Macrophage Activation in Type 1 Diabetic Patients With Catheter Obstruction During Peritoneal Insulin Delivery With an Implantable Pump

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OBJECTIVE — The purpose of this study was to evaluate the activation of macrophages in type 1 diabetic patients during peritoneal insulin delivery with an implantable pump against two types of insulin: that which was collected from the pump reservoir and that which came straight from the bottle (i.e., vial insulin). Macrophage activation was studied in patients with and without catheter obstruction and compared with activation in healthy subjects.

RESEARCH DESIGN AND METHODS — Human insulin (21PH, 400 U/ml; Hoescht) was collected from the pump reservoir (Minimed) of diabetic patients with (n = 3) or without (n = 7) catheter obstruction, as assessed by histological examination of the catheter tip. Monocytes were obtained from venous blood samples from both kinds of diabetic patients and from healthy subjects (n = 5) and were differentiated into monocyte-derived macrophages in culture. Their chemotaxis and tumor necrosis factor-α (TNF-α) release were studied with respect to both types of insulin, as previously stated. Formyl-methionyl-leucyl-phenylalanine (fMLP) and lipopolysaccharide (LPS) were used as controls.

RESULTS — Neither insulin recovered from the pump reservoir nor vial insulin proved chemotactic to macrophages from either healthy subjects or those diabetic patients with and without catheter obstruction. The migration toward IMLP of macrophages from patients presenting a catheter obstruction was significantly higher than that observed with macrophages from either diabetic patients without obstruction or healthy subjects; the chemotactic index (mean ± SD) was 3.81 ± 0.36 vs. 2.30 ± 0.89 and 2.60 ± 0.80, respectively (P < 0.05). LPS significantly stimulated the TNF-α secretion of macrophages from diabetic subjects with a catheter obstruction, whereas both native and reservoir-recovered insulin had no effect on this release (144.83 ± 67.25 vs. 5.15 ± 2.93 and 5.27 ± 2.43 pg/ml, P < 0.001).

CONCLUSIONS — The human insulin used in implantable pumps, regardless of how long it had remained in the pump reservoir, did not induce macrophage activation in diabetic patients treated through intraperitoneal insulin delivery. In some of these diabetic patients, catheter obstruction could be explained by their high capacity of macrophage chemotaxis.


For the last decade, intraperitoneal insulin infusion (IPII) with implantable systems has been intensively investigated to improve the metabolic control in diabetic patients (1–6). Significant improvements in blood glucose stabilization have been reported, suggesting that this technique could provide long-term benefits to the quality of life of patients under intensified subcutaneous insulin treatment who are prone to severe hypoglycemia (7). However, the peritoneal catheter remains the weakest link in the infusion system. The most frequent complications are catheter obstructions caused by the deposition of insulin aggregates inside the lumen and by fibrin clotting at the peritoneal tip. The frequency of intraperitoneal catheter obstruction ranges between centers from 15 to 31 cases per 100 patients per year (4,8). This problem is associated with increased catheter resistance, decreasing the insulin flow and consequently leading to a deterioration in metabolic control. The mechanisms responsible for these events remain elusive, but the presence of amorphous deposits reacting with anti-insulin antibodies surrounded by macrophages have been described (4,9,10). The presence of peritoneal macrophages together with insulin deposits at the catheter tip plead for the predominant role of macrophages at the onset of the peritoneal response to the catheter.

Macrophages are cells involved both in nonspecific inflammation and in specific immunological reactions. Investigations have demonstrated that macrophages play a pivotal role not only in the destruction of β-cells at the onset of the disease, but also in the genesis of micro- and macrovascular complications (11–15). A previous study reported on amyloid deposits caused by the formation of insulin fibrils in subcutaneous tissues in diabetic patients treated through repeated insulin injections. Such deposits may preserve the mitogenic properties of insulin and activate fibrosis around the catheter through the stimulation of omental cells (16). We hypothesized that the exposure of human insulin to the reservoir sur-
faced the high frequency of catheter blockages in diabetic patients and previous peritoneal insulin infusion either by external or implantable pumps also suggests a persistent chronic immune system activation (9).

The aim of this work was to evaluate the state of activation of macrophages from type 1 diabetic patients undergoing IPII with an implantable pump toward both insulin collected from the pump reservoir and vial insulin. Macrophage activation was studied in patients with or without catheter obstruction and compared with that of healthy subjects.

