The Effects of Vitamin D Binding Protein-Macrophage Activating Factor and Colony-Stimulating Factor-1 on Hematopoietic Cells in Normal and Osteopetrotic Rats

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Osteopetrosis is a heterogeneous group of bone disorders characterized by the failure of osteoclasts to resorb bone and by several immunological defects including macrophage dysfunction. Two compounds, colony-stimulating factor-1 (CSF-1) and vitamin D-binding protein-macrophage activating factor (DBP-MAF) were used in the present study to evaluate their effects on the peritoneal population of cells and on cells within the bone marrow microenvironment in normal and incisors absent (ia) osteopetrotic rats. Previous studies in this laboratory have demonstrated that administration of DBP-MAF to newborn ia animals results in a substantial increase in bone marrow cavity size due to upregulated osteoclast function. To study the effects of these compounds on the macrophage/osteoclast precursors, DBP-MAF, CSF-1, and the combination of these compounds were given to newborn ia and normal littermate animals. Both the normal and mutant phenotypes responded similarly when treated with these compounds. Rats exhibited a profound shift toward the macrophage lineage from the neutrophil lineage when compared with vehicle-treated control animals after treatment with these compounds. In the in vivo peritoneal lavage study, animals received injections of CSF-1, DBP-MAF or DBP-MAF/CSF-1 over a 4-week period. The various types of cells in the peritoneal cavity were then enumerated. The in vitro study consisted of cells isolated from the bone marrow microenvironment and cultured on feeder layers of CSF-1, DBP-MAF, or DBP-MAF/CSF-1 for colony enumeration. The increase in macrophage numbers at the expense of neutrophil numbers could be seen in both the in vivo and in vitro experiments. The macrophage/osteoclast and neutrophil lineages have a common precursor, the granulocyte/macrophage colony-forming cell (GM-CFC). With the addition of CSF-1, the GM-CFC precursor may be induced into the macrophage/osteoclast lineage rather than the granulocyte lineage. This increased pool of cells in the macrophage/osteoclast lineage can be functionally upregulated with the subsequent addition of DBP-MAF to perform the activities of phagocytosis and bone resorption. The in vitro data also showed that DBP-MAF did not support colony development as in CSF-1 or the combination treatment. The recruitment and activation of cells into the macrophage/osteoclast lineage may help to correct the bone and immune defects found in diseases demonstrating a significant lack of myeloid cells, as well as neutrophilia disorders and the disease, osteopetrosis.

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VITAMIN D-BINDING PROTEIN is a novel serum protein that, through a series of enzymatic cleavages, can be converted to a potent macrophage activating factor, vitamin D-macrophage protein-macrophage activating factor (DBP-MAF). The parent, precleaved compound, vitamin D-binding protein, has been implicated in many physiological events. In contrast, the recently discovered DBP-MAF has only been characterized to a limited extent.

The DBP, also known as group-specific component or Gc-globulin, has one vitamin D-binding site that binds vitamin D metabolites in plasma. The name vitamin D-binding protein may be a misnomer given that the protein also binds actin and other agents with equal affinities. The parent DBP compound circulates at a 30- to 50-fold excess over vitamin D and the metabolites it carries, with a plasma concentration of 5 to 8 × 10⁻⁹ mol/L. Physical characteristics of the human protein show a 58,000 molecular weight molecule that can be divided into three domains. The third domain (nearest to the C-terminus portion of the DBP molecule) contains an important glycosylation site. This O-linked glycosylation contains a galactose and a sialic acid residue that can be cleaved by an inducible β-galactosidase enzyme produced by B cells and an inducible sialidase enzyme produced by T cells in response to inflammation. When this modification of the DBP molecule occurs, the resulting protein becomes a potent activator of macrophages, DBP-MAF.

DBP-MAF was originally found to play a role in the activation of macrophages during the inflammatory response. More recently, DBP-MAF has been shown to have an effect on the skeleton as well. The osteoclast is the cell responsible for bone resorption and is a descendant of the same precursor cell that gives rise to the mononuclear/macrophage lineage. Even though the mature progeny of this lineage, macrophages and osteoclasts, differ greatly with respect to structure and function, the effects of DBP-MAF were evaluated in an osteopetrotic mutant showing defective osteoclastic activity.

