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Tuning Cytokines Enriches Dendritic Cells and Tregs in the Periodontium

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Abstract

Background: Periodontal disease results from the pathogenic interactions between the tissue, immune system, and microbiota; however, standard therapy fails to address the cellular mechanism underlying the chronic inflammation. Dendritic cells (DC) are key regulators of T-cell fate, and biomaterials that recruit and program DC locally can direct T-cell effector responses. We hypothesized that a biomaterial that recruited and programmed dendritic cells toward a tolerogenic phenotype could enrich regulatory T-cells within periodontal tissue, with the eventual goal of attenuating T-cell mediated pathology.

Methods: The interaction of previously identified factors that could induce tolerance, granulocyte-macrophage colony stimulating factor (GM-CSF) and thymic stromal lymphopoietin (TSLP), with the periodontitis network was confirmed *in silico*. The effect of the cytokines on DC migration was explored *in vitro* using time-lapse imaging. Finally, regulatory T-cell enrichment in the dermis and periodontal tissue in response to alginate hydrogels delivering TSLP and GM-CSF was examined *in vivo* in mice using immunohistochemistry and live-animal imaging.

Results: The GM-CSF and TSLP interactome connects to the periodontitis network. GM-CSF enhances DC migration *in vitro*. An intradermal injection of an alginate hydrogel releasing GM-CSF enhanced DC numbers and the addition of TSLP enriched FOXP3+ regulatory T-cells locally.

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CONFLICT OF INTEREST

David Mooney, R. Warren Sands, Eduardo A. Silva, and Toshihisa Kawai have intellectual property related to *in situ* programming of DC for tolerogenic therapies: US patent number: US8728456B2.

Injection of a hydrogel with GM-CSF and TSLP into the periodontal tissue in mice increased DC and FOXP3+ cell numbers in the tissue, FOXP3+ cells in the lymph node, and IL-10 in the tissue.

Conclusion: Local biomaterial-mediated delivery of GM-CSF and TSLP can enrich DC and FOXP3+ cells and holds promise for treating the pathologic inflammation of periodontal disease.

Summary Sentence:

This study describes a biomaterial approach to tune the periodontal microenvironment locally by enriching and evoking tolerogenic responses in DC to promote regulatory T cells with the ultimate goal of preventing pathogenic inflammation in periodontal disease.

Keywords

immunotherapy; adult periodontitis; dendritic cell; regulatory T cells; biomaterials

INTRODUCTION

Periodontitis is an inflammatory disease of the tissues surrounding the tooth, including the cementum, periodontal ligament, alveolar bone, and gingiva, which leads to the destruction of periodontal tissue[1]. The majority of the adult population has periodontitis with 5–15% having severe generalized periodontitis[2]. More generally, periodontitis has been associated with systemic illnesses including cancer[3]. It results from the pathogenic interactions between bacterial flora, host immunity, and periodontal tissue and can lead to tooth loss. The pathophysiology of the disease is complex and the T cell effector response defies straightforward Th1, Th2, and Th17 subtype classification[4]. Synthesizing the results from the clinic as well as preclinical animal models reveals many seemingly contradictory reports[5], suggesting that numerous cells, cytokines and other bioactive factors often have protective or pathogenic roles depending upon their type, timing, quantity, duration, and localization. Thus, the development of curative immunomodulatory therapies has been slow. The current mainstay of treatment, antibiotics and mechanical disruption of the bacterial biofilms[6], can limit disease progression, but does not treat the underlying chronic inflammation.

