Macrophage-mediated neuroprotection and neurogenesis in the olfactory epithelium


1Department of Physiology, University of Kentucky College of Medicine; 2Department of Statistics, University of Kentucky; 3Department of Anatomy and Neurobiology, University of Kentucky College of Medicine; 4Sanders-Brown Center on Aging, University of Kentucky, Lexington, Kentucky; 5Department of Molecular Cell Biology, Vrije Universiteit, VUMC, Amsterdam, The Netherlands; and 6Department of Microbiology, Immunology, and Molecular Genetics, University of Kentucky College of Medicine, Lexington, Kentucky

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Borders AS, Hersh MA, Getchell ML, van Rooijen N, Cohen DA, Stromberg AJ, Getchell TV. Macrophage-mediated neuroprotection and neurogenesis in the olfactory epithelium. Physiol Genomics 31: 531–543, 2007. First published September 11, 2007; doi:10.1152/physiolgenomics.00008.2007.— Resident and recruited olfactory epithelial macrophages participate in the regulation of the survival, degeneration, and replacement of olfactory sensory neurons (OSNs). We have reported that liposome-encapsulated clodronate (Lip-C) induced selective and statistically significant depletion of macrophages in the OE of sham and 48 h OBX mice (38 and 35%, respectively) that resulted in increased OSN apoptosis and decreased numbers of mature OSNs and proliferating basal cells compared to controls (Lip-O). The aim of this study was to identify molecular mechanisms by which the selective depletion of macrophages in the OE resulted in these cellular changes by using a microarray expression pattern analysis. A 2×2 ANOVA identified 4,085 overall significantly (P < 0.01) regulated genes in the OE of Lip-O and Lip-C sham and 48 h OBX mice, and further statistical analysis using pairwise comparisons identified 4,024 genes that had either a significant (P < 0.01) treatment main effect (n = 2,680), main group effect (n = 778), or interaction effect (n = 980). The mean hybridization signals of immune response genes, e.g., Cxcr4, and genes encoding growth factors and neurogenesis regulators, e.g., Hdgf and Neurod1, respectively, were primarily lower in Lip-C mice compared with Lip-O mice. Apoptosis genes, e.g., Bak1, were also differentially regulated in Lip-C and/or OBX mice. Expression patterns of selected genes were validated with real-time RT-PCR; immunohistochemistry was used to localize selected gene products. These results identified the differential regulation of several novel genes through which alternatively activated macrophages regulate OSN progenitor cell proliferation, differentiation, and maturation, and the survival of OSNs.

clodronate; liposomes; microarray; immune response

THE DYNAMIC ENVIRONMENT of the olfactory epithelium (OE), where olfactory sensory neurons (OSNs) undergo apoptosis and are replaced from a pool of basal progenitor cells throughout life (9, 41, 49), continues to provide insight into mechanisms of neuroprotection, neuronal apoptosis, and neurogenesis. More specifically, recent studies (6, 23, 33) on the interaction between the immune system and neurogenesis in the OE have strengthened the case for the participation of macrophages in the regulation of OSN survival and the proliferation, differentiation, and maturation of OSN basal progenitor cells in the unperturbed OE and during OE remodeling as a result of olfactory bulbectomy (OBX)-induced OSN apoptosis.

OBX induces the synchronous retrograde apoptosis of mature OSNs (mOSNs) via activation of the caspase pathway (12, 16). The apoptotic mOSNs are phagocytosed by macrophages (53) that secrete chemokines, including macrophage inflammatory protein-1α (MIP-1α) and monocyte chemoattractant protein-1 (MCP-1), to recruit additional macrophages into the degenerating OE (26, 33). Furthermore, OBX-induced mOSN apoptosis results in an increase in mitotic activity of basal cells in the OE, some of which differentiate into new OSNs (8, 11, 48). The temporal increase in basal cell proliferation following OBX coincides with the infiltration of macrophages into the OE (42).

In addition to their phagocytic capacity, macrophages secrete a number of molecules that perform neuroprotective functions in the nervous system (55), including the OE. For example, following OBX, the levels of expression of leukemia inhibitory factor (LIF) and its receptor (LIFR) were increased (24, 25, 42). LIF was expressed by macrophages and mOSNs, and LIFR was expressed by globose basal cells (GBCs) and olfactory ensheathing cells (OECs). LIF has also been demonstrated to promote basal cell proliferation in vivo and in vitro (3, 31). Additionally, growth factors that are secreted by macrophages (43) as well as other cell types in the OE have been implicated in regulating basal cell proliferation and differentiation as well as OSN survival. These include members of the fibroblast growth factor (FGF), transforming growth factor-α and β (TGF-α, TGF-β), insulin-like growth factor (IGF), and platelet-derived growth factor (PDGF) families (reviewed in Refs. 29, 36, 49), thus supporting the evidence for macrophage-mediated neurogenesis in the OE.

We previously introduced the use of liposome-encapsulated clodronate (Lip-C), which induces apoptosis selectively in macrophages, to deplete resident and recruited macrophages in the OE (6). Analogous to OBX, where mOSNs are selectively depleted through induced retrograde apoptosis, intranasal administration of Lip-C selectively depletes macrophages in the nasal mucosa. Following phagocytosis of Lip-C by macrophages, phospholipases within the macrophages degrade the phospholipid bilayer of the liposome, resulting in the release of free clodronate molecules inside the cell to induce macrophage apoptosis (58). Studies utilizing Lip-C-induced macrophage depletion have defined tissue protective/remodeling roles of macrophages in experimental models ranging from retinal...
vasculogenesis (13) and central nervous system remyelination (32) to suppression of experimental colitis (46).

We demonstrated that combined intranasal and intravenous administration of Lip-C induced a significant 38 and 35% reduction in resident and recruited macrophages in the OE of sham and 48 h OBX mice, respectively (6). Macrophage depletion in the OE resulted in a decrease in OE thickness and the numbers of olfactory marker protein (OMP)+ mOSNs and 5-bromo-2-deoxyuridine (BrdU)+ proliferating basal cells in sham and 48 h OBX mice. Additionally, we demonstrated that macrophage depletion in the OE led to an increase in activated caspase-3-mediated apoptosis of OSNs at 48 h following OBX. However, the molecular mechanisms that underlie this macrophage-mediated neuroprotection and neurogenesis in the OE are not completely understood.