The incisors absent (ia) rat mutation shows defects in both osteoclast activity and in some aspects of the immune response. Similar to the childhood form of the disease, this osteopetrotic animal shows a diminished bone marrow cavity because of the near absence of osteoclast function. The ia rat mutation is an autosomal recessive disorder whose phenotype improves with 1,25-dihydroxyvitamin D₃ treatment, interleukin (IL)-2 treatment, and hematopoietic stem cell transplantation. Colony-stimulating factor-1 (CSF-1) is a growth factor on which mononuclear phagocytes are dependent for their survival. This growth factor has been characterized as to its effects on the cells in the mononuclear phagocytic lineage.
Additionally, CSF-1 has been used in the treatment and partial cure of two osteopetrotic animal mutations, the osteopetrotic (op) mouse,24-26 and the toothless (df) rat.27,28 However, to date, a CSF-1 defect has not been found in osteopetrotic children. Therefore, therapy using CSF-1 alone has not been warranted.29

Several osteopetrotic mutations show defects in cell populations that are important immunologically. Defects in lymphocyte populations,18,30,31 mast cells,26 neutrophils,26,32 as well as macrophages23,35 have all been reported. Therefore, these cells, which normally populate the peritoneal cavity, were isolated to study the effects of DBP-MAF, CSF-1, and the combination of these treatments on cells that play a role in immune function, as well as on cells that have common precursors to the osteoclast.

The ia rat mutation was used in these studies because, unlike other osteopetrotic mutations, this animal has abundant levels of all of these cells, as well as osteoclasts, whereas other mutations lack one or more of these cellular populations (for review, see Seifert).36 In the ia mutation, osteoclasts are present, but lack a ruffled border, the site of active bone resorption.16 In a previous study, ia rats were treated with ex vivo generated DBP-MAF, which resulted in a significant increase in bone marrow cavity size when compared with the sham-injected control animals. Additionally, the presence of a ruffled border adjacent to the bone surface appeared with DBP-MAF treatment. Superoxide production by osteoclasts in osteopetrotic ia animals was substantially reduced. When ia rats were treated with DBP-MAF, this reduction was corrected to normal levels.37

It was hypothesized that the addition of CSF-1 to the system (administered before the DBP-MAF) would enhance macrophage precursor numbers, which could then be directed in their differentiation and activation by DBP-MAF to elevate both bone resorption and immune function in these mutants. This combination was used to determine the skeletal effects of the therapy in a previous study. When CSF-1 was administered in combination with DBP-MAF, enhanced osteoclast activity was observed that resulted in an increased bone marrow cavity size.38 The increase in marrow cavity size with the combination treatment was greater than the increase observed with either compound alone. In fact, in the ia mutation, CSF-1 had no effect on the skeletal system.

The peritoneal cavity provides a unique environment of cells that can be easily isolated and quantitated. Therefore, these cells were studied to determine the effects of CSF-1, DBP-MAF, and the combination of the two on cells in the CFU-GM (colony-forming unit-granulocyte/macrophage) hematopoietic pathway. Additionally, colony-forming assays were performed to evaluate the effects of these compounds on bone marrow stem cells in vitro.

The studies reported here show that all three treatment groups, DBP-MAF, CSF-1, and the combination of these treatments have a similar effect on the differentiation of the hematopoietic cells. With treatment, the GM-CFC is induced in the macrophage lineage pathway. As this population increases, the neutrophil population subsequently decreases. Although all treatments elicited a significant effect, CSF-1 and the combination of DBP-MAF and CSF-1 exhibited the most profound effect on these cellular populations.
mm sterile petri plates using Iscove’s media plus 10% FBS, 5% penicillin-streptomycin and 0.5% agar. This media was supplemented with 200 pg/mL DBP-MAF, 10,000 U/mL CSF-1, a combination of DBP-MAF and CSF-1 at the aforementioned concentrations and control plates with no supplementation. One milliliter of feeder agar was placed in a 35-mm petri plate and allowed to solidify at room temperature. The femur of 4-week old normal and ia mutant animals was isolated and the epiphyses removed. Bone marrow cells were removed by aspiration of one end of the shaft using a 20-gauge needle and a solution of PBS (70%) and FBS (30%). Ficoll-hypaque centrifugation was then performed on the isolated cells and the interface removed. Cells were counted using trypan blue exclusion for viability. Cells were resuspended at a concentration of 0.2 × 10⁶ cells/mL in Iscove’s media with 10% FBS, 5% penicillin/streptomycin, and 0.3% agar. One milliliter of cell suspension was gently layered onto the feeder layer, and the plates were allowed to incubate at 37°C at 5% CO₂ for 7 days. The plates were then removed, and the cells were fixed with 100% methanol and stained with Giemsa stain. Colonies were enumerated and phenotypically analyzed at the light microscopic level using cellular and nuclear morphological criteria. A colony was defined as a cellular cluster composed of 50 or more cells. All assay parameters were performed in triplicate. Comparisons between treatment groups were performed using the Bonferroni multiple comparison test.

