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Total absence of colony-stimulating factor 1 in the macrophage-deficient osteopetrotic (*op/op*) mouse

(macrophage growth factor/mouse mutant/osteopetrosis/macrophage deficiency)

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ABSTRACT Osteopetrotic (*op/op*) mutant mice suffer from congenital osteopetrosis due to a severe deficiency of osteoclasts. Furthermore, the total number of mononuclear phagocytes is extremely low in affected mice. Serum, 11 tissues, and different cell and organ conditioned media from *op/op* mice were shown to be devoid of biologically active colony-stimulating factor 1 (CSF-1), whereas all of these preparations from littermate control +/+ and +/*op* mice contained the growth factor. The deficiency was specific for CSF-1 in that serum or conditioned media from *op/op* mice possessed elevated levels of at least three other macrophage growth factors. Partial correction of the *op/op* defect was observed following intraperitoneal implantation of diffusion chambers containing L929 cells, which in culture produce CSF-1 as their sole macrophage growth factor. No rearrangement of the CSF-1 gene in *op/op* mice was detected by Southern analysis. However, in contrast to control lung fibroblasts, which contained 4.6- and 2.3-kilobase CSF-1 mRNAs, only the 4.6-kilobase species was detected in *op/op* cells. An alteration in the CSF-1 gene is strongly implicated as the primary defect in *op/op* mice because they do not contain detectable CSF-1, their defect is correctable by administration of CSF-1, the *op* locus and the CSF-1 gene map within the same region of mouse chromosome 3, their CSF-1 mRNA biosynthesis is altered, and the *op/op* phenotype is consistent with the phenotype expected in a CSF-1 deficient mouse.

Mice homozygous for the *op* mutation mapping on chromosome 3 suffer from congenital osteopetrosis due to a severe deficiency of osteoclasts (1, 2) and macrophages (3). They are smaller and more stubby in appearance than their normal littermates, possessing extensive skeletal deformities and a lower body weight. They also have a lower life-span and very poor breeding performance (2). The unimpaired ability of macrophage progenitors from osteopetrotic (*op/op*) mice to generate macrophages *in vitro* when incubated with macrophage growth factors (3) suggests that the deficiency of macrophages *in vivo* is caused by the absence or deficiency of a macrophage growth factor and/or an overabundance of macrophage growth inhibitor. Significantly, impairment of macrophage colony-stimulating factor 1 (CSF-1) production by cultured calvaria of *op/op* mice has been recently reported (4). If the defect in the *op/op* mouse could eventually be pinpointed to a systemic deficiency of a specific macrophage growth factor or to elevated levels of a growth factor inhibitor, the *op/op* mouse could serve as an excellent model in which to pursue the biology of growth factor or growth factor inhibitor expression.

In this paper, we report that *op/op* mice have a systemic and specific deficiency of CSF-1. No inhibitor of macrophage growth could be detected and the deficiency of macrophages, their progenitors, and osteoclasts in *op/op* mice could be partially corrected by implantation of diffusion chambers containing CSF-1-producing cells. These findings, in conjunction with an observed alteration in CSF-1 mRNA splicing, and other published data (5–7), suggest that the deficiency of CSF-1 may be the primary cause of osteopetrosis and macrophage deficiency in the *op/op* mouse and support previous evidence (7, 8) indicating that CSF-1 is the macrophage growth factor primarily responsible for normal macrophage and osteoclast maturation *in vivo*.

MATERIALS AND METHODS

Production of *op/op* Mice and +/+ and +/*op* Control Mice. White-bellied agouti (C57BL/6J-A^{W-J}/A^{W-J} × C3HeB/FeJ)F_1 females bearing transplanted nonagouti, osteopetrotic (*a/a op/op*) ovaries and nonagouti males (C57BL/6J × C3HeB/FeJ-a/a)F_1 were mated to produce known +/*op* heterozygotes which were purchased as breeding pairs from The Jackson Laboratory. These heterozygotes were then crossed to produce *op/op* and normal littermate controls (+/+ or +/*op*). The *op/op* mice, which are distinguished from their phenotypically normal +/+ and +/*op* littermates by an absence of incisors and a characteristic skull deformation (2), were maintained after weaning on a diet of wet mesh chow.