The aim of this study was to identify macrophage-mediated molecular mechanisms that regulate OSN survival as well as the proliferation and differentiation of OSN progenitors. We used a microarray pattern analysis to analyze differential gene expression levels in the OE of sham- or OBX-treated mice that received intranasal/intravenous Lip-C or empty liposomes to identify genes whose expression was modulated by selective macrophage depletion. Molecular validation of the expression patterns of selected genes and the cellular localization of selected gene products in the OE contributed to a proposed model of the role of macrophages in these processes.

METHODS

Animals. Twenty-four 6-wk-old C57BL/6J male mice obtained from Jackson Labs (Bar Harbor, ME) were maintained on a 12:12 h light-dark cycle in the Department of Laboratory Animal Research facilities and were given food and water ad libitum. All protocols were implemented in accordance with National Institutes of Health guidelines and approved by the University of Kentucky Institutional Animal Care and Use Committee.

Macrophage depletion. Lip-C was used to deplete both resident macrophages and blood monocytes, which are recruited into the OE following OBX. Liposomes were prepared as previously described (58). Clodronate was a gift from Roche Diagnostics (Mannheim, Germany). Overlapping series of injections of either Lip-C (n = 12) or empty liposomes without clodronate (Lip-O, n = 12) were administered locally and systemically (Table 1) as previously described (6). This injection paradigm was derived from our previous study (6) that reported an 89% decrease in the number of macrophages in the lungs of Lip-C mice compared with Lip-O mice, which was comparable to published data (34).

OBX. Mice were anesthetized with Avertin. Six mice per group (Lip-O, Lip-C; n = 12) underwent bilateral OBX, and six mice per group (n = 12) underwent bilateral sham OBX on the second day of the injection series (Table 1); surgeries were performed as previously described (24).

Tissue harvesting. Mice that were used for microarray and real-time RT-PCR analysis (n = 12, 3/group/surgery) were euthanized by CO₂ asphyxiation 48 h following either OBX or sham OBX. Olfactory nasal mucosae, lungs, spleens, and brains were rapidly microdissected using RNase-free techniques, weighed, flash-frozen in liquid N₂, and stored at −80°C. Mice used for histological and immunohistochemical studies (n = 12, 3/group/surgery) were perfused with 3% paraformaldehyde, followed by microdissection of olfactory nasal mucosae, lungs, spleens, and brains that were processed and sectioned as previously described (33).

All reagents used in animal preparation and tissue collection were molecular biology grade. Surgical instruments and disposables were RNase-free, and surgery and tissue collection were performed using RNase-free techniques.

RNA isolation. Total RNA was isolated from the olfactory mucosa of each mouse in TRI Reagent (Molecular Research Center, Cincinnati, OH) under RNase-free conditions as previously described (23, 33). The yield and purity of each total RNA sample was analyzed spectrophotometrically (DU 640 Spectrophotometer; Beckman Coulter, Fullerton, CA) and with a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). All samples had A₂₆₀/A₂₈₀ ratios >2.0 and had two sharp peaks corresponding to 18S and 28S RNA on Bioanalyzer electropherograms.

High-density oligonucleotide arrays. Microarray analysis using Affymetrix Murine Genome (MG) U430 2.0 GeneChips (Affymetrix, Santa Clara, CA) was performed in the University of Kentucky Microarray Core Facility. The microarray dataset was deposited into the Gene Expression Omnibus database; the accession number is GSE6540. Twelve GeneChips were used: three each for Lip-O sham, Lip-O OBX, Lip-C sham, and Lip-C OBX mice. Each GeneChip was hybridized with unpooled total RNA isolated from the olfactory mucosa of a single mouse. Hybridization, normalization, and signal acquisition were performed as previously described according to the Affymetrix protocol (23, 33). Hybridization signals were normalized across all chips and analyzed using Affymetrix Microarray Suite Version 5 (MAS5). Each normalized hybridization signal was assigned an “Absolute Call” of Present, Marginal, or Absent by MAS5. The annotated probe sets that were identified as Absent on every chip as well as expressed sequence tags, quality control, and nonannotated probe sets were excluded from the statistical analysis.

Statistical analysis of microarray data. To determine which of the Present annotated probe sets were significantly regulated by Lip-C, OBX, or both, the mean hybridization signals were analyzed using a 2×2 analysis of variance (ANOVA) assuming equal variance, with

### Table 1. Experimental schedule

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Injection Route/Surgery</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lip-O (n = 12)</td>
<td>in (50 µl)</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
<td>euthanize</td>
</tr>
<tr>
<td></td>
<td>iv (200 µl)</td>
<td>0 µl</td>
<td>sham or OBX</td>
<td>200 µl</td>
<td></td>
</tr>
<tr>
<td>Lip-C (n = 12)</td>
<td>in (50 µl)</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
<td>euthanize</td>
</tr>
<tr>
<td></td>
<td>iv (200 µl)</td>
<td>0 µl</td>
<td>sham or OBX</td>
<td>200 µl</td>
<td></td>
</tr>
</tbody>
</table>

Volumes and days of injections, along with days of surgery and tissue harvesting. Six mice from each treatment were used for microarray analysis, while the other 6 mice from each treatment were used for histology. Six mice from each treatment (3 each for the microarray analysis and histology) underwent either sham surgery or OBX. Abbreviations: Lip-O, empty liposomes; Lip-C, liposomes containing clodronate; in, intranasal; iv, intravenous; OBX, olfactory bulbectomy.

Modified from Ref. 6.
group (Lip-O, Lip-C) as one factor and treatment (sham, OBX) as the other factor. The overall significant (P < 0.01) probe sets were further analyzed by pair-wise comparisons to characterize the probe sets as either: 1) main effect genes, defined as having a statistically significant difference (P < 0.01) between treatments, groups, or both, with no significant interaction (P ≥ 0.01); 2) interaction effect genes, defined as having a statistically significant interaction (P < 0.01) between treatment and group; or 3) genes that did not have a statistically significant pair-wise effect (P ≥ 0.01). The fold changes of the expression levels were calculated as the mean hybridization signals of Lip-C vs. Lip-O for group and OBX vs. sham for treatment. Data were analyzed using SAS (SAS Institute, Cary, NC) and SigmaPlot (SPSS, Chicago, IL).

