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High Level Monocyte Chemoattractant Protein-1 Expression in Transgenic Mice Increases Their Susceptibility to Intracellular Pathogens[1]

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We have constructed transgenic mice in which the mouse mammary tumor virus long terminal repeat controls the expression of murine monocyte chemoattractant protein-1 (MCP-1). Several independently derived lines of transgenic mice constitutively expressed MCP-1 protein in a variety of organs. Protein extracts from these organs had substantial in vitro monocyte chemoattractant activity that was neutralized by an anti-MCP-1 Ab, indicating that transgenic MCP-1 protein is biologically active. However, no transgenic mouse at any age displayed monocyte infiltrates in MCP-1-expressing organs. Two transgenic lines had circulating MCP-1 levels of 13 to 26 ng/ml, which is a concentration sufficient to induce maximal monocyte chemotaxis in vitro. These transgenic lines showed a 1 to 1.5 log greater sensitivity to infection with *Listeria monocytogenes* and *Mycobacterium tuberculosis*. A third transgenic line had lower serum levels of MCP-1 and was resistant to *L. monocytogenes*. The results suggest that this transgenic model is one of monocyte nonresponsiveness to locally produced MCP-1 due to either receptor desensitization or neutralization of a chemoattractant gradient by high systemic concentrations of MCP-1. Regardless of the mechanism, the data indicate that constitutively high levels of MCP-1 expression do not induce monocytic infiltrates, and that MCP-1 is involved in the host response to intracellular pathogens. *The Journal of Immunology,* **1995, 155: 4838–4843.**

Leukocyte infiltration into tissues is the result of a complex set of molecularly distinguishable signals (1, 2). First, a circulating leukocyte must adhere loosely to the vascular endothelium, then in the presence of an appropriate stimulus, this adhesion becomes firm. Finally, the leukocyte will follow a concentration gradient of chemoattractant as it diapedeses to enter the inflammatory site. The fact that particular leukocyte subsets appear in an infiltrate to the exclusion of others suggests that the signals have target cell specificity. Although some specificity may be exerted through adhesion molecules with restricted patterns of leukocyte expression, there is increasing evidence that chemoattractant signals are also specific for leukocyte subsets.

Recently, a family of leukocyte-specific chemoattractants, called chemokines, has been described whose members may play important roles in generating inflammatory cell infiltrates (3, 4). Chemokines are low m.w. proteins that share a high degree of structural and genetic similarity, in particular four cysteines in conserved positions. Within the large chemokine family, two subfamilies can be distinguished based on the presence or the absence of an intervening amino acid between the two cysteines nearest the N-terminals. The former subfamily is denoted C-X-C, and the latter family is C-C. This structural distinction is reflected at a genetic level, since, to date, all of the genes that encode C-X-C chemokines map to the long arm of chromosome 4, while all of the C-C chemokine genes map to the long arm of chromosome 17.

There is also a partial functional distinction between the chemokine subfamilies. Although there are exceptions, most C-X-C chemokines, e.g., IL-8 and the GRO proteins, specifically attract neutrophils. In contrast, most C-C chemokines attract monocytes and memory T lymphocytes and, in some cases, eosinophils and basophils. Among the C-C chemokines, MCP-1[4] has been the most intensively studied. In vitro, MCP-1 attracts monocytes and memory T cells at subnanomolar concentrations (5–7) and is a potent stimulator of histamine release from mast cells (8, 9). Its expression has been documented in several diseases that are characterized by monocyte-rich inflammatory cell infiltrates, leading to the suggestion that MCP-1 expression may play a pathogenetic role in diseases as diverse as rheumatoid arthritis and atherosclerosis (10–12). To date, however, it has been difficult to demonstrate conclusively that abnormally regulated MCP-1 expression is responsible for disease.