RESEARCH DESIGN AND METHODS — We included 10 type 1 diabetic patients (C-peptide–negative), after informed consent, and treated them for at least 6 months through IPII with an implantable pump (Minimed Implantable Pump 2001, Minimed Technologies, Sylmar, CA). Pump catheters were made out of silicone-coated polysulfone. The infused insulin was 21 PH neutral semisynthetic human insulin (Hoechst, Frankfurt, Germany) at a concentration of 400 U/ml, stabilized by a glycol-polyethylene-polypropylene surface-active agent (Genapol). Refilling was performed under aseptic conditions every 6 weeks. The pumps were emptied, and the residual insulin was collected for chemotaxis and cytotoxicity analysis.

Group 1 was composed of three diabetic patients (two men and one woman) aged 37–53 years (mean 45.6) who had been suffering from diabetes for 20.6 years on average (range 14–28). During the year after pump implantation, they presented with at least one episode of irreversible catheter obstruction, which led to the removal of the catheter. Catheter obstruction was clinically suspected when the patients presented with hyperglycemia and/or required increasing doses of insulin for several days. This was confirmed by celioscopy, which was used to identify material surrounding the catheter tip. Histology and immunocytochemistry revealed the presence of a fibrous capsule made of several collagen layers with fibroblasts, macrophages, and a few lymphocytes. Amorphous deposits reactive to anti-insulin antibodies were surrounded with giant epithelioid cells and macrophages (4).

Group 2 included seven diabetic patients who did not have catheter obstruction, but who were comparable with group 1 patients in terms of age, diabetic history, body weight, and insulin requirement (Table 1).

Monocytes isolation and culture

Blood samples (150 ml) from diabetic patients were obtained from venous puncture during routine clinical examination. Peripheral mononuclear cells were separated from heparinized blood using Ficoll gradient centrifugation (d = 1.077) and purified with immunomagnetic beads coated with anti-CD14 monocyte antibody.

Mononuclear cells were collected by leukopheresis from five anonymous healthy donors (group 3) and purified through countercurrent centrifugal elutriation (J6M-B, Beckman Coulter, Fullerton, CA). Monocyte purity was checked by morphological examination after Wright-Giemsa staining and by flow cytometry analysis with fluorescein isothiocyanate (FITC)-labeled antibodies directed against CD14. We found 85–90% of the cells were CD14+ by using the forward versus sideward light scattering pattern in combination with anti–CD14-FITC antibody. Monocyte viability was checked using the trypan exclusion method.

Purified monocytes were suspended in an RPMI-1640 medium containing glucose (5.5 mmol/l), L-glutamine (2 mmol/l), and 25 mmol/l HEPES. It was supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin (Life Technologies, Paisley, U.K.), and 10% sterile-filtered heat-inactivated (30 min at 56°C) pooled human AB serum (Etablissement de Transfusion Sanguine, Strasbourg, France). To induce macrophage differentiation, 50 U/ml recombinant human granulocyte-macrophage colony-stimulating factor (Life Technologies) was added to the culture medium. The cells were cultured for 7 days at a concentration of 2 × 10^6 cells/ml in gas-permeable hydrophobic Teflon bags (Polylabo, Strasbourg, France) at 37°C under a 5% CO2 humidified atmosphere. After a 7-day culture, monocytes were differentiated into monocyte-derived macrophages (MDMs) and counted after trypan blue dye staining. MDMs were plated at a concentration of 10^5 MDMs per well in a 12-well cell culture cluster for 90 min at 37°C (Costar, Cambridge, MA) before chemotaxis and cytotoxicity analyses.

All cell preparations and culture media were endotoxin-free. Endotoxin in the media, additives, and all disposable materials were quantitated using a limulus amebocyte lysate assay (LAL) from Sigma (St Louis, MO). The sensitivity of LAL was 0.015 endotoxin unit/ml using Escherichia coli standard endotoxin. Disposable materials were endotoxin-free, and all glassware was dry-heated at 220°C for 6 h.