RESULTS

In Vivo Peritoneal Lavage Studies

Treatment of normal and ia mutant animals with DBP-MAF alone. DBP-MAF was administered to newborn animals at a concentration of 200 pg/animal per injection. The animals were injected every 4 days for 4 weeks. This injection scheme was derived from studies regarding maximal macrophage activation after treatment with alkylglycerols to initiate the cascade of the DBP cleavage to DBP-MAF (for review, see Yamamoto et al.).

When both normal and mutant animals were treated with DBP-MAF alone, there was a significant increase in the percentage of macrophages present in the peritoneal cavity when compared with control, sham-injected animals (see Fig 1). These increases are considered extremely significant by the Bonferroni multiple comparison test.

The other cell population greatly affected by this treatment was the neutrophil. In contrast to the increased percentages in peritoneal macrophages, the neutrophil population decreased significantly when compared with untreated control animals (see Fig 2). This decrease is also considered extremely significant by the same statistical tests. Other cell populations studied were not significantly affected with DBP-MAF treatment (data not shown).

Treatment of normal and ia animals with CSF-1 alone. When animals were treated with CSF-1 for 4 weeks at a concentration of 1 × 10⁶ U per injection, there were again changes in cell populations within the peritoneal cavity. The increase in the macrophage population was similar to that observed with the DBP-MAF alone (see Fig 1). The difference in percentages from the CSF-1–treated animals was extremely significant when compared with vehicle control animals. A significant decrease in the neutrophil population could be seen in the CSF-1 treatment as well (see Fig 2). Again, the other cells evaluated within the peritoneum did not constitute a significant difference in cell percentages from the control study.

Treatment of normal and ia animals with combined DBP-MAF and CSF-1 therapy. A combined CSF-1/DBP-MAF study was performed to study cell populations in the peritoneal cavity. This experiment was performed for 28 days, and animals were injected every alternate day with CSF-1 (100,000 U/injection) and DBP-MAF (200 pg/injection). The increase in the macrophage cell populations is extremely significant when compared with that in the vehicle control study (see Fig 1). Conversely, there was an extremely significant decrease in the percentage of neutrophils after treatment when compared with that in the vehicle control studies (see Fig 2). None of the other cell populations were significantly affected by this treatment (data not shown).

In these peritoneal studies, a significant increase in the percentage of macrophage cell numbers was observed in all treatment groups. The percentage of neutrophil cell numbers decreased with all treatments.

Total cell numbers within the peritoneal cavity. The total cell numbers within the peritoneum were calculated from the cell mass of each animal. Cell numbers were conducted for 28 days and were calculated from the
same lavage as the previous studies. The 28-day sham injection values were $3.01 \times 10^7 \pm 4.86 \times 10^6$ for the normal animals and $2.86 \times 10^7 \pm 3.51 \times 10^6$ for the mutant animals.

The total cellular population within the peritoneal cavity was not significantly altered by any of the treatments (Fig 3). None of the total cell numbers obtained in these experiments represents a significant difference from the vehicle control studies for total peritoneal cell numbers. Therefore, the effects of these compounds was not to increase the total cell numbers within the peritoneal cavity, but instead, recruit progenitor cells away from the neutrophil lineage and into the macrophage/monocyte lineage. These cells then migrate to and populate the peritoneal cavity in a normal manner.

