Human Pancreatic Islets Produce and Secrete MCP-1/CCL2: Relevance in Human Islet Transplantation

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We investigated the capacity of human islets to produce monocyte chemoattractant protein-1 (MCP-1). Primary cultures of pancreatic islets expressed and secreted MCP-1, as determined by Northern blot, immunohistochemistry, in situ hybridization, and enzyme-linked immunosorbent assay. The produced MCP-1 was biologically active as it attracted monocytes in chemotaxis assay, and chemotactic activity was almost abrogated by a neutralizing anti–MCP-1 monoclonal antibody. Expression of MCP-1 was increased by primary inflammatory cytokines (interleukin-1β, tumor necrosis factor-α) and lipopolysaccharide at both the mRNA and protein levels but not by glucose. However, MCP-1 did not modulate insulin secretion. MCP-1 secreted by pancreatic islets plays a relevant role in the clinical outcome of islet transplant in patients with type 1 diabetes. In fact, low MCP-1 secretion resulted as the most relevant factor for long-lasting insulin independence. This finding opens new approaches in the management of human islet transplantation. Finally, the finding that MCP-1 appears constitutively present in normal human islet β-cells (immunohistochemistry and in situ hybridization), in the absence of an inflammatory infiltrate, suggests that this chemokine could have functions other than monocyte recruitment and opens a new link between the endocrine and immune systems. *Diabetes* 51: 55–65, 2002

Chemokines form a superfamily of small (8–10 kDa), inducible, secreted chemotactic cytokines that play a crucial role in inflammation, infection, and immunity (1–4). Chemokines are divided into four subfamilies (C, CC, CXC, and CX3C) on the basis of their genetically conserved cysteine motif, important for the structure and function of the proteins. Monocyte chemoattractant protein-1 (MCP-1) is a member of the CC chemokine family and is produced by endothelial cells (5,6), vascular smooth muscle cells (7), keratinocytes (8), fibroblasts (9), mesangial cells (10,11), tubular epithelial cells (12), lymphocytes, and monocyte/macrophages (13) in response to proinflammatory stimuli, including tumor necrosis factor-α (TNF-α), γ-interferon (IFN-γ), lipopolysaccharide (LPS), interleukin-1β (IL-1β), platelet-derived growth factor (PDGF), and oxidized LDL. MCP-1 has been shown to have a variety of functions. In vitro, it is able to induce chemotaxis of monocytes at subnanomolar concentration (14,15) and to recruit a subset of T-cells (16,17) and IL-2–activated natural killer cells (18,19). In monocytes, MCP-1 induces not only chemotaxis but also respiratory burst, rapid induction of arachidonic acid release, and changes in Ca^{2+} concentration (20,21). Because of its target cell specificity, MCP-1 was postulated to play a pathogenic role in a variety of diseases characterized by mononuclear cell infiltration, including atherosclerosis, neoplasm, immunoinflammatory diseases, and human immunodeficiency infection (1–4).

Human pancreatic islet is a main target for autoimmune attack as in type 1 diabetes. There is ample experimental evidence to support a role of macrophage in type 1 diabetes (22). Isolated macrophages are highly cytotoxic to pancreatic islet cells in vitro (23,24), and transfer of peritoneal macrophages from nonobese diabetic (NOD) mice can accelerate the onset of diabetes in prediabetic NOD recipients (25). Administration of the macrophage-toxic substance silica in animal models of spontaneous type 1 diabetes prevents diabetes development (26) and prevents adoptive transfer of diabetes (27). Moreover, islet histopathology in experimental models of diabetes (low-dose streptozotocin–treated mice, BB rats, NOD mice) shows that macrophages are the first cells that infiltrate the islets at the onset of the disease. (28–31). Heavy macrophage infiltration was found also in two patients with type 1 diabetes with acute onset, indicating that macrophages may play a relevant role even in humans (32,33). Other evidence of the strict relation between human pancreatic islets and macrophages emerges from the clinical experience of human pancreatic islet transplantation. Human islet transplantation is a minimally invasive approach to restore glucose homeostasis in patients with type 1 diabetes. Despite its great potential, the success rate of this practice in patients with type 1 diabetes, already immunosuppressed for a kidney graft, is

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DAB, diaminobenzidine; ELISA, enzyme-linked immunosorbent assay; GAD, glutamic acid decarboxylase; IFN-γ, γ-interferon; IL, interleukin; ISH, in situ hybridization; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; MIF, migration inhibitory factor; PDGF, platelet-derived growth factor; RPA, RNAse protection assay; TNF-α, tumor necrosis factor-α

still low, with a large percentage of graft primary nonfunction and limited insulin independence of recipients (34). Islet allotransplantation engraftment has been considered one of the principal obstacles for subsequent graft function. Only part of the transplanted β-cell mass survives after the infusion (35), so islets from two or more pancreata are required to normalize glycemia in one recipient (36). In the early days after transplantation, the islets suffer hypoxia and inflammatory response to the graft, which lead to islet dysfunction, apoptosis, and a reduction in β-cell mass, followed by tissue remodeling. Macrophages play an important role in early islet graft loss (37–41), and their depletion by gadolinium or clodronate improved graft survival in a rodent model of islet transplantation (42).

We hypothesized a role of MCP-1 in macrophage recruitment by human pancreatic islets. In this study, we investigated whether human islet cells are able to attract monocyte/macrophages and whether MCP-1 plays a role in this interaction. Using primary cultures, we showed that human islets are able to produce and secrete biologically active MCP-1. Data on clinical islet transplantations in patients with type 1 diabetes suggest a relevant role of MCP-1 secreted by islets in the possibility to obtain insulin independence. This finding opens a new perspective in other endocrine pancreatic disease.

RESEARCH DESIGN AND METHODS

Pancreas procurement and islet preparations. Pancreata were obtained from heart-beating cadaveric multiorgan donors through the North Italian Transplant Organization. Islets were isolated according to a modification of the automated method (43,44). Samples of the pancreas and samples of isolated islets were taken for histological examination and for in vitro study. The purification was assayed by computerized morphometric method (Leica Imaging System LDD, Cambridge, U.K.).

The purified islets were cultured in a sterile flask containing 25 ml of M199 medium (Seromed Biochrom, Berlin, Germany) supplemented with 10% fetal calf serum, 1% L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin (complete medium). The islets were incubated at 30°C in 5% CO_2 and 95% humidified air. Islet viability was assessed by trypan blue and by the evaluation of the basal cytosolic calcium concentrations ($[Ca^{2+}]_i$) in fura-2–loaded islets by digital microscopy. Fluorescence images were collected at a middle plane of the islets by an intensified charged-coupled device camera and fed into a digital image processor, where video frames were digitized and integrated (typically 10 consecutive frames) in real time (45). Measurements were done in at least 30 individual islets from each preparation. Images were then processed to convert fluorescence data in $[Ca^{2+}]_i$ images (340/380 nm excitation wavelength ratio method). In five islet preparations, the assessment of islet viability, with double staining for propidium iodide and acridine orange, also was applied. Criteria for islet viability were the following: trypan blue exclusion <10% of cells; $[Ca^{2+}]_i$ as 340/380 <0.7 that means $[Ca^{2+}]_i$ >350 nmol/l; propidium iodide <10%, acridine orange >90%. Islets were initially tested for sterility and endotoxin (<0.05 EU/ml in all tested preparations; Chromogenic LAL test; Bio Whittaker, Walkersville, MD) and mycoplasm (Mycoplasma detection kit; Boehringer Mannheim, Indianapolis, IN) content.