Therapies that target dendritic cells have been broadly explored to activate or attenuate innate and adaptive immunity by directing T cell fate, including strategies that enrich for regulatory T cells (Treg) that are able to dampen pathogenic Th1, Th2, and Th17 immunity[7]. In the setting of periodontal disease, Treg have been shown to reduce disease severity[8–11]. Granulocyte macrophage colony stimulating factor (GM-CSF) is a potent dendritic cell enrichment factor that, depending upon the dose, location, and kinetics of its delivery, may either evoke immunity or tolerance[12–14]. Controlled delivery of GM-CSF has been used widely to enhance the number of DC locally, and to potentiate vaccine efficacy[15, 16]. The function of thymic stromal lymphopoeitin (TSLP) is diverse, as it acts on DC to elicit a Th2/Treg inducing phenotype, but also in certain circumstances has been found to inhibit or have no effect on regulatory T cell function[17–21]. In the NOD diabetes mouse model, treatment of dendritic cells with TSLP followed by adoptive transfer has been used to prevent

autoimmunity via the induction of regulatory T cells[17]. In another study, TSLP enhanced Treg expansion through the effects of dendritic cells[21].

Biomaterials may be useful in the development of immunotherapies against periodontitis. To date, biomaterials have mainly been used in periodontal disease as physical barriers or devices to augment tissue regeneration[22]; however, they have been used more recently to increase local Treg cell numbers[23, 24]. In general, biomaterials can be used to recruit and program DC *in situ* to direct T effector responses without the limitations of adoptive transfer and may provide an additional level of control that cannot be achieved through T cell targeting alone. Biomaterials can release bioactive agents at a controlled rate over a specified period of time, and create a tailored local microenvironment, shielding cells from a pathogenic milieu while providing signals to direct cell fate[15]. Alginate based biomaterials are commonly used in dentistry as an impression material and have been used in the clinic as components of FDA approved therapies[25]. Alginate hydrogels can be injected in a minimally invasive manner and are biocompatible, modifiable, and capable of delivering a wide variety of drugs locally over a sustained period of time[26, 27]. For example, a single injection of an alginate hydrogel containing VEGF led to increased levels of VEGF in the surrounding tissue for 15 days, with minimal cytokine present in the serum[27].

This study was based on the hypothesis that a single injection of an alginate hydrogel containing GM-CSF and TSLP could recruit and program DC in the periodontal tissue, enrich for regulatory T cells, and a create a local immunosuppressive milieu. In order to test this hypothesis, GM-CSF and TSLP were encapsulated into alginate hydrogels that could be readily injected into tissues using a needle and syringe, and the hydrogels were administered into the dermis and periodontal tissue of healthy mice. *In vivo* therapeutic validation was assessed by quantifying DC and T cell numbers, as well as local IL-10 production.

MATERIALS AND METHODS

In silico network analysis

The GM-CSF, TSLP, and periodontal disease associated protein network was constructed using Ingenuity Pathway Analysis software.* Specifically, TSLP, GM-CSF, and periodontal disease related molecules were combined to create a new pathway. Chemical compounds were then removed (trimmed) from the selected periodontal disease related molecules. Next, the Path Explorer tool in Ingenuity Pathway Analysis and the underlying literature database were used to create connections between GM-CSF or TSLP and the periodontal associated genes and proteins.

In vitro alginate release of GM-CSF and TSLP and atomic force microscopy

Hydrogels were fabricated as previously described[27]. In short, hydrogels (50–100 μl) were composed of 2% ultrapure MVG alginate† consisting of 75% low molecular weight and 25% high molecular weight oxidized alginate (both 1% oxidized with NaIO4), and were allowed to gel with either 3 μg of GM-CSF or 1 μg of TSLP with trace ^{125}I radiolabeled

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GM-CSF or TSLP, respectively[‡]. The gels were incubated at 37°C on a rocker in PBS supplemented with 0.1 g/L $MgCl_2 \cdot 6H_2O$ and 0.132 g/L of $CaCl_2 \cdot 2H_2O$. In the GM-CSF study the media was also supplemented with 1% BSA. Media was collected at predefined intervals and the release was quantitated with a gamma counter (WIZARD Automatic Gamma Counter[§]) and normalized to the total amount of compound encapsulated. Atomic force microscopy (AFM) images of alginate hydrogel disks were obtained in tapping mode using a Multimode Scanning Probe microscope with a Nanoscope IIIa controller at the Eastern Regional Research Center of the Agricultural Research Service Imaging Core Facility following an adapted protocol of Fishman[28].

