Neutrophils and oral squamous cell carcinoma: lessons learned and future directions

Marco A. O. Magalhaes,*,†,‡ J Judah E. Glogauer,* and Michael Glogauer*

*Matrix Dynamics Group and †Department of Oral Pathology and Oral Medicine, Faculty of Dentistry, University of Toronto, Ontario, Canada


ABSTRACT
The role of cells of the innate immune system in the pathogenesis of squamous cell carcinoma has been the subject of intense research in recent years. In particular, neutrophils have been shown recently to have either a pro-tumor or anti-tumor phenotype in different cancers. Here, we review the role of neutrophils as tumor microenvironment and signaling modulators of OSCC and their possible role as biomarkers of OSCC prognosis. Current evidence supports a pro-tumor role for neutrophils in OSCC, but more research is needed to clarify the precise mechanisms involved.


Introduction
Oral and pharyngeal cancer is the sixth most common cancer in the world [1], and the overall 5-year relative survival rate has changed minimally from 1992 to 2008 [2]. A total of 42,440 new cases and 8390 deaths is estimated in the United States in 2014 alone [3, 4]. The high mortality rate of patients with oral cancer is associated with late detection and the presence of regional and distant disease at the time of diagnosis [4]. The relative 5-year survival rate for oral cancer is 62%, dropping to 36% for patients with metastatic disease [4]. Recent advances have been made in understanding the genetic changes and molecular mechanisms underlying OSCC progression, including the role of neutrophils in this process.

Neutrophils are key mediators of the innate immune system. Neutrophil activation is essential to protect the host system against infections and promote normal healing [5, 6]. For many decades, leukocytosis has been associated with a poor prognosis in different types of malignancies [7–10]. Despite this, the specific role of neutrophils and macrophages in the pathogenesis of cancer has only recently become the subject of intense research [11, 12], with special focus on the association between inflammation and cancer progression. This idea was initiated by Pekarek et al. [13], who showed that neutrophils can induce tumor angiogenesis, prompting a significant number of research groups to investigate possible neutrophil pro-tumor or anti-tumor roles, as well as their potential uses as diagnostic and prognostic markers. Neutrophils are important players in cancer biology [14, 15], and different neutrophil subpopulations may have opposing roles (anti-tumor or pro-tumor) in cancer progression. Experimental observations by Fridlender et al. [16] hypothesized a polarization of neutrophils, where N1 neutrophils (anti-tumor) are characterized by reduced MMP9 expression, increased ROS formation, and apoptosis after IFN-β stimulation. The N2 neutrophils develop a pro-tumor phenotype after TGF-β stimulation, leading to increased arginase, MMP9 and collagenase expression, protein kinase B activation, and promotion of leukocyte recruitment [15]. This N2 phenotype is particularly relevant for OSCC patients, as OSCCs show increased expression of IL-1β, IL-6, and TGF-β [17]. For clarity and consensus in the terminology, we will use the terms “pro-tumor” and “anti-tumor” to describe the opposing neutrophil population. These proposed opposing roles for neutrophils suggest that they may be important biomarkers for OSCC and perhaps targets to control cancer progression [18].

During the past 4 years, an increasing number of publications have shown that neutrophils are present in a variety of tumors, including renal cell carcinomas and HNSCC [19], and that they may contribute to tumor progression [20, 21], metastasis [22], and extracellular trap (NET)-dependent tumor metastasis [23]. The general roles of neutrophils in cancer pathogenesis and prognosis have been reviewed elsewhere [12, 18, 24, 25], but the particular roles of neutrophils in the diagnosis, prognosis, and progression of OSCCs have not yet been


1. Correspondence: Matrix Dynamics Group, Faculty of Dentistry, University of Toronto, 241-150 College St., Toronto, Ontario, Canada M5S 3E2. E-mail: marco.magalhaes@utoronto.ca

Received June 13, 2014; Revised July 25, 2014; Accepted July 27, 2014. DOI: 10.1189/jlb.4RU0614-294R
reviewed. With the consideration of the constant presence of neutrophils in the oral tissues as a result of the oral biofilms, there is an increased interest in analyzing how the presence of neutrophils modulates OSCC behavior. The literature on OSCCs and neutrophils is limited compared with other cancers, and we added publications on other types of HNSCCs to this discussion.

