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Transgenic Monocyte Chemoattractant Protein-1 (MCP-1) in Pancreatic Islets Produces Monocyte-Rich Insulitis Without Diabetes

Abrogation by a Second Transgene Expressing Systemic MCP-1[1]

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Monocyte chemoattractant protein-1 (MCP-1) is a CC chemokine that attracts monocytes and T lymphocytes in vitro; however, its in vivo functions are poorly understood. To address this question, we constructed transgenic mice expressing MCP-1 controlled by an insulin promoter. These mice developed a chronic insulitic infiltrate composed of F4/80$^+$ monocytes with minor populations of CD4$^+$, CD8$^+$, and B220$^+$ cells. Despite persistent transgene expression, the insulitis never progressed, and blood glucose levels remained normal. Thus, MCP-1 alone is sufficient to elicit a monocytic infiltrate, but not to activate elicited cells. These results differ from those obtained with another transgenic model using the mouse mammary tumor virus long terminal repeat, in which mice expressed substantial MCP-1 in several organs but had no infiltrates. However, mice expressing both transgenes had minimal insulitis, indicating that high systemic levels of MCP-1 prevented monocytes from responding to local MCP-1. Thus, the ability of MCP-1 to elicit monocytic infiltration depends on its being expressed at low levels in an anatomically restricted area. *The Journal of Immunology,* **1997, 159: 401–408.**

Monocyte chemoattractant protein-1 (MCP-1)[3] is a member of the chemokine family of proinflammatory cytokines (1, 2). Many chemokines were first isolated on the basis of their ability to attract specific leukocyte subtypes in in vitro chemotaxis assays and are considered as a group because of their significant amino acid sequence homology. In particular, nearly all chemokines have four cysteines in highly conserved positions. However, the approximately 25 known chemokines can be divided into four subgroups based on structural and genetic considerations. In the proteins of one subgroup the two cysteines nearest their amino termini are adjacent, and the subgroup has been denoted CC to reflect this structural feature. In the other major subgroup, the two cysteines are separated by a single amino acid, and the subgroup is denoted CXC. In one chemokine, lymphotactin, a single cysteine occurs in the N-terminal domain, and it has been suggested that this chemokine belongs to a third subfamily, called C (3); another chemokine, CX_3C, has three amino acids between the first two cysteines (4). Chemokine genes also cluster along subfamily lines: CC chemokine genes map to 17q11.2–12, and CXC chemokine genes to 4q13; lymphotactin and the CX_3C chemokine map elsewhere.

MCP-1 is a CC chemokine that was originally cloned as the *JE* gene, a platelet-derived growth factor-inducible gene in 3T3 cells (5, 6). It specifically attracts monocytes and memory T lymphocytes in vitro and stimulates the release of histamine from basophils (7–11). MCP-1 expression has been documented in a variety of human diseases that have inflammatory components, including atherosclerosis, multiple sclerosis, asthma, and rheumatoid arthritis among many others (12–15). However, a direct role for MCP-1 in the pathogenesis of these diseases has not yet been demonstrated. Nonetheless, passive immunization experiments in a handful of pulmonary and renal inflammatory models in rodents have clearly shown that MCP-1 can play a part in experimental inflammation (16, 17).

Defining a role for MCP-1 in disease depends on a thorough understanding of its in vivo properties, about which some fundamental questions remain. Direct injection of MCP-1 into rodent skin has produced conflicting results, from inducing a mild influx of mononuclear cells (18) to no detectable infiltrate (19). The ability of MCP-1 to attract monocytes in vivo was suggested by experiments in which tumor cells engineered to express MCP-1 were injected into mice and elicited a monocyte-rich inflammatory infiltrate in association with tumor rejection (20, 21).

Transgenic approaches have also produced conflicting results. In one model, murine MCP-1 was expressed in several organs under the control of the mouse mammary tumor virus long terminal repeat (MMTV-LTR) (22). Despite achieving high levels of expression of biologically active transgenic protein, no monocyte infiltrates were observed in expressing organs. These mice had serum

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[3] Abbreviations used in this paper: MCP-1, monocyte chemoattractant protein-1; MMTV-LTR, mouse mammary tumor virus long terminal repeat; RIP, rat insulin promoter; MCA, monocyte chemoattractant activity.

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levels of MCP-1 well above the K_d for the MCP-1 receptor on blood monocytes, and it was hypothesized that circulating MCP-1 had desensitized its receptor on monocytes, rendering them incapable of responding to locally produced MCP-1. Alternatively, high levels of MCP-1 could have physically negated the chemoattractant concentration gradient arising from expressing organs. Consistent with either idea was the greater susceptibility of these mice to intracellular pathogens, including *Listeria monocytogenes* and *Mycobacterium tuberculosis*. In contrast, a second transgenic model put MCP-1 expression under the control of the myelin basic protein promoter (23). Levels of expression were low and temporally limited, and in contrast to the MMTV-LTR model, these mice displayed a mild perivascular infiltrate in the brain.

In the current study, we attempted to address the question of the ability of MCP-1 to elicit infiltrates in vivo by using a well-studied model of transgenically induced inflammation in pancreatic islets of Langerhans. This model uses the rat insulin promoter, and it has been employed to express several proinflammatory cytokines with results that demonstrate the feasibility of using the islet as a model of cytokine-induced chronic inflammation (24–28). We have constructed transgenic mice expressing MCP-1 under the control of the insulin promoter and have used them to investigate the conditions required for MCP-1 to elicit monocytic infiltration in vivo.

Materials and Methods

Transgenic mouse construction

Murine MCP-1 genomic DNA was modified to remove the first 20 bases of exon 1, as previously described (22), and cloned downstream from the rat insulin II promoter in the pRIP-SK-K plasmid (27) to generate the plasmid pRIP-mMCP-1. Purified RIP-mMCP-1 DNA was used to make transgenic mice in a (C57Bl/6 × C3H)F_2 background as previously described (29). Transgenic mice were identified by Southern blotting of DNA extracted from tail snips using standard techniques (30). Digestion of genomic DNA with *Bam*HI and probing with a *Nae*I-*Hpa*I fragment of genomic MCP-1 DNA identified an 8000-bp fragment derived from wild-type MCP-1 alleles and a 2300-bp fragment derived from the RIP-MCP-1 allele. For crosses with MMTV-LTR-MCP-1 transgenic mice, the MMTV-LTR-MCP-1 transgene was detected by Southern blotting as a 1700-bp *Bam*HI fragment using the same probe (22).