Cytokine and chemokine measurements. Aliquots of pancreatic islet preparations (500 islets/ml) were cultured in complete medium in a 24-well plate. Culture supernatants were harvested at different time points and stored at −80°C. To investigate regulation of MCP-1 secretion by inflammatory cytokines, we added IL-1β (Pepro Tech EC, London, U.K.; specific activity 1×10^7 units/mg), TNF-α (specific activity $>2 \times 10^7$ units/mg; Pepro Tech EC), INF-γ, and LPS (Sigma, St. Louis, MO) at different concentrations and times. Human MCP-1, TNF-α, IL-1β, and IL-8 were detected using a sandwich enzyme-linked immunosorbent assay (ELISA) as described (46,47). ELISA for MCP-1 is specific for human MCP-1 and does not detect the mouse equivalent or the closely related human chemokines MCP-2 and MCP-3 (47).

Immunohistochemistry and in situ hybridization. Immunohistochemistry for MCP-1 was performed on frozen and paraffin-embedded 5-μm-thick sections obtained from surgical pancreatic specimens and from isolated pancreatic islets. Serial sections were immunostained for MCP-1 (clones 5D3, 2A1, 9G6, 3A3 1 μg/ml; the clones recognize different epitopes as shown by competitive binding with peptide aa 43–65 and aa 63–85 of MCP-1) and insulin (clone E2E3, from Signet, prediluted) to localize Langerhans islets better, and CD45 (clone T29/33 from Dako, dilution 1:200) to exclude potential synthesis of MCP-1 by lymphocytes by using an avidin-biotin-complex peroxidase method (Vector), using diaminobenzidine (DAB) as chromogen. In situ hybridization (ISH) detection of MCP-1-mRNA was performed on sections obtained from surgical pancreatic specimens previously embedded in OCT compound and snap-frozen in liquid nitrogen. Briefly, 5-μm cryostat sections were fixed for 20 min in 4% paraformaldehyde, washed in buffer, dehydrated in alcohol, and then hybridized with a cocktail of biotin-labeled MCP-1 probe (R&D Systems, Minneapolis, MN) at a concentration of 2 ng/μl in hybridization solution at 37°C for 3 h. After stringent washes, the sections were incubated in an avidin-biotin-peroxidase complex (Vector) for 30 min at room temperature. The labeling was revealed by DAB. Negative controls were performed by omission of the probe or by using an indifferent biotinylated probe.

Northern blot analysis and RNAse protection assay. Total cellular RNA was prepared from hand-picked human pancreatic cultured islets. Total RNA was extracted by the guanidinium thiocyanate method, blotted, and hybridized as previously described (48). MCP-1 and CCR2B cDNAs were obtained by PCR amplification of the reported sequences (49). RNAse protection assay (RPA) was performed using the RiboQuant Multi-Probe template sets (hCK-5), following the manufacturer's instructions (PharMingen, San Diego, CA).

Chemotaxis assay. Cell migration was evaluated using a chemotaxis microchamber technique as previously described (50). Supernatants from different pancreatic islet preparations were used as such or mixed with 1 μg of anti–MCP-1 antibody (clone 24822.111; R&D Systems, Minneapolis, MN). Recombinant human MCP-1 (PeproTech, Rocky Hill, NJ) was used as reference chemoattractant. Results are expressed as the mean number of migrated cells counted in five microscope high-power fields (magnification ×1,000).

In vitro regulation of MCP-1 secretion. After 2 days of culture, the insulin released in response to glucose and to human MCP-1 (300 ng/ml) was assessed by static incubation with a Krebs-Ringer solution buffered with HEPES plus 0.1% bovine serum albumin. After 30 min of equilibration with 3.3 mmol/l glucose, human islets were stimulated for 30 min with 16.7 mmol/l glucose. MCP-1 (300 ng/ml) was added to both 3.3 mmol/l and 16.7 mmol/l glucose to assess the effect in both basal and stimulated conditions. All samples were frozen at −20°C, and insulin was assessed by RIA using a commercial kit (Incstar, Stillwater, MN). Data are expressed as the mean ± SD $[Ca^{2+}]_i$ changes after 300 ng/ml–1 μg/ml MCP-1 was assessed in islets loaded with fura-2 and examined by digital microscopy as described previously (45).

Islet transplantation into patients with diabetes. Preparations were considered adequate for transplantation according to the following criteria: *1*) number of equivalent islets >5,000/kg body wt, *2*) purity >20% (computerized morphometric determination of islet/total purified preparation; Leica Imaging System LDD), and *3*) islet viability. Transplantation was performed between 24 and 72 h after isolation. The islet preparation was suspended in 100 ml of Hanks' solution (Clinical Grade, SALF, Bergamo, Italy) containing 1,000 units of heparin and 2% human albumin. Percutaneous trans-hepatic injection (under local anesthesia) was performed according to the protocol approved by the local ethics committee. All patients were already under immunosuppression therapy with steroids and cyclosporine for a previous kidney transplant (44). After islet transplantation, the therapy was changed as follows: antilymphocyte globulin (125 mg/day for 10 days; IMTIX, Marseille, France), cyclosporine (7.5 mg · kg^{-1} · day^{-1}), mycophenolate mofetil (2 g/day), and methylprednisone (500 mg immediately before surgery, 0.25 mg · kg^{-1} · day^{-1} for 2 months after surgery, then lowered to 5 mg/day). All recipients were negative for C-peptide (Dako, Cambridgeshire, U.K.), with the exception of patients 30 (C-peptide = 0.39 nmol/l, insulin requirement 34 units/day) and 32 (0.26 nmol/l, insulin requirement 27 units/day), in whom C-peptide values were not responsive to arginine test (data not shown). Before transplantation, patients 30, 32, and 35 had autoantibodies to glutamic acid decarboxylase (GAD) and tyrosine phosphatase-like protein (IA-2), measured by radiobinding assay (51). Patients 29, 34, 42 had autoantibody only to GAD; patient 41 had autoantibody only to IA-2. During the first 10 days posttransplantation, blood glucose was maintained between 4.4 and 7.0 mmol/l by continuous insulin infusion.

RESULTS

MCP-1 production by cultures of human pancreatic islets. We investigated the capacity of human pancreatic islets to secrete MCP-1. Supernatants from 20 different primary cultures of human pancreatic islets were studied (Fig. 1*A*). Every islet preparation (210,000 ± 49,000 equiv-