Part of the difficulty in ascribing pathogenetic roles to MCP-1 is uncertainty about its in vivo properties. In some animal models, intradermal injection of human MCP-1 produces an accumulation of monocytes at the inoculation site (13, 14). However, we have been unable to induce infiltrates in mouse skin in response to pure murine MCP-1 (15). One reason for the absence of infiltrates may have been an inability to supply high concentrations of MCP-1 at a local site for sufficiently long periods of time. To create a model that might mimic sustained MCP-1 synthesis during an inflammatory response, we constructed transgenic mice in which murine

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[4] Abbreviations used in this paper: MCP-1, monocyte chemoattractant protein-1; MMTV-LTR, mouse mammary tumor virus long terminal repeat; CAPS, 3-[cyclohexylamino]-1-propanesulfonic acid.

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MCP-1 expression was controlled by a constitutive heterologous promoter. As it turned out, this was a poor model of physiologic MCP-1 release at local sites, since these mice produced high levels of MCP-1 in several organs throughout the animals' lifetimes. Nonetheless, the phenotype of these transgenic mice is one of increased susceptibility to intracellular pathogens, and they are, thus, informative as a model of monocyte dysfunction due perhaps in part to desensitization to MCP-1. These results point to the involvement of MCP-1 in physiologic pathways that are required for the elimination of intracellular pathogens.

Materials and Methods

Transgenic mice

Genomic MCP-1 DNA (pGMJE-1) (16) was modified by substituting the *Xba*I to *Ppu*MI fragment from murine MCP-1 cDNA (pcJE-1) (16) for the *Xba*I to *Ppu*MI fragment in pGMJE-1. This manipulation removed genomic MCP-1's mRNA cap site and placed an *Eco*RI site at the 5′ end of the remaining MCP-1 DNA, a position +20 relative to the cap site. The *Eco*RI fragment from this construct (which contains genomic MCP-1 from +20 to +2000 bp beyond the polyadenylation signal) was placed downstream from the *Ava*I to *Bam*HI fragment of the MMTV-LTR (17) to yield pMMJE+20, in which the MMTV-LTR cap site is 121 bp 5′ to the first nucleotide of modified genomic MCP-1. Thus, the final construct contains the MMTV-LTR promoter and its mRNA cap fused to genomic murine MCP-1 at a point 20 bp downstream from its own cap site. There were no manipulations of MCP-1's coding sequences.

Linearized transgene DNA, trimmed of most of its plasmid vector sequences, was used to inject oocytes from FVB/N mice according to standard techniques (18). After transfer to foster mothers, progeny were tested for the presence of the transgene at 3 wk of age by Southern blot analysis. DNA was extracted from tail samples (19), and Southern blots were probed with a radiolabeled *Nae*I-*Hpa*I fragment derived from genomic murine MCP-1 DNA. Transgene copy number was estimated by comparing the intensity of a transgene signal (as determined by laser densitometry (Pharmacia, Piscataway, NJ)) to that of an endogenous gene signal. Mice were killed at various ages, and tissue sections were prepared for routine hematoxylin and eosin staining and for immunohistochemistry.

RNA analysis

Organs were harvested and homogenized in 1 ml of 4 M guanidine isothiocyanate, 25 mM sodium citrate (pH 7.0), and 100 mM 2-ME. RNA was purified from the homogenate by centrifugation through a cushion of 5.7 M cesium chloride, followed by precipitation with ethanol (20). Twenty micrograms of RNA was fractionated by electrophoresis through a 1.5% agarose gel containing 2.2 M formaldehyde and transferred to nylon-based membranes. Gels were stained with ethidium bromide and photographed to document the intensity of ribosomal RNA in each lane. Membranes were probed using standard techniques (21) with murine MCP-1 cDNA (16) radiolabeled with [^{32}P]deoxynucleotides by nick translation.

Immunoblot analysis

Organs were placed in 1 ml of 60 mM Tris-HCl (pH 6.8), 100 mM DTT, and 2% SDS; boiled for 5 min; sonicated; and clarified by centrifugation. Proteins were separated on 15% SDS-PAGE, then blotted to Immobilon filters (Millipore Corp., Danvers, MA) in 10 mM CAPS (pH 11.0) and 10% methanol. MCP-1 protein was identified using rabbit anti-mouse MCP-1 serum as previously described (15, 22).