Measurement of MCP-1 synthesis

Pancreata were placed in 0.3 ml of cold 50 mM Tris-HCl (pH 7.5) and 0.2 mM EDTA and homogenized. Homogenates were clarified by centrifugation at 15,000 × *g* for 5 min, and protein determinations were made by Bradford assay. One aliquot of pancreas extract was used for MCP-1 ELISA determinations using hamster anti-mouse MCP-1 mAbs (31) for capture (18241D, PharMingen, San Diego, CA) and detection (biotinylated 18272D, PharMingen) as previously described (22). (The same ELISA system was also used to measure serum levels of MCP-1.)

A second aliquot of pancreas extract was serially diluted in Gey's balanced salt solution with 0.02% BSA and tested for its ability to attract human monocytes in a 48-well micro-Boyden chamber apparatus as previously described (32). The concentration of monocyte chemoattractant activity (MCA) in MCA units per milliliter was defined as the inverse of the dilution demonstrating half-maximal activity (33). Immunoadsorption of MCP-1 was performed using rabbit anti-mouse MCP-1 (34) as previously described (22, 35).

Immunohistochemistry and assessment of insulitis

Pancreata were either frozen in OCT embedding compound (Miles Laboratories, Elkhart, IN) on dry ice, or fixed in 10% formalin before embedding in paraffin. Paraffin-embedded tissues were sectioned and stained with hematoxylin-eosin and analyzed by light microscopy for the presence of insulitis. Frozen sections were analyzed by immunohistochemistry as previously described (27), using biotinylated mAbs directed against F4/80 (Serotec, Oxford, U.K.), CD4 (Life Technologies, Grand Island, NY), CD8 (Life Technologies), B220 (Caltag, South San Francisco, CA), NK-1.1 (PharMingen), insulin (BioGenex, San Ramon, CA), and a polyclonal anti-glucagon Ab (BioGenex).

Results

Construction and analysis of transgenic mice

Genomic murine MCP-1 DNA was cloned downstream from the rat insulin promoter II, and the chimeric gene was used to construct transgenic mice. Progeny were genotyped by Southern blot analysis of DNA extracted from tail snips, and two founder mice (lines 17 and 21) were identified that had distinct transgene insertion sites and approximately one to five copies of integrated transgene per genome (see Fig. 5 for a direct comparison of the copy number of RIP-MCP-1 transgenes and MMTV-LTR-MCP-1 transgenes which were also present at one to five copies per genome (22)). These mice were bred with C57Bl/6 mice, and their progeny were interbred. Mice that did not inherit the transgene from these crosses were maintained and interbred to provide a population of nontransgenic control mice with the same strain background as the transgenic mice.

Expression of transgenic MCP-1

To document transgene expression, pancreata were isolated, and protein extracts were prepared as described in *Materials and Methods*. Immunoblot analysis of whole pancreas extracts and immune precipitation of material secreted from islet preparations in vitro did not detect transgenic MCP-1 protein. However, as shown in Figure 1*A*, extracts from transgenic pancreata had more MCA than extracts from nontransgenic tissue. Normalized for total protein content, the transgenic pancreas had 15.7 MCA units/mg total protein compared with the wild-type pancreas's 0.02 MCA units/mg protein. Taking the specific activity of murine MCP-1 as approximately 10^6 MCA units/mg (19), the transgenic pancreas had 15.7 ng MCP-1/mg total protein. Furthermore, Figure 1*B* shows that this activity was specific for MCP-1, since about 90% could be adsorbed with anti-MCP-1 Abs.

Transgenic MCP-1 production was also quantified by ELISA using hamster anti-mouse MCP-1 mAbs (Table I). When normalized for total protein content in the extract, pancreata of transgenic mice from both lines expressed 6- to 10-fold more MCP-1 than those of nontransgenic mice from the same lines. Levels of transgenic MCP-1 expression determined by ELISA were similar to those inferred from bioactivity. For unknown reasons, however, basal MCP-1 levels in nontransgenic pancreata were somewhat higher in ELISA than they were in bioassays. Expression of transgenic MCP-1 was detected as early as 13 days after birth (the first time point measured) and continued unabated for at least 12 mo. RIP-MCP-1 mice had undetectable serum levels of MCP-1 by ELISA.

Inflammatory phenotype of RIP-MCP-1 mice

All mice bearing the RIP-MCP-1 transgene developed insulitis in the majority of their islets. The infiltration was often in a bull's eye pattern, with inflammatory cells in the center of the islet and around its periphery (Fig. 2, *B–D*). In other cases, there was no specific infiltrative pattern, but a dispersed cellular collection (Fig. 2*E*). In general, however, islet architecture remained intact. The infiltrates were detected by 13 days of age (mice were not examined earlier) and did not increase in severity throughout a transgenic mouse's life span.

Infiltrates were comprised of a monotonous collection of cells with the morphologic appearance of monocytes. Occasional apoptotic figures were present, but whether they derived from pancreatic cells or infiltrative cells could not be determined. Immunohistochemical analysis (Fig. 3) showed that most of the infiltrate stained with F4/80, a monocyte/macrophage-specific surface marker. In addition, however, there were small populations of