In the next sections, we will analyze our current knowledge about the roles of neutrophils in the progression of OSCC in three main categories: biomarkers, microenvironment changes, and intercellular signaling (Table 1).

**NEUTROPHILS AS BIOMARKERS OF OSCC**

Significant efforts are being made to identify new diagnostic and prognostic markers to manage OSCC better. Neutrophils are highly proteolytic and motile cells, allowing them to have direct contact with various cells of the tumor microenvironment. It is possible that the proteins they release can directly or indirectly be used for early detection, staging, and prognosis of OSCC lesions. As a result of their accessibility in peripheral blood and saliva, neutrophils are excellent candidates to develop new biomarkers for oral cancer. Here, we describe the role of neutrophils and neutrophil-derived proteins as biomarkers of OSCC.

**Neutrophil infiltration**

Similar to the literature on other tumors, recent reports have shown that high neutrophil infiltration in OSCC is associated with poor clinical outcomes. Trellakis et al. [26] used a retrospective histological analysis of HNSCCs to show an association between PMN infiltration and squamous cell carcinomas prognosis. Increased neutrophil infiltration, as detected by CD66b

<table>
<thead>
<tr>
<th>Role</th>
<th>Studies</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>biomarker</td>
<td>Neutrophil infiltration</td>
<td>[26] OSCC</td>
</tr>
<tr>
<td></td>
<td>NLR</td>
<td>[27] OSCC—tongue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[28] HNSCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[29] HNSCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[30] OSCC</td>
</tr>
<tr>
<td></td>
<td>Neutrophil-secreted proteins</td>
<td>[31] OSCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[32] OSCC—tongue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[33] OSCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[34] OSCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[35] OSCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[36] OSCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[37] OSCC</td>
</tr>
<tr>
<td></td>
<td>ROS</td>
<td>[38] Review</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[39] OSCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[40] OSCC</td>
</tr>
<tr>
<td>Cancer microenvironment</td>
<td>MMPs</td>
<td>[41] HNSCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[42] HNSCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[43] Fibrosarcoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[44] Cervical</td>
</tr>
<tr>
<td></td>
<td>Angiogenesis</td>
<td>[45] Fibrosarcoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[46] Pancreatic ductal adenocarcinoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[47] Fibrosarcoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prostate</td>
</tr>
<tr>
<td></td>
<td>NETs</td>
<td>[48] Lung</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[22] Liver</td>
</tr>
<tr>
<td></td>
<td>Microbiome, neutrophils, and cancer</td>
<td>[49] OSCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[50] OSCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[51] OSCC—gingival</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[52] Colorectal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[53] HNSCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[54] HNSCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[55] Orohypharingeal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[56] HNSCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[57] HNSCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[58] Multiple tumors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[26] OSCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[59] Chronic myelogenous leukemia</td>
</tr>
</tbody>
</table>

The roles of neutrophils are academically divided in: biomarkers, cancer microenvironment remodeling, and modulation of cellular function and signaling. References based on other cancers were added in cases where OSCC publications are lacking.
immunostaining, correlated with poor patient survival. These findings were consistent with a recent report by Wang et al. [27], who showed that tongue squamous cell carcinomas with neutrophil infiltration displayed increased lymph node metastasis, a higher clinical stage, and increased chances of tumor recurrence.

**NLR**

The NLR is a well-established marker of advanced disease and poor prognosis in various conditions, including cardiovascular diseases [60] and cancers [61–63] that include HNSCC [28]. An increase in NLR is indicative of an ongoing inflammatory process with a decrease in regulatory pathways. Millrud and coworkers [29] have correlated the activation pattern of leukocytes and survival of HNSCC patients, where the prognostic markers (CD71, CD98, CD4/8 ratio, CD16/14) and a high NLR correlated with poor prognosis. Perisanidis et al. [30] evaluated 97 patients with biopsy-proven OSCC, who received preoperative chemoradiotherapy and showed that a high NLR (>1.9) is an independent marker for shorter disease-specific survival in OSCC patients. Although this finding suggests that the NLR may be an important OSCC prognostic biomarker, larger prospective studies are needed to clarify further the use of NLR and the mechanisms and relevance behind the changes in lymphocyte and neutrophil counts in OSCC.