Categorical analysis. Expression Analysis Systematic Explorer (EASE) was used to identify overrepresented functional gene categories based on Gene Ontology (GO) Biological Process, Cellular Component, and Molecular Function annotations within the main and interaction effect genes. Up- and downregulated main effect groups, and interaction genes were analyzed separately against the genes that were overall significantly regulated (P < 0.01) between treatments, groups, or both, with no significant interaction (P ≥ 0.01); 2) interaction effect genes, defined as having a statistically significant interaction (P < 0.01) between treatment and group; or 3) genes that did not have a statistically significant pair-wise effect (P ≥ 0.01). The fold changes of the expression levels were calculated as the mean hybridization signals of Lip-C vs. Lip-O for group and OBX vs. sham for treatment. Data were analyzed using SAS (SAS Institute, Cary, NC) and SigmaPlot (SPSS, Chicago, IL).

Real-time RT-PCR. Real-time RT-PCR (qPCR) was performed to validate the microarray expression patterns of seven selected genes using the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). cDNA from 1 µg of total RNA from each of the same unpoled RNA samples used for microarray analysis (n = 12) was reverse-transcribed using the GeneAmp RNA PCR Core Kit (Applied Biosystems) according to the manufacturer’s protocol; samples without reverse transcriptase were run to ensure that there was no DNA contamination. The qPCR reactions were run using Power Sybr Green PCR Master Mix (Applied Biosystems) according to the manufacturer’s protocol with 0.5 µl each of forward and reverse primer (15 µM, Invitrogen) and 1 µl unpoled sample cDNA (10 ng). Primer sequences were either obtained from the literature or designed using Primer Express (Applied Biosystems). cDNA for the derivation of standard curves and no-template controls using Master Mix without cDNA to confirm the absence of nonspecific amplification and primer-dimer formation were run in duplicate. Table 2 lists the primer sequences and references, annealing temperature of primers, expected amplicon size (bp), and melting temperature of amplicon for each qPCR amplicon. Reaction parameters were as follows: 2 min at 50°C; 15 min at 95°C; and 40 cycles of 15 s at 94°C, 30 s at the appropriate annealing temperature for each primer, and 1 min at 72°C. All 12 samples were run in triplicate. A dissociation curve to verify the presence of a single amplicon with the appropriate melting temperature and a 2% agarose gel stained with 1× Sybr Green I (Invitrogen) in dH₂O to verify the presence of a single amplicon of the correct size were performed for each amplicon. Samples without reverse transcriptase had no amplification after 40 cycles, confirming the absence of DNA contamination.

Immunohistochemistry. Immunohistochemistry was performed to localize HDGF (hepatoma-derived growth factor) and SDF-1 (stromal cell-derived factor 1, CXCL12) in the OE. The primary antibodies used in our study were a polyclonal goat anti-human HDGF (1:100, sc-28062; Santa Cruz Biotechnology, Santa Cruz, CA), a polyclonal goat anti-human SDF-1 (1:250, sc-6193; Santa Cruz), and a monoclonal rat anti-mouse CD68 (1:50, MCA 1957 GA; Serotec, Raleigh, NC). For the nuclear antigen HDGF, sections were rehydrated with 0.1 M phosphate-buffered saline (PBS, pH = 7.4) and pretreated with 2.5% preheated trypsin (37°C) for 2 min followed by three 5-min rinses in PBS; sections were then incubated in 2 N HCl at 37°C for 40 min followed by two 5-min rinses in borate buffer (pH 7.4) and three 5-min rinses in PBS. To compare the distribution of immunoreactivity for SDF-1 and CD68, a macrophage marker, adjacent sections were rehydrated with PBS without pretreatment.

All sections were incubated in a preosak solution (1% BSA with 0.5% Tween 20 in PBS for HDGF, 2% BSA with 0.4% Triton X-100 in PBS for SDF-1 and CD68) for 30 min at room temperature, and then in either the primary antibody diluted in preosak or preosak alone overnight at 4°C. To test the specificity of the HDGF antibody, a blocking peptide (1:10 antibody-peptide; Santa Cruz Biotech) was incubated with the primary antibody overnight at 4°C, followed by centrifugation for 15 min; the supernatant from the blocking peptide solution was used in place of the primary antibody. Species-specific rhodamine red X-conjugated secondary antibodies (1:150; Jackson Immunoresearch, West Grove, PA) were applied to tissue sections for 1 h at room temperature in the dark. Sections that were incubated with preosak alone or with the blocking peptide supernatant showed no specific staining. Additionally, positive control tissues were used to compare immunoreactivity for each antibody to published data, which corresponded in each case.

Table 2. Primers used for real-time RT-PCR validation

<table>
<thead>
<tr>
<th>Target</th>
<th>Sequence</th>
<th>Tm, °C</th>
<th>Size, bp</th>
<th>Tm, °C</th>
<th>Reference (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omp</td>
<td>F: 5′-GACATTCTTCTAGGTGTCC-3′</td>
<td>50</td>
<td>348</td>
<td>85</td>
<td>(7)</td>
</tr>
<tr>
<td></td>
<td>R: 5′-CACGTTTGGCCAAGGGG-3′</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crabp1</td>
<td>F: 5′-TGAAAGGGCATGTCGAAGAA-3′</td>
<td>50</td>
<td>266</td>
<td>85</td>
<td>Primer Express</td>
</tr>
<tr>
<td></td>
<td>R: 5′-CAAGGCCCTGAAAGGTTGTC-3′</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ccxr4</td>
<td>F: 5′-GTCCTCGAGACTAGTGAATCC-3′</td>
<td>50</td>
<td>525</td>
<td>85</td>
<td>(57)</td>
</tr>
<tr>
<td></td>
<td>R: 5′-CACAGATGTACCTGTGATCC-3′</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurod1</td>
<td>F: 5′-ACAGACCTCTCTGTGAAAGGTTG-3′</td>
<td>50</td>
<td>366</td>
<td>88</td>
<td>(15)</td>
</tr>
<tr>
<td></td>
<td>R: 5′-GCGGATGTTTGGTGTGAAGG-3′</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ccd2</td>
<td>F: 5′-CCCACACCAAAAGGAAAGAATCT-3′</td>
<td>50</td>
<td>94</td>
<td>81</td>
<td>(17)</td>
</tr>
<tr>
<td></td>
<td>R: 5′-CCGCTTAAGGTGAAAACTGG-3′</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soc5</td>
<td>F: 5′-AAGCGGGGGCAAGAGATCTTCC-3′</td>
<td>50</td>
<td>158</td>
<td>82</td>
<td>(56)</td>
</tr>
<tr>
<td></td>
<td>R: 5′-TCTGGCGCAACATTTGGT-3′</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pdap1</td>
<td>F: 5′-AGAAGGTCAGGAACTGATCC-3′</td>
<td>50</td>
<td>167</td>
<td>81</td>
<td>Primer Express</td>
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<tr>
<td></td>
<td>R: 5′-TGTTTCTTCAGGATGTCGCA-3′</td>
<td></td>
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</tbody>
</table>