Studies involving mice

All animal studies were conducted according to an IACUC approved animal use protocol that followed institutional guidelines for the husbandry, care, and use of animals. The animals were purchased as specific-pathogen free animals and underwent routine testing per the supplier and animal care facility. The animals were housed in standard caging with a 12 hour light and dark cycle and fed standard irradiated chow typically with an automatic waterer and otherwise housed per institutional protocols. Given studies were completed by multiple investigators at different institutions, animals were bred for portions of this research, and protocols had to be established for this and other studies it is difficult to ascertain the direct number of animals attributed to this work. With these caveats we estimate approximately 65 mice were used for this study.

BMDC culture

DC were obtained from day 7–12 cultures following precursor isolation from tibia and femur bone marrow isolates from C57BL/6J[**] mice[29]. Typical purity was greater than 85% as observed with flow cytometry staining with anti-CD11c antibodies. Reagents were obtained from Sigma[††] and consumables were obtained from VWR[‡‡] or Thermo Fisher[§§] unless otherwise noted. In brief, bone marrow isolates were obtained from mice, washed, and re-plated in RPMI-1640 supplemented with 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml), 2-mercaptoethanol (50 μM), and 20 ng/ml GM-CSF[***] in 100 mm bacteriologic cell culture plates at a concentration of 0.2×10^6 cells/ml (10 ml). On day 3, 10 ml of additional media was added. On days 6 and 8, 10 ml of media was gently aspirated from the wells, centrifuged down, and to the pellet 10 ml of fresh media was added prior to re-plating. On day 10 the procedure was repeated, except that media with 10 ng/ml of GM-CSF was added per the reference protocol. Non-adherent cells were used in subsequent assays.

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In vitro BMDC migration

Alginate gels with or without 1 μg GM-CSF were excised from molds using a punch biopsy[27]. The gel disk was then placed into a glass bottom petri dish and a liquid collagen solution containing 1 x 10^6 fluorescent transgenic BMDC/ml was then circumferentially aliquoted around the disk to fully cover it with collagen solution. The gels were allowed to mature for 1 hour and then covered in media. Petri dishes containing gels were then placed in a microscopic viewing chamber at 37°C and 5% CO_2. An arbitrary spatial coordinate within the collagen gel was selected as the imaging window for each alginate/collagen gel system (n = 3) and its radial and tangential orientation with respect to the inner alginate gel was noted. Snapshots at these locations were acquired over the course of 8 hours using an Olympus IX81 inverted microscope. Imaris software[†††] was used to capture and analyze the motion of the cells. Average velocity was determined as the final minus the initial position of the cell's path divided by the overall time the path was traced (an individual cell that came in and out of the plane of focus may have generated multiple paths).

Injection of alginate hydrogels and quantitation of DC and regulatory T cells

The alginate hydrogels and the bioluminescent CD11c+ cells recruited to the hydrogels were imaged using the IVIS Spectrum imaging system.[‡‡‡] To image the hydrogels, 40 μl of 2% alginate hydrogels as described above were labeled with trace PE-Cy7 microbeads[§§§] and injected into the dermis of euthanized mice. To image the cells, blank control or GM-CSF (3 μg) containing 2% alginate hydrogels were injected into the dermis of C57BL/6J CD11c-Cre-EGFP x FVB Stop/loxP Luc mice.[****] Seven days later the animals were injected i.p. with 150 mg/kg luciferin[††††] and imaged during the signal plateau phase to quantitate radiance, and therefore CD11c cellular localization. A paired student's t-test was completed with InStat statistical software[‡‡‡‡] to compare the bioluminescence between GM-CSF and blank hydrogels seven days after injection.