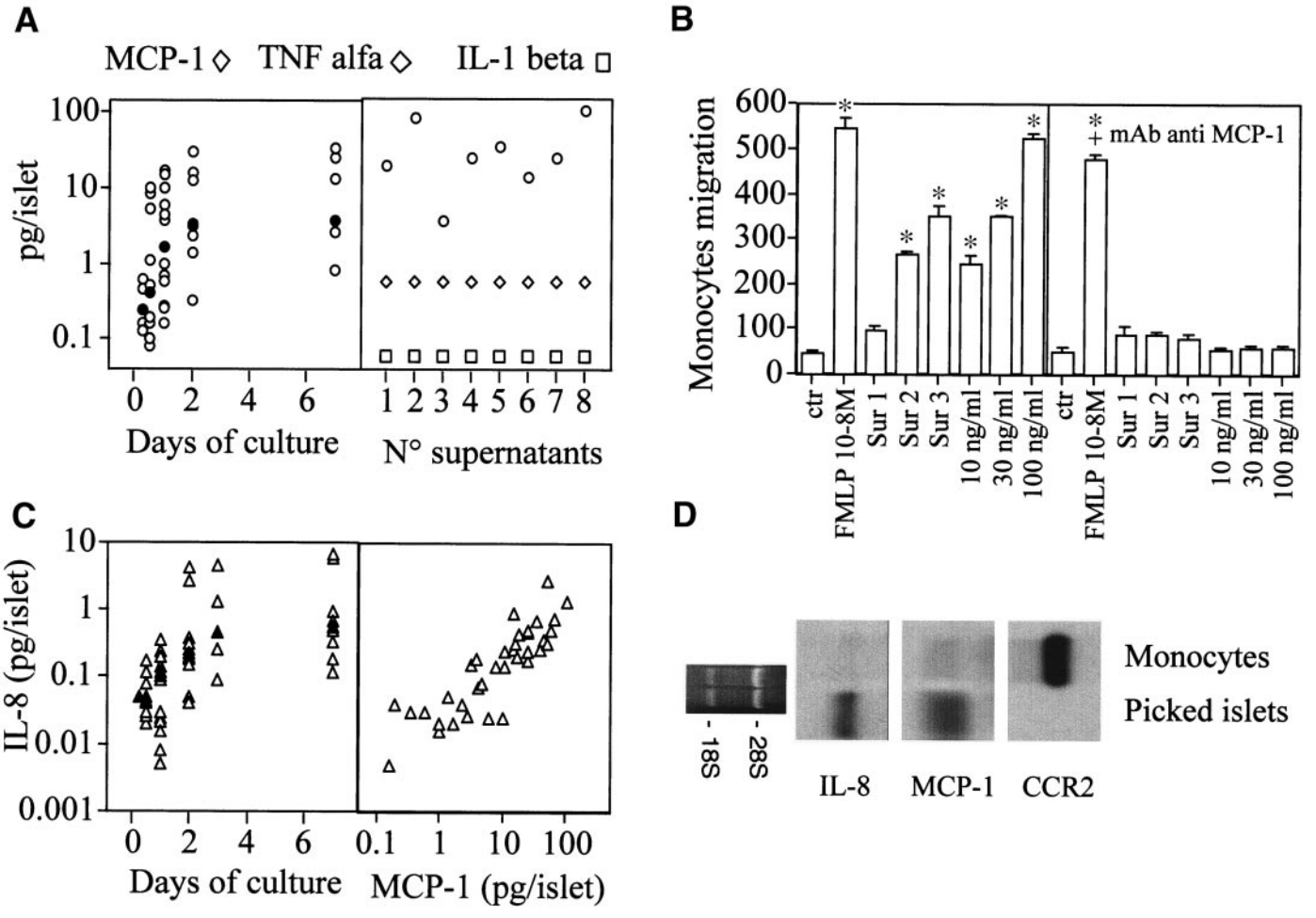

FIG. 1. Human pancreatic islets produce and secrete MCP-1. *A*: Supernatants from 20 different primary cultures of human pancreatic islets were tested by ELISA. *Left*: High levels of MCP-1 were detected, which increased over time. Black dots are median values. *Right*: Supernatants from eight cultured islets were tested for TNF-α and IL-1β at day 7. No detectable levels of TNF-α (<0.6 pg/ml) or IL-1 were present (<0.06 pg/islet). *B*: Chemotactic activity of MCP-1 secreted by human pancreatic islets. *Left*: Undiluted supernatants from three different cultured islet preparations were used as chemoattractants for human monocytes. By ELISA, supernatants contained 0.08 ng/ml (sur 1), 20.3 ng/ml (sur 2), and 33.7 ng/ml (sur 3) of MCP-1. *Right*: Preincubation of supernatants with 1 μg/ml anti–MCP-1 monoclonal antibody. Results are expressed as number of migrated monocytes in five microscope high-power fields. fMLP was used at 10^{-8}M. The picture shown is representative of three similar experiments from an independent donor. *P < 0.05 versus control (Student's t test). *C*: *Left*: Pancreatic islet supernatants contained low levels of IL-8. Black dots are median values. *Right*: Thirty-eight supernatants of pancreatic islets were tested by ELISA for MCP-1 and IL-8. Production of IL-8 correlates with MCP-1 (P = 0.001; regression). *D*: Northern blot analysis of MCP-1, IL-8, and CCR2 RNA in human pancreatic islets. Hand-picked purified human islets express mRNA for MCP-1 and IL-8; no detectable expression of CCR2 was present. The picture shown is representative of three similar experiments from an independent donor.

alent islets) remained sterile (anaerobic, aerobic, fungal, mycoplasm determination), with low endotoxin content (<0.05 EU/ml) and viable (trypan blue and fura-2 assays) for the culture period (7 days). The purification of the islet preparation was 35 ± 11% (islet/total purified preparation) with 67 ± 8% of β-cells (computerized morphometric analysis; Leica Imaging System LDD). In five different islet preparations, islet viability was confirmed also by double staining with propidium iodide (<10% positive) and with acridine orange (>90% positive). High levels of MCP-1 were detected in the culture medium. The secretion seemed to be time-dependent and with a wide range of concentration (Fig. 1*A*). Eight supernatants were also studied for the presence of TNF-α and IL-1. No detectable levels of TNF-α or IL-1 were present even after 7 days of culture (Fig. 1*A*). MCP-1 expression was analyzed by Northern blotting. Total cellular RNA was prepared from hand-picked human islets from three different preparations. High levels of MCP-1 mRNA were detected, whereas expression of mRNA for CCR2, the MCP-1 receptor, was not found (Fig. 1*D*). To investigate the biological activity of MCP-1 secreted by human pancreatic islets, we tested supernatants as chemoattractant for monocytes in vitro. Islet supernatants were able to attract human monocytes in a classical chemotaxis assay (Fig. 1*B*). Three different supernatants with different determination of MCP-1 (<0.08, 20.3, and 33.7 ng/ml) induced migration of 99 ± 8, 266 ± 4, and 351 ± 24 monocytes, respectively. The chemotactic activity of a recombinant MCP-1 used as standard was strikingly similar to that of islet-derived MCP-1. Migration was near totally inhibited by pretreatment with a blocking anti–MCP-1 antibody, indicating that MCP-1 is the major chemokine for monocytes in the supernatants of cultured pancreatic islets. We also tested the chemotactic activity of islet supernatants with polymorphonuclear cells and phytohemagglutinin (PHA)-activated T-cells and did not find significant migration. Modulation of MCP-1 production by cultured human pan-