Abbreviations: Ccd2, cyclin D2; Soc5, suppressor of cytokine signaling 5; Pdap1, PDGFA associated protein 1; Neurod1, neurogenic differentiation 1; Omp, olfactory marker protein; Crabp1, cellular retinoic acid binding protein 1; Ccxr4, chemokine (C-X-C motif) receptor 4; F, forward primer; R, reverse primer; Tm, primer annealing temperature; Tm, amplicon melting temperature.

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RESULTS

Data analysis and significance tests. Among the 27,664 annotated probe sets on the Affymetrix MG U430 2.0 GeneChip, an Absolute Call analysis identified 21,954 probe sets (79%) that were Present on at least one of the 12 GeneChips. Of these, a 2-way ANOVA identified 4,085 overall significantly regulated genes ($P < 0.01$, 19%). Pair-wise comparisons of the 4,085 overall significantly regulated genes identified 4,024 genes (99%) that had a significant ($P < 0.01$) pair-wise main or interaction effect. These included 3,044 genes (76%) whose mean hybridization signals had a significant main effect ($P < 0.01$) of treatment ($n = 2,266$, 74%), group ($n = 364$, 12%), or both ($n = 414$, 14%), and 980 genes (24%) that had a significant interaction effect ($P < 0.01$) between group (Lip-O, Lip-C) and treatment (sham, OBX).

The 3,044 significant main effect genes are those genes whose response to OBX was not statistically significantly different between the Lip-O and Lip-C mice. Of the main effect genes, 2,680 genes (88%) had mean hybridization signals that were significantly different between the sham and OBX mice (treatment effect), 1,347 were significantly upregulated, and 1,333 were significantly downregulated at 48 h following OBX. For example, the mean hybridization signal of Msr1 (macrophage scavenger receptor 1; Fig. 1A), a mediator of phagocytosis of apoptotic cells, was not significantly different between Lip-O and Lip-C sham mice as determined by a two-sample t-test ($P \geq 0.01$). At 48 h following OBX, the mean group (combined Lip-O and Lip-C) expression level of Msr1 increased significantly ($P < 0.01$, 1.8-fold). Msr1 was expressed at a marginally lower level in the Lip-C mice compared with the Lip-O mice at 48 h following OBX ($P = 0.05$, 1.2-fold).

Of the 3,044 main effect genes, the mean hybridization signals of 778 genes (26%) had a statistically significant difference ($P < 0.01$) between Lip-C and Lip-O mice (group effect). For example, the mean hybridization signal of Sox2 (SRY-box containing gene 2; Fig. 1B), a neurogenesis transcription factor, was significantly lower in the Lip-C sham and OBX mice compared with the Lip-O mice ($P < 0.01$; 1.4- and 1.2-fold, respectively), while the group mean hybridization signal increased marginally at 48 h following OBX ($P = 0.02$, 1.2-fold). Of the 778 main effect group genes, the mean hybridization signals of 432 genes (56%) were significantly greater in the Lip-C mice compared with the Lip-O mice, while 346 genes (44%) were significantly lower in the Lip-C mice compared with the Lip-O mice.

There were 414 genes that overlapped the 778 main effect group genes and 2,680 main effect treatment genes. These 414 genes had statistically significant differences between OBX and sham (treatment) and Lip-C and Lip-O (group). For example, the mean hybridization signal of Il13ra1 (interleukin 13 receptor, alpha 1; Fig. 1C), a marker of alternative activation of macrophages, was significantly greater in the Lip-C compared with the Lip-O sham mice ($P < 0.01$, 1.4-fold). At 48 h following OBX, the mean hybridization signal of Il13ra1 increased significantly (Lip-O, $P \geq 0.01$, 1.2-fold; Lip-C, $P < 0.01$, 1.4-fold; overall $P < 0.01$, 1.3-fold), while the expression level in the Lip-C mice was less than in the Lip-O mice at 48 h following OBX ($P \geq 0.01$, 1.1-fold).

The 980 interaction genes are those whose expression levels in the Lip-C mice had a statistically significantly different response to OBX than in the Lip-O mice. For example, the mean hybridization signal of Hdgf (Fig. 1D), a neuronal survival and proliferative growth factor, was not significantly different between the Lip-O and Lip-C sham mice. However, at 48 h following OBX, the expression of Hdgf increased significantly ($P < 0.01, 1.2$-fold) in the Lip-O mice and remained relatively unchanged in the Lip-C mice ($P = 0.3$). The expression of Hdgf was significantly greater in the Lip-O mice compared with the Lip-C mice at 48 h following OBX ($P < 0.01, 1.2$-fold).

The expression levels of these significantly regulated genes displayed a variety of patterns indicative of their main or interaction effects, which provided evidence for diverse functional roles in the OE and a basis on which to select relevant genes for further analysis.

**Molecular validation using qPCR.** Seven genes (Table 2) were carefully selected for qPCR validation based on their microarray expression patterns and the increase in OSN apoptosis and decrease in OSN progenitor cell proliferation associated with macrophage depletion reported in our previous study (6). The microarray hybridization patterns of the main effect genes Omp (treatment), Crabp1 (cellular retinoic acid binding protein $1$; group), and Cxcr4 [chemokine (C-X-C motif) receptor 4; group/treatment]; and of the interaction genes Neurod1 (neurogenic differentiation $1$), Ccdn2 (cyclin D2), Socs3 (suppressor of cytokine signaling $5$), and Pdap1 (PDGFA-associated protein $1$) were validated by qPCR mean quantity patterns. For each gene, the graphical patterns of the qPCR mean quantities closely resembled that of the microarray mean hybridization signals (examples shown in Fig. 2). Group and treatment fold changes from the microarray and qPCR experiments were also statistically similar, with the exception of three cases in which the fold changes from the two experiments were not statistically similar but were in the same direction and of similar magnitude (Table 3).