Control blank alginate hydrogels or hydrogels containing 3 μg GM-CSF were injected i.d. into C57BL/6J mice. Seven days later the gels were resected, fixed, sucrose-infiltrated, frozen, embedded in OCT, cut, and stained with DAPI and/or antibodies against CD11c and MHC II[30]. In a similar manner, alginate hydrogels containing 3 μg GM-CSF alone or 3 μg GM-CSF and 1 μg of TSLP and FOXP3. Image analysis was completed using ImageJ and a student's t-test was performed using InStat.

Periodontal injection of alginate hydrogels and quantitation of DC, regulatory T cells, and IL-10 elaboration

C57BL/6 mice received no injection (control) or a 1.5 μl injection of an alginate hydrogel containing 1 μg of GM-CSF and TSLP into their periodontal tissue. Seven days later the animals were sacrificed and the periodontal tissue was stained with anti-CD11c and anti-

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[‡‡‡]Caliper Life Sciences, Hopkinton, MA.
[§§§]Invitrogen, Carlsbad, CA.
[****]Jackson Laboratories, Bar Harbor, ME.
[††††]Gold Biotechnology, Olivette, MO.
[‡‡‡‡]GraphPad, San Diego, CA.

IL-10 antibodies. Similarly, FOXP3-EGFP-KI mice on the C57BL/6J background were injected with alginate gels containing 1 μg GM-CSF or 1 μg of both GM-CSF and TSLP. After seven days the periodontal tissue from the injected animals and wild-type, naïve, controls was resected, processed, and imaged using fluorescent confocal microscopy. The cervical lymph nodes were also harvested, purified through a nylon column, and enumerated by flow cytometry.

RESULTS

Periodontal disease and TSLP and GM-CSF interacting network

To assess whether TSLP and GM-CSF could potentially attenuate periodontitis, proteins associated with periodontal disease were identified using Ingenuity Pathway Analysis. Interacting terms between GM-CSF (CSF-2) or TSLP and the proteins associated with periodontal disease were then generated using Ingenuity pathway analysis (Figure 1). 17 possible interactions between GM-CSF or TSLP and the periodontitis associated proteins are observed, suggesting likely interactions between the cytokines and the proteins involved with periodontitis and GM-CSF/TSLP signaling.

Hydrogel delivery of GM-CSF and TSLP

Alginate consists of linear polysaccharides composed of (1,4)-linked α-L-guluronic and β-D-mannuronic acid monomers (Figure 2A), and creates nanoporous gels following addition of calcium (Figure 2B). These nanopores serve to entrap factors present during gelation. These gels can be injected in a minimally invasive manner using a large gauge syringe (Figure 2C). Drug release from the gels *in vitro* is biphasic, with 90 percent of the GM-CSF (2D) and TSLP (2E) delivered within the first 24 hours, and the remainder released within one week. This is occurring in the setting of prior *in vitro* biodegradation studies that demonstrate approximately 50% degradation of the gels by day 6[27].

GM-CSF impact on DC migration in vitro

In order to evaluate the effects of GM-CSF released from gels on DC motility, an *in vitro* model was designed to track DC movement. This 3D system was intended to mimic the implantation of a hydrogel containing GM-CSF into tissue containing DC (Figure 3). The speed of DC in the collagen abutting the control alginate hydrogels was approximately one-half that found in the samples with GM-CSF releasing hydrogels (1.12 + 0.08 μm/min vs. 2.09 + 0.03 μm/min, respectively; $p < 0.01$). However, the mean velocity for the control and GM-CSF hydrogels was equivalent (−0.02 + 0.06 and 0.01 + 0.05 μm/min in the radial direction and 0.00 + 0.02 and 0.01 + 0.07 μm/min in the tangential direction for the control and GM-CSF containing hydrogels, respectively). For both the control and experimental conditions, the net average velocity of zero was reflected in the symmetry of the plots (Figures 3B and 3C). These results suggest minimal chemotactic, but a pronounced chemokinetic effect of GM-CSF on DC in this model system at these GM-CSF concentrations.