In vitro monocyte chemotaxis

Salivary glands from control or transgenic mice were placed in 1 ml of cold 50 mM Tris-HCl (pH 7.5)-0.2 mM EDTA and homogenized on ice. Extracts were clarified by centrifugation at 15,000 × *g* for 30 min, followed by a second centrifugation at 100,000 × *g* for 45 min. Serial dilutions were tested for human monocyte chemoattractant activity using a 48-well microchamber apparatus as previously described (23). Concentration of monocyte chemoattractant activity in monocyte chemoattractant activity units per ml was defined as the inverse of the dilution demonstrating half-maximal activity (24). Immunoadsorption of MCP-1 from the extracts was performed using rabbit anti-mouse MCP-1 as previously described (25).

Listeria monocytogenes *infection*

Mice were injected i.p. with the indicated inocula of virulent *L. monocytogenes* (strain 1778^{+}, American Type Culture Collection designation 43251, obtained from Dr. Eric Pamer, Yale University, New Haven, CT). Mice were observed daily and killed if they were unable to right themselves or reach water.

Mycobacterium tuberculosis infection

Mice were injected i.v. with 8×10^4 CFU of strain H37Rv. Forty days after inoculation, mice were killed, and dilutions of homogenates from lungs, livers, and spleens were plated on enriched agar plates (Middlebrook 7H11, Difco Laboratories, Detroit, MI). Colonies were counted after 3 to 4 wk.

IL-1 challenge

Animals were anesthetized using inhaled methoxyflurane, and 200 ng of murine IL-1β (Genzyme, Boston, MA) was injected intradermally into the left footpad using a 27-gauge needle. An equivalent volume of sterile PBS was injected into the right footpad. Twenty-four and forty-eight hours after injection, animals were killed by CO_2 inhalation, and footpads were processed for histologic analysis.

Murine MCP-1 ELISA

Plates (Nunc MaxiSorp "C" bottom 96-well plates, VWR, Boston, MA) were coated with 0.2 μg of MCP-1 capture Ab (18241D, PharMingen, San Diego, CA), diluted in 0.1 M $NaHCO_3$ overnight at 4°C, then blocked with 3% BSA-PBS. Samples and standards were added in duplicate, and the plate was incubated for 4 h at room temperature. After washing, biotinylated detecting Ab (18272D, PharMingen) was added at 0.2 μg/well for 45 min, followed, after washing, by the addition of 0.25 μg/well avidin-peroxidase (Sigma Chemical Co., St. Louis, MO) for 30 min. The ABTS microwell peroxidase substrate system (Kirkegaard and Perry, Gaithersburg, MD) was used for detection. Results were read at 405 nm using a microtiter plate reader (Molecular Devices, Sunnyvale, CA), and a standard curve was generated. The minimum detection limit for MCP-1 in the assay was 0.50 to 0.75 ng/ml.

Results

Expression of MCP-1 in transgenic mice

As described in *Materials and Methods*, we constructed a transgene in which murine genomic MCP-1 DNA was placed downstream from a fragment of the MMTV-LTR. Using Southern blotting, we identified 11 founder lines, most of which had one to five copies of the transgene per diploid genome, as determined by scanning analysis of Southern blot autoradiographs. However, one line, denoted Tg33, had at least 30 copies of the transgene, and this high copy number was stably inherited.

Of the 11 founder lines, 5 lines (Tg21C, Tg24, Tg25, Tg33, and Tg59) expressed transgene mRNA that was distinguishable from wild-type mRNA by its increased size (data not shown) and because MCP-1 mRNA is not expressed constitutively in any organ of wild-type FVB mice. Figure 1 shows expression of transgenic MCP-1 mRNA in four of these lines. The pattern of expression was consistent with the reported properties of the MMTV-LTR, namely high levels of expression in the salivary glands of three lines (17). Expression in other organs, in particular lung, also occurred in some lines. The pattern of expression in line Tg33 (the line with a high number of tandem insertions) was remarkable for its low levels of expression in salivary gland and high levels in kidney.