**Neutrophil-secreted proteins**

Numerous studies have used neutrophil-secreted products as diagnostic and prognostic markers of cancer. Among these, human defensins (HNP), TNF family proteins, and NGAL have been studied in the OSCC patients.

Human defensins (HNP1, HNP2, and HNP3) are known to induce cytotoxic effects in numerous target cells, including squamous cell carcinomas [31]. In a search for a possible role for HNPs in cancer progression, Lundy et al. [32] investigated the presence of HNPs in OSCC and reported a two- to 12-fold increase in their presence in localized tumor areas. This finding also correlated with an increase in neutrophil infiltrates. HNPs were also elevated in the saliva of OSCC patients, but the clinical significance of this finding has yet to be clarified [33, 34, 64].

Members of the TNF superfamily of secreted proteins, including APRIL [35], TRAIL, and DR5 [36], were also investigated as possible oral cancer biomarkers. APRIL has been shown to regulate tumor cell survival and proliferation through binding to heparin sulfate side-chains of proteoglycans or to the calcium modulator and cyclophilin ligand interactor [65]. Jablonska et al. [36] analyzed the expression of APRIL in peripheral blood neutrophils of patients with OSCC and found a correlation between high expression and poor prognosis. TRAIL is a soluble TNF-α family ligand produced by numerous cells and may be a promising candidate for cancer suppression. TRAIL induces apoptosis by activation of DRs, including DR1 and DR5, and its expression and release are decreased in late-stage squamous cell carcinoma. As many cells in the tumor microenvironment may secrete TRAIL, further studies are needed to understand better the role and the prognostic importance of neutrophil-derived TRAIL in OSCC progression.

NGAL is a regulator of iron and hydrophobic-compound transport and has an important role in protecting MMP9 from degradation. Recent studies have described a pro-tumor role for NGAL in different tumors, including breast and esophageal cancers [66]. NGAL has also been shown to be up-regulated in well-differentiated OSCC, whereas poorly differentiated tumors showed a weak expression, suggesting a possible role for this protein in tumor staging [37].

**ROS**

ROS have an increasing number of roles in different cellular processes, including phagocyte killing, chemotaxis, apoptosis, and intracellular signaling [38]. The formation of ROS by neutrophils is decreased in most cancers [15]. Peripheral blood neutrophils from patients with stage II and stage II OSCC had lower iNOS production and phospho-p38 MAPK, whereas stage IV patients had an increase in iNOS [39] and reduced apoptosis [19]. This mechanism is dependent on the p38 MAPK pathway. Similarly, Jablonska and others [40] showed that iNOS expression and NO production are reduced significantly in peripheral blood neutrophils of OSCC patients. Although ROS are not considered biomarkers of cancer progression, future studies will clarify the signaling changes in TNs and the reduction of ROS in these cells. This may contribute to the understanding of the cross-talk between cancer cells and TNs.

**NEUTROPHILS REMODELING THE CANCER MICROENVIRONMENT**

One of the most interesting recent findings suggests that neutrophils are recruited to the niches of distant cancer metastasis and may be a key player in the establishment of metastatic spread [22]. Similarly, Huh et al. [67] found that neutrophils were recruited and increased the metastatic spread of melanoma through an IL-8-dependent mechanism.