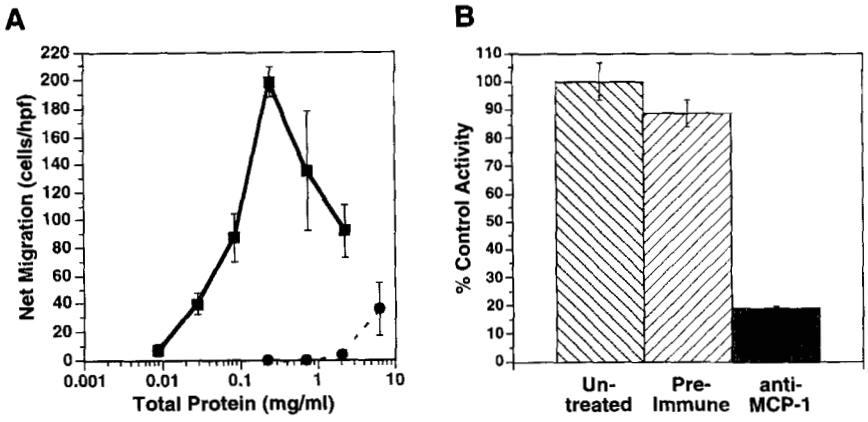

FIGURE 1. Biologic activity of transgenic MCP-1. *A*, Pancreas extracts from a transgenic mouse (■) and a nontransgenic littermate control (●) were serially diluted and tested for their ability to attract human monocytes in vitro as described in *Materials and Methods*. Cells were counted in five high power fields (hpf) in duplicate Boyden chamber wells. Error bars indicate the SD of the mean of cell counts from duplicate wells. This experiment is representative of three performed. *B*, Pancreas extract from the transgenic mouse analyzed in *A* was processed by passing over a column of preimmune rabbit serum immobilized on protein A-Sepharose (Pre-immune) or a column of rabbit anti-mouse MCP-1 similarly immobilized (anti-MCP-1). Serial dilutions of the effluent were tested for MCA as described in *A*. The inverse of the dilution producing half-maximal activity provided the concentration of MCA (MCA units per milliliter). Concentrations were then normalized to the concentration of MCA in untreated material. Error bars indicate the SD of duplicate measurements.

Table I. *MCP-1 expression in transgenic and nontransgenic pancreata determined by ELISA*[a]

Line	Transgenic (ng MCP-1/mg Protein)	Nontransgenic (ng MCP-1/mg Protein)	p^b
17	11.79 ± 1.34 (10)[c]	1.77 ± 0.50 (7)	≤0.005
21	15.70 ± 1.70 (4)	1.43 ± 0.26 (3)	≤0.025

[a] Extracts of pancreata from transgenic mice or nontransgenic mice of identical background were tested by ELISA for murine MCP-1 content as described in *Materials and Methods*. All mice were at least 50 days old. Values are nanograms of MCP-1 per milligram of total pancreas extract protein.
[b] *Student's t test.*
[c] Values are means ± SE. Numbers in parentheses are the numbers of mice analyzed.

$CD4^+$, $CD8^+$, and $B220^+$ cells. Infiltrating cells in the islets did not stain with NK-1.1, while control cells in spleen sections on the same slides showed positive NK-1.1 staining (data not shown).

Despite the presence of a significant and persistent insulitis, mice remained nondiabetic. Blood sugars never rose significantly above those of nontransgenic littermates, and except for areas obscured by inflammatory cells, transgenic islets displayed nearly normal immunohistochemical staining for insulin and glucagon (Fig. 4). An attempt was made to activate infiltrating monocytes by administering a single dose of 100 μg of LPS i.p. However, this manipulation did not produce hyperglycemia when serum glucose was measured 72 h later, nor did it alter the morphologic appearance of the islets or their infiltrates.

Modulation of the inflammatory phenotype by an MMTV-MCP-1 transgene

We had reported earlier that transgenic mice expressing MCP-1 under the control of the MMTV-LTR displayed no monocytic infiltration in organs that expressed the transgene (22). The explanation was that high serum levels of MCP-1 rendered circulating monocytes incapable of responding to locally produced MCP-1. We tested this hypothesis by mating RIP-MCP-1 mice with MMTV-LTR-MCP-1 mice, predicting that the local effects of MCP-1 from the RIP promoter would be masked by the high levels of serum MCP-1 derived from the MMTV-LTR.

F1 mice were analyzed from the mating of one of the RIP-MCP-1 lines (line 17) to the Tg24 line of MMTV-LTR mice, which are homozygous (22). Since the MMTV-LTR mice are in an FVB background, the progeny from this cross were compared with progeny from a mating of the same RIP-MCP-1 line to a wild-type FVB mouse. The two transgene alleles were easily distinguished in a single Southern blot analysis (Fig. 5). In both cases, mice that inherited the RIP-MCP-1 transgene expressed 20 ng MCP-1/mg of pancreas extract as determined by ELISA. However, while the RIP-MCP-1 × FVB mice carrying the RIP-MCP-1 transgene showed the typical monocytic insulitis shown in Figure 2, the RIP-MCP-1 × MMTV-LTR-MCP-1 mice carrying both transgenes showed little if any infiltrate (Fig. 6). Similar results were observed in five mice carrying both transgenes.

Discussion

By expressing murine MCP-1 under the control of the rat insulin promoter, we have demonstrated that MCP-1 by itself induces leukocyte infiltration in vivo. The infiltrate was comprised almost entirely of F4/80-positive mononuclear cells with the morphologic appearance of blood monocytes rather than activated macrophages. In addition, $CD4^+$ and $CD8^+$ T lymphocytes as well as $B220^+$ cells made up a smaller proportion of the infiltrate. The $B220^+$ cells are likely to be B lymphocytes rather than NK cells, since they did not stain with NK-1.1.

Our results are somewhat similar to those of Fuentes et al., who expressed MCP-1 in the brain under the control of the myelin basic protein promoter (23). They also observed an $F4/80^+$ mononuclear cell infiltrate in perivascular and meningeal regions coincident with transgene expression. However, the results in the current study differ from theirs in a few respects. First, the islet infiltrates are much more intense than those in the brain. While neither we nor Fuentes et al. quantified infiltrating cells, a direct comparison of histologic patterns shows more leukocytes per parenchymal nucleus in the islets than in the brain. Whether this reflects differences in the local tissue environment, e.g., expression of adhesion molecules or matrix proteases, is unknown. Also unknown is