We documented MCP-1 protein expression in some of the organs that expressed transgene mRNA. Figure 2 shows an immunoblot analysis of salivary gland, lung, and testis extracts from a control mouse and three transgenic mice demonstrating high levels of the fully glycosylated forms of murine MCP-1 (15) in transgenic salivary glands. (MCP-1 protein expression was also documented in the salivary glands of the remaining two transgenic lines that expressed transgenic mRNA (data not shown).) Lower amounts of MCP-1 protein were expressed in lungs and testes of two of the three lines. We have never detected constitutive MCP-1 protein expression in any organ from nontransgenic mice. This analysis was performed on boiled extracts from transgenic organs

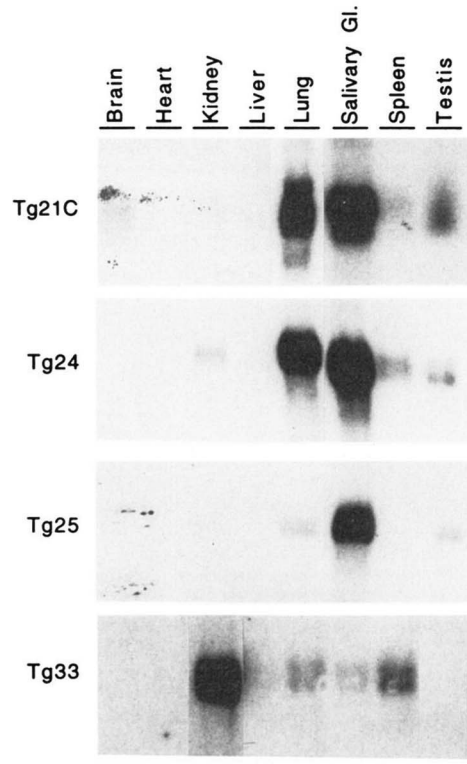

FIGURE 1. RNA blot analysis of MCP-1 expression in transgenic mice. RNA was purified from the indicated organs of four transgenic mouse lines and analyzed by Northern blotting as described in *Materials and Methods*. The analysis of Tg21C is a composite of two blots, with the lung lane taken from another blot in which the levels of lung RNA more closely matched the RNA levels from other organs, as determined by the intensity of ethidium bromide-stained ribosomal RNA. By the same criteria, the kidney lane from Tg33 was four- to fivefold overloaded compared with the other lanes.

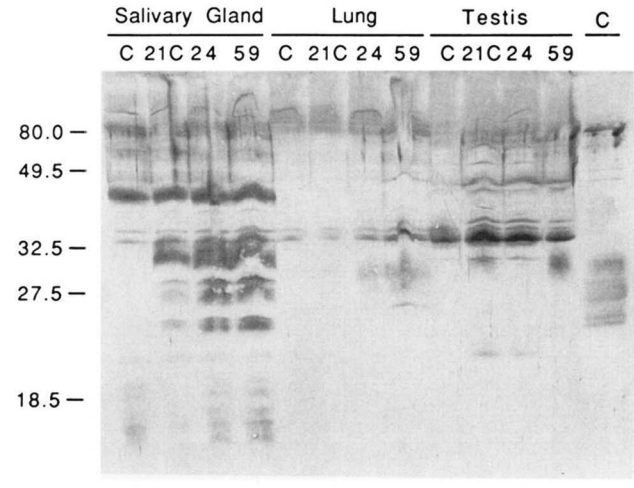

FIGURE 2. Immunoblot analysis of MCP-1 expression in transgenic mice. Protein was extracted from salivary gland, lung, and testis of a nontransgenic mouse (C) and one mouse from each of three transgenic lines (Tg21C, Tg24, and Tg59). Immunoblot analysis was performed as described in *Materials and Methods*. The lane labeled C contains recombinant murine MCP-1 produced in Chinese hamster ovary cells as a positive control (15) and shows the heterogeneously glycosylated MCP-1 proteins of approximately 23,000 to 29,000 M_r. There is no detectable MCP-1 expression in the organs of nontransgenic mice. Transgenic MCP-1 is expressed in the salivary glands of Tg21C, Tg24, and Tg59; in the lung of Tg24 and Tg59; and in the testis of Tg21C and Tg59.

to insure that material bound to connective tissue elements would be released. However, experiments on nondenatured extracts (next section) demonstrated that substantial amounts of MCP-1 were freely soluble.