Macrophage chemotaxis

We evaluated macrophage chemotaxis toward human insulin using modified Boyden chambers composed of two compartments, one placed on top of the other (17). The upper compartment consisted of a culture insert (Transwell Insert; Costar, Cambridge, MA) equipped with an 8-µm polycarbonate filter. The lower one was filled with 900 µl insulin solution (0.5 U/ml) containing either insulin that was collected from the pump reservoir of the diabetic patients in both groups or insulin that came directly from the bottle (vial insulin). The upper chamber was filled with a cell suspension containing 3 × 10^6 MDMs from diabetic or healthy subjects. After incubation for 90 min under a humidified atmosphere at 37°C, the upper compartment was

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Developed obstruction</th>
<th>No obstruction</th>
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<tbody>
<tr>
<td>n</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>2/1</td>
<td>5/2</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.6 ± 6.2</td>
<td>43.7 ± 3.4</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>24.6 ± 2.8</td>
<td>23.7 ± 3.7</td>
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<tr>
<td>Diabetes duration (years)</td>
<td>20.6 ± 7.2</td>
<td>19.4 ± 3.6</td>
</tr>
<tr>
<td>Insulin requirements (U/day)</td>
<td>71.6 ± 10.5</td>
<td>59.3 ± 6.2</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>7.4 ± 0.6</td>
<td>8.1 ± 0.6</td>
</tr>
<tr>
<td>Anti-insulin antibodies (%)</td>
<td>23.13 ± 26.63</td>
<td>35.2 ± 31.42</td>
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</table>

Data are n or means ± SD.
Macrophage activation during IPII

Figure 1—Migration of macrophages toward pump reservoir insulin (A), vial insulin (B), and fMLP (C) from diabetic patients who developed (A) or did not develop (B) catheter obstruction during IPII with an implantable pump and from healthy subjects (C). **P < 0.01; ***P < 0.001.

The migration response was defined as the mean number of MDMs per field (n = 3). The chemotactic effect of both kinds of insulin was determined by the migration index expressed as the following ratio: the number of MDMs attracted to either native insulin or insulin collected from the pump reservoir divided by the number of MDMs attracted to the control medium.

In each experiment, the chemotactic peptide formyl-methionyl-leucyl-phenylalanine (fMLP) from Sigma was used as a reference. It was shown that 10^{-8} mol/l was the concentration at which the chemotactic response of MDM was optimal. The migration of MDMs toward the culture medium was considered as a random migration and was used as negative control.

Tumor necrosis factor-α release
The MDMs of diabetic and healthy subjects were cultured for 16 h in the culture medium described above containing vial insulin or insulin collected from the reservoir at a concentration of 0.5 U/ml. Under the same conditions, the stimulation of MDMs with 50 ng/ml E. coli lipopolysaccharide (LPS) from Sigma served as the control. After centrifugation at 10,000g for 5 min at 4°C, the supernatants were harvested for tumor necrosis factor-α (TNF-α) determination using the enzyme-linked immunosorbent assay method (Immuno-tech kit, Beckman Coulter) (n = 3) with a detection limit of 5–10 pg/ml. The working range was 10–1,000 pg/ml with inter- and intra-assay variations of 5.4 and 1.6%, respectively.

Statistical analysis
All data are expressed as means ± SD. A statistical analysis of macrophage migration indexes and cytokine release in diabetic patients of both groups and healthy subjects was performed using one-way analysis of variance. Significance is shown in Figs. 1 and 2.

RESULTS
Influence of human insulin on macrophage activation
Neither insulin recovered from the pump reservoir nor vial insulin stimulated the migration of macrophages from diabetic patients without catheter obstruction. In response to both kinds of insulin, the chemotactic indexes for these macrophages were 0.86 ± 0.15 and 0.81 ± 0.07, respectively. These values were similar to those obtained for macrophages from diabetic patients with catheter obstruction and healthy subjects (Fig. 1).

The migration toward fMLP of macrophages from diabetic patients exhibiting catheter obstruction during IPII was significantly higher compared with that obtained in the presence of either native or reservoir-recovered insulin (3.81 ± 0.36 vs. 1.07 ± 0.03 and 1.09 ± 0.05, P < 0.001). Similar data were found with macrophages from diabetic patients having no catheter obstruction and from healthy subjects (Fig. 1).

LPS significantly stimulated the secretion of TNF-α by macrophages from diabetic patients with catheter obstruction, whereas neither vial nor reservoir-recovered insulin had any effect on the release of this substance (144.83 ± 67.25 vs. 5.15 ± 2.93 and 5.27 ± 2.43 pg/ml [P < 0.001]). Similar results were found with macrophages from diabetic patients without catheter obstruction and healthy subjects (Table 2). When macrophages from diabetic patients or healthy subjects were incubated in the presence of either kind of insulin, the release of TNF-α was similar to that obtained with culture medium alone.