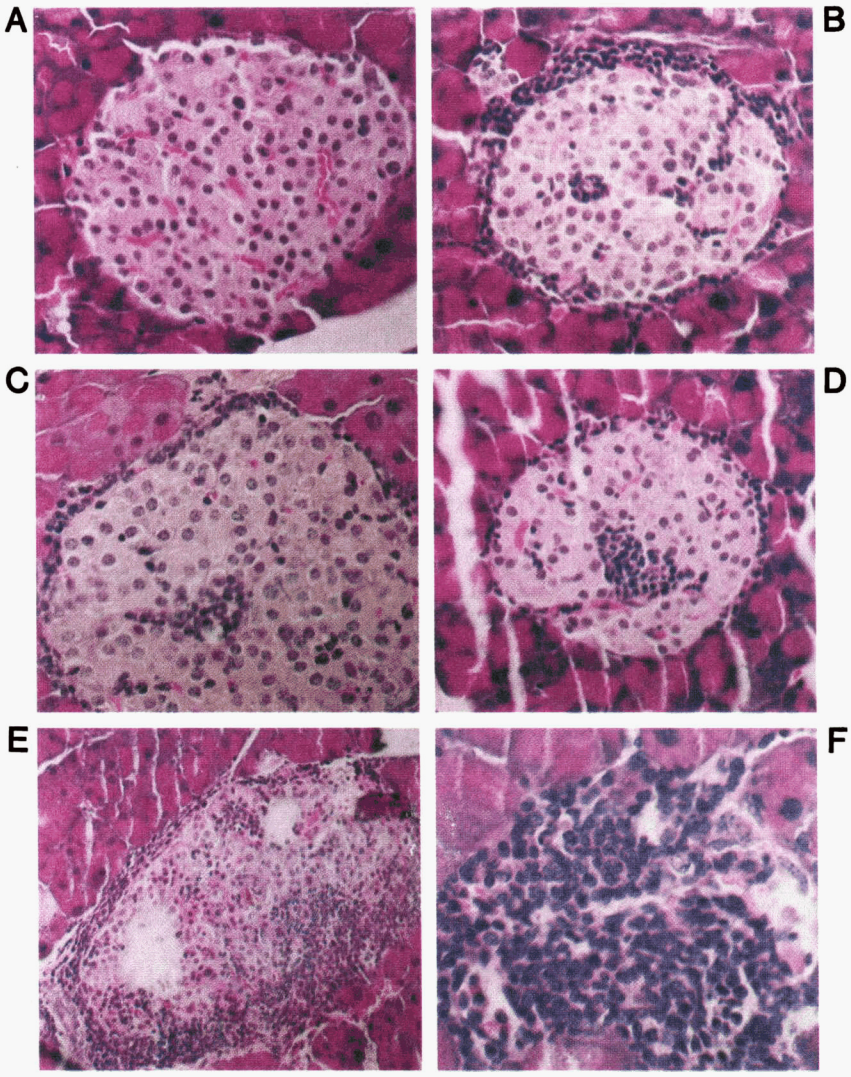

FIGURE 2. RIP-MCP-1-induced insulitis. The indicated mice were killed, and their pancreata were fixed in formalin, sectioned, and stained with hematoxylin and eosin. *A*, Nontransgenic mouse, aged 252 days (×400). *B*, Transgenic littermate of the mouse in *A*, aged 252 days (×400). *C*, Transgenic mouse, aged 345 days (×400). *D*, Transgenic mouse, aged 136 days (×400). *E*, Transgenic mouse, aged 52 days (×400). (Other islets in this 52-day-old mouse appeared similar to those in *B–D*.) *F*, Transgenic mouse, aged 136 days (×800).

whether this difference is due to varying levels of transgene expression, since Fuentes et al. did not quantify the product of the MBP-MCP-1 transgene.

A second difference is the clear presence of T lymphocytes in the islet infiltrates. Fuentes et al. detected no $CD4^+$ or $CD8^+$ cells either in the brains of MBP-MCP-1 mice or in the thymi of *lck* promoter-driven MCP-1 transgenic mice (23). Again, whether this is due to differences in microenvironment or levels of transgene expression cannot presently be determined. However, it is notable that despite MCP-1 being a potent chemoattractant for T lymphocytes in vitro, none of these MCP-1 transgenic models produced an intense lymphocytic infiltrate in expressing organs. While it is possible that MCP-1 is not a very potent T cell chemoattractant in vivo, a more likely explanation for the relative absence of T cells is that the expression of CCR2, an MCP-1 receptor, is up-regulated by activation, which may not occur in these systems (36). Alternatively, T cells may have greater difficulty entering tissue spaces than monocytes, perhaps due to the absence of appropriate adhesion molecules in the models examined to date.

Finally, the presence of B lymphocytes in MCP-1-induced insulitis is surprising and was not seen by Fuentes et al. MCP-1 has no reported effects on B lymphocyte chemotaxis in vitro, and CCR2 has not been found to be expressed by B cells. Thus, either B cells have an as yet undescribed receptor for murine MCP-1, or MCP-1 expression in the islet secondarily induces a B lymphocyte chemoattractant. Although B220 is expressed by NK cells, which also express CCR2, there were no detectable NK cells in the infiltrates. As with T lymphocytes, high levels of CCR2 expression may require NK cell activation. We did not examine infiltrates for the presence of dendritic cells, which also express CCR2.

This is now the fifth transgenic model of MCP-1 expression to be reported. The *lck*-MCP-1 and MBP-MCP-1 models (described above) showed a mild mononuclear cell infiltration, as did a third model in which MCP-1 expression was controlled by the K14 keratin promoter (37). The latter mice demonstrated an increased number of Langerhans-like cells in their skin and responded to contact hypersensitivity challenges with an exaggerated accumulation of monocytes and T lymphocytes. These models along with the RIP-MCP-1 model reported herein, which produced the most intense inflammatory picture reported to date, clearly demonstrate that MCP-1 is capable of inducing predominantly monocytic infiltration in vivo.

Another transgenic model, also reported from one of our laboratories, expressed MCP-1 at high levels in several organs under the control of the MMTV-LTR (22). Despite expression of biologically active transgenic MCP-1, no organs had infiltrates. These mice had high serum levels of MCP-1 (≈20 ng/ml), and it was