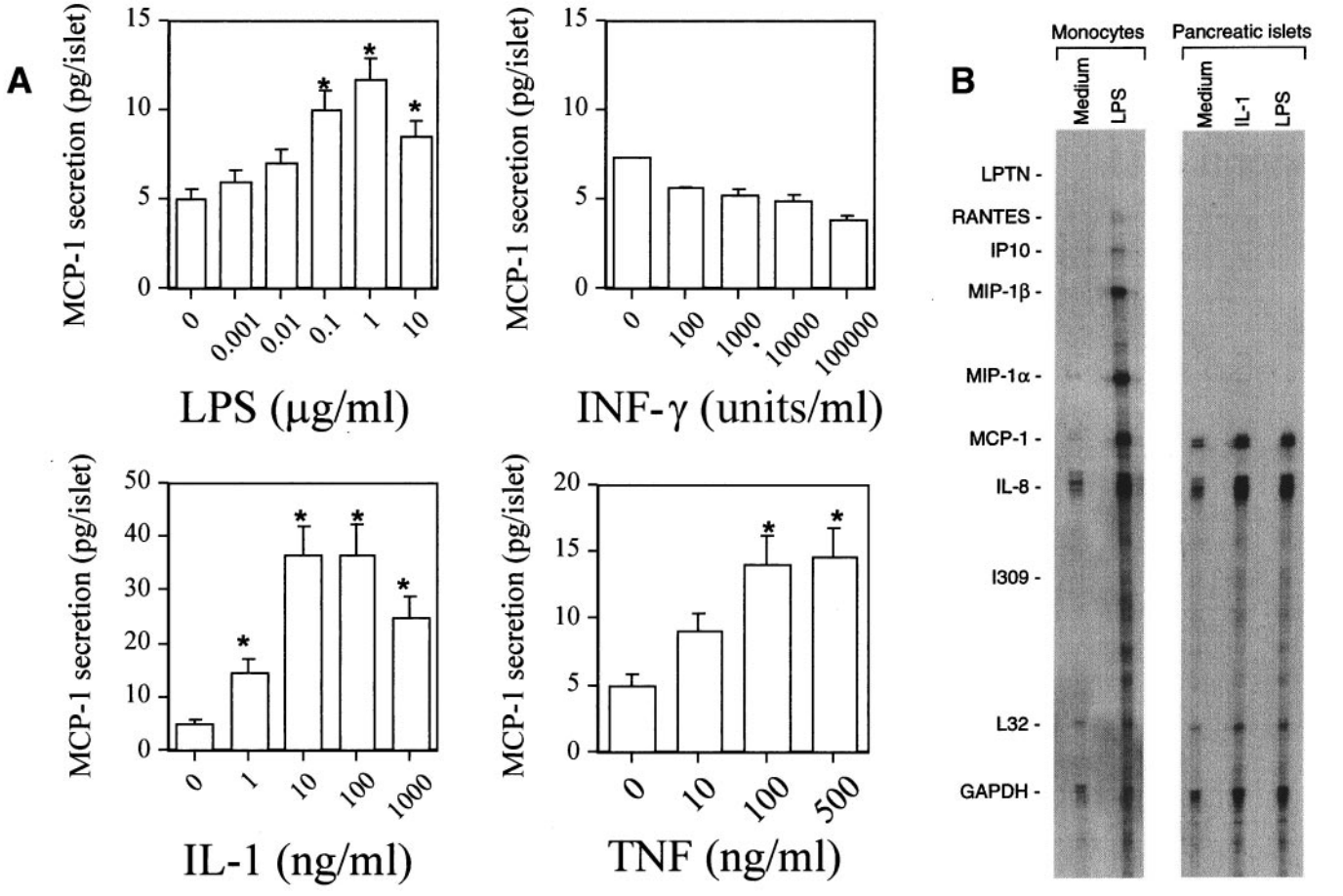

FIG. 2. Modulation of MCP-1 expression and secretion by inflammatory cytokines. *A*: Secretion of MCP-1 was upregulated by IL-1β, TNF-α, and LPS. INF-γ induced only a small decrease in the secretion at the highest concentration used. IL-1β, TNF-α, and LPS enhanced the production of MCP-1 in a dose-dependent manner. Results refer to 24-h exposure to stimuli; $n = 6$; *$P < 0.05$ versus control (Student's t test). *B*: RPA for different chemokines was performed with RNA from hand-picked purified human islets. No mRNA for lymphotactin (LPTN), MIP-1α, MIP-1β, IP-10, and RANTES was detected in resting condition or after exposure to IL-1β or LPS. Pancreatic islets constitutively expressed IL-8 and MCP-1 mRNA, which increased after IL-1β or LPS stimulation. The picture shown is representative of three similar experiments from independent donors.

creatic islets from six different preparations in the presence of inflammatory stimuli was investigated (Fig. 2*A*). IL-1β, TNF-α, and LPS enhanced the production of MCP-1 in a dose-dependent manner. INF-γ induced only a small decrease in the secretion, at the highest concentration used. The upregulatory effects of IL-1β and TNF-α on the production of MCP-1 were already demonstrable at 24 h and increased over time. To study whether other chemokines are secreted by human islets especially after stimulation with inflammatory stimuli, we performed an RPA from three different islet preparations (Fig. 2*B*). As expected, MCP-1 expression was increased after IL-1β or LPS stimulation. No mRNA for macrophage inflammatory protein (MIP)-1α, MIP-1β, interferon inducible protein 10 (IP-10), or RANTES was detected in resting condition or after exposure to IL-1β or LPS. In contrast, a positivity for IL-8 mRNA was present, which was increased after stimulation. By ELISA, low levels of IL-8 were detected in the culture supernatants of pancreatic islet (Fig. 1*C*). Mean values of IL-8 in 20 different preparations were 0.1 and 1.75 pg/islet at 24 h and 7 days of culture, respectively. It should be noted that levels of IL-8 were 10 times lower than MCP-1 levels. It is interesting that islet supernatants with highest MCP-1 content also showed the highest IL-8 production (Fig. 1*C*).

MCP-1 secretion, insulin secretion, and glucose stimulation. The observation that MCP-1 is produced from endocrine cells of human islets suggested a possible role for MCP-1 in modulating islet endocrine secretion. An early intracellular event in leukocytes activated by MCP-1 is a rise in $[Ca^{2+}]_i$. Although no expression of CCR2 mRNA was detected in pancreatic islet, it was not possible to rule out the presence of another (previously unidentified) MCP-1 receptor. Therefore, it was of interest to evaluate $[Ca^{2+}]_i$ in human islet after exposure to MCP-1. In a range of concentrations from 100 ng/ml to 1 μg/ml, MCP-1 did not induce an increase in $[Ca^{2+}]_i$ in fura-2–loaded human islets ($n = 5$ from three different preparations; Fig. 3*A*). Next, we evaluated the possibility that MCP-1 might influence insulin secretion in a system of static incubation. Human islets ($n = 6$ from six different islet preparations) exposed for 30 min to 100 ng/ml MCP-1 did not change their insulin release at either 3 (basal condition) or 16 mmol/l (stimulated condition) of glucose (Fig. 3*B*). Conversely, to assess the capacity of glucose to modulate MCP-1 secretion, we studied MCP-1 release after exposure