**Categorical analysis.** EASE analysis was performed using GO Biological Process, Cellular Component, and Molecular Function annotations to identify functional biological themes within the significant main and interaction effect genes (Table 4). Of the 2,680 genes with a significant treatment main effect and therefore whose changes in expression levels were primarily regulated by OBX, there were 1,347 genes that were significantly upregulated at 48 h following OBX compared with sham mice, among which EASE identified 35 overrepresented categories (EASE score $< 0.05$). These 35 categories included $12$ categories associated with the cytoskeleton and cell adhesion, consisting of genes encoding actins, laminins, vimentins, collagens, and other cytoskeleton and extracellular matrix molecules. There were eight overrepresented categories of membrane-associated genes that included scavenger receptor, CD antigen, G protein-coupled receptor, growth factor receptor, and aquaporin genes. These results are consistent with the increase in the OE immune response and tissue remodeling activity occurring at 48 h following OBX. Among the 1,333 main effect treatment genes that were significantly downregulated at 48 h following OBX compared with sham mice, there were 43 overrepresented categories. Eight of the 43 overrepresented categories were olfactory-associated gene categories, which included $11$ olfactory receptor genes (Olfr15, 16, 17, 64, 68, 69, 140, 701, 749, 870, and 976) and Omp. The identification of overrepresented categories of downregulated genes containing olfactory, transport, ion channel, and neurosynaptic genes is consistent with the decrease in the number of mature OSNs resulting from an increase in OSN apoptosis at 48 h following OBX.

The EASE analysis of the genes with a significant group main effect was of special interest because the expression levels of these genes had a statistically significant Lip-C effect and thus were regulated primarily by the decrease in the number of macrophages in the OE. Of the 778 main effect group genes, the 432 genes whose mean hybridization signals were significantly greater in Lip-C mice compared with Lip-O mice had 23 overrepresented functional gene categories, including one growth factor activity category that included Fgf21 (fibroblast growth factor $21$) and Bmp6 (bone morphogenetic protein $6$). Among the $346$ main effect group genes whose mean hybridization signals were significantly lower in the Lip-C mice compared with Lip-O mice, there were $14$ overrepresented functional gene categories. Three of these $14$ categories were functionally associated with the immune response, which included Cxcr4, and two categories were associated with the cell cycle, which included Cdk4 (cyclin-dependent kinase $4$), consistent with the depletion of macrophages in the OE and the decrease in basal cell proliferation, respectively, reported in Lip-C sham and OBX mice compared with Lip-O mice.

Among the $980$ interaction genes, there were $37$ overrepresented GO categories. Of particular interest was the overrepresentation of $12$ nucleic acid activity/transcription categories that included genes involved in the promotion and regulation of the cell cycle, e.g., Neurod1 and Cdk9 (cyclin-dependent kinase $9$). Additionally, apoptosis genes, e.g., Bcl2 (B-cell leukemia/lymphoma $2$), were present among the five overrepresented metabolism categories.

We next identified specific statistically significant pairwise effect genes that were functionally associated with the identified overrepresented immune response, growth factor activity, and cell cycle and neurogenesis categories, in addition to apoptosis genes, to identify macrophage-mediated mechanisms in the OE that result in the induction of OSN survival, turnover, and maturation following OBX.

**Immune response genes.** Numerous immune response genes (Supplemental Table S1) had significant pairwise effects. As indicated by the EASE results, nearly all of the main effect immune-associated genes had mean hybridization signals that were either significantly ($P < 0.01$) or marginally ($0.01 < P < 0.05$) lower in the Lip-C mice, upregulated at 48 h following OBX, or both. These results are consistent with the decrease in the number of macrophages in the OE of Lip-C mice and the parallel increase in the number of macrophages in the OE of Lip-O and Lip-C mice at 48 h following OBX.

There were three scavenger receptor genes that had significant main effects: Cd36, Scarb1 (scavenger receptor, class B $1$), and the aforementioned Msr1. Scavenger receptors mediate the binding of apoptotic cells to macrophages to induce phagocytosis. Cd36 and Scarb1 were identified as significant group and treatment main effect genes that were expressed at a significantly lower level in the Lip-C mice compared with the

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1 The online version of this article contains supplemental material.
Fig. 2. Validation of the microarray expression patterns of 3 genes with significant main effects (A–F) and 2 genes with significant interactions (G–J) using real-time RT-PCR (qPCR). Patterns of the mean hybridization signals ± SD from the microarray analysis (left column) for each gene were positively validated by the mean quantities (ng) ± SD determined by qPCR (right column). A, B: Omp, olfactory marker protein (treatment main effect). C, D: Crabp1, cellular retinoic acid binding protein 1 (group main effect). E, F: Cxcr4, chemokine (C-X-C motif) receptor 4 (group and treatment main effect). G, H: Neurod1, neurogenic differentiation 1 (interaction effect). I, J: Ccnd2, cyclin D2 (interaction effect). Dotted lines, empty liposome-treated (Lip-O) mice; solid lines, liposome-encapsulated clodronate-treated (Lip-C) mice.
Lip-O mice ($P < 0.01$, 1.4-fold), and were both significantly upregulated following OBX ($P < 0.01$, 1.5-fold).

Interestingly, binding of apoptotic cells to scavenger receptors on macrophages has been reported to lead to the alternative activation of macrophages, a pathway that promotes tissue restoration and remodeling. Accordingly, a number of alternative activation markers were identified as genes with significant main effects, including 

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Lip-C/Lip-O</th>
<th>OBX/Sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stat6</td>
<td>1.11</td>
<td>−1.08</td>
</tr>
<tr>
<td>Cxcr4</td>
<td>−1.56</td>
<td>1.26</td>
</tr>
<tr>
<td>Interation Genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurod1</td>
<td>−2.99</td>
<td>1.27</td>
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The major overrepresented categories for each pattern are shown along with examples of genes in each category. Functionally related Gene Ontology (GO) Biological Process, Cellular Component, and Molecular Function categories were grouped into broadly defined categories; the number of grouped categories is in parentheses. Group, Lip-C vs. Lip-O; Treatment, OBX vs. sham.
The mean hybridization signals for all of the identified growth factor genes that had significant interaction effects were lower in Lip-C mice compared with Lip-O mice, which is consistent with the apparent decrease in neuroprotection observed in Lip-C mice compared with Lip-O mice at 48 h following OBX. For example, Hdgf was identified as a gene with a significant interaction as previously discussed. Another growth factor gene with a significant interaction was Tgfbr1 (transforming growth factor, beta receptor 1). Tgfbr1 and Tgfbr2 comprise the receptor for TGF-β. Tgfbr2 was identified as a significant treatment main effect gene that was upregulated at 48 h following OBX (1.1-fold, \( P = 0.08 \)) at 48 h following OBX. The expression level of the main effect treatment and group gene Ascl1 (apoptosis inhibitor 5), an antiapoptosis regulator, was 1.2-fold significantly \( (P < 0.01) \) lower in Lip-C and OBX mice.