DC and regulatory T cell accumulation in the dermis

The effects of alginate hydrogels containing GM-CSF and TSLP on DC and regulatory T cell numbers *in vivo* were next examined in dermal tissue. First, either blank control or GM-CSF releasing alginate hydrogels were injected into the dermis of transgenic mice that express luciferase under the control of the CD11c promoter (Figure 4A, B, and E) or wild type C57BL/6J mice (Figure 4C–D). At day 7, gels releasing GM-CSF exhibited approximately 20% higher radiance at the site of the hydrogel injection, in comparison to blank controls. The number of local FOXP3+ cells was also evaluated. Alginate gels that released both GM-CSF and TSLP led to qualitatively higher number of FOXP3+ cells adjacent to gels, in comparison to GM-CSF alone (Figure 4F–G).

DC and regulatory T cells in periodontal tissue and local lymph nodes

To determine if GM-CSF and TSLP could enrich for DC and regulatory T cells in periodontal tissue, alginate hydrogels delivering GM-CSF and TSLP were injected into the periodontal tissue of mice (Figure 5). Similar to what was observed in the skin, the number of CD11c+ cells was enhanced at the injection site, as was the elaboration of IL-10 by cells in animals that received an injection of the hydrogel containing GM-CSF and TSLP, as compared to control animals (Figure 5 B, C, L and M). Next, alginate hydrogels were injected into the periodontal tissue of FOXP3-EGFP-KI mice, and the effects on regulatory T cells was evaluated. A marked infiltration of FOXP3+ cells was observed in the mice receiving alginate hydrogels containing GM-CSF or GM-CSF and TSLP at 7 days, while few FOXP3+ cells were detected in the tissue of control mice (Figure 5 D–M). Interestingly, FOXP3+ cells and DC appeared to co-localize in the periodontal tissue of animals that received alginate hydrogels containing both GM-CSF and TSLP (Figure 5 M, yellow staining).

To examine if the effects extended beyond the local site of gel injection, FOXP3+ cells in the draining lymph nodes were enumerated. An increase in the proportion of FOXP3+EGFP+ regulatory T cells was observed in the cervical lymph nodes of mice that received hydrogels releasing GM-CSF and TSLP, whereas no increase was seen with alginate gels releasing GM-CSF alone, compared to the control blank hydrogels (Figure 5: N–P).

DISCUSSION

In this report we demonstrated that controlled delivery of GM-CSF and TSLP could enrich for DC and regulatory T cells locally and elicit regulatory responses. *In vitro* GM-CSF enhanced DC migration and *in vivo* it increased recruitment. When alginate hydrogels delivering GM-CSF and TSLP were injected into the skin or periodontal tissue of mice, increased numbers of regulatory T cells were observed locally and in the draining lymph nodes in concert with elevated IL-10 expressing cells in the periodontal tissue.

This work builds upon previous studies that demonstrate the tolerogenicity of TSLP and GM-CSF in certain contexts while extending past results to the murine periodontal tissue model. The combination of TSLP and GM-CSF likely elicit the Th2/Treg phenotype in DC reported previously[17, 18, 31]. Of note, murine allergy and asthma models have shown that

TSLP can elicit pathogenic Th2 mediated immunity[18, 19, 32] and controlled delivery likely functions as a rheostat to modulate dendritic cells and therefore T cells. The effects of GM-CSF are similarly complex and depending upon the dose and kinetics of GM-CSF delivery either immunity or tolerance can be evoked[12, 13, 33]. Although Th2 immunity is not directly investigated here, by delivering TSLP and GM-CSF from a material system the location, dose, duration, and kinetics of delivery can be tightly controlled, potentially making it possible to design a system that promotes regulatory T cells while reducing unwanted Th2 type immune responses, increasing the overall specificity and reducing off-target effects. This delivery approach may also allow for TSLP to be used as a tolerogenic factor in humans, where TSLP appears to direct a more Th2 biased response[34]. Still, the inflammatory milieu in periodontitis is complex and more biomarker assays and functional studies are necessary to fully demonstrate tolerance induction or immunosuppression and the overall benefit of this approach, particularly given the possibility of a negative feedback loop inducing the tolerogenic milieu. This would also include deep quantitative phenotyping of the enriched DC and T cell populations including subsets and deep cytokine profiling.