Only the release of TNF-α by macrophages from healthy subjects and the chemotaxis of macrophages toward insulin collected from the pump reservoir of diabetic patients with catheter obstruction have been represented (Fig. 1C and Table 2). Similar data have been obtained with insulin collected from the pump reservoir of diabetic patients without obstruction.

Influence of IPII events on macrophage activation
When monocytes from diabetic patients exhibiting catheter obstruction were differentiated into macrophages, their chemo-
Chemotactic index toward both kinds of insulin was significantly higher than that of macrophages from diabetic patients without catheter obstruction (1.09 ± 0.03 vs. 0.86 ± 0.15 \( P < 0.05 \)) for reservoir-recovered insulin and 1.07 ± 0.03 vs. 0.81 ± 0.07 for vial insulin). In response to native insulin, the chemotactic indexes of macrophages from diabetic patients without obstruction and healthy subjects were similar (Fig. 2A and B).

In the same way, the migration toward fMLP of macrophages from patients with catheter obstruction was significantly higher than that observed in macrophages from diabetic patients without obstruction and healthy subjects (3.81 ± 0.36 vs. 2.30 ± 0.89 and 2.60 ± 0.80, \( P < 0.05 \)) (Fig. 2C).

In response to LPS, the secretion of TNF-\(\alpha\) by macrophages from diabetic subjects with or without catheter obstruction reached 144.83 ± 67.25 and 130.77 ± 54.32 pg/ml, respectively. These values were not significantly different from those observed in macrophages from healthy subjects (114.81 ± 32.60 pg/ml). However, neither insulin collected from the pump reservoir nor vial insulin stimulated the release of TNF-\(\alpha\) by macrophages from the diabetic patients of both groups and the healthy subjects.

Highly purified semisynthetic Genapol-stabilized insulin proved to be nonchemotactic toward the macrophages of both diabetic and healthy subjects. Using a modified chemotactic chamber, Josefsen et al. (18) also demonstrated that human insulin exhibited no chemotactic effect on human monocytes. Our results also show that Genapol, which is used as a surfactant to avoid insulin denaturation and aggregation, was devoid of any effect on macrophage migration.

A previous in vitro study demonstrated that a temperature of 37°C and constant shaking led to the formation of a few insulin secondary products (e.g., desamido insulin and derived high–molecular weight molecules) (19). After 4 weeks, 95% of the biological potency remained. However, the antigenicity of these products was not documented. Several studies have reported on the formation of amyloid deposits reacting with anti-insulin antibodies at the catheter tip (4, 9, 10). Moreover, these aggregates were colonized by fibroblasts and macrophages. The authors speculated that the amyloid deposits could be composed of insulin products that had undergone major modifica-

**Table 2** — Release of TNF-\(\alpha\) (pg/ml) by macrophages from diabetic patients who developed or did not develop catheter obstruction during IPII with an implantable pump and from healthy subjects in the presence of pump reservoir or vial insulin and LPS

<table>
<thead>
<tr>
<th></th>
<th>Pump insulin</th>
<th>Native insulin</th>
<th>LPS</th>
<th>( P )</th>
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<tr>
<td>Group 1 (developed obstruction)</td>
<td>5.15 ± 2.93</td>
<td>5.27 ± 2.43</td>
<td>144.83 ± 67.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group 2 (no obstruction)</td>
<td>6.31 ± 2.16</td>
<td>5.45 ± 2.18</td>
<td>130.77 ± 54.32</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group 3 (healthy subjects)</td>
<td>6.35 ± 2.98</td>
<td>6.21 ± 2.13</td>
<td>114.81 ± 32.60</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are means ± SD.
Macrophage activation during IPII

As shown by the HbA1c levels, there was no difference in the glycemic control in both groups, with and without catheter obstruction. In our study, the stimulation of macrophage activation concerned only macrophage chemotaxis and not cytokine release. Indeed, regardless of the stimulation (i.e., LPs or reservoir-collected or vial insulin), the level of TNF-α released by macrophages was comparable in diabetic patients (with and without catheter obstruction) and healthy subjects.

In conclusion, human insulin used for intraperitoneal insulin delivery with implantable pumps is not involved in catheter obstruction through the mechanism of macrophage activation. However, the high level of macrophage chemotaxis observed in diabetic patients having developed catheter obstruction suggests an individual sensitivity to peritoneal insulin delivery and might explain the recurrence of this complication that is observed in some of these patients.

References