Activity of transgenic MCP-1

The functional integrity of transgenic MCP-1 protein was determined by testing salivary gland extracts in a monocyte chemotaxis assay. (Figure 3, *left*) compares monocyte chemoattractant activity in an extract from a Tg24 salivary gland to activity in an extract from a nontransgenic littermate. The transgenic extract had over 8000 times the monocyte chemoattractant activity of the nontransgenic extract (22.2 U/mg total protein compared with 0.0026 U/mg total protein). Furthermore, immunoadsorption with an anti-MCP-1 Ab removed nearly 90% of the monocyte chemoattractant activity from the transgenic extract (Fig. 3, *right*).

Histologic and hematologic analysis of transgenic mice

We examined organs from transgenic mice, aged 2 to 52 wk. Despite the expression of high levels of biologically active MCP-1, there were no histologic abnormalities in any organ of any transgenic mouse that were not also present in nontransgenic littermates. In particular, we observed no monocyte infiltrates in any organs either by examination of routine hematoxylin and eosin sections or by immunohistochemical analysis using the murine monocyte/macrophage-specific Ab F4/80 (26).

A small proportion (<10%) of Tg33 mice developed a fatal disorder characterized by profound leukocytosis with extramedullary hematopoiesis and ulcerative proctocolitis. Some mice developed WBC counts of 100,000/μl, comprised almost entirely of mature neutrophils. The occurrence of this phenotype in a single line suggests that it may have been due to insertional mutagenesis, although this line also had the highest number of transgene copies and the highest levels of circulating MCP-1 (see below). The basis for this phenotype is still under investigation. All other transgenic lines had normal blood counts.

In vivo challenges

There are several possible explanations for the lack of monocyte infiltration in organs that constitutively expressed transgenic MCP-1. First, MCP-1 expression could be necessary, but not sufficient to elicit an infiltrate. To test this hypothesis, we administered varying doses of LPS or IL-4 to transgenic mice, but neither of these costimulants produced monocyte infiltration in expressing organs. Second, transgenic mice may be incapable of mounting any sort of inflammatory cell response. To test this, we challenged transgenic and nontransgenic mice by injection of murine IL-1β into the footpad. Figure 4 shows that transgenic and nontransgenic mice responded to IL-1β injection with cellular infiltrates of equal intensity, comprised of neutrophils and mononuclear cells.

Another explanation for the apparent absence of an abnormal phenotype is that transgenic mice secreted such high levels of MCP-1 that their circulating monocytes could not respond, because of either monocyte desensitization or neutralization of the chemoattractant concentration gradient. This model would predict measurable levels of MCP-1 in the blood of transgenic mice. In fact, Table I shows that Tg24 and Tg33 mice had serum levels of MCP-1 ranging from 13 to 26 ng/ml; however, a third line, Tg25, had only 4 ng/ml. As expected, control mice had less than 1.0 ng/ml. The levels of circulating MCP-1 in Tg24 and Tg33 mice are