Cell cycle and neurogenesis genes. EASE analysis identified several cell cycle gene categories that were overrepresented, consistent with the decrease in basal cell proliferation in the OE of Lip-C sham and OBX mice compared with Lip-O mice. Among the cell cycle and neurogenesis genes that had significant pair-wise effects (Supplemental Table S3) were several transcription factors, including the basic helix-loop-helix (bHLH) transcription factors Ascl1 (Mash1), Hes6, Ngn1, and Neurod1, which promote the proliferation and/or differentiation of OSN progenitor cells. Hes6 and Ngn1 were identified as significant treatment and group main effect genes that were significantly upregulated at 48 h following OBX \( (P < 0.01; 1.3-\) and 1.4-fold, respectively) and expressed at a significantly lower level in the Lip-C mice compared with the Lip-O mice \( (P < 0.01; 1.7-\) and 2.9-fold, respectively). Neurod1 was identified as a significant interaction gene that was expressed at a significantly lower level in the Lip-C mice compared with the Lip-O mice \( (P < 0.01; 3.6\)-fold) and significantly upregulated in the Lip-O mice \( (P < 0.01, 1.4\)-fold) but relatively unchanged in the Lip-C mice at 48 h following OBX. Ascl1 was a significant group gene that was expressed at a significantly lower level in the Lip-C mice compared with the Lip-O mice \( (P < 0.01, 7.9\)-fold). Sox2, a neurogenesis transcription factor, was identified as a significant group main effect gene as previously discussed.

Additional cell cycle regulatory genes that had significant pair-wise effects included several cyclin-associated genes. These genes included the cell-cycle promoters Ccnb1 (cyclin B1; group) and the catalytic partner of G1/S-phase specific D-type cyclins Cdk4 (cyclin-dependent kinase 4; group and treatment). The cyclin-associated genes were generally expressed at a significantly or marginally lower level in the Lip-C mice compared with the Lip-O mice and were significantly or marginally upregulated at 48 h following OBX.

Apoptosis genes. The OBX-induced apoptosis of mOSNs was confirmed by the significant downregulation at 48 h following OBX of 11 odorant receptor genes, in addition to Omp, that were identified as significant treatment main effect genes. Our microarray analysis also identified pro- and antiapoptosis genes that had significant main and interaction effects with a variety of expression patterns (Supplemental Table S4). The regulation of these genes indicated the involvement of both the extrinsic and intrinsic apoptosis signaling pathways in the OE. For example, Bak1 (BCL2-antagonist/killer 1), the proapoptotic antagonist of the antiapoptosis gene Bcl2 (B-cell leukemia/lymphoma 2), was identified as a 1.3-fold significantly upregulated main effect group gene that was upregulated \( (1.1\)-fold, \( P = 0.08 \)) at 48 h following OBX. The expression level of the main effect treatment and group gene Api5 (apoptosis inhibitor 5), an antiapoptosis regulator, was 1.2-fold significantly \( (P < 0.01) \) lower in Lip-C and OBX mice.

Immunohistochemical localization of selected proteins. Since macrophages are known to express and secrete SDF-1, which is the ligand for CXCR4, we localized SDF-1 and the macrophage-specific marker CD68 (Fig. 3) on adjacent tissue sections. OE delimited by arrowheads. Scale bar = 50 μm.
sections. Based on the localization and distribution of SDF-1 and CD68 immunoreactivity in adjacent sections (Fig. 3, B, D, F, and H), SDF-1 appeared to be expressed by a subpopulation of CD68+ macrophages. The amount of SDF-1 immunoreactivity appeared similar between Lip-O and Lip-C sham mice in the representative sections shown in Fig. 3, A and E. At 48 h following OBX, immunoreactivity for SDF-1 appeared to increase in both Lip-O and Lip-C mice (Fig. 3, C and G), consistent with OBX-induced macrophage infiltration.

The expression pattern of the gene product of the significant interaction gene Hdgf was investigated on the protein level using immunohistochemistry (Fig. 4). Comparable to the microarray expression pattern (Fig. 1D), the amount of HDGF immunoreactivity appeared similar in representative sections of the OE of Lip-O and Lip-C sham mice (Fig. 4, A and C). At 48 h following OBX, HDGF immunoreactivity appeared to increase greatly in the OE of Lip-O mice and minimally in the OE of Lip-C mice (Fig. 4, B and D). HDGF immunoreactivity was localized in the basal cell layer of Lip-O and Lip-C sham mice. At 48 h following OBX, HDGF immunoreactivity was localized in the OSN and basal cell layers. Because HDGF is a nuclear antigen, we counterstained HDGF-stained sections with 6-diamidino-2-phenylindole dihydrochloride (DAPI) (Fig. 4, E–G) to demonstrate that the HDGF immunoreactivity was nuclear. HDGF was localized to the nucleus as observed in the merged image (Fig. 4G) of the HDGF+ cell (Fig. 4E) and the DAPI+ nucleus (Fig. 4F).

**DISCUSSION**

The results of this study demonstrate that: 1) clodronate-induced macrophage depletion in the OE resulted in significantly altered expression levels of genes with complex patterns of regulation between treatment (sham, OBX) and group (Lip-O, Lip-C); 2) immune response, growth factor activity, nucleotide activity, cell cycle, and cytoskeleton were among the GO functional categories of genes that were significantly overrepresented within the genes that had significant pair-wise effects; and 3) cellular localization of SDF-1 and HDGF characterized novel macrophage-mediated molecular mechanisms regulating OSN survival and basal cell proliferation. The categorical analyses, patterns of expression of specific significantly regulated genes in response to OBX and macrophage depletion, and localization of selected gene products suggested several molecular pathways by which macrophage depletion in the OE may lead to increased OSN apoptosis, and decreased proliferation, differentiation, and maturation of OSN progenitor cells in response to OBX as illustrated in our proposed working model (Fig. 5).