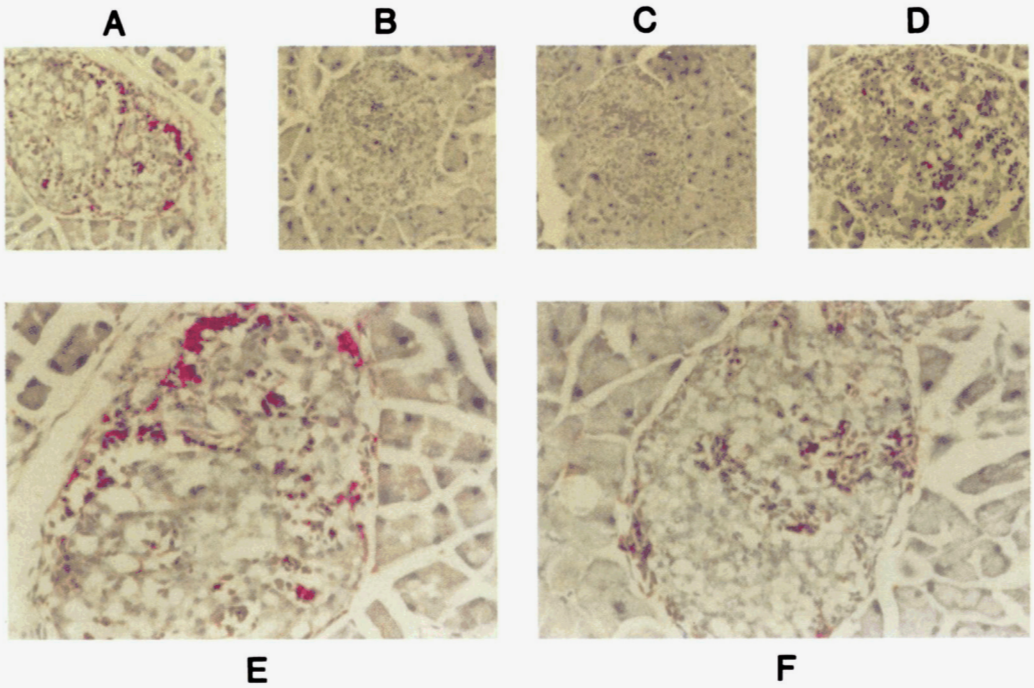

FIGURE 3. Immunohistochemical analysis of MCP-1-induced insulitis. Islets from a transgenic mouse, aged 143 days, stained for: *A*, F4/80 (×200); *B*, CD4 (×200); *C*, CD8 (×200); *D*, B220 (×220); *E*, F4/80 (×400); and *F*, F4/80 (a different islet from *E*; ×400).

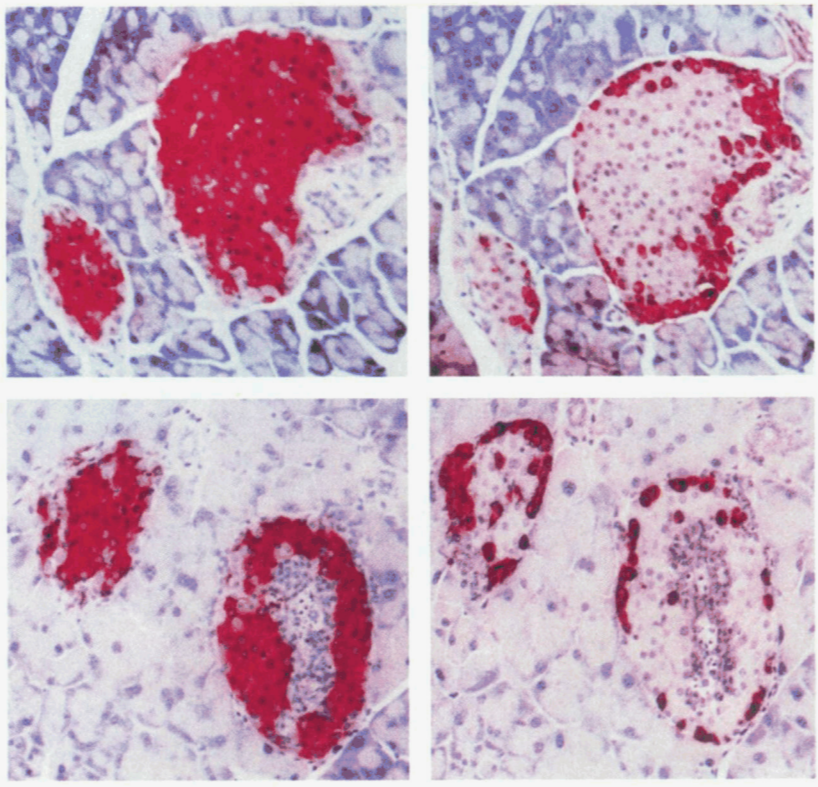

FIGURE 4. Islets from a nontransgenic mouse (*top*), aged 143 days, stained for insulin (*left*) and glucagon (*right*), and islets from a transgenic mouse (*bottom*), aged 162 days, stained for insulin (*left*) and glucagon (*right*) are shown.

hypothesized that this either physically disrupted the chemoattractant gradient of MCP-1 emanating from expressing organs or desensitized circulating monocytes, preventing them from responding to local MCP-1. Confirmation of that hypothesis was obtained in the current study by mating RIP-MCP-1 mice (which have no detectable circulating MCP-1) to MMTV-MCP-1 mice and observing a substantial amelioration of insulitis in mice expressing both transgenes. Thus, leukocyte infiltration in response to chemokines such as MCP-1 requires expression at low levels in anatomically restricted areas. That our results may be generalized to other chemokines is suggested by the fact that systemically administered IL-8 similarly blunts the chemoattractant activity of locally delivered IL-8 (38). The levels of MCP-1 in the serum of MMTV-MCP-1 transgenic mice are similar to those in patients with septic shock or in human volunteers given endotoxin, but much higher than levels in patients with glomerulonephritis

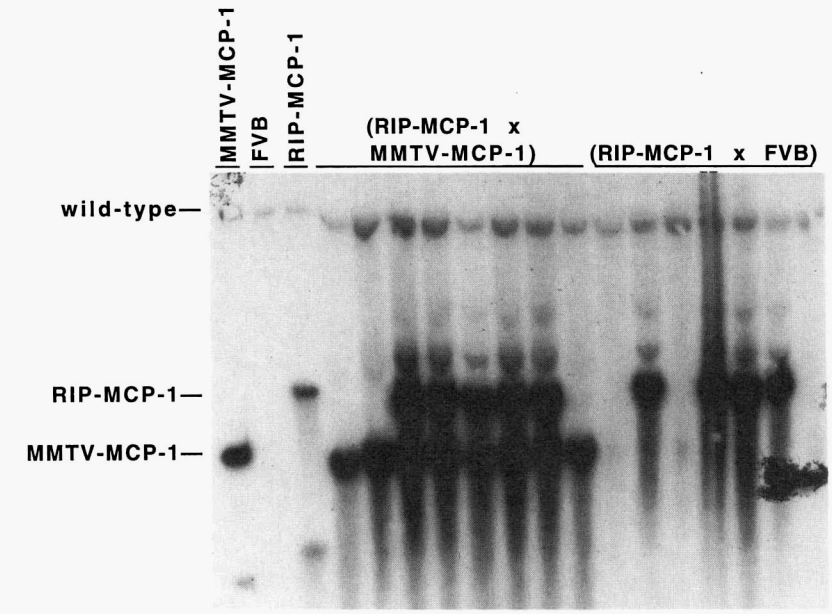

FIGURE 5. Southern blot analysis of transgenic mice. RIP-MCP-1 mice were bred either to homozygous MMTV-LTR-MCP-1 transgenic mice (which are in an FVB background) or to wild-type FVB mice. DNA was extracted from tail snips of progeny, and *Bam*HI-digested material was examined by Southern blotting using a murine MCP-1 probe. The sizes of fragments derived from the wild-type, RIP-MCP-1, and MMTV-LTR-MCP-1 alleles are indicated.