Preparation of Sera, Conditioned Media (CM), and Tissue Extracts. L929 cell (American Type Culture Collection) CM (LCM) and WEHI-3BD cell (American Type Culture Collection) CM (WCM) were prepared by growing cells to confluence and 1 × 10^6 cells per ml, respectively, in medium containing 10% fetal calf serum (GIBCO). Endotoxin (E) serum was collected from the blood of mice injected 4 hr previously with E (*Escherichia coli* B4:0111, 10 μg per mouse) (9). E lung, E bone, and E skin CM were prepared by culturing for 48 hr lungs, marrow-depleted bones, or skin, respectively, from individual mice that were injected with E 4 hr prior to killing (9). Pokeweed mitogen spleen CM (PWM SCM) was prepared by culturing splenocytes in the presence of pokeweed mitogen for 7 days (9). Fibroblast CM were obtained from confluent secondary and tertiary cultures of bone or skin fibroblasts. Tissue extracts were prepared as described (10). Except where otherwise indicated, materials

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Abbreviations: CSF-1, colony-stimulating factor 1; CM, conditioned medium(ia); LCM, L929 cell CM; WCM, WEHI-3BD cell CM; E, endotoxin; PWM SCM, pokeweed mitogen spleen CM; GM-CSF, granulocyte/macrophage CSF; G-CSF, granulocyte CSF; IL-3, interleukin 3; IL-2, interleukin 2; IL-4, interleukin 4.

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tested for colony-stimulating activity were derived from four to six individual mice of each genotype.

Assays. Effector bone marrow or spleen cells for macrophage colony formation were obtained from 4-week-old *op/op* or littermate control mice and cultured with the appropriate preparations in a semisolid agar system for 7 days (11). Cultures were then fixed with glutaraldehyde, stained with hematoxylin/eosin, and evaluated for the presence of pure macrophage colonies.

Concentrations of granulocyte/macrophage CSF (GM-CSF), interleukin 3 (IL-3), granulocyte CSF (G-CSF), and interleukin 2/interleukin 4 (IL-2/IL-4) were determined using cell lines that selectively respond to particular growth factors. Results are expressed in units obtained from standard curves derived from cultures containing known concentrations of recombinant growth factors (Genzyme). GM-CSF concentrations were determined using DA-3 indicator cells (obtained from J. E. Ihle, Saint Jude Children's Research Hospital, Memphis, TN) in a cell survival assay (12). As these cells also respond to IL-3, the response was attributed to GM-CSF only when IL-3 was not detected in a parallel assay of the particular sample. IL-3 concentrations were determined using a subline of FDCP-1 cells (13) that responds exclusively to this factor in a mitogenesis assay (14). Similarly, IL-2/IL-4 levels were determined using HT-2 cells in a mitogenesis assay (14). Although HT-2 cells respond to IL-2 and IL-4, values are expressed in IL-2 units. G-CSF concentrations were determined using a differentiation induction assay (15, 16) involving WEHI-3BD+ cells (obtained from M. A. S. Moore, Memorial Sloan–Kettering Cancer Center, New York). Mouse CSF-1 radioimmunoassays were performed as described (17); 1 unit of CSF-1 is equivalent to 0.44 fmol or 12 pg of protein (18).

Analyses of DNA and RNA. For Southern analysis (19), 15 μg of DNA extracted (20) from the brains of *op/op* or normal littermate control mice was digested with the appropriate restriction enzyme, subjected to 1% agarose gel electrophoresis in 40 mM Tris-acetate/1 mM EDTA, blotted, and probed with a randomly primed ^{32}P-labeled insert of a 2.3-kilobase (kb) cDNA clone of mouse CSF-1 mRNA (pGEM2mCSF-53) (21). Northern analysis of RNA prepared from mouse L929 cells and lung fibroblasts was carried out as described (22).