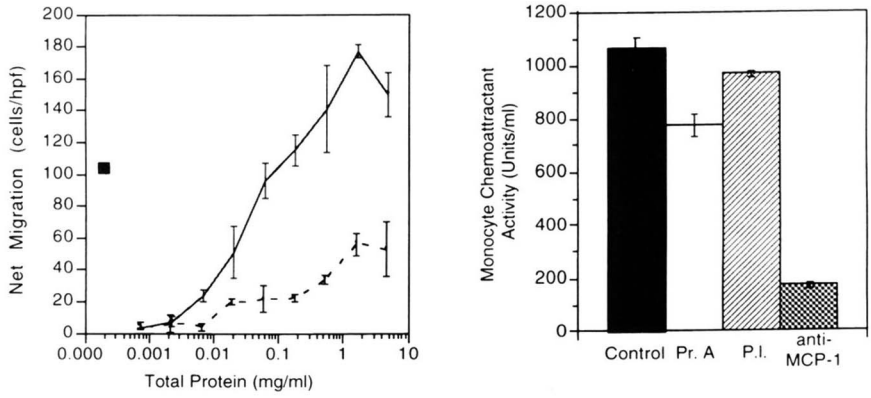

FIGURE 3. Biologic activity of transgenic MCP-1. *Left,* Protein was extracted from the salivary glands of a Tg24 mouse and a nontransgenic littermate. Serial dilutions of each extract were tested for their ability to attract human monocytes in vitro as described in *Materials and Methods.* Solid line, Tg24 extract; dotted line, nontransgenic extract; square, response to 10^{-8} M FMLP. Error bars are the SD of two data sets that are the average of counts in five high power fields. The experiment shown is representative of two. *Right,* Aliquots of protein extract from a Tg24 salivary gland were applied to microcolumns containing protein A-Sepharose beads (Pr. A), preimmune Ig adsorbed to protein A-Sepharose (P.I.), or anti-MCP-1 Ig bound to protein A-Sepharose (anti-MCP-1). Eluates were tested for monocyte chemoattractant activity as described in *Materials and Methods* and compared with untreated extract (Control). Error bars represent the SD of two data sets that are the average of counts in five high power fields. The experiment shown is representative of two.

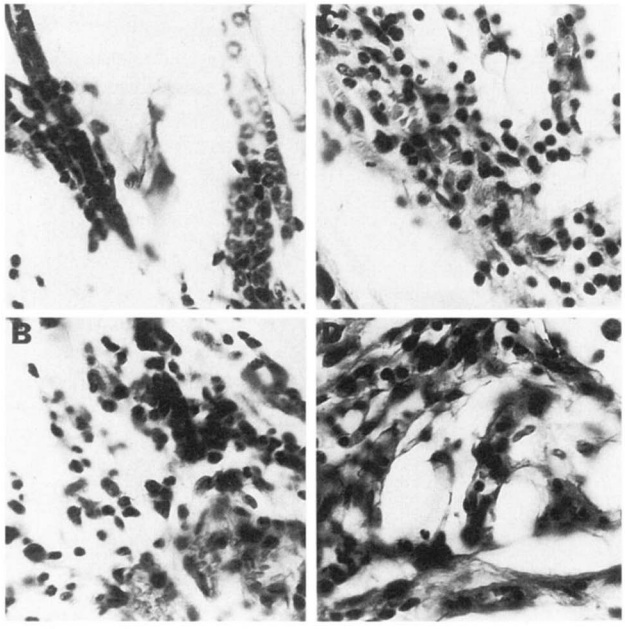

FIGURE 4. Cellular response to IL-1β. Footpads of transgenic and nontransgenic mice were injected with 200 ng of murine IL-1β and analyzed histologically 24 and 48 h later. These are hematoxylin- and eosin-stained sections at ×200 magnification. *A,* Nontransgenic at 24 h; *B,* nontransgenic at 48 h; *C,* Tg24 at 24 h; *D,* Tg24 at 48 h. Note the presence of polymorphonuclear and mononuclear cells in all infiltrates. Similar results were obtained in four transgenic and four nontransgenic animals.

the same as those that elicit a maximal monocyte chemotaxis response in vitro, and the level in Tg25 mice is near the EC_{50} (15).