**MMPs**

The cancer microenvironment is a complex niche that is constantly being remodeled by fibroblasts and inflammatory and cancer cells. Neutrophils secrete MMP8 (collagenase), MMP9 (gelatinase), elastase, cathepsin G, proteinase 3, and other matrix proteinases that contribute to the remodeling of the tumor microenvironment. These proteases can degrade the extracellular matrix directly and facilitate the invasion of cancer cells [41] or alternatively, activate membrane type 1-MMP in the tumor cells and facilitate cancer progression indirectly [43]. Moilanen et al. [42] showed that MMP8 is also secreted by HNSCCs, including oral cancers. MMP9 is also known to regulate tumor angiogenesis, a key factor in tumor progression (see below). In tumors where macrophages are the main source of MMP9, inhibition of macrophage recruitment induces a compensatory response, increasing the recruitment of MMP9+ neutrophils that have a pro-tumor phenotype [44]. Further studies are needed to verify the significance of this mechanism in OSCC.
Angiogenesis

Neutrophils are known to promote tumor angiogenesis through up-regulation of MMP9 and VEGF [45]. Bausch and others [46] have shown that MMP9 is a VEGF-independent angiogenic factor with an additive effect to VEGF-induced angiogenesis in hepatocellular carcinoma. Complete inhibition of angiogenesis requires both MMP9 and VEGF inhibition.

Unlike other cells, neutrophils secrete pro-MMP9 without the inhibitor TIMP1, providing a readily active MMP9, which is critical for tumor angiogenesis and invasation. Inhibition of IL-8 decreases neutrophil recruitment to the tumor, angiogenesis, and metastasis [47]. The combination of TIMP (MMP9 inhibitor) and anti-IL-8 antibody induces a substantial decrease in local angiogenesis and invasation of tumor cells in a given area [47]. This is a fundamental link between inflammation and cancer progression, highlighting the importance of neutrophils in cancer spread. The role of MMP9 in the progression and spread of OSCC is not yet completely understood.

NETs

Recent evidence shows that neutrophils may also contribute to the metastatic spread of cancers by facilitating the seeding of circulating cancer cells [48]. Several mechanisms have been described to explain the neutrophil-mediated metastatic spread, including NETs, which are composed of extruded neutrophilic DNA that can be seen under normal systemic inflammatory/infectious responses. Cools-Lartigue et al. [23] have showed that NETs sequester circulating cancer cells and increase the formation of liver micrometastasis. There are no publications describing the role of NETs in OSCC metastasis. Further studies are needed to understand the clinical relevance of this mechanism in OSCC.

ORAL MICROBIOME, NEUTROPHILS, AND CANCER

There is significant evidence linking bacteria to the pathogenesis and progression of cancers, particularly after the groundbreaking work describing the link between Helicobacter pylori and gastric cancers [68]. With the consideration of the oral microenvironment, numerous studies have investigated the relationship between oral biofilms and OSCC, as reviewed recently by Whitmore and Lamont [49]. In this section, we will focus on oral bacteria, neutrophil activation, and oral cancer.

The oral biofilm is a complex, dynamic, multispecies system that in certain circumstances, can promote chronic inflammatory diseases, including periodontal disease. Among these species, Pg is the most well-studied oral bacteria with a pro-tumor role. Pg is commonly seen in periodontitis [69], and studies have shown an increase in Pg in OSCC [49–51] relative to healthy oral tissues. The mechanisms underlying the pro-tumor role of Pg include decreased apoptosis [70, 71] and suppression of p53 in epithelial cells [72]. Fusobacterium nucleatum may also participate in the progression of cancer by modulating E-cadherin/B-catenin signaling [52] and inducing motility, survival, and expression of MMPs in epithelial cells [73–75].

In addition to the above-described mechanisms, current evidence supports the theory that oral bacteria can modulate neutrophil recruitment and function to promote a pro-tumor phenotype. Several oral pathogens associated with periodontal disease recruit and modulate neutrophil activation and apoptosis [76, 77]. Pg is known to increase neutrophil migration, decrease apoptosis [78], induce ROS formation [79], promote TIMP1 degradation [80], and increase production of MMP-stabilizing NGAL [80]. Aggregatibacter (Actinobacillus) actinomycetemcomitans is also commonly found in periodontal disease and is known to induce MMP8 secretion and degranulation of neutrophils [81, 82]. Similarly, Streptococcus sanguinis and Fusobacterium nucleatum induce the release of MMP8, IL-1β, and ROS production by neutrophils [83].