Diffusion Chamber Implantation Experiments. Diffusion chambers, each consisting of two 20-μm (pore size) polycarbonate membranes (Nucleopore) cemented on each side of a lucite ring (Millipore) with cement (Millipore no. 1), were prepared as described (23) and filled with either medium (control) or 1×10^5 viable L929 cells (experimental) and the filling holes were sealed with paraffin wax. Chambers were implanted into the peritoneal cavities of 6-month-old *op/op* mice (six mice per group). Mice were evaluated 1 month later. Histochemical staining for acid phosphatase and tartrate-resistant acid phosphatase was carried out as described in kit no. 387 obtained from Sigma.

RESULTS

Effect of Sera and CM from *op/op* Mice on Macrophage Colony Formation by Progenitor Cells from *op/op* and Control Mice. Previous studies showed that, although the total number of bone marrow macrophage progenitors in *op/op* is only 10% of the number in control mice, the ability of these progenitors to generate macrophages is unimpaired *in vitro* (3). Initially, we sought to confirm this observation using three different sources of macrophage growth factors. The results, shown in Table 1, indicate that the frequency of bone marrow macrophage progenitor cells responding to either LCM, WCM, or PWM SCM was not decreased in *op/op* mice compared with littermate controls (+/+ or +/*op*). Interestingly, the frequency of *op/op* cells responding to PWM SCM was twice that of control mice. However, since the bone marrow cellularity of the *op/op* mice is 10% of normal (3), the total number of progenitor cells responding to the growth factors is reduced, as reported earlier (3). In the case of the spleen, however, in which total cellularity is only slightly reduced in the *op/op* mice (3), there was an increase in the frequency and total number of macrophage progenitor cells responding to all three growth factor preparations, again more marked in the case of PWM SCM. Thus in agreement with the earlier observations, *op/op* mice have a reduced number of macrophage progenitors in bone marrow that parallels the decrease in its cellularity, whereas the number of these cells is differentially increased in the spleen.

Table 1. Effect of different CM on macrophage colony formation by progenitor cells from *op/op* and control mice

	Colonies per 1×10^5 cells			
	Bone marrow		Spleen	
CM, %	Control	*op/op*	Control	*op/op*
LCM, 5	334 ± 38	412 ± 45	17 ± 3	101 ± 23
WCM, 5	313 ± 54	348 ± 58	18 ± 4	96 ± 17
PWM SCM, 10	310 ± 40	630 ± 87	8 ± 3	104 ± 23

Values are presented as means ± SD (n = 3).

To investigate whether the failure of macrophage progenitors to proliferate and differentiate in *op/op* mice is due to a macrophage growth factor deficiency or to the production of a macrophage growth factor inhibitor, we tested the ability of sera and media conditioned by cells prepared from *op/op* mice to stimulate macrophage colony formation (Table 2). Unlike E serum from control mice, *op/op* E serum did not contain detectable macrophage colony- (or cluster, data not shown) stimulating activity. This effect was not due to the presence of colony inhibitory activity in *op/op* serum, as this serum does not prevent, but rather enhances, colony formation stimulated by either LCM or WCM (plating efficiencies for control E serum alone, 0.401 ± 0.049; for LCM alone, 0.334 ± 0.038; for LCM plus *op/op* E serum, 0.623 ± 0.054; for LCM plus control E serum, 0.587 ± 0.037; for WCM alone, 0.313 ± 0.054; for WCM plus *op/op* E serum, 0.459 ± 0.043; for WCM plus control E serum, 0.427 ± 0.027). Media conditioned by fibroblasts from bone, skin (Table 2), and peritoneum (data not shown) of *op/op* mice were similarly devoid of macrophage colony-stimulating activity, whereas the corresponding CM from control mice possessed high activity. However, E lung CM, PWM SCM (Table 2), and lung CM (data not shown) from *op/op* mice contained as high or higher macrophage colony-stimulating activity than the corresponding CM from normal mice, indicating that not all sources of macrophage growth factor from *op/op* mice were biologically inactive.