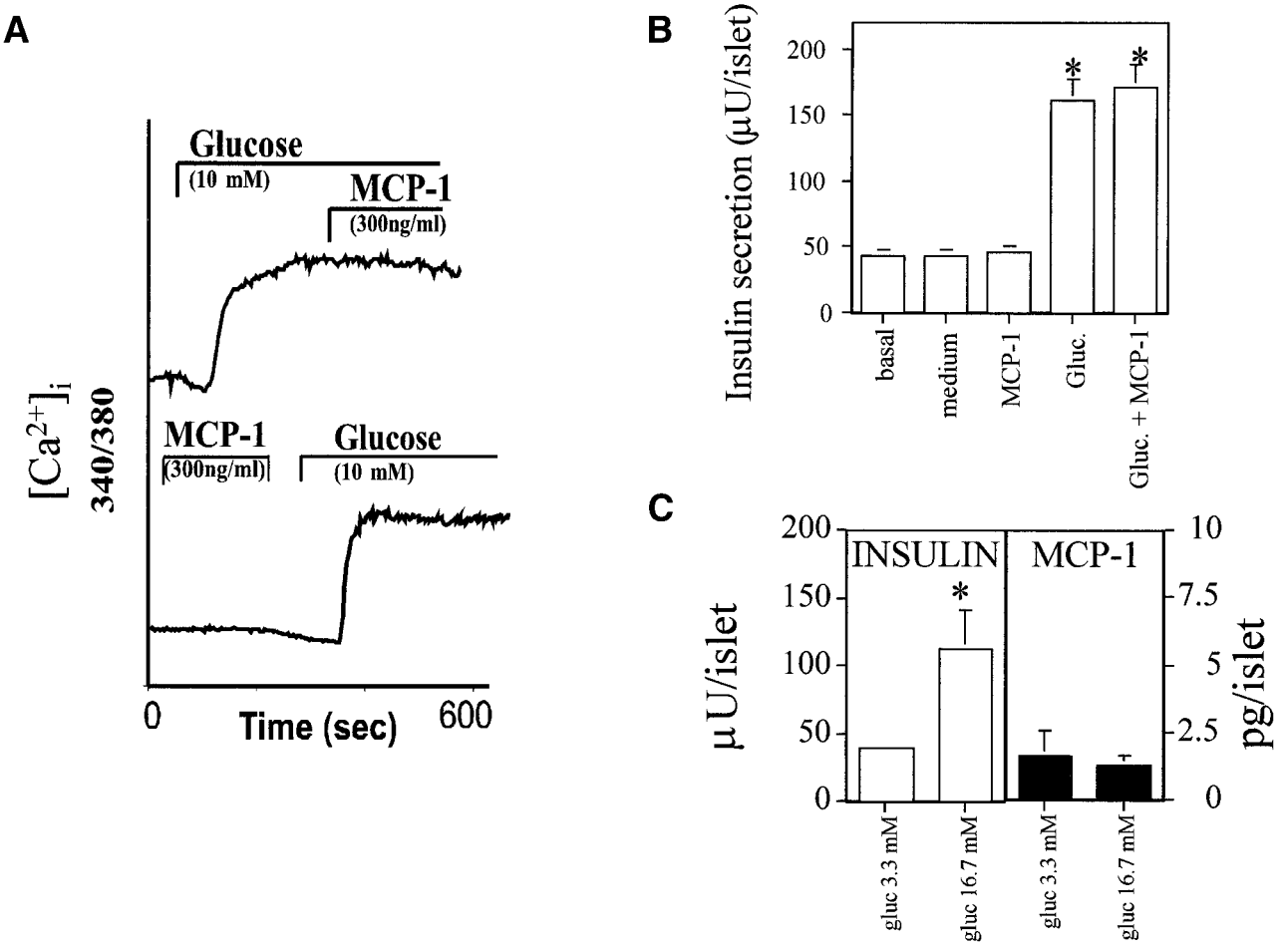

FIG. 3. Modulation of MCP-1 or insulin secretion. *A*: Evaluation of $[Ca^{2+}]_i$ in fura-2–loaded pancreatic human islets after exposure to MCP-1 (300 ng/ml). MCP-1 did not induce increase in $[Ca^{2+}]_i$ up to 1 μg/ml. *B*: Human pancreatic islets exposed to MCP-1 (100 ng/ml) did not change insulin release at either 3 (basal condition) or 16 mmol/l (stimulated condition) of glucose; *$P < 0.05$ versus control (Student's t test). *C*: Human pancreatic islets exposed to 16 mmol/l glucose did not increase MCP-1 secretion, whereas insulin was increased. Samples were done in duplicate, and the data presented are the means of six experiments; *P < 0.05 versus control (Student's t test).

to 16.7 mmol/l glucose. As expected, insulin secretion was increased whereas MCP-1 release was not affected (Fig. 3*C*). These results suggest that MCP-1 production by pancreatic islets is not associated and is not modulated by the endocrine activity of islets.

Ex vivo expression of MCP-1 in pancreatic islets. Standard methodology of immunohistochemistry was used to identify MCP-1 in human islets (Fig. 4). To evaluate MCP-1 expression before enzymatic digestion, we studied whole pancreas from surgery specimen (tumor, $n = 3$; chronic pancreatitis, $n = 3$; pancreas from explanted organs, $n = 8$). MCP-1 was visualized within the cytoplasm of islet cells that were also positive for insulin (Fig. 4*A* and *B*). MCP-1–positive cells did not react with a specific anti-CD45 antibody (Fig. 4*C*), indicating that MCP-1 staining was not due to infiltrating leukocytes. A less intense positivity was also seen in ductal cells (in particular, small ducts), whereas exocrine tissue seemed to be negative. To confirm these results, we used three other anti–MCP-1 monoclonal antibodies, recognizing different epitopes of MCP-1, with identical pattern of positivity (not shown). Immunoelectron microscopy confirmed the cytoplasmic positivity for MCP-1 of endocrine cells in human islet (not shown). By ISH, MCP-1 mRNA was detected in endocrine cells of human islets (Fig. 4*D*). Insulinoma and glucagonoma were also analyzed, and MCP-1 immunostaining was detected in the endocrine cells, confirming their capacity to secrete MCP-1 (data not shown). Whole pancreas from surgery specimen was also studied for IL-8 expression. IL-8 was not detected by immunohistochemical analysis within the cytoplasm of islet cells or other pancreatic components (not shown).

Islet transplantation into patients with diabetes. Twenty islet preparations were transplanted into 14 patients with type 1 diabetes, who were already immunosuppressed for a previous kidney graft. Patients' characteristics are detailed in Tables 1 and 2. To reach the minimum number of 5,000 IE/kg body wt, 6 of the 14 patients received two islet preparations, either within 10 days from the first preparation (30, 33, 34, 38, 40) or 4 months later (41). Starting from the second month after transplant, insulin requirement reduction correlates inversely with the MCP-1 secretion capacity of islets (Fig. 5*A*). Patients were divided into two groups according to values of MCP-1 released by the islet preparations during a 24-h culture. Cutoff value was the 50th percentile, 4.56 pg · $islet^{-1}$ · 24 h^{-1}. Group 1 (low MCP-1) received islet preparations below the 50th percentile, (MCP-1 mean value, 2.04 ± 0.51 pg · $islet^{-1}$ · 24 h^{-1}); group 2 (high MCP-1) received islet preparations above the 50th percen-