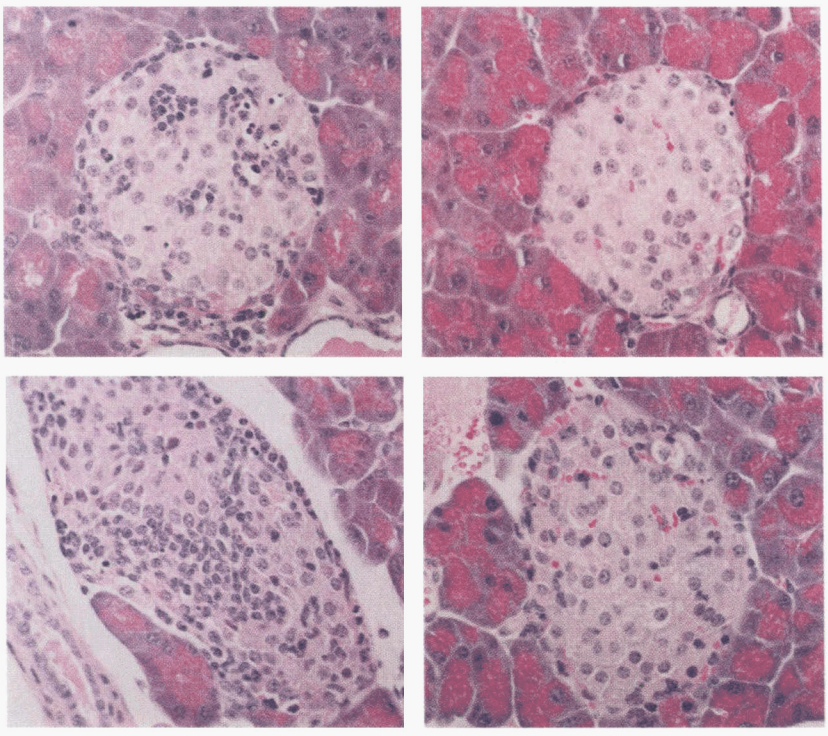

FIGURE 6. Insulitis in (RIP-MCP-1 × MMTV-LTR-MCP-1)F_1 mice. Two progeny from a cross of RIP-MCP-1 × FVB mice (*left*) and RIP-MCP-1 × MMTV-LTR-MCP-1 inheriting both transgenes (*right*) were analyzed for the presence of insulitis as described in Figure 2. Similar results were noted in three additional RIP-MCP-1 × MMTV-LTR-MCP-1 mice. Magnification, ×400.

(39–43). High levels of circulating MCP-1 in septic patients might contribute to their generalized immunodeficiency by a mechanism similar to that observed in our transgenic models.

It is interesting to note that RIP-MCP-1 mice were not diabetic. A similar absence of autoimmune reactivity was observed in mice expressing TNF-α or TNF-β in their islets despite the presence of an intense lymphocyte-rich infiltrate (26, 27, 44). The TNF-recruited lymphocytes could, however, be activated when B7–1 was also expressed on islet cells, suggesting that costimulation is a necessary part of this autoimmune model (45). In the MCP-1-expressing model, elicited monocytes might be capable of costimulation through their own B7–1 if it were expressed at sufficiently high levels, but in this case the infiltrate is nearly devoid of lymphocytes. Thus, one might predict that a combination of TNF expression to recruit lymphocytes and MCP-1 to recruit monocytes would produce diabetes. Alternatively, since TNF-α expression in islets of nonobese diabetic (NOD) mice prevents diabetes and reduces the autoreactivity of splenic lymphocytes, TNF-α may induce anergy in the lymphocytes it so efficiently recruits to the islets (46). Again, expression of B7–1 by MCP-1-elicited monocytes may overcome this effect.

The infiltrates produced in RIP-MCP-1 mice and in several of the models mentioned above indicate that some, but not all, of the in vitro properties of MCP-1 predict its in vivo behavior. For example, although the monocyte chemoattractant properties of MCP-1 in vitro are recapitulated in the islets, there are reports of equipotent T lymphocyte chemoattractant activity that was not apparent here, perhaps for the reasons cited above (11). In addition, MCP-1 has been reported to stimulate a respiratory burst in monocytes in vitro (47), which might be taken as surrogate evidence of monocyte activation, but apparently this did not occur in vivo. Thus, the function of MCP-1 in inflammation may be to attract monocytes, but additional signals are necessary to activate them.

Nonetheless, monocytes must first be attracted to a tissue site before they can be activated there, and this suggests that MCP-1 and/or its receptor may be a reasonable therapeutic target in diseases characterized by monocyte-rich infiltration and MCP-1 expression. Furthermore, despite continual MCP-1 expression throughout the life span of transgenic mice, infiltrates became no more intense than they were in the first few weeks of life. This may suggest the presence of a homeostatic mechanism that dampens the effects of MCP-1 either by blocking the influx of more monocytes or by inducing the departure or apoptosis of resident monocytes to match the numbers of incoming cells. Understanding this counter-regulatory mechanism may provide new methods of interfering with monocyte infiltration in disease.