In Vitro Colony-Forming Assay

Statistical analysis of total colony numbers. In the colony-forming assay, bone marrow cells from both normal and mutant animals were plated in soft agar on feeder layers containing DBP-MAF, CSF-1, a combination of DBP-MAF/CSF-1 and control layers containing no added factors. After incubating for 7 days, the colonies were counted using Giemsa stain for visualization of cellular and nuclear morphology. An overview of the total colonies present after 7 days shows that there was no statistical difference in the number of macrophage, neutrophil, or mixed colonies present on the control plates when compared with that of the DBP-MAF seeded plates for either phenotype ($P > .05$, data not shown). For the normal animals, there was a significant increase in the number of macrophage colonies present on the CSF-1 (18.0 ± 3.61 vs 60.3 ± 12.5) and the combination treatment (18.0 ± 3.61 vs 69.7 ± 5.13) over both the control plates, as well as the DBP-MAF plates (24.3 ± 3.74, $P < .001$ for all). In the mutant animals, the total macrophage colony population also increased significantly with CSF-1 (28.0 ± 6.56 vs 51.3 ± 5.03) and combination treatment (28.0 ± 6.56 vs 41.0 ± 8.08).

Additionally, there were no significant differences in total colony numbers between the cells receiving CSF-1 and the cells receiving the combination DBP-MAF/CSF-1 supplementation ($P > .05$). This was true for all colony types.

In the neutrophil population, addition of CSF-1 did not significantly alter the total number of these colonies ($P > .05$, data not shown). When the combination DBP-MAF and CSF-1 was added to the feeder layers, the normal animals showed a very significant decrease (18.7 ± 3.06 vs 7.67 ± 1.53, $P < .01$) in the neutrophil colonies. In response to the combination treatment, the mutant $ia$ animals showed a significant decrease in the total number of neutrophil colonies as well (17.0 ± 6.93 vs 3.33 ± 2.08, $P < .05$). The total number of mixed colonies was not affected by any treatment in either normal or mutant animals ($P > .05$, data not shown).

Analysis of the percentage of macrophage colonies. When macrophage colonies were enumerated, there was no significant difference between normal control and the $ia$ mutant control plates (see Fig 4). There were significant increases in the percentages of macrophage colonies with the addition of CSF-1 and the combination of these compounds in colony assays of both normal and mutant cells. In the normal animals, there was an extremely significant increase over control plates when DBP-MAF was added. Conversely, the difference between control plates and DBP-MAF-treated plates was not significant in the mutant animals (see Fig 4). There was also no significant difference in the percentage of colonies in the CSF-1 treated plates compared with the combination plates in either phenotype.

Analysis of the percentage of neutrophil colonies. Comparing the percentage of neutrophil colonies present in the control assay, no significant difference in peritoneal cells can be found ($P > .05$ for all comparisons to the normal experimental values using the Bonferroni multiple comparison test).
and mutant animals. Cells from the normal animal bone marrow, when exposed to DBP-MAF, also showed a very significant decrease in the percentage of colonies; however, mutant cells failed to respond in a similar manner. There was also no significant difference in the percentage of colony numbers in the CSF-1 plates when compared with that of the combination plates of either mutant or normal animals (see Fig 5).

Analysis of the percentage of mixed colonies. Mixed colonies are colonies that form with cells from both the macrophage lineage and the neutrophil lineage. There was no difference in mixed colony numbers between the mutant and the normal control assay plates (see Fig 6). Additionally, no significant difference was observed between controls and any of the treatment groups of the normal animals. The mutant animals cells that formed mixed colonies on the DBP-MAF/CSF-1 feeder layers decreased significantly in the percentage of colonies when compared with all other treatment groups. In the in vitro culture assay, the combination of DBP-MAF/CSF-1 elicited the greatest increase of the macrophage population when compared with other treatment groups. However, CSF-1 alone had an extremely significant effect on this population as well. In the neutrophil population, the combination treatment had the most profound effect on the decrease of this population. However, both DBP-MAF and CSF-1 elicited effects on this population, as well as in the normal animals, but DBP-MAF did not significantly affect any of the macrophage or neutrophil colony percentages in the mutant cultures.