Measurement of GM-CSF, IL-3, G-CSF, and IL-2/IL-4 in Preparations from *op/op* and Control Mice. To determine

Table 2. Effect of sera and CM from *op/op* and control mice on macrophage colony formation by control mouse bone marrow progenitor cells

	Colonies per 1×10^5 cells	
Preparation, %	Control	*op/op*
E serum, 2.5	401 ± 49	0
Bone fibroblast CM, 10	311 ± 35	0
Skin fibroblast CM, 10	241 ± 34	0
E lung CM, 10	73 ± 17	105 ± 23
PWM SCM, 10	310 ± 40	523 ± 57

Values are presented as means ± SD from duplicate determinations of individual samples from four to six mice.

which growth factors might be produced in lower amounts in *op/op* mice, the preparations listed in Table 2 were tested for the presence of specific growth factors by using cell lines known to exhibit different growth factor requirements (Table 3). The growth factors assayed in this fashion, with the exception of G-CSF, which is produced by cultured macrophages, have direct effects on macrophage proliferation *in vitro*. Depending on the preparation tested, the *op/op* mice exhibited either increased or slightly impaired production of GM-CSF and G-CSF. Interestingly, PWM SCM-stimulated lymphoid cells from *op/op* mice exhibited an increased capacity to produce IL-3 and IL-2/IL-4 compared with lymphoid cells from control mice. Thus the macrophage deficiency in *op/op* mice could not be ascribed to any of these growth factors.

Absence of Biologically Active CSF-1 in Sera, Tissues, and CM Prepared from *op/op* Mice. The absence of detectable macrophage colony-stimulating activity in *op/op* E serum and fibroblast CM (Table 2), which in normal mice contain high concentrations of CSF-1, suggested that a lack of this macrophage-specific growth factor might be associated with the *op/op* phenotype. Sera, tissue extracts, and CM derived from *op/op* and control mice were therefore assayed for CSF-1 using a specific radioimmunoassay that only detects biologically active CSF-1 and that is slightly more sensitive than the biological assay for CSF-1 (17). As shown in Table 4, neither serum, E serum, nor any of the 11 tissues of *op/op* mice contained any CSF-1 by this assay, in contrast to control mice. Furthermore, even when *op/op* derived CM were concentrated up to 18-fold by ultrafiltration, it was not possible to detect any CSF-1 by radioimmunoassay (Table 5). These data demonstrate the existence of an absolute CSF-1 deficiency in the sera and tissues of the *op/op* mouse.

Analysis of *op/op* DNA and RNA. To determine whether the mutation in the *op/op* mouse was associated with a major alteration in the CSF-1 gene, Southern analysis of DNA from *op/op* and control mice was carried out using a mouse CSF-1 cDNA probe. As shown in Fig. 1 *a–c*, there was no difference in the banding patterns between *op/op* and control DNA that was restricted with *Hin*dIII, *Eco*RI, or *Bam*HI, indicating that there is no major rearrangement of the CSF-1 gene in *op/op* mice. To determine whether the *op/op* mutation was associated with decreased levels of CSF-1 mRNA, RNA from *op/op* and control lung fibroblasts was subjected to Northern analysis. As shown in Fig. 1*d*, compared with RNA from control mouse L cells (lane 1) or control mouse lung fibroblasts (lane 2), which contained normal levels of 4.6- and 2.3-kb CSF-1 mRNAs, the *op/op* lung fibroblasts contained normal levels of only the 4.6-kb species (lane 3). These results could be explained by a differential alteration in CSF-1 gene transcription, mRNA splicing, or mRNA stability.

Partial Correction of the *op/op* Defect by Implantation of Diffusion Chambers Containing CSF-1-Producing Cells. Correction of the mutant phenotype by CSF-1 administration would provide some evidence that the primary defect in the *op/op* mouse is due to a CSF-1 deficiency. However, circulating CSF-1 has a half-life of only 10 min (24). Thus, a method for the continual administration of CSF-1 over the prolonged period necessary to demonstrate correction was required. Since CSF-1 is the only macrophage growth factor produced by cultured mouse L929 cells (25), 6-month-old *op/op* mice were implanted with diffusion chambers containing either CSF-1-producing L929 cells or control medium without cells. After 1 month, the mice were killed for determination of cell or macrophage numbers in the bone marrow, spleen, and periphery. Mice that received L929 cells contained on average 650 units of CSF-1 per ml of plasma compared with 0 units/ml in mice that received diffusion chambers containing medium alone. Thus 1 month after their implantation, the L929 cells were still producing CSF-1 and the circulating concentration was almost half that of a normal mouse (1453 units/ml, Table 4). In addition, as shown in Table 6, *op/op* mice that received L929 cells exhibited a