Acknowledgments

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References

1. Rollins, B. J. 1996. Monocyte chemoattractant protein 1: a potential regulator of monocyte recruitment in inflammatory disease. *Mol. Med. Today 2:198.*
2. Oppenheim, J. J., C. O. C. Zachariae, N. Mukaida, and K. Matsushima. 1991. Properties of the novel proinflammatory supergene "intercrine" family. *Annu. Rev. Immunol. 9:617.*
3. Kelner, G. S., J. Kennedy, K. B. Bacon, S. Kleyensteuber, D. A. Largaespada, N. A. Jenkins, N. G. Copeland, J. F. Bazan, K. W. Moore, T. J. Schall, and A. Zlotnik. 1994. Lymphotactin: a cytokine that represents a new class of chemokine. *Science 266:1395.*
4. Bazan, J. F., K. B. Bacon, G. Hardiman, W. Wang, K. Soo, D. Rossi, D. R. Greaves, A. Zlotnik, and T. J. Schall. 1997. A new class of membrane-bound chemokine with a CX_3C motif. *Nature 385:640.*
5. Cochran, B. H., A. C. Reffel, and C. D. Stiles. 1983. Molecular cloning of gene sequences regulated by platelet-derived growth factor. *Cell 33:939.*
6. Rollins, B. J., E. D. Morrison, and C. D. Stiles. 1988. Cloning and expression of *JE*, a gene inducible by platelet-derived growth factor and whose product has cytokine-like properties. *Proc. Natl. Acad. Sci. USA 85:3738.*
7. Matsushima, K., C. G. Larsen, G. C. DuBois, and J. J. Oppenheim. 1989. Purification and characterization of a novel monocyte chemotactic and activating factor produced by a human myelomonocytic cell line. *J. Exp. Med. 169:1485.*
8. Yoshimura, T., E. A. Robinson, S. Tanaka, E. Appella, and E. J. Leonard. 1989. Purification and amino acid analysis of two human monocyte chemoattractants produced by phytohemagglutinin-stimulated human blood mononuclear leukocytes. *J. Immunol. 142:1956.*
9. Alam, R., M. A. Lett-Brown, P. A. Forsythe, D. J. Anderson-Walters, C. Kenamore, C. Kormos, and J. A. Grant. 1992. Monocyte chemotactic and activating factor is a potent histamine-releasing factor for basophils. *J. Clin. Invest. 89:723.*
10. Kuna, P., S. R. Reddigari, D. Rucinski, J. J. Oppenheim, and A. P. Kaplan. 1992. Monocyte chemotactic and activating factor is a potent histamine-releasing factor for human basophils. *J. Exp. Med. 175:489.*
11. Carr, M. W., S. J. Roth, E. Luther, S. S. Rose, and T. A. Springer. 1994. Monocyte chemoattractant protein 1 acts as a T-lymphocyte chemoattractant. *Proc. Natl. Acad. Sci. USA 91:3652.*
12. Barker, J. N. W. N., M. L. Jones, C. L. Swenson, V. Sarma, R. S. Mitra, P. A. Ward, K. J. Johnson, J. C. Fantone, V. M. Dixit, and B. J. Nickoloff. 1991. Monocyte chemotaxis and activating factor production by keratinocytes in response to IFN-γ. *J. Immunol. 146:1192.*
13. Koch, A. E., S. L. Kunkel, L. A. Harlow, B. Johnson, H. L. Evanoff, G. K. Haines, M. D. Burdick, R. M. Pope, and R. M. Strieter. 1992. Enhanced production of monocyte chemoattractant protein-1 in rheumatoid arthritis. *J. Clin. Invest. 90:772.*
14. Nelken, N. A., S. R. Coughlin, D. Gordon, and J. N. Wilcox. 1991. Monocyte chemoattractant protein-1 in human atheromatous plaques. *J. Clin. Invest. 88:1121.*
15. Yla-Herttuala, S., B. A. Lipton, M. E. Rosenfeld, T. Sarkioja, T. Yoshimura, E. J. Leonard, J. L. Witztum, and D. Steinberg. 1991. Expression of monocyte chemoattractant protein 1 in macrophage-rich areas of human and rabbit atherosclerotic lesions. *Proc. Natl. Acad. Sci. USA 88:5252.*
16. Jones, M. L., M. S. Mulligan, C. M. Flory, P. A. Ward, and J. S. Warren. 1992. Potential role of monocyte chemoattractant protein 1/JE in monocyte/macrophage-dependent IgA immune complex alveolitis in the rat. *J. Immunol. 149:2147.*
17. Flory, C. M., M. L. Jones, and J. S. Warren. 1993. Pulmonary granuloma formation in the rat is partially dependent on monocyte chemoattractant protein 1. *Lab. Invest. 69:396.*
18. Zachariae, C. O. C., A. O. Anderson, H. L. Thompson, E. Appella, A. Mantovani, J. J. Oppenheim, and K. Matsushima. 1990. Properties of monocyte chemotactic and activating factor (MCAF) purified from a human fibrosarcoma cell line. *J. Exp. Med. 171:2177.*
19. Ernst, C. A., Y. J. Zhang, P. R. Hancock, B. J. Rutledge, C. L. Corless, and B. J. Rollins. 1994. Biochemical and biological characterization of murine MCP-1: identification of two functional domains. *J. Immunol. 152:3541.*
20. Rollins, B. J., and M. E. Sunday. 1991. Suppression of tumor formation in vivo by expression of the *JE* gene in malignant cells. *Mol. Cell. Biol. 11:3125.*
21. Bottazzi, B., S. Walter, D. Govoni, F. Colotta, and A. Mantovani. 1992. Monocyte chemotactic cytokine gene transfer modulates macrophage infiltration, growth, and susceptibility to IL-2 therapy of a murine melanoma. *J. Immunol. 148:1280.*
22. Rutledge, B. J., H. Rayburn, R. Rosenberg, R. J. North, R. P. Gladue, C. L. Corless, and B. J. Rollins. 1995. High level monocyte chemoattractant protein-1 expression in transgenic mice increases their susceptibility to intracellular pathogens. *J. Immunol. 155:4838.*
23. Fuentes, M. E., S. Durham K., M. R. Swerdel, A. C. Lewin, D. S. Barton, J. R. Megill, R. Bravo, and S. A. Lira. 1995. Controlled recruitment of monocytes/macrophages to specific organs through transgenic expression of MCP-1. *J. Immunol. 155:5769.*
24. Sarvetnick, N., J. Shizuru, D. Liggitt, L. Martin, B. McIntyre, A. Gregory, T. Parslow, and T. Stewart. 1990. Loss of pancreatic islet tolerance induced by beta-cell expression of interferon-γ. *Nature 346:844.*
25. DiCosmo, B. F., D. Picarella, and R. A. Flavell. 1994. Local production of human IL-6 promotes insulitis but retards the onset of insulin-dependent diabetes mellitus in non-obese diabetic mice. *Int. Immunol. 6:1829.*
26. Picarella, D. E., A. Kratz, C. B. Li, N. H. Ruddle, and R. A. Flavell. 1993. Transgenic tumor necrosis factor (TNF)-α production in pancreatic islets leads to insulitis, not diabetes: distinct patterns of inflammation in TNF-α and TNF-β transgenic mice. *J. Immunol. 150:4136.*
27. Picarella, D. E., A. Kratz, C. B. Li, N. H. Ruddle, and R. A. Flavell. 1992. Insulitis in transgenic mice expressing tumor necrosis factor beta (lymphotoxin) in the pancreas. *Proc. Natl. Acad. Sci. USA 89:10036.*
28. Lee, M. S., R. Mueller, L. S. Wicker, L. B. Peterson, and N. Sarvetnick. 1996. IL-10 is necessary and sufficient for autoimmune diabetes in conjunction with NOD MHC homozygosity. *J. Exp. Med. 183:2663.*
29. Hogan, B., F. Costantini, and E. Lacy. 1986. *Manipulating the Mouse Embryo: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
30. Laird, P. W., A. Zijderveld, K. Linders, M. A. Rudnicki, R. Jaenisch, and A. Berns. 1991. Simplified mammalian DNA isolation procedure. *Nucleic Acids Res. 19:4293.*
31. Luo, Y., J. Laning, M. Hayashi, P. R. Hancock, B. Rollins, and M. E. Dorf. 1994. Serologic analysis of the mouse β chemokine JE/monocyte chemoattractant protein-1. *J. Immunol. 153:3708.*
32. Falk, W., R. H. J. Goodwin, and E. J. Leonard. 1980. A 48-well micro chemotaxis assembly for rapid and accurate measurement of leukocyte migration. *J. Immunol. Methods 33:239.*
33. Yoshimura, T., and E. J. Leonard. 1990. Secretion by human fibroblasts of monocyte chemoattractant protein-1 (MCP-1), the product of gene JE. *J. Immunol. 144:2377.*
34. Rollins, B. J., P. Stier, T. E. Ernst, and G. G. Wong. 1989. The human homologue of the *JE* gene encodes a monocyte secretory protein. *Mol. Cell. Biol. 9:4687.*
35. Leonard, E. J., and A. Skeel. 1985. Disposable microliter immunoabsorbent columns: construction and operation. *J. Immunol. Methods 82:341.*
36. Loetscher, P., M. Seitz, M. Baggiolini, and B. Moser. 1996. Interleukin-2 regulates CC chemokine receptor expression and chemotactic responsiveness in T lymphocytes. *J. Exp. Med. 184:569.*
37. Nakamura, K., I. R. Williams, and T. S. Kupper. 1995. Keratinocyte-derived monocyte chemoattractant protein 1 (MCP-1): analysis in a transgenic model demonstrates MCP-1 can recruit dendritic and Langerhans cells to skin. *J. Invest. Dermatol. 105:635.*
38. Hechtman, D. H., M. I. Cybulsky, H. J. Fuchs, J. B. Baker, and M. A. Gimbrone. 1991. Intravascular IL-8: inhibitor of polymorphonuclear leukocyte accumulation at sites of acute inflammation. *J. Immunol. 147:883.*