GM-CSF is known to influence the migration of neutrophils, eosinophils monocytes and DC[35–37]. To our knowledge this is the first time that chemokinesis has been demonstrated for murine DC in response to GM-CSF. In the current study, cellular migration was examined *in vitro* over the course of 8 hours. During most of this period, the rapid release of GM-CSF may lead to local concentrations greater than the (presumed) Kd of the GM-CSF receptor, which could inhibit chemotaxis as the receptors would be saturated (and theoretically limit cellular enrichment). If the experimental duration was changed, lower doses of GM-CSF were incorporated into the alginate, GM-CSF release kinetics were slowed, or an alternative experimental setup (e.g. transwells or a microfluidics) was used, chemotaxis may also have been observed. The *in vitro* migration studies and the GM-CSF release assays together suggest that delayed GM-CSF release kinetics may enhance dendritic cell recruitment. Alginate systems can be facilely modified to deliver drugs with a range of kinetics and additional studies correlating DC migration and enrichment with a range of delivery kinetics is an important next step for pre-clinical optimization.

In addition to its effects *in vitro*, GM-CSF has been used in many studies to increase the number of innate cells *in vivo*[38] and a similar result is observed in this study following intradermal (i.d.) or periodontal injection of alginate gels containing GM-CSF (Figures 4 and 5). GM-CSF also has immunostimulatory and immunomodulatory roles and local delivery via a material system may allow one to optimize its effects to further attenuate periodontal disease[12, 13, 39–42]. It is important to note that a variety of other factors, including Flt3L, MCP-1 (CCL2), MIP-3α (CCL20), fractalkine (CX_3CL1), or CCL22 could be incorporated into this system as recruitment factors for DC, or in the case of CCL22, T regulatory cells[23, 24, 43, 44]. Further, in future studies it would be interesting to explore the effects of GM-CSF on gingival fibroblasts and the ECM given the expression of GM-CSFR on these cells[45].

Co-delivery of GM-CSF and TSLP led to increased FOXP3+ regulatory T cells locally and regionally in the draining lymph node (Figure 5). Regulatory T cells have been found to play an important role in reducing inflammation in periodontal disease[8] suggesting that a strategy

of locally recruiting and programming DC toward a regulatory phenotype in the periodontal tissue may attenuate disease severity and should be the subject of future investigations. A single dose and release kinetics for GM-CSF and TSLP were tested in these studies, and optimization of these variables through the design of more sophisticated delivery systems[46] may further enhance regulatory T cell accumulation. Also, future studies to evaluate whether a causal relationship exists between the increased number of local regulatory T cells and the elevated IL-10 concentration are warranted.

In summary, the results of this study demonstrated the ability of a locally administered, alginate based biomaterial to enhance DC number, promote immunosuppressive cytokines in periodontal tissue, and enrich for regulatory T cells. Quantitative and dynamic control of DC may allow one to dial in the correct balance between effector and tolerogenic pathways, and restore the balance required to relieve the chronic inflammation of periodontitis that cannot be achieved with standard immunotherapies. This approach could be combined in the future with bone regeneration strategies to not only slow/reverse inflammation, but also promote regeneration. More generally, we anticipate that this strategy of enhancing DC numbers locally and programming them to elicit a specific T cell response may be broadly applicable to many other inflammatory diseases.

CONCLUSION

Local, controlled delivery of GM-CSF and TSLP in an alginate hydrogel delivery platform can enrich DC and FOXP3+ regulatory T cells in periodontal tissue. These findings hold the promise for local immunotherapy to treat the pathologic inflammation of periodontal disease.