Table 3. Concentrations of biologically active GM-CSF, IL-3, G-CSF, and IL-2/IL-4 in sera and CM prepared from control and *op/op* mice

	Growth factor activity, units/ml			
Preparation	GM-CSF ($\times 10^{-1}$)	IL-3 ($\times 10^{-3}$)	G-CSF ($\times 10^{-3}$)	IL-2/IL-4 ($\times 10^{-2}$)
E serum				
Control	2.3 ± 0.5	ND	16.7 ± 2.6	ND
op/op	4.7 ± 0.8	ND	8.2 ± 1.7	ND
E lung CM				
Control	43.0 ± 8.0	ND	4.6 ± 8.8	ND
op/op	87.3 ± 10.0	ND	6.2 ± 1.2	ND
PWM SCM				
Control	NE	0.4 ± 0.1	NT	0.7 ± 0.2
op/op	NE	6.1 ± 1.2	NT	2.3 ± 0.5
E bone CM				
Control	11.0 ± 2.0	ND	7.8 ± 0.8	ND
op/op	5.7 ± 1.5	ND	14.3 ± 1.3	ND
E skin CM				
Control	2.3 ± 0.5	ND	16.7 ± 2.6	ND
op/op	4.7 ± 0.8	ND	8.2 ± 1.7	ND

Values are presented as means ± SD of duplicate assays of individual preparations from four or five mice. NE, not evaluable: the presence of IL-3 did not allow determination of GM-CSF using DA-3 cells; ND, not detectable; NT, not tested.

Table 4. CSF-1 concentration in sera and tissues of *op/op* and control mice

	CSF-1, units/ml or units/mg of tissue	
Source	Control	*op/op*
Serum	1453 ± 173 (4)	50 ± 45 (6)
E serum	3425 (2)	0 (2)
Lung	3.33 ± 0.44 (6)	0.03 ± 0.02 (4)
Liver	0.43 ± 0.13 (6)	0.01 ± 0.02 (4)
Spleen	1.61 ± 0.06 (6)	0.14 ± 0.10 (4)
Kidney	2.29 ± 0.35 (6)	0.06 ± 0.02 (4)
Brain	1.27 ± 0.58 (6)	0.05 ± 0.02 (4)
Bone	0.60 ± 0.27 (6)	0.03 ± 0.03 (4)
Heart	1.12 ± 0.16 (6)	0.16 ± 0.08 (4)
Muscle	0.47 ± 0.14 (6)	0.03 ± 0.01 (4)
Skin	0.74 ± 0.20 (6)	0.10 ± 0.05 (4)
Thymus	0.86 ± 0.35 (6)	0.16 ± 0.07 (4)
Peritoneum	0.62 ± 0.21 (6)	0.05 ± 0.03 (4)

Values are presented as the means ± SD of results of duplicate assays on samples derived from individual mice. The number of mice sampled per group is given in parentheses. All results for *op/op* mice were not significantly different from results obtained in blank tubes not containing CSF-1.

Table 5. CSF-1 concentration in media conditioned by tissues

	CSF-1, units/ml	
CM	Control	*op/op*
Femurs and tibia	196 (1)	0* (1)
Calvaria	316 (2)	0 (2)
Bone fibroblasts	44 (1)	0* (1)
Peritoneum	448 (2)	0* (2)
Skin	360 ± 136 (5)	0 (5)
Lung	73 ± 65 (5)	0 (3)
E Lung	333 (2)	0 (2)

Results are expressed as described in the legend to Table 4.
*The same result was obtained when samples were concentrated 5- to 18-fold prior to assay.