All combined, the effects of oral bacteria on neutrophils are consistent with a pro-tumor phenotype. Hypothetically, by increasing the release of MMPs by neutrophils and protecting them through the release of NGAL and degradation of TIMP1, oral bacteria may promote invasion and negatively affect cancer prognosis. This may explain the significant literature linking periodontal disease, neutrophils, and cancer [53, 54, 84].

MODULATION OF CELL FUNCTION AND SIGNALING

Both neutrophils and cancer cells influence the behavior of each other in the cancer microenvironment [41, 59]. Recent key studies have identified potential pathways involved in the cross-talk between cancer cells and neutrophils. We will focus this discussion on the regulation of cell function and signaling between OSCC and neutrophils.

Dumitru and coworkers [55] recently showed that TANs increased cortactin phosphorylation in oropharyngeal squamous cell carcinomas, promoting cancer migration, leading to a poor prognosis. This is a promising result that links the presence of TAN with cytotoxic changes in the cancer cells. More importantly, cortactin is an essential actin-binding protein, regulating leading-edge formation and invadopodia formation in cancer cells [85].

Trellakis and coworkers [26] performed a functional analysis of peripheral blood neutrophils to show that the peripheral blood of HNSCC patients had an increase in PMN, CXCL8, CCL4, and CCL5. In vitro analysis showed an increased migration and increased survival of PMN exposed to FaDu cells or FaDu cell supernatants. This effect was reduced significantly by CXCL8 inhibition. Stimulation of PMN with the FaDu supernatant also increased the secretion of CCL4, MMP9, and lactoferrin. These results show that HNSCCs establish a feedback loop with neutrophils, leading to increased inflammation.

Cancer cells can also change the activation state of neutrophils. With the use of a protein phosphorylation array, Dumitru et al. showed that neutrophils challenged with FaDu cancer cells showed a strong activation of p38/MAPK, CREB, and p27. The activation of p38/MAPK was associated with an increase in chemotaxis and cell survival and secretion of CCL4 and CXCL8. p27 and CREB also regulated the release of MMP9 by neutrophils. The authors also demonstrated an in-
crease in expression of MMP9 and CCLA by CD66b-positive cells (neutrophils) in HNSCC [56]. Neutrophils show increased survival when exposed to supernatants from different tumors, and several mechanisms are implicated in this process, including the release of cytokines and hyaluronan by tumor cells and other cells in the microenvironment [57, 58]. The MIF was recently shown to modulate the activation and increase survival of neutrophils [57]. MIF significantly increased neutrophil chemotaxis, reduced apoptosis, and increased the expression of CCL4 and MMP9 through a CXCR2-dependent mechanism. Samples from cancer patients were also used to show that MIF expression in tumors correlates with neutrophil recruitment and poor survival. The observation that neutrophils survive longer in the tumor microenvironment challenges the initial understanding that neutrophils, as extremely short-lived cells, could not participate effectively in tumor progression that occurs over an extended period of time. Also, increased neutrophil survival translates into sustained inflammation and secretion of neutrophil inflammatory mediators that may contribute to tumor progression.

CONCLUDING REMARKS

Increasing roles of innate immune cells in cancer biology

Considering the increased interest in the development of targeted therapies, immune cells may become a primary target for prognosis and possible treatment of cancers, and this is valid for almost all malignancies, including OSCC. The dogma of the short-lived, “nonspecific” neutrophil in cancer is disappearing and is being replaced by the concept of the neutrophil as a protagonist in cancer progression.

**Figure 1.** The role of neutrophils in the progression of OSCC. Neutrophils have important roles in OSCC, including the remodeling of the cancer microenvironment, modulating cell function and signaling, and a potential biomarker. This figure summarizes the available information on each of these categories.
There are many reasons that support this hypothesis. First, the neutrophil has extremely efficient motility machinery, allowing it to be recruited quickly to areas of early cancer development and interact with tumor cells in the very early steps of malignant transformation. Neutrophils can move in and out of the tumor microenvironment, conveying important signaling cues, and can also be detected in the peripheral circulation. Second, contrary to initial beliefs, neutrophils in contact with cancer cells have a prolonged life cycle and can be a resident of the tumor microenvironment for extended periods of time. Third, neutrophils are equipped with a variety of matrix-remodeling proteinases that can help shape the tumor microenvironment. The roles of neutrophils in OSCC are illustrated in Fig. 1.