Response to intracellular pathogens

Transgenic mice (except for the small proportion of Tg33 mice) are healthy and long-lived in pathogen-free conditions, and they are capable of mounting histologically normal foreign body responses to material in surgical wounds (data not shown). This suggests that their monocytes are not completely inactivated. However, some of the transgenic mice are deficient in their responses

Table I. *Serum concentrations of MCP-1* [a]

	MCP-1 (ng/ml)[b]
Wild-type (2)[c]	<1.0
Tg24 (3)	13.1 ± 4.9
Tg25 (2)	4.1 ± 4.7
Tg33 (3)	25.6 ± 1.2

[a] Serum concentration of MCP-1 measured by ELISA, as described in *Materials and Methods.*
[b] Concentration ± standard deviation.
[c] Number of mice tested in each group.

to intracellular pathogens. Figure 5 shows survival curves for wild-type, Tg24, and Tg33 mice after *L. monocytogenes* inoculation. The LD_{50} for both transgenic lines ($\sim 5 \times 10^4$) is more than 10-fold lower than that for wild-type mice. Tg25 mice survived *L. monocytogenes* challenge as well as wild-type mice.

To test whether the increased susceptibility of Tg24 mice could be generalized to other intracellular pathogens, we challenged them with *M. tuberculosis* inoculation, then enumerated bacteria in several organs 40 days after injection. Table II shows that the number of CFU in the organs of transgenic mice was 0.5 to 1.5 logs greater than that in wild-type mice.

Discussion

To correlate the chemoattractant properties of MCP-1 in vitro with in vivo observations and to determine whether MCP-1 might have other activities, we constructed the transgenic mice described in this report. Surprisingly, despite the expression of high levels of biologically active protein, organs that expressed MCP-1 constitutively were not ravaged by monocytic infiltration. One explanation is that the MMTV-LTR drove transgene expression at such high levels that MCP-1 entered the bloodstream and inactivated monocyte responses to locally produced MCP-1. The correlation we observed between high levels of circulating MCP-1 and increased sensitivity to *L. monocytogenes* and *M. tuberculosis* supports this idea. However, one transgenic line (Tg25) with lower levels of circulating MCP-1 and wild-type *Listeria* resistance did not display monocyte infiltrates despite high levels of locally produced MCP-1. Although its levels were lower than those in Tg24 and Tg33, this line did have circulating

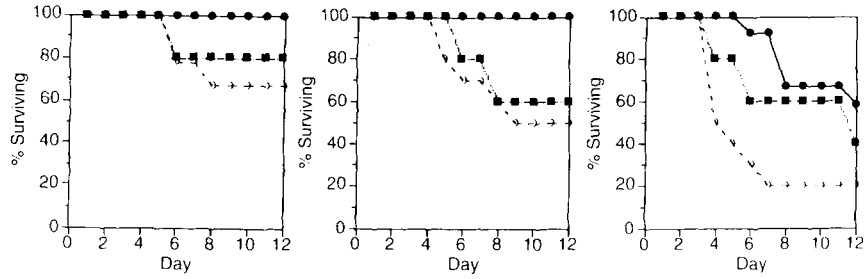

FIGURE 5. Survival of transgenic mouse strains after *L. monocytogenes* inoculation. Transgenic (lines Tg24 and Tg33) and nontransgenic mice of similar ages were administered three different inocula of virulent *L. monocytogenes* i.p., and survival was monitored daily. *Left*, Inoculum of 5×10^3 bacteria, 9 nontransgenic mice, 9 Tg24 mice, and 5 Tg33 mice. *Middle*, Inoculum of 5×10^4 bacteria, 11 nontransgenic mice, 10 Tg24 mice, and 5 Tg33 mice. *Right*, Inoculum of 5×10^5 bacteria, 12 nontransgenic mice, 10 Tg24 mice, and 5 Tg33 mice. ●, Nontransgenic; ■, Tg24; ○, Tg33. The survival of transgenic mice was significantly worse that that of wild-type mice at all inocula by application of the exact version of the Breslow-Day test ($p = 0.0004$ for Tg24; $p = 0.05$ for Tg33) (35).

