile in all cases.

For comparison with the loosened group, eight
further patients with unilateral cemented total hip arthroplasty, followed over an average period of ten years (six to 12), who showed neither clinical nor radiological signs of aseptic loosening, were also assessed immunologically. There were five women and three men with a mean age of 58 years (36 to 72) at the time of hip arthroplasty. All had osteoarthritis, two secondary to femoral neck fracture and none had rheumatoid arthritis. In these eight cases, a Charnley–Müller prosthesis had been implanted.

Lymphocytes were isolated from 10 ml of venous blood. Cell cultures were performed in NUNC plates (Flow Laboratories, McLean, Virginia) up to a concentration of $1 \times 10^6$ cells/ml. Either phytohaemagglutinin or the other test substances were added to 100 µl of cellular suspension at a concentration of 1 to 10 µg/10^6 cells. For the transformation test to PMMA, the monomer was first diluted in ethanol and then adjusted for molarity with physiological saline solution. In this form, the monomer solution does not modify the viability of the cell culture.

The immunological studies included a patch test against PMMA, a lymphoblast transformation test (LTT) and analysis of the phenotype of B and T lymphocytes and the monocytic series. The patch test was performed on the patients' back, following the recommendations of the International Contact Dermatitis Research Group. As possible allergens, we applied 10% PMMA and the stabilisers used in the cement, namely, 1% hydroquinone and 5% dimethyl p-toluidine, all dispersed in petrolatum. Patch tests were read at 72 hours, when erythema, induration and/or papules were recorded as a positive test.

To investigate the systemic immunological response, LTT to PMMA and bone cement stabilisers were compared with the spontaneous LTT, and to that induced by phytohaemagglutinin, considered as the control highest response. Tests were performed according to a modification of the Orphanos and Bicker technique (Hudson and Hay 1980; Lobera, Sanz and Subirá 1982). In plates used for testing spontaneous transformation, 100 µl of RPMI-1640 culture medium (MA Bioproducts, Walkersville, Maryland) was added to the cell suspension. Plates were incubated for 24 hours at 37°C in a humid atmosphere with 5% CO₂ and 10 µl of tritiated thymidine (0.5 µCi/ml) added to the cultures before final assay. After 72 hours of incubation, cells were recovered, washed and the uptake of thymidine measured in counts per minute in a beta counter. We used specific monoclonal antibodies (Coulter Immunology Co, Hialeah, Florida) to identify lymphocyte clusters of differentiation (CD) as follows: CD2 (total T cells); CD4 (inducer/helper T cells); CD8 (suppressor/cytotoxic T cells); CD22 (pan-B mature lymphocytes); CD25 (interleukin-2 receptor cells or activated T-B cells); and CD35 (B cells, granulocytes and monocytes). Values were given in percentage of cells in relation to the total number of lymphocytes and monocytes obtained by Ficoll-paque gradient from 8 ml of blood.
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Fig. 2
Histology of an interface membrane showing an area of aggressive granulomatosis characterised by a histiocytic-monocytic reaction with giant multinucleated cells containing particles of PMMA (arrows).

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Control group (n = 8)</th>
<th>Positive patch test (n = 13)</th>
<th>Negative patch test (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous transformation</td>
<td>497 (75)</td>
<td>662 (141)</td>
<td>491 (100)</td>
</tr>
<tr>
<td>Phytohaemagglutinin</td>
<td>35 026 (9551)</td>
<td>48 746 (11281)</td>
<td>39 474 (7797)</td>
</tr>
<tr>
<td>PMMA</td>
<td>353 (108)†</td>
<td>4048 (1033)★★</td>
<td>1316 (352)*</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>416 (81)</td>
<td>477 (106)</td>
<td>1159 (797)</td>
</tr>
<tr>
<td>Toluidine</td>
<td>290 (102)</td>
<td>820 (382)</td>
<td>644 (263)</td>
</tr>
</tbody>
</table>

*difference significant at p < 0.01
†p < 0.001 (two-tailed t-test)

Table I. Results of the lymphoblast transformation test (LTT), as mean values (standard error) in counts per minute

For comparative statistical analysis, we used the two-tailed t-test and the Anova test with the Statview 512+ package (Brain Power Inc, Calabasas, California).