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References

1. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. Nat Rev Dis Primers 2017;3:17038. [PubMed: 28805207]
2. Eke PI, Dye BA, Wei L, Thornton-Evans GO, Genco RJ, CDC Periodontal Disease Surveillance workgroup: James Beck GDRP. Prevalence of periodontitis in adults in the United States: 2009 and 2010. J Dent Res 2012;91:914–920. [PubMed: 22935673]
3. Nwizu N, Marshall J, Moysich K, et al. Periodontal disease and incident cancer risk among postmenopausal women: results from the Women's Health Initiative observational cohort. Cancer Epidemiol Biomarkers Prev 2017;26 1255–1265. [PubMed: 28765338]
4. Cekici A, Kantarci A, Hasturk H, Van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontol 2000 2014;64:57–80. [PubMed: 24320956]
5. Ebersole JL, Dawson DR, Morford LA, Peyyala R, Miller CS, Gonzalez OA. Periodontal disease immunology: 'double indemnity' in protecting the host. Periodontol 2000 2013;62:163–202. [PubMed: 23574466]
6. Heitz-Mayfield LJA, Lang NP. Surgical and nonsurgical periodontal therapy. Learned and unlearned concepts. Periodontol 2000 2013;62:218–231. [PubMed: 23574468]

7. Phillips BE, Garciafigueroa Y, Engman C, Trucco M, Giannoukakis N. Tolerogenic dendritic cells and T-regulatory cells at the clinical trials crossroad for the treatment of autoimmune disease; emphasis on type 1 diabetes therapy. Front Immunol 2019;10:148–148. [PubMed: 30787930]
8. Garlet GP, Cardoso CR, Mariano FS, et al. Regulatory T cells attenuate experimental periodontitis progression in mice. J Clin Periodontol 2010;37:591–600. [PubMed: 20642629]
9. Wilensky A, Segev H, Mizraji G, et al. Dendritic cells and their role in periodontal disease. Oral Dis 2014;20:119–126. [PubMed: 23656605]
10. Arizon M, Nudel I, Segev H, et al. Langerhans cells down-regulate inflammation-driven alveolar bone loss. Proc Natl Acad Sci USA 2012;109:7043–7048. [PubMed: 22509018]
11. Cafferata EA, Jerez A, Vernal R, Monasterio G, Pandis N, Faggion CM. The therapeutic potential of regulatory T lymphocytes in periodontitis: A systematic review. J Periodontal Res 2019;54:207–217. [PubMed: 30474205]
12. Gaudreau S, Guindi C, Menard M, Besin G, Dupuis G, Amrani A. Granulocyte-macrophage colony-stimulating factor prevents diabetes development in NOD mice by inducing tolerogenic dendritic cells that sustain the suppressive function of CD4(+)CD25(+) regulatory T cells. J Immunol 2007;179:3638–3647. [PubMed: 17785799]
13. Cheatem D, Ganesh BB, Gangi E, Vasu C, Prabhakar BS. Modulation of dendritic cells using granulocyte-macrophage colony-stimulating factor (GM-CSF) delays type 1 diabetes by enhancing CD4+CD25+regulatory T cell function. Clin Immunol 2009;131:260–270. [PubMed: 19171501]
14. Lam RS, O'Brien-Simpson NM, Hamilton JA, et al. GM-CSF and uPA are required for Porphyromonas gingivalis-induced alveolar bone loss in a mouse periodontitis model. Immunol and Cell Bio 2015.
15. Ali OA, Huebsch N, Cao L, Dranoff G, Mooney DJ. Infection-mimicking materials to program dendritic cells in situ. Nat Mater 2009;8:151–158. [PubMed: 19136947]
16. Dranoff G GM-CSF based cancer vaccines. Cancer Immun 2005;5:26.
17. Besin G, Gaudreau S, Menard M, Guindi C, Dupuis G, Amrani A. Thymic stromal lymphopoietin and thymic stromal lymphopoietin-conditioned dendritic cells induce regulatory T-cell differentiation and protection of NOD mice against diabetes. Diabetes 2008;57:2107–2117. [PubMed: 18477807]
18. Al-Shami A, Spolski R, Kelly J, Keane-Myers A, Leonard WJ. A role for TSLP in the development of inflammation in an asthma model. J Exp Med 2005;202:829–839. [PubMed: 16172260]
19. Shi LY, Leu SW, Xu F, et al. Local blockade of TSLP receptor alleviated allergic disease by regulating airway dendritic cells. Clin Immunol 2008;129:202–210. [PubMed: 18757241]
20. Lei LY, Zhang YL, Yao WG, Kaplan MH, Zhou BH. Thymic stromal lymphopoietin interferes with airway tolerance by suppressing the generation of antigen-specific regulatory T cells. J Immunol 2011;186:2254–2261. [PubMed: 21242516]
21. Leichner TM, Satake A, Harrison VS, et al. Skin-derived TSLP systemically expands regulatory T cells. J Autoimmun 2017;79:39–52. [PubMed: 28126203]
22. Ramseier CA, Rasperini G, Batia S, Giannobile WV. Advanced reconstructive technologies for periodontal tissue repair. Periodontol 2000 2012;59:185–202. [PubMed: 22507066]
23. Francisconi CF, Vieira AE, Biguetti CC, et al. Characterization of the protective role of regulatory T cells in experimental periapical lesion development and their chemoattraction manipulation as a therapeutic tool. J Endod 2016;42:120–126. [PubMed: 26589811]
24. Garlet GP, Sfeir CS, Little SR. Restoring host-microbe homeostasis via selective chemoattraction of Tregs. J Dent Res 2014;93:834–839. [PubMed: 25056995]
25. Lee KY, Mooney DJ. Alginate: Properties and biomedical applications. Prog Polym Sci 2012;37:106–126. [PubMed: 22125349]
26. Lee KY, Bouhadir KH, Mooney DJ. Controlled degradation of hydrogels using multi-functional cross-linking molecules. Biomaterials 2004;25:2461–2466. [PubMed: 14751730]
27. Silva EA, Mooney DJ. Spatiotemporal control of vascular endothelial growth factor delivery from injectable hydrogels enhances angiogenesis. J Thromb Haemost 2007;5:590–598. [PubMed: 17229044]
28. Fishman ML, Cooke PH, Coffin DR. Nanostructure of native pectin sugar acid gels visualized by atomic force microscopy. Biomacromolecules 2004;5:334–341. [PubMed: 15002992]