**OBX-induced macrophage activation.** OBX-induced OSN apoptosis results in activation of resident OE macrophages. Macrophage activation in the OE is regulated in part through the binding of exposed ligands, including phosphatidylserine, on the apoptotic OSNs to scavenger receptors (SRs) on the macrophage plasma membrane (23), most notably MSR-1, CD36, and SCARB-1 as indicated in our proposed model (Fig. 5). A previous study from our laboratory using a transgenic mouse model in which the Msr1 gene was disrupted demonstrated decreased macrophage infiltration into the OE at multiple time points following OBX (23). Additionally, immune response genes, including other phagocytosis-related genes, were expressed primarily at lower levels in the transgenic mice compared with wild-type mice and were upregulated at multiple time points following OBX, which was confirmed and extended in this study as indicated by the EASE results. Interestingly, binding of apoptotic cells to scavenger receptors has been reported to result in the alternative activation of macrophages (18). In this study, two markers of alternative activation were identified as significantly regulated:

*Il13ra1*, which mediates anti-inflammatory mechanisms in macrophages and upregulates the expression of the mannose receptor; and *Mrc1*, which is involved in cell adhesion and upregulates the expression of arginase, leading to the production of anti-inflammatory mediators (27). The data from this study and...
Macrophage-mediated OSN survival. Since we previously reported that partial macrophage depletion in the OE resulted in increased OSN apoptosis at 48 h following OBX and decreased numbers of mOSNs in sham and OBX mice (6), we hypothesized that upon alternative activation following OBX-induced OSN apoptosis, macrophages secrete growth and survival factors to delay or prevent apoptosis in OSNs. Based on our microarray analysis, these factors may include IGF-1 and/or RA, both of which have been reported to promote the survival of OSNs in vivo and in vitro (28, 45, 47), while IGF-1R (IGF-1 receptor) has been localized to OSNs and basal cells (40, 54). Igf1 was a significantly upregulated main effect treatment gene that was expressed at a marginally lower level in Lip-C mice compared with Lip-O mice, while a number of IGF signaling proteins were significantly upregulated at 48 h following OBX. Crabp1, a signaling molecule for RA that has been reported to promote neuronal survival (39, 59), may also be a macrophage-derived mediator of OSN survival following OBX.

Chemoattraction and maintenance of structure. It was previously reported that macrophage activation in the OE following OBX subsequently results in the secretion of chemoattractants, including MIP-1α and MCP-1, leading to the recruitment of additional macrophages into the OE (26, 33). Both Mip1α and Mcp1 were expressed at a lower level in the Lip-C mice compared with the Lip-O mice (P ≥ 0.01; 3.3- and 2.1-fold, respectively). Based on the decrease in OE thickness in the Lip-C sham and OBX mice compared with the Lip-O mice, it is also possible that the activated macrophages secrete molecules or enzymes that regulate the expression of cytoskeletal and extracellular matrix components to promote the trafficking of leukocytes into the OE as well as the migration of leukocytes and newly generated OSNs and their progenitors within the OE. Consistent with this hypothesis was the overrepresentation of structure-associated genes, including genes that encode actins, tubulins, and laminins, among the significantly upregulated main effect treatment genes identified by the EASE analysis, which is consistent with previous findings that demonstrated that contact signaling genes, e.g., integrins and cadherins, were differentially regulated at multiple time points following OBX (24). Additionally, the overrepresentation of others from our laboratory (6, 23) strongly suggest that alternative activation of OE macrophages leads to survival of OSNs and promotes OE remodeling following OBX, as discussed below.

Fig. 5. Proposed working model of macrophage-mediated molecular mechanisms of olfactory sensory neuron (OSN) neuroprotection and neurogenesis. At 48 h following OBX, apoptotic OSNs activate resident macrophages (red) via scavenger receptors (SR; for example, MSR-1, CD36, SCARB-1). Binding of apoptotic OSNs to scavenger receptors on the macrophages upregulates the expression of II13ra1 and Mrec1(MR). The activated macrophages then secrete a number of potential growth and survival factors that may include IGF-1, RA, and HDGF, which act upon OSNs to delay/prevent apoptosis. Additionally, macrophages that are recruited into the OE by MIP-1α and MCP-1 induce globose basal cell proliferation through LIF binding to its receptor, LIFR, resulting in the upregulation of cyclin-related genes, the neurogenesis bHLH TFs HES6, NGN1, and NeuroD1 and the neurogenesis TF SOX2. The daughter cells of globose basal cell division that are committed to the OSN lineage express HDGF as a survival or proliferative factor. Macrophages also likely secrete a number of molecules, including SDF-1, TGF-β, and RA to promote the survival, differentiation, and maturation of OSN progenitor cells. Abbreviations: mOSN, mature olfactory sensory neuron; GBC, globose basal cell; OB, olfactory bulb; OBX, olfactory bulbectomy; OE, olfactory epithelium; SR, scavenger receptor; MSR-1, macrophage scavenger receptor-1; SCARB-1, scavenger receptor class B-1; II13ra1, chemokine (C-X-C motif) receptor 4; IGF-1, insulin-like growth factor-1; RA, retinoic acid; HDGF, hepatoma-derived growth factor; MIP-1α, macrophage inflammatory protein-1α; MCP-1, monocyte chemoattractant protein-1; LIF, leukemia inhibitory factor; LIFR, LIF receptor; bHLH, basic helix-loop-helix; TF, transcription factors; HES6, hairy and enhancer of split 6; NGN1, neurogenin 1; NeuroD1, neurogenic differentiation 1; SOX2, SRY-box containing gene 2; SDF-1, stromal cell-derived factor-1; CRABP1, cellular retinoic acid binding protein 1; GFBP, insulin-like growth factor binding protein; MIP-1α, macrophage inflammatory protein-1α; MCP-1, macrophage chemotactic protein-1; LIF, leukemia inhibitory factor; LIFR, LIF receptor; bHLH, basic helix-loop-helix; TF, transcription factors; HES6, hairy and enhancer of split 6; NGN1, neurogenin 1; NeuroD1, neurogenic differentiation 1; SOX2, SRY-box containing gene 2; SDF-1, stromal cell-derived factor-1; TGF-β, transforming growth factor beta; TGFβR, transforming growth factor beta receptor; CXCR4, chemokine (C-X-C motif) receptor 4.
cytoskeleton-related genes, including genes that encode actins and microtubule-associated proteins, among the significant interaction genes strongly suggests that macrophages regulate the structural remodeling of the OE following OBX-induced OSN apoptosis.