Neutrophils, cancer, and the oral microenvironment
Considering the oral microenvironment, the constant presence of neutrophils in the saliva may be an important factor in early malignant transformation, signaling, and most importantly, a marker for disease progression that is easily accessible with a mouth rinse without the need of blood collection [77, 86]. Also, there are numerous conditions that are characterized by changes in the neutrophil population in the mouth, including periodontal disease [77, 87]. These changes in neutrophils may be involved in the observed increase in the rate of oral epithelial malignant transformation in chronic inflammatory states [88, 89].

Certainly, more research is needed to address these topics. Another important question is whether the modulation of neutrophil activity can influence the development or prognosis of oral cancers. There is evidence to suggest that the maintenance of good oral hygiene and therefore, low neutrophil counts in the saliva correlates with a small prevalence of oral cancer [84, 90]. Finally, oral cancers are amenable to treatments that modulate neutrophil function locally, as the oral cavity is a readily accessible site.

Future directions
There are still many unanswered questions regarding the role of neutrophils in cancer pathogenesis in general. The literature on OSCC and neutrophils is limited compared with other models. New results show that neutrophils may have different roles in the primary tumor and metastatic sites [91] and recruit T regulatory cells that may contribute to a decreased antitumor immunity: understanding the specific roles of Rac1 and Rac2. Ph.D. thesis, University of Toronto.

Certainly, more research is needed to answer these questions. Another important question is whether the modulation of neutrophil activity can influence the development or prognosis of oral cancers. There is evidence to suggest that the maintenance of good oral hygiene and therefore, low neutrophil counts in the saliva correlates with a small prevalence of oral cancer [84, 90]. Finally, oral cancers are amenable to treatments that modulate neutrophil function locally, as the oral cavity is a readily accessible site.

ACKNOWLEDGMENTS
This work is supported by a Dental Research Institute grant and a Canadian Institutes of Health Team Grant TBO-122068 (to M.G.). The authors thank Dr. Grace Bradley for the helpful discussions and support of this work.

REFERENCES

AUTHORSHIP
M.A.O.M. designed and prepared the manuscript. J.E.G. and M.G. contributed to the manuscript.

DISCLOSURES
The authors have no conflict of interest to disclose.


KEY WORDS: prognosis · tumor microenvironment · inflammation · innate immunity · cancer immunology
Neutrophils and oral squamous cell carcinoma: lessons learned and future directions

Marco A. O. Magalhaes, Judah E. Glogauer and Michael Glogauer

*J Leukoc Biol* 2014 96: 695-702 originally published online August 21, 2014
Access the most recent version at doi:10.1189/jlb.4RU0614-294R

<table>
<thead>
<tr>
<th>References</th>
<th>This article cites 89 articles, 16 of which can be accessed free at: <a href="http://www.jleukbio.org/content/96/5/695.full.html#ref-list-1">http://www.jleukbio.org/content/96/5/695.full.html#ref-list-1</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>Subscriptions</td>
<td>Information about subscribing to <em>Journal of Leukocyte Biology</em> is online at <a href="http://www.jleukbio.org/site/misc/Librarians_Resource.xhtml">http://www.jleukbio.org/site/misc/Librarians_Resource.xhtml</a></td>
</tr>
<tr>
<td>Permissions</td>
<td>Submit copyright permission requests at: <a href="http://www.jleukbio.org/site/misc/Librarians_Resource.xhtml">http://www.jleukbio.org/site/misc/Librarians_Resource.xhtml</a></td>
</tr>
<tr>
<td>Email Alerts</td>
<td>Receive free email alerts when new an article cites this article - sign up at <a href="http://www.jleukbio.org/cgi/alerts">http://www.jleukbio.org/cgi/alerts</a></td>
</tr>
</tbody>
</table>

© 2014 Society for Leukocyte Biology