DISCUSSION
Previously, the combination CSF-1/DBP-MAF treatment was performed in the ia and op osteopetrotic rat mutations to assess the effects of this therapy on the skeletal manifestations of the disease. Treatment of either osteopetrotic muta-

![Fig 4. Enumeration of macrophage colonies present on different feeder layers of soft agar assays. The combination DBP-MAF/CSF-1 treatment showed a significant increase in the percentage of macrophage colonies present over DBP-MAF and control plates (P < .001). The difference in the percentage of macrophage colonies on the CSF-1-treated plates and the combination treated plates was not significant (P > .05).](image)

![Fig 5. Enumeration of neutrophil colonies present on different feeder layers of soft agar assays. When normal or mutant cells were seeded on feeder layers containing a combination of DBP-MAF/CSF-1, as well as CSF-1 alone, the number of neutrophil colonies present decreased significantly (P < .001) over the control plates, as well as the DBP-MAF-seeded plates. Cells from normal animals on the DBP-MAF treated plates showed a very significant (P < .01) decrease in the percentage of neutrophil colonies when compared with control cultures; however, the mutant cells did not significantly decrease (P > .05).](image)

![Fig 6. Enumeration of mixed colonies present on different feeder layers of soft agar assays. No difference in the number of mixed colonies can be seen in any of the normal assay plates. In the mutant plates, a significant decrease (P < .05) in mixed colonies in the combination DBP-MAF/CSF-1 assay can be observed.](image)
tion with CSF-1 alone did not ameliorate any skeletal defects including the osteoclast dysfunction. Alternatively, treatment with DBP-MAF alone had a positive influence on the skeletal abnormalities. Combined treatment increased bone resorption over either therapy alone. Further studies in this laboratory have shown that treatment of ia mutant rats with small dosages of DBP-MAF (200 pg/rat) return the defective oxidative metabolism of isolated macrophages to within the normal parameters. Following this finding, it was hypothesized that the combination CSF-1/DBP-MAF treatment might stimulate the formation, differentiation and activation of macrophages and osteoclasts in the ia rat more so than individual treatment with either compound.

When rats were treated in vivo with CSF-1, DBP-MAF, and the combination of these two compounds, the population of cells within the peritoneal cavity shifts toward the macrophage lineage, at the expense of the neutrophil population. Additional evidence of the phenomenon in the CSF-1 and combination treatment is seen in the in vitro soft agar culture system. Total colony numbers increased significantly from control cultures in both the normal and mutant animals when cells were seeded on either CSF-1 or the DBP-MAF/CSF-1 combination. When colonies are broken down by cell type, this increase can be attributed to the macrophage-type colony (see Fig 4). However, in these colony-forming assays, DBP-MAF was not as effective in shifting the cellularity from the neutrophil population to the macrophage population. The observation of the increase in the percentage of macrophage colonies present in the CSF-1 and the combination DBP-MAF/CSF-1 feeder layers and the decrease in the percentage of neutrophil colonies in these cultures is further demonstration of the shift from cells directed toward the neutrophil lineage to cells of the macrophage/osteoclast lineage. In both the in vitro and the in vivo study, DBP-MAF did not show the profound effects that CSF-1 and the combination therapy showed. It is quite possible that DBP-MAF acts at the level of the terminally differentiated osteoclast and macrophage rather than at an earlier level of this hematopoietic lineage. Incisors-absent osteopetrotic animals receiving DBP-MAF show accentuated bone resorption and macrophage activity; however, the monocyte/macrophage population was not significantly affected by DBP-MAF in both the bone marrow study, as well as the peritoneal study.

The soft agar culture assay is comprised of cells of many stages of the hematopoietic lineages, from the pluripotent stem cell to more terminally differentiated cells. Given the fact that there are no more colonies in the control cultures than in the DBP-MAF cultures, DBP-MAF is clearly not exerting proliferative effects on the stem cells. Therefore, the effects of DBP-MAF on the hematopoietic lineage are occurring after the self-renewing stem cell, and possibly even as late as the terminally differentiated cells of the mononuclear phagocyte lineage and osteoclasts.

The common precursor of the macrophage and the osteoclast is at the stage of the GM-CFC or later. Evidence of this common precursor is demonstrated in the osteopetrotic (op/op) mouse. CSF-1 has been shown to play a key role in the formation of macrophage/monocyte colonies. In the osteopetrotic (op) mouse, it has been shown that the lack of CSF-1 in these animals is vital to the progress of the disease. The deficiency of macrophage and osteoclast precursors explains the total lack of a bone marrow cavity. When CSF-1 is given to these animals, macrophages and osteoclasts appear with the subsequent appearance of the bone marrow cavity. This finding would suggest that the formation of these cells is compromised in the op animals, a condition that can be corrected by the addition of CSF-1.