**Induction of basal cell proliferation, differentiation, and maturation.** The temporal recruitment and infiltration of macrophages into the OE following OBX coincides with the increase in basal cell proliferation as determined by BrdU labeling and with the upregulation of LIFR, the receptor for the neuroepoietic cytokine LIF, on basal cells (42). LIF mRNA was localized in macrophages as well as in OSNs following OBX (25), indicating that macrophages are not the only regulators of basal cell proliferation. There is additional evidence that OSNs themselves and other cells regulate the proliferation and differentiation of OSN progenitors through the expression of BMPs 2, 4, 7, and 12, and FGFs 2 and 8 (10, 37, 44, 51). While these molecules were not differentially expressed in our model (i.e., \( P \geq 0.01 \)), several Fgf-related genes had significant pair-wise effects, namely Fgf9, Fgf12, Fgf21, Frag1 (FGF receptor activating protein 1), Fgfrl, Fgfr2, and Fgfr11. Most of these FGF-related genes were expressed at lower levels in the Lip-C mice and upregulated following OBX. Taken together, these data suggest that while macrophages may not be the sole source of these regulators of basal cell proliferation, they may regulate the expression of BMP- and FGF-related genes by other cell types in the OE through signaling of cytokines or other growth factors.

A result of the extracellular molecular regulation of basal cell proliferation is the differential expression of cell cycle promoters and transcription factors that promote neurogenesis. As expected, the majority of these genes, including the cell-cycle promoters Ccnd2, Ccnb1, and Cdk4, were significantly upregulated at 48 h following OBX and expressed at significantly lower levels in the OE of Lip-C mice compared with Lip-O mice, which was confirmed by the EASE results. Consistent with the decrease in basal cell proliferation associated with macrophage depletion, transcription factors associated with neurogenesis in the OE were expressed at significantly lower levels in Lip-C mice compared with Lip-O mice and upregulated at 48 h following OBX. For example, the bHLH transcription factors Ascl1, Hes6, Ngn1, and Neurod1 that are involved in the proliferation and differentiation of OSN progenitors (38, 50, 52) and Sox2, a transcriptional promoter of neurogenesis (19), had significantly decreased levels of expression in the OE of Lip-C mice compared with Lip-O mice. This indicates that the normal signaling mechanisms needed for basal cell proliferation are decreased as a result of macrophage depletion in the OE of Lip-C mice.

Following division of GBCs, the resulting daughter cells may commit to one of three possible cellular pathways: 1) the cell may return to the GBC population, 2) the daughter cell may become an immediate OSN precursor, or 3) the daughter cell may become a sustentacular cell (4, 14). Based on the similarity of the microarray data and the immunohistochemical expression pattern of Hdgf, as well as the localization of HDGF to basal cells and OSNs, we hypothesize that the daughter cells of GBC division that are committed to the OSN lineage express HDGF as a survival or proliferative factor. HDGF is primarily a nuclear autocrine survival and proliferative factor for neurons (39, 59). Therefore, it is possible that, either directly or indirectly, macrophages regulate the expression of Hdgf in basal cells to promote proliferation. Once the OSN precursor becomes postmitotic, Hdgf may be expressed as a survival factor. The characterization of Hdgf and its protein product in the OE provides novel insight into additional molecular mechanisms that may regulate proliferation and differentiation of OSN progenitors and survival of OSNs.

Several of the genes whose expression patterns were altered by macrophage depletion may regulate the differentiation and/or maturation of OSN progenitors. TGF-β has been reported to promote the differentiation and maturation of OSN progenitors (29, 49). Our microarray data indicated that the two components of the TGF-β receptor, Tgbr1 (interaction) and Tgbr2 (treatment), had significant pair-wise effects. Both genes were expressed at lower levels in Lip-C mice compared with Lip-O mice, while Tgbr1 was significantly downregulated and Tgbr2 was significantly upregulated at 48 h following OBX. Interestingly, immature and mature OSNs, and possibly basal cells, were reported to express TGF-βII in vivo (22). Another gene that may be involved in OSN maturation is Ccxd4. Based upon the immunohistochemical localization of SDF-1 and the microarray pattern of Ccxd4, our hypothesis is that activated macrophages express and secrete SDF-1 that binds to CXCR4 (5) on another cell type within the OE, possibly other macrophages and/or OSNs, to promote the recruitment of macrophages into the OE as well as the maturation of newly generated OSNs.

While our microarray data, combined with molecular and cellular validation of selected genes, provide strong evidence for the regulatory mechanisms associated with macrophage infiltration, OSN survival and replacement, and remodeling of the OE, we have to take into consideration that macrophages are not the sole source of these signaling molecules. As noted above, it has been established that other cell types provide trophic support for basal cells and OSNs (49). Several studies have provided evidence that OSNs, basal cells, sustentacular cells, and OECs express molecular regulators of the proliferation and differentiation of OSN progenitors, including TGF-β, FGFs, LIF, IGF-1, and OMP (20, 21, 25, 30). Our study provides evidence that macrophages also contribute to the regulation of the survival of OSNs and to the proliferation, differentiation, and maturation of OSN progenitors through the expression of growth, survival, and proliferative factors.

In conclusion, our results characterized the altered gene expression profiles in the OE of sham and 48 h OBX mice as a result of liposome-encapsulated clodronate-induced selective macrophage depletion. In confirming and building upon previous studies from our laboratory (6, 23, 33), these gene expression profiles indicate that macrophages promote OSN survival, the proliferation and differentiation of OSN progenitors, and olfactory epithelial remodeling through the regulation of the expression of novel and traditional immune modulators, growth factors, cell cycle promoters, and pro- and antiapoptosis signaling molecules.

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