RESULTS

The patients with aseptic loosening did not differ from the control patients as regards sex or age distribution, or the time interval between the first arthroplasty and the immunological study. Radiographically, there were no obvious technical errors or mechanical failures of the implant which could have been responsible for the aseptic loosening. In 15 cases radiographs showed an aggressive granulomatous type of loosening, with large ovoid osteolytic areas around the components, especially at the calcar and the tip of the stem (Fig. 1). The other 11 had simple loosening, characterised by regular radiolucency around the femoral stem, with no major osteolytic areas.

Histological examination of the tissue removed from the interfaces around the cemented stems showed two different patterns, which corresponded closely to the two radiographic appearances. Thus, in the 15 patients with extensive lytic zones, the extracted tissue had a high number of histiocytic-monocytic reactive areas with giant multinucleated cells, occasionally containing particles of PMMA (Fig. 2). By contrast, the interface membrane from the 11 patients with simple or linear aseptic loosening contained dense connective tissue with low cellularity and an abundant extracellular collagenous matrix.

In the immunological studies, the cutaneous patch test against PMMA was positive in 13 of the 26 patients with aseptic loosening. Those against hydroquinone and toluidine were negative. The state of immune reactivity measured by the LTT in patients with aseptic loosening is compared with that in the control group in Table I. Except for hydroquinone, a higher transformation rate was found in patients with aseptic loosening and a positive PMMA patch test, including spontaneous lymphoblast transformation. The differences between groups were statistically significant only for the LTT induced by PMMA. Thus, patients with loosening and a positive patch test showed higher LTT values against PMMA than patients with loosening and a negative skin reaction (p < 0.01), and the control group (p < 0.001). Patients with aseptic loosening and a negative patch test
also showed an increased reactivity against PMMA and the stabilisers than the control group, but the difference was only significant for the monomer (p < 0.05). In patients with no prosthetic loosening, spontaneous lymphoblast transformation was not stimulated by PMMA.

As regards lymphocytic phenotype, there was an overall increase in the immunological response in patients with loose hip prostheses (Table II). Only CD2, CD22 and CD25 lymphocytes, however, showed a statistically significantly higher average than in the control group (p = 0.002, p = 0.04 and p = 0.02 respectively). Patients with prosthesis loosening and a positive or negative patch test against PMMA showed no differences, except for CD25 lymphocytes (p < 0.001). Higher amounts of interleukin-2 receptor-positive T lymphocytes, CD25, were found in patients with positive patch tests (Fig. 3). In patients with aseptic loosening, there was a close correlation between the detected amount of CD25 lymphocytes and the severity of the lymphoblast transformation induced by PMMA (F-test: 21.4, p < 0.0001) (Fig. 4).

Except for the CD4 T lymphocyte subset, which was higher (p = 0.03), patients with bilateral hip replacement did not differ from those with unilateral arthroplasty in terms of reactive lymphocytic phenotype and LTT values. The type of aseptic loosening, simple or aggressively granulomatous, was not related to any particular characteristic immune response (Table III).

**DISCUSSION**

Our results indicate that a lymphocyte-mediated immune response is activated in patients with aseptic loosening of cemented total hip prostheses. The most significant alterations were the high immune reactivity induced by the monomer of PMMA measured by the LTT, and the increase in total T lymphocytes (CD2 cluster), especially those displaying the interleukin-2 receptor (CD25) which is an early marker for lymphocyte activation.

Our histological findings on the interface membrane agree with previous reports (Harris et al 1976; Freeman et al 1982; Goldring et al 1983; Tallroth et al 1989; Santavirta et al 1990). In two-thirds of our cases, the infiltration of multinucleated giant cells and monocyte-macrophages loaded with cement particles induced a granulomatous reaction similar to that found in the presence of a foreign body (Chambers 1978). It has been suggested that foreign particles may cause a continuous stimulus to the macrophages, but this reaction itself does not activate the lymphocyte-mediated immune response (Santavirta et al 1991). According to our results, the immunopathological character of this reaction could be mediated by the effects of interleukin-2, a monocyte stimulating factor.