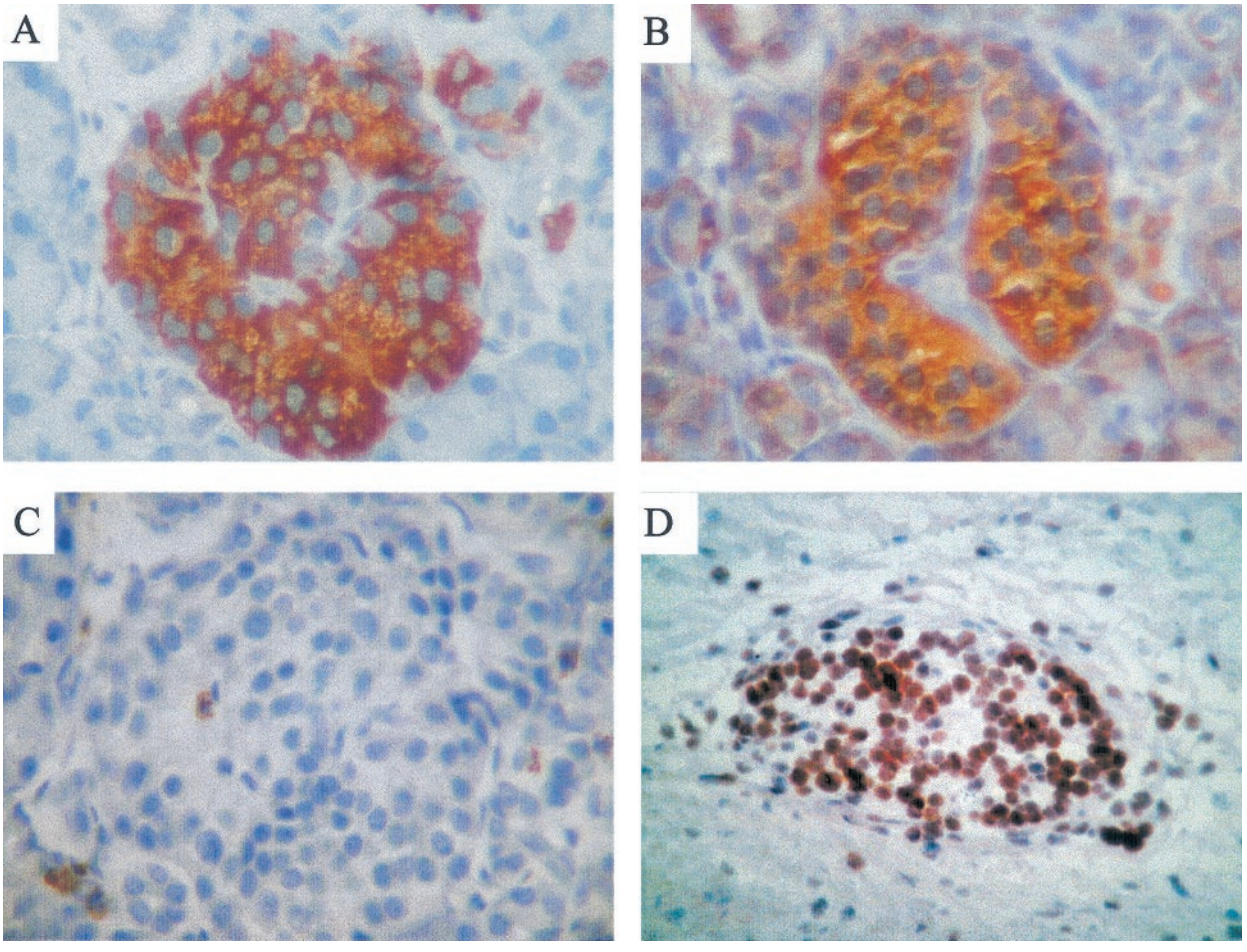

FIG. 4. Immunohistochemical localization of MCP-1 and ISH in human pancreatic islets. *A–C*: Serial sections of the same specimen. Insulin-positive β-cells (*A*) are positive also for MCP-1. *B*: Anti–MCP-1 (5D3-F7) stains the cytoplasm of islet β-cells. Specificity for MCP-1 was confirmed with three other monoclonal antibodies able to recognize different epitopes of MCP-1; all of the antibodies showed the same pattern of positivity (not shown). *C*: MCP-1–positive cells do not react with a specific anti-CD45 antibody, indicating that the MCP-1 staining is not due to infiltrating leukocytes. *D*: By in situ hybridization, MCP-1 mRNA is detected in endocrine cells of human pancreatic islets.

tile (MCP-1 mean value, $34.03 \pm 12.47\ pg \cdot islet^{-1} \cdot 24\ h^{-1}$). Islet preparations in the two groups of patients did not differ in viability, purity, number, and timing of culture before transplant (Table 1). Patients did not differ in terms of the following parameters: age, weight, time of diabetes, pretransplant insulin requirement, and metabolic control (Table 2). If we analyze the mean insulin requirement reduction in the two groups during the follow-up, patients in the high MCP-1 group showed a decrease in their insulin requirement that was significantly lower than that of patients in low MCP-1 group starting from the second month after the transplant (Fig. 5*B*). Glycosylated hemoglobin was significantly lower in the low MCP-1 group already 6 months from the transplant ($5.7 \pm 0.3\%$ vs. $7.7 \pm 0.8\%$; $P < 0.05$).

We evaluated islet transplants according the Islet Transplant Registry parameters (Fig. 5*C*) (34). Six patients in the low MCP-1 group became insulin-independent with a mean follow-up of insulin independence of 7 ± 3 months. All patients but one (31) are still insulin-free. In contrast, only two patients in the high MCP-1 group became insulin-independent for 2 ± 1 months; both patients are not still insulin-independent. One patient in the low MCP-1 group and three patients in the high MCP-1 group reduced their insulin requirement by more than 50% (partial function). Two patients in the high MCP-1 group and no patients in the low MCP-1 group showed a reduction of less than 50% (graft failure). No primary nonfunction was observed in our patients.

DISCUSSION

This is the first report demonstrating that human pancreatic islets prepared for a clinical transplant constitutively produce MCP-1 in the absence of detectable infections or endotoxin contamination. The produced MCP-1 is biologically active because it chemoattracts monocytes in chemotaxis assay and its activity (referred as ng/ml measured in ELISA) correlated well with that of a recombinant MCP-1 used as standard. Moreover, the chemotactic activity for monocytes was almost abrogated by a neutralizing anti–MCP-1 monoclonal antibody, indicating that no other chemotactic factors were present in the supernatants. In fact, RPA did not reveal expression of MIP-1α, MIP-1β, RANTES, or IP-10 even after LPS/IL-1β stimulation. We did find mRNA for IL-8, and protein levels were measured in pancreatic islet supernatants even if it is almost 1 log less than the release of MCP-1. In chemotaxis assay, pancreatic islet supernatants were unable to induce migration of human polymorphonuclear leukocytes (PMNs) (data not

TABLE 1
Characterization of islet preparations

Donor data			Islet preparation characterization						
	Age (sex)	BMI (kg/m^2)	EI	EI/kg body wt	Islet/whole preparation (%)	β-cell/ islet (%)	Basal Ca^{2+} (340/380)	Secretion index	MCP-1 pg · islet^{-1} · 24 h^{-1}
Low MCP-1 group									
31	33 (M)	27.7	355,800	7,412	29	73	0.301	3.2	0.6
41	64 (F)	25.8	491,000	9,702	46*	67*	0.331*	1.3*	0.7*
	54 (F)	23.4	324,000						
34	28 (F)	25.7	267,500	16,682	43*	76*	ND	6.0*	1.7*
	38 (M)	24.1	600,000						
35	55 (F)	25.7	430,000	7,818	70	64	0.320	10.1	2.0
30	59 (M)	24.4	400,000	13,020	13*	59*	0.425*	2.7*	2.3*
	60 (F)	25.4	290,000						
29	45 (M)	29.1	495,000	6,718	11	73	0.475	1.3	2.6
45	52 (F)	34.9	411,000	7,473	ND	ND	ND	ND	4.6
Mean	49 ± 12 6F/4M	26.6 ± 3.4	525,042 ± 185,000	9,281 ± 4,000	35 ± 22	69 ± 7	0.363 ± 0.1	4.05 ± 3.4	2.04 ± 1.35
High MCP-1 group									
34b	50 (M)	ND	459,000	7,650	ND	ND	0.315	ND	4.9
33	53 (M)	ND	245,000	6,090	21*	87*	ND	ND	14.4*
	59 (F)	22.2	230,000						
32	61 (M)	26.0	447,200	7,450	36	58	0.430	ND	15.1
40	51 (F)	ND	274,000	9,870	30*	59*	0.311*	2.1*	29.2*
	20 (F)	23.4	407,000						
42	40 (M)	26.3	592,000	12,081	46	75	0.445	2.5	32.6
38	39 (M)	22.6	438,000	10,550	21*	55*	0.355*	2.8*	38.0*
	63 (F)	20.8	195,000						
43	57 (M)	24.7	346,750	5,418	35	70	0.416	ND	104.0
Mean	49 ± 12 6F/4M	23.7 ± 2.0	517,678 ± 117,449	8,406 ± 2503	32 ± 10	67 ± 12	0.348 ± 0.1	2.46 ± 0.35	34.03 ± 33