Table II. *Colony-forming units in the organs of mice inoculated with* M. tuberculosis[a]

	Log CFU		
	Spleen	Liver	Lung
Wild-type (5)[b]	5.45 ± 0.17[c]	5.93 ± 0.11	5.59 ± 0.43
Transgenic (6)	7.01 ± 0.12[d]	6.79 ± 0.12[d]	6.50 ± 0.06[d]

[a] Mice were inoculated intravenously with 8×10^4 CFU of *M. tuberculosis* (strain H37Rv), and sacrificed on day 40, at which time CFU were enumerated in their lungs, livers, and spleens.
[b] Number of mice per group in parentheses.
[c] CFU ± standard deviation.
[d] Difference from wild-type significant at $p \leq 0.0005$ by Student's *t*-test.

levels of MCP-1 which were at the EC_{50} for MCP-1-mediated monocyte chemotaxis in vitro. This amount may have been sufficient to prevent monocyte infiltration, but not high enough to produce the phenotype of increased *L. monocytogenes* susceptibility.

We have not determined whether the systemic inactivation of monocytes in some lines is due to receptor desensitization or simply to the physical loss of a chemoattractant gradient. In the former case, high levels of MCP-1 produced in specific organs may place monocytes on the descending limb of the bell-shaped chemoattractant response curve. One way to distinguish between these possibilities would be to test transgenic monocytes for their ability to respond chemotactically to MCP-1 in vitro. Because of the small number of circulating monocytes in the mouse, we have been unable to perform this experiment. However, regardless of the mechanism, our results are analogous to those of Simonet et al., who generated transgenic mice with circulating levels of IL-8 and diminished capacity to respond to inflammatory stimuli (27). These results are also reminiscent of the ability of i.v. injected IL-8 to prevent the accumulation of neutrophils in response to locally injected IL-8 (28, 29). In contrast, Lira et al. generated mice in which KC (a neutrophil-specific C-X-C chemokine) was expressed only in thymus and led to neutrophil infiltration in that organ (30). This suggests that lower levels of MCP-1 expression restricted to a single anatomical site might also induce monocyte infiltration. In fact, we are aware of three transgenic models in which highly localized production of MCP-1 produces monocyte infiltration in the thymus, brain (S. Lira, personal communication), and pancreas (B. R. Rutledge, R. M. Flavell, and B. J. Rollins, manuscript in preparation).

Our model does permit some inferences to be made about the physiologic functions of MCP-1 in vivo. The greater sensitivity to intracellular pathogens in transgenic mice suggests that MCP-1 is involved in critical monocyte- or T lymphocyte-dependent activities required for ridding the host of these organisms. It is currently unclear whether these activities involve leukocyte trafficking to infected sites or intracellular processes necessary for killing bacteria. Either way, MCP-1 appears to play only a partial role in these responses and may be compensated by other chemokines. For example, the magnitude of the increased sensitivity of MCP-1 transgenic mice to *L. monocytogenes* is similar to that of IFN-γ receptor-negative mice (31) or that of IL-12-neutralized mice (32). In contrast, mice lacking the 55-kDa TNF receptor-1 are 4 to 5 logs more sensitive (33, 34). Thus MCP-1 contributes to natural immunity, but in this context, it appears to do so to a lesser extent than TNF.

The in vitro properties of chemokines have suggested their pathogenetic involvement in diseases characterized by inflammatory cell infiltrates. Their in vivo properties have been more difficult to determine, but the current studies suggest that MCP-1 may be involved in fundamental aspects of monocyte-mediated host defense. Although we suggest that this transgenic model is one of monocyte desensitization to MCP-1, a more reliable model of unresponsiveness to MCP-1 will be MCP-$1^{-/-}$ mice created by targeted gene disruption. However, the phenotype of MMTV-MCP-1 transgenic mice may be distinct from the MCP-$1^{-/-}$ model to whatever extent the former mice may have desensitized their MCP-1 receptors. In this case, the transgenic overexpressors will be desensitized to MCP-3 and any other as yet uncharacterized chemokines that activate the MCP-1 receptor. Targeted disruption of MCP-1 should generate a much more limited defect, which may have a different phenotype from that of the mice described in this report.

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