**Table II. Percentage of different lymphocytic phenotypes (mean ± SD)**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Normal values*</th>
<th>Control group (n = 8)</th>
<th>Positive patch test (n = 15)</th>
<th>Negative patch test (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD2</td>
<td>70 to 80</td>
<td>61.5 ± 11</td>
<td>71.1 ± 8†</td>
<td>71.6 ± 8†</td>
</tr>
<tr>
<td>CD4</td>
<td>25 to 50</td>
<td>34.5 ± 9</td>
<td>38 ± 10</td>
<td>36.4 ± 12</td>
</tr>
<tr>
<td>CD8</td>
<td>17 to 37</td>
<td>19.7 ± 3</td>
<td>20 ± 6</td>
<td>22.5 ± 7</td>
</tr>
<tr>
<td>CD22</td>
<td>2 to 11</td>
<td>9.5 ± 2</td>
<td>12 ± 3‡</td>
<td>12.3 ± 2‡</td>
</tr>
<tr>
<td>CD25</td>
<td>0 to 6</td>
<td>5.6 ± 2</td>
<td>11.1 ± 3‡</td>
<td>6.3 ± 3‡</td>
</tr>
<tr>
<td>CD35</td>
<td>12 to 20</td>
<td>19 ± 1</td>
<td>21.6 ± 8</td>
<td>21.3 ± 4</td>
</tr>
</tbody>
</table>

* according to suppliers of the monoclonal antibodies
†p < 0.005
‡p < 0.05

Percentage levels of interleukin-2 receptor-positive T lymphocytes, CD25, in the control group and those with aseptic loosening (AL), with (+) or without (-) a positive patch test to PMMA.

Santavirta et al (1990) analysed the local immunopathological response at the cement–bone interface in a small group of patients with aggressive granulomatosis, comparing it with that seen in association with non-granulomatous aseptic loosening. In aggressive granulomatosis, CD2-positive T lymphocytes were detected occasionally and did not stain with the monoclonal antibody for interleukin-2, indicating that the local T cells were not activated. No cells of the lymphoid series were identified in the interface membrane of patients with non-granulomatous loosening. Their findings contrast with our results; we analysed the lymphocyte-mediated immune response at systemic level and showed an increase in both total and activated T lymphocytes but this activation was not related to the presence or absence of aggressive granulomatous lesions at the interface.
In addition to a predominance of monocyte-macrophages, a relative lack of activated fibroblasts has been found in aggressive granulomatous lesions (Santavirta et al 1990). Activated fibroblasts are the principal cell component at the cement–bone interface in cases of non-granulomatous aseptic loosening, and it has therefore been suggested that aggressive granulomatosis after cemented arthroplasty and non-granulomatous aseptic loosening should be considered as two different conditions (Santavirta et al 1990). This hypothesis is not supported by our immunological study: we found no differences between granulomatous and non-granulomatous prosthetic loosening regarding patch test reactivity, lymphoblast transformation induced by PMMA, or lymphocyte subtypes. We believe that both histopathological patterns may express a single systemic immunological response, but one of variable severity. Local or subjective factors, however, responsible for the different histological appearance of the cement–bone interface remain to be elucidated.

Although we did not perform immunological studies at the cement–bone interface membrane, the increase in total T lymphocytes, especially those displaying the interleukin-2 receptor, suggests the occurrence of a type IV immunological hypersensitivity reaction at that level (cell-mediated response or contact sensitivity). The high rate of lymphoblast transformation produced by PMMA indicates that only this substance, and not the bone cement stabilisers, acts as the allergen.

Our findings with the patch test were contradictory since, with a T-lymphocyte-mediated hypersensitivity to PMMA, all the patch tests would be expected to be positive, yet half our patients with aseptic loosening had negative tests to PMMA. These patients, moreover, showed no increase in activated CD25 lymphocytes and, in comparison with those displaying a positive skin reaction, were low responders to PMMA in the LTT. Similar findings have recently been reported in a group of patients who underwent revision for failed uncemented total hip replacements with titanium-alloy components (Lalor et al 1991). In this series, monoclonal antibody labelling of the interface membrane showed abundant macrophages and T lymphocytes, suggesting sensitisation to titanium (type IV contact hypersensitivity reaction). All patients patch tested with dilute titanium salt solutions had surprisingly negative results, which could be explained, like our skin reaction findings, by the lack of suitably standardised procedures for testing both titanium and PMMA sensitivity. Both methods, the LTT and patch testing, assess the same type IV immunological hypersensitivity reaction, but the LTT is more sensitive. This could explain a negative skin reaction to PMMA in the presence of a not very high lymphoblast transformation rate.

The continuing use of PMMA cement would seem to justify further study of the place of pre-operative tests to determine those patients at risk of an unfavourable immunological response to PMMA, and of its possible treatment with immunoregulating drugs.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

REFERENCES