Data of pancreas donor and of in vitro parameters analyzed after 24 h of culture. Islet preparations were divided into two groups according to values of MCP-1 released during a 24-h culture (50th percentile = 4.56 pg/islet): group 1 (low MCP-1) released MCP-1 below the 50th percentile (mean MCP-1 value 2.04 ± 0.5 pg/islet); group 2 released MCP-1 above the 50th percentile (mean MCP-1 value 34.03 ± 12.4 pg/islet). No differences were observed between these two groups according to the donor data and all of the other in vitro parameters considered. Patients 30, 33, 34, 38, 40, and 41 received two islet preparations: *the mean of the in vitro parameters of each preparation, normalized according to their corresponding number of equivalent islets. EI, equivalent islet.

shown). A major goal of this study was to correlate the clinical outcome of patients who receive allogeneic transplants of pancreatic islets with biological parameters. Primary cultures of pancreatic islets displayed similar characteristics but differed in the production of MCP-1. Follow-up demonstrated that patients who received islets that produced high levels of MCP-1 (>4.56 pg · islet^{-1} · 24 h^{-1}) were not able to induce long-term insulin independence, as two of seven patients reached insulin independence and only for a short period, whereas five patients had graft failure or partial graft function. In contrast, six of seven patients who received islets that produced low levels of MCP-1 reached long-lasting insulin independence. A possible interpretation is that high titers of MCP-1 at the graft site (liver parenchyma) attracts monocyte-macrophages locally. The recruited macrophages, activated by the inflammatory reaction, eventually lead to the destruction of the grafted organ. In support of this is the demonstration that elimination of macrophages improves graft survival in a rodent model of islet transplantation (42). The demonstration that islet MCP-1 release interferes with the engraftment may open new approaches in human islet transplantation. First, the identification of factors and/or drugs that are able to regulate the in vitro MCP-1 secretion could suggest strategies to identify new immunosuppressive approaches. Second, a screening of islet preparations based on their MCP-1 release before transplant may increase the success rate of transplantation.

Another major and intriguing finding of this study is the immunoreactivity for MCP-1 and also specific RNA by ISH in pancreatic sections from surgical specimen (pancreatic adenocarcinomas, chronic pancreatitis, pancreas from multiorgan donors). Although none of these clinical settings is perfectly normal, sections were carefully selected for lack of evident necrosis or hypoxia or hemorrhages. In all pancreata studied, islets stained positive for MCP-1 with four different anti–MCP-1 monoclonal antibodies. MCP-1 was produced by β-islet cells because both mRNA and protein stained insulin-producing cells. In addition, no CD45+ leukocytes were found within the islets. The presence of MCP-1 in pancreatic islets raises the question of which role MCP-1 might play, different from leukocyte recruitment, in an endocrine organ. Our data seem to exclude a role in the endocrine metabolism. In fact, MCP-1 does not affect the release of insulin or vice versa, and high glucose concentrations do not modulate MCP-1 release. MCP-1 does not induce elevation of free $[Ca^{2+}]_i$ (like glucose). It has long been known that chemokines also

TABLE 2
Clinical data of the recipients before the transplant

	Age (sex)	Weight (kg) (BMI, kg/m^2)	Years of diabetes	Pretransplant unit of insulin (HbA_{1c}, %)	Creatinine (mg/dl)	Autoantibody IA-2-GAD	
Low MCP-1 group							
31	43 (F)	48 (19)	31	26 (8)	0.9	−	−
41	47 (M)	84 (28)	29	64 (8.3)	1.6	+	−
34	41 (F)	52 (21)	23	41 (6.6)	1.0	−	+
35	44 (F)	55 (21)	38	53 (8.5)	1.0	+	+
30	48 (F)	53 (21)	45	34 (7.9)	1.0	+	+
29	41 (M)	64 (22)	22	65 (7.4)	0.9	−	+
45	37 (F)	55 (22)	28	68 (9.2)	0.9	−	−
Mean	43 ± 4	60 ± 12 (22 ± 3)	31 ± 8	50 ± 16 (8.6 ± 1.5)	1.1 ± 0.2	3/7	4/7
High MCP-1 group							
34b	51 (M)	60 (23)	19	62 (8.4)	1.0	−	−
33	61 (M)	78 (27)	31	45 (8.5)	2.2	−	−
32	41 (M)	60 (21)	19	27 (7.1)	2.6	+	+
40	52 (M)	69 (24)	48	74 (7.0)	1.0	−	−
42	42 (F)	64 (23)	9	36 (6.5)	1.6	−	−
38	42 (F)	60 (23)	35	53 (9.5)	0.7	−	+
43	34 (M)	49 (21)	32	40 (10.4)	0.9	−	−
Mean	46 ± 9	63 ± 9 (23 ± 2)	23 ± 2	48 ± 16 (8.2 ± 1.4)	1.4 ± 0.7	1/7	2/7

Patients were divided into two groups according to values of MCP-1 released by islet preparations during a 24-h culture (50th percentile = 4.56 pg/islet). Group 1 (low MCP-1) received islet preparations below the 50th percentile (mean MCP-1 value 2.04+0.5 pg/islet); group 2 received islet preparation above the 50th percentile (mean MCP-1 value 34.03+12.4 pg/islet). Patients in the two groups did not differ for the following characteristics: age, weight, time of diabetes, pretransplant insulin requirement creatinine, and presence of autoantibodies.

have no chemotactic functions, including control of angiogenesis, collagen production, and regulation of hematopoietic precursor proliferation. MCP-1, in particular, increases deposition of collagen type 1 by fibroblasts and upregulates endogenous expression of TGF-β, an important fibrogenic cytokine (52). The role of MCP-1 could facilitate the formation of the peri-islet capsule, which is severely damaged during in vitro isolation procedure (53) and is of crucial importance in vivo to separate the endocrine cells from the exocrine pancreas producing highly reactive enzymes. MCP-1 null mice did not show any gross alteration of organ structures, but organization of pancreatic islet architecture in these mice was not specifically addressed. The real function of MCP-1 in pancreatic islets, therefore, remains elusive. Other tissues produce MCP-1, among them are many tumors, from which MCP-1 was first identified. In human ovarian carcinomas, levels of MCP-1 correlate with infiltrating macrophages (54). In contrast, MCP-1 expression in pancreatic islets is not associated with an inflammatory infiltrate. This is reminiscent of regulatory mechanisms that may shut off the recruitment of potentially dangerous leukocytes. β-cells in pancreatic islets produce migration inhibitory factor (MIF), an old cytokine with inhibitory effects on monocyte migration (55). It might be that its presence with β-cells encompasses also the limitation of infiltrating monocytes. It is worth noting that some studies recently demonstrated that MIF also inhibited the MCP-1–induced migration of monocytes (56,57). In addition, we do not know which is the amount of MCP-1 released by islet in vivo and if in physiological condition is sufficient to induce monocyte recruitment. Finally, the monocyte recruitment is a multistep process that occurs through complex interactions of adhesion molecules and chemoattractants and their receptors and that involves endothelial cells. Some of our preliminary data (not shown) showed that islets are able to produce molecules that inhibit endothelia permeability and activation from inflammatory stimuli. A regulatory role to inducing a resting state on endothelial cells may explain the absence of a strong monocyte infiltration despite a low secretion of MCP-1.