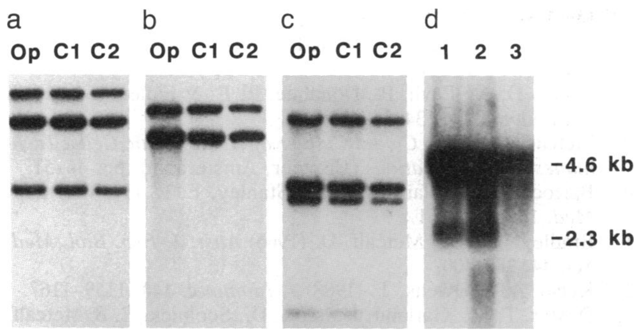

FIG. 1. Analysis of DNA and RNA from *op/op* mice. (*a–c*) Southern analysis of brain DNA from an *op/op* mouse (Op) and two control sibling mice (C1, C2). DNA was digested with *Hin*dIII (*a*), *Eco*RI (*b*), or *Bam*HI (*c*). (*d*) Northern analysis of RNA from mouse L929 fibroblasts (lane 1), littermate control lung fibroblasts (lane 2), and *op/op* lung fibroblasts (lane 3). Blots were probed with a mouse CSF-1 probe.

significantly elevated cellularity in the bone marrow and peritoneal cavity, including an 80-fold increase in the number of peritoneal macrophages, in comparison to *op/op* mice that received diffusion chambers containing medium alone. The cellular composition of peritoneal lavage from control mice was 80% lymphocytes, 15% granulocytes, and 5% macrophages, compared with the peritoneal lavage from mice receiving L929 cells, which contained 19% lymphocytes, 15% granulocytes, 66% macrophages, and 1% erythroblasts. Interestingly, 10% of the macrophages from the peritoneal cavities of *op/op* mice receiving L929 cells were positive for the osteoclast-specific marker (26) tartrate-resistant acid phosphatase, whereas peritoneal lavages from control *op/op* mice or normal mice were devoid of these cells. Similarly, there were 10.5 ± 3.7 acid phosphatase-positive cells per 1 × 10^4 cells in the bone marrow of these mice, whereas the bone marrows from control *op/op* and normal mice were devoid of these cells. These results are consistent with the suggestion that the primary defect in the *op/op* mouse is the failure to produce biologically active CSF-1.

DISCUSSION

We have shown that the tissues, sera, and CM, which from normal littermate control mice contain biologically active CSF-1, specifically lack this macrophage growth factor if obtained from *op/op* mice. Our results suggest that the CSF-1 deficiency in *op/op* mice is absolute. Several findings suggest that this particular growth factor deficiency may be the primary defect in *op/op* mice. An important result is the observation reported here that the *op/op* phenotype can be partially corrected by administration of CSF-1. Although the diffusion chamber implantation experiments provide indirect evidence and the correction of the defect was partial, this was to be expected from administration of the growth factor to older *op/op* mice (see below). It is significant that the *op* mutation and the CSF-1 gene both map within the same region on chromosome 3—i.e., between *Amy* and *Hao-2* (5, 6). Our Southern analyses of the CSF-1 gene in *op/op* mice and the normal size and level of expression of the 4.6-kb CSF-1 mRNA in lung fibroblasts from *op/op* mice suggest that there is no major rearrangement of the CSF-1 gene. However, the presence of this transcript in the absence of detectable CSF-1 protein implies that the defect prevents the production of an active translation product. Interestingly, the failure to detect the 2.3-kb CSF-1 mRNA in *op/op* lung fibroblasts indicates that there is also a pretranslational alteration in CSF-1 gene expression. Any delineation of the molecular basis of the defect(s) in the *op/op* mouse must therefore account for both of these observations. Clearly the results presented here strongly implicate the existence of alterations within the CSF-1 gene in *op/op* mice. Studies are necessary to determine the molecular basis of the *op* defect(s). Finally, the *op/op* phenotype itself is consistent with a specific CSF-1 deficiency, as CSF-1 has been implicated in the generation of osteoclasts (7) and it is the only mononuclear phagocyte-specific growth factor described to date.