Recently, work has been done by Nilsson et al studying granulocyte-macrophage colony-stimulating factor (GM-CSF) in the op/op osteopetrotic mouse. The op mice have been observed to undergo an age-related correction of the macrophage and skeletal defects associated with this mutation. Beginning around 6 weeks of age, increased cellularity in the bone marrow microenvironment can be observed in the op mouse, which progresses to about 22 weeks where cellularity within the cavity is present at normal levels. Because the gene defect cannot spontaneously correct, there could be another factor with CSF-1–like activity that compensates for the CSF-1 deficiency. This agent apparently would exert its effect on the skeletal system later in life. The age-related correction of the skeletal and hematopoietic defects seen in the op mouse were still present despite the absence of GM-CSF studied in a GM-CSF knockout expressed in the op mouse mutation. It is, therefore, plausible that another compound could be responsible for the age-related correction of these defects seen in the op/op mouse mutation. DBP-MAF could represent a candidate for an alternative compensatory factor that might upregulate macrophage and osteoclast function later in life in the op mouse mutation.

A possible alternate solution to the age-related correction of the op mouse osteopetrotic phenotype was recently put forth. The investigators suggest that the correction may be due to a variant form of CSF-1 in these animals caused by an alternate splice site that has yet to be discovered (for review, see Hume et al). This new form of CSF-1 would not be as physiologically active as the more common CSF-1 types and, therefore, would account for the slow correction of the phenotype. Whether the age-related correction of osteopetrosis in the op mouse is due to a CSF-1 compensatory molecule or an alternative splice site of CSF-1 remains to be shown.

The results of this study and our previous studies in the ia rat indicate that DBP-MAF, unlike CSF-1, appears to affect more terminally differentiated cells such as the macrophage and the osteoclast. Additionally, DBP-MAF does not appear to have any stimulatory effect on the hematopoietic stem cell population to proliferate and generate colonies. In the op/op and ia/ia rat osteopetrotic mutations, macrophages and osteoclasts are present; however, these cells have reduced function. Vitamin D binding protein-macrophage activating factor has previously been shown to activate macrophages even at very low doses. When DBP-MAF was given to ia and op rats, the macrophage defect present in these animals was overcome and activation of these cells occurred. Additionally, a loss of the excessive bone was observed. Although a novel DBP-MAF receptor has not
yet been found on the terminally differentiated osteoclast, it has been shown that DBP-MAF does have an effect on these cells. On “isolated” rat osteoclasts in culture, the addition of DBP-MAF upregulates osteoclast activity in a dose-dependent manner when compared with control osteoclast cultures.20 The results of these preliminary data suggest that there is an interaction of DBP-MAF with the osteoclast; however, a DBP-MAF receptor on the osteoclast has yet to be discovered. Therefore, whether DBP-MAF has a direct or indirect effect on osteoclast function has to be resolved by future investigations that are ongoing in our laboratory.

Implications of these findings may be found in disease states other than osteopetrosis. Diseases that result in either detrimental neutrophilia or decreased macrophage production/activation could potentially benefit from CSF-1, DBP-MAF, or combination treatment. Notably, the defective monocyte function found in patients with systemic lupus erythematosus could conceivably benefit from increased macrophase function.21

These results have exceptional implications in the childhood disease osteopetrosis. The stimulation via CSF-1 and subsequent activation via DBP-MAF of the macrophage/osteoclast precursor cells (as shown in previous studies) will allow a greater number of cells to be recruited to perform the activities of both phagocytosis and bone resorption, given their common precursor. The recruitment and activation of these cells may help to correct the bone and immune defects found in the disease osteopetrosis. Children who are diagnosed with the infantile, malignant form of osteopetrosis could be screened for defects in DBP and/or DBP-MAF. Affected children could be treated with ex vivo generated DBP-MAF. Additionally, the effects of DBP-MAF could be enhanced using a combined CSF-1/DBP-MAF therapy, as in the previous animal studies.38 Unlike the il−/− and op−/− mouse, no CSF-1 defect has been detected in osteopetrotic children.29 In light of this, treatment might be most effective if the combined CSF-1/DBP-MAF therapy were employed for some human forms of the disease.

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The effects of vitamin D binding protein-macrophage activating factor and colony-stimulating factor-1 on hematopoietic cells in normal and osteopetrotic rats

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