Another major question is whether MCP-1 plays any role in diabetes onset. Pathological examinations in NOD mice have demonstrated that pancreatic islets are infiltrated early with cells of the monocyte-macrophage lineage and that this event precedes an inflammatory reaction (insulitis) and subsequent organ destruction. It was shown recently that the NOD genetic background is characterized by the presence of MCP-1 expression by pancreas-resident cells even in the absence of infiltrated mononuclear cells (58). If the constitutive production of MCP-1 in pancreatic islets does not result in insulitis, then it is reasonable to speculate that in the presence of primary inflammatory cytokines and increased levels of MCP-1, increased leukocyte recruitment may occur. It should be noted that MCP-1 transgenic mice, in which the transgene was under the control of the insulin promoter, have normal glycemia (59). These mice developed a monocytic infiltrate but never became diabetic, and there was no evidence for any local tissue destruction. This evidence strengthens the concept that, in vivo, MCP-1 functions mainly as a chemotactic factor without inducing cell activation, indicating that locally produced MCP-1 could recruit but not activate monocytes. It is very likely that other inflammatory signals (e.g., IL-1, TNF, LPS) are required to obtain the full activation of leukocytes. Of course, a possible involvement of MCP-1 in the pathogenesis of diabetes needs to be further analyzed. Other works recently reported the expression of MCP-1 mRNA and protein in rat and human islet cells exposed to cytokines (60,61). Similar to our

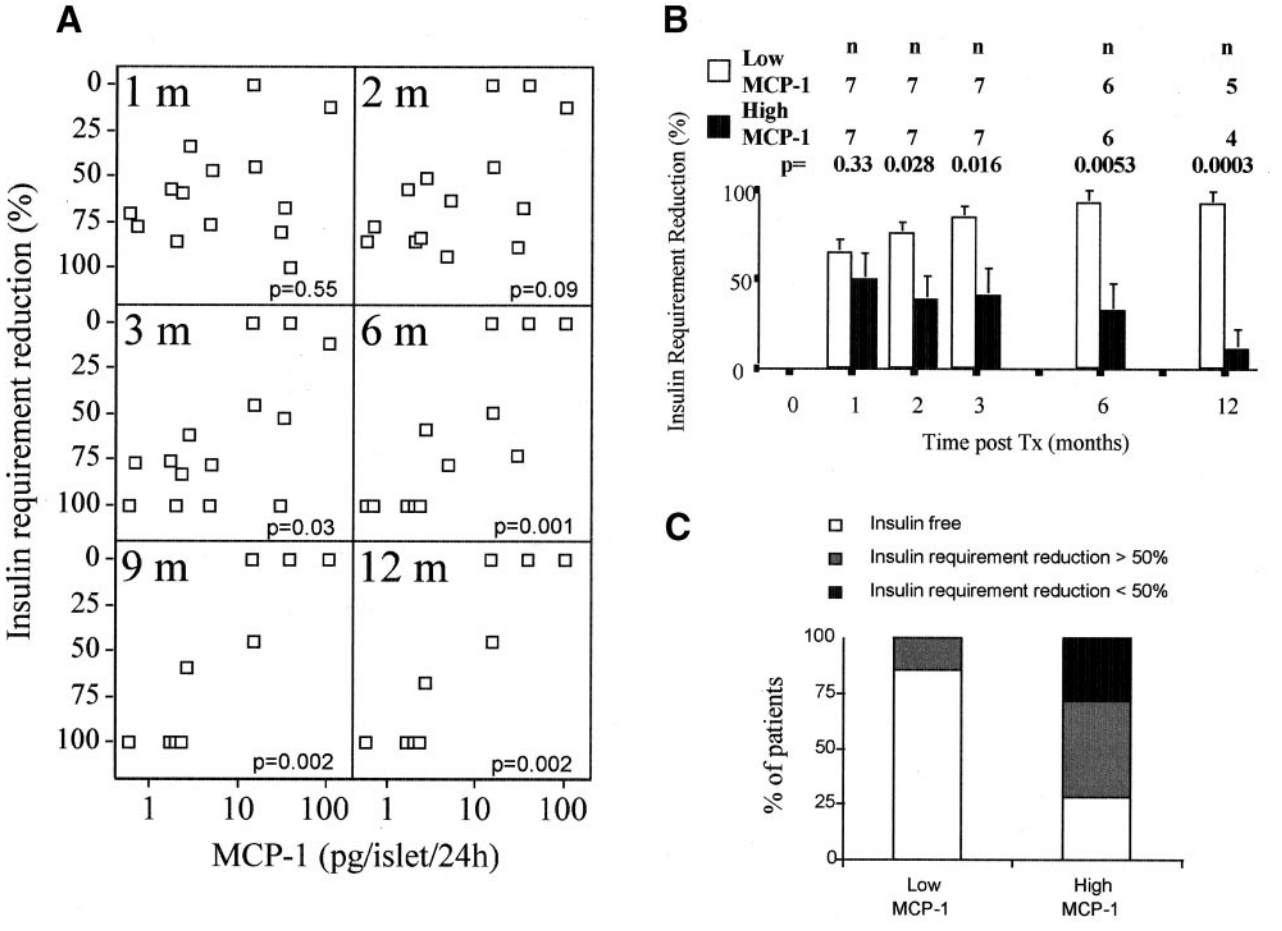

FIG. 5. Role of MCP-1 produced by pancreatic islets transplanted into patients with diabetes. Twenty islet preparations were transplanted into 14 patients with type 1 diabetes who were already immunosuppressed for a previous kidney graft. MCP-1 secretion by human islets was evaluated after a 24-h culture. *A*: Insulin requirement reduction correlated inversely with MCP-1 secretion capacity by transplanted islets (Kendal Rank correlation). *B*: Analysis of the mean insulin requirement reduction during the follow-up. Patients were divided into two groups according to values of MCP-1 released by islet preparations, during a 24-h culture (50th percentile = 4.56 pg/islet). Group 1 (low MCP-1) received islet preparations below the 50th percentile (mean MCP-1 value 2.04 ± 0.5 pg/islet); group 2 received islet preparations above the 50th percentile (mean MCP-1 value 34.03 ± 12.4 pg/islet). *C*: Analysis of graft function during the follow-up of the patients. Insulin free:insulin independence for >1 week; partial function:insulin requirement reduction >50% for >1 week; graft failure:insulin requirement reduction <50%.

results, they reported in human islet a great increase of MCP-1 release after IL-1β exposure and only a slight increase after TNF-α (not significant in the study of Chen et al. [60] but significant in our study). Furthermore, different from our data, the authors reported MCP-1 mRNA in human islets only after exposure to IL-1β and not in a constitutive situation. It must be noted that in the work of Chen et al., even when mRNA for MCP-1 is not present without IL-1β stimulation, protein production analyzed by ELISA showed that MCP-1 is also present in control unstimulated human islets. So the absence of mRNA in unstimulated islets was probably due to the sensitivity of the method.

In conclusion, this study is the first to report that unstimulated human pancreatic islets produce a high level of MCP-1, which plays a relevant role in the clinical outcome of islet transplantation in patients with type 1 diabetes. In fact, low MCP-1 secretion resulted as the more relevant factor for successful engraftment and long-lasting insulin independence. This finding opens new approaches in the management of human islet transplantation. MCP-1 production by pancreatic islets is strongly increased by inflammatory cytokines, suggesting a possible involvement of this chemokine in the induction of insulitis and pathogenesis of pancreatic endocrine diseases. Finally, the finding that MCP-1 is constitutively present in normal human islet cells, in the absence of an inflammatory infiltrate, suggests that this chemokine could have functions other than monocyte recruitment, and it opens a new link between the endocrine and immune systems.

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