Despite the correction of bone marrow and peritoneal cellularity in *op/op* mice, incisors failed to appear and there was no correction of pleural cavity cellularity in those mice given diffusion chambers containing L929 cells. These results imply that local or developmentally regulated expression of CSF-1 is important in the development of certain responsive cell populations. Despite the anticipated technical problems, injection of purified CSF-1 into younger, neonatal mice could therefore result in a more complete correction of the *op/op* defect. Furthermore, this approach may enable situations to be delineated in which local rather than humoral expression of CSF-1 is required for normal development. Obviously, such studies might also be complemented and developed by appropriate transgenic experiments.

In normal mice, sinusoidally located Kupffer cells and splenic macrophages remove 90% of the circulating CSF-1 by CSF-1 receptor-mediated internalization and intracellular destruction (24). Thus macrophages, whose production is regulated by CSF-1, play an important role in negatively regulating the concentration of CSF-1 in the circulation. The circulating concentration of GM-CSF was elevated in *op/op* serum and the concentrations of GM-CSF, IL-3, and IL-2/IL-4 in CM were elevated in media conditioned by certain *op/op* cells and tissues. These elevations may therefore be explained by compensatory increases in the synthesis of these macrophage growth factors or, alternatively, by a decreased rate of their internalization and destruction by target cells due to the decreased number of macrophages in the tissues of *op/op* mice. Further investigation of these possibilities utilizing the *op/op* mouse may illuminate the mechanisms by which these macrophage growth factors are regulated *in vivo*.

CSF-1 has been found to stimulate the formation of osteoclast-like multinucleated cells in long-term bone marrow cultures of baboon cells (7). However, others have reported that CSF-1 inhibits the growth of osteoclast precursor-like cells (27) and bone resorption by osteoclasts disaggregated from rat bone (28). Although it is difficult to explain these latter findings on the basis of the data presented here, it is possible that CSF-1 has an additional role of stimulating the production of cytokines (e.g., monokines) that actually inhibit these processes. Clearly studies in the *op/op* mouse will be useful in resolving these and other questions relating to the

Table 6. Partial correction of macrophage deficiency in *op/op* mice implanted with L cells in diffusion chambers

Cell type	Cell number × 10^{-6} Control DC	L929 cell DC
Peritoneal lavage		
Cells	1.6 ± 0.3	13.1 ± 2.7*
Macrophages	0.1 ± 0.1	8.4 ± 2.7*†
Pleural lavage		
Cells	0.8 ± 0.4	0.7 ± 0.4
Macrophages	0.4 ± 0.2	0.4 ± 0.2
Femoral cells	4.9 ± 1.0	10.8 ± 1.9*
Tibial cells	3.7 ± 0.9	7.6 ± 1.1*
Splenic cells	89.1 ± 15.0	95.0 ± 23.0

Values are presented as means ± SD (six mice per group). DC, diffusion chambers.
*Significantly different, $P \leq 0.001$.
†One hundred percent of cells phagocytic for India ink.

roles of cytokines in osteoclast production and bone resorption.

Apart from more clearly understanding the role of CSF-1 in the regulation of bone resorption, the identification of the *op/op* mouse as a CSF-1-deficient mutant provides a unique opportunity for studies of CSF-1 action. Precise definition of the range of mononuclear phagocyte and progenitor cell deficiencies in various organs of the *op/op* mouse should allow the CSF-1 requirement of cell populations in different tissues to be determined. As indicated earlier, by carefully timing CSF-1 administration in reconstitution experiments it should be possible to identify processes that are developmentally regulated by CSF-1. The *op/op* mouse will be particularly useful in studying the role of processes in which regulation by CSF-1 does not appear to involve mononuclear phagocytes or osteoclasts—e.g., placental development (10, 22, 29).

Note Added in Proof. Sequencing of polymerase chain reaction products using oligonucleotide primers specific for the coding region of the *op/op* CSF-1 gene revealed the presence of a nonsense mutation at the codon for amino acid no. 277. Although this mutation can explain the absence of biologically active CSF-1 in the *op/op* mouse, it is not clear whether it is the only mutation in the *op/op* CSF-1 gene and, if so, whether it is sufficient to cause the absence of the 2.3-kb CSF-1 mRNA species.

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