39. Bossink, A. W., L. Paemen, P. M. Jansen, C. E. Hack, L. G. Thijs, and J. Van Damme. 1995. Plasma levels of the chemokines monocyte chemotactic proteins-1 and -2 are elevated in human sepsis. *Blood 86:3841.*

40. Sylvester, I., A. F. Suffredini, A. J. Boujoukos, G. D. Martich, R. L. Danner, T. Yoshimura, and E. J. Leonard. 1993. Neutrophil attractant protein-1 and monocyte chemoattractant protein-1 in human serum: effects of intravenous lipopolysaccharide on free attractants, specific IgG autoantibodies and immune complexes. *J. Immunol. 151:3292.*

41. Noris, M., S. Bernasconi, F. Casiraghi, S. Sozzani, E. Gotti, G. Remuzzi, and A. Mantovani. 1995. Monocyte chemoattractant protein-1 is excreted in excessive amounts in the urine of patients with lupus nephritis. *Lab. Invest. 73:804.*

42. Rovin, B. H., N. Doe, and L. C. Tan. 1996. Monocyte chemoattractant protein-1 levels in patients with glomerular disease. *Am. J. Kidney Dis. 27:640.*

43. Wada, T., H. Yokoyama, S. B. Su, N. Mukaida, M. Iwano, K. Dohi, Y. Takahashi, T. Sasaki, K. Furuichi, C. Segawa, Y. Hisada, S. Ohta, K. Takasawa, K. Kobayashi, and K. Matsushima. 1996. Monitoring urinary levels of monocyte chemotactic and activating factor reflects disease activity of lupus nephritis. *Kidney Int. 49:761.*

44. Higuchi, Y., P. Herrera, P. Muniesa, J. Huarte, D. Belin, P. Ohashi, P. Aichele, L. Orci, J. D. Vassalli, and P. Vassalli. 1992. Expression of a tumor necrosis factor-α transgene in murine pancreatic beta cells results in severe and permanent insulitis without evolution towards diabetes. *J. Exp. Med. 176:1719.*

45. Guerder, S., D. E. Picarella, P. S. Linsley, and R. A. Flavell. 1994. Costimulator B7–1 confers antigen-presenting-cell functions to parenchymal tissue and in conjunction with tumor necrosis factor α leads to autoimmunity in transgenic mice. *Proc. Natl. Acad. Sci. USA 91:5138.*

46. Grewal, I. S., K. D. Grewal, F. S. Wong, D. E. Picarella, C. A. J. Janeway, and R. A. Flavell. 1996. Local expression of transgene encoded TNFα in islets prevents autoimmune diabetes in NOD mice by preventing the development of autoreactive islet specific T cells. *J. Exp. Med. 184:1963.*

47. Rollins, B. J., A. Walz, and M. Baggiolini. 1991. Recombinant human MCP-1/JE induces chemotaxis, calcium flux, and the respiratory burst in human monocytes. *Blood 78:1112.*