29. Lutz MB, Kukutsch N, Ogilvie ALJ, et al. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. J Immunol Methods 1999;223:77–92. [PubMed: 10037236]
30. Basic Methods in Microscopy: Protocols and Concepts from Cells: A Laboratory Manual: Cold Spring Harbor Laboratory Press; 2006.
31. Shi YF, Liu CH, Roberts AI, et al. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and T-cell responses: what we do and don't know. Cell Res 2006;16:126–133. [PubMed: 16474424]
32. Zhang F, Huang G, Hu B, Song Y, Shi Y. A soluble thymic stromal lymphopoietin (TSLP) antagonist, TSLPR-immunoglobulin, reduces the severity of allergic disease by regulating pulmonary dendritic cells. Clin Exp Immunol 2011;164:256–264. [PubMed: 21352203]
33. Jinushi M, Nakazaki Y, Dougan M, Carrasco DR, Mihm M, Dranoff G. MFG-E8-mediated uptake of apoptotic cells by APCs links the pro- and antiinflammatory activities of GM-CSF. J Clin Invest 2007;117:1902–1913. [PubMed: 17557120]
34. Soumelis V, Reche PA, Kanzler H, et al. Human epithelial cells trigger dendritic cell-mediated allergic inflammation by producing TSLP. Nat Immunol 2002;3:673–680. [PubMed: 12055625]
35. Wang JM, Colella S, Allavena P, Mantovani A. Chemotactic activity of human recombinant granulocyte-macrophage colony-stimulating factor. Immunology 1987;60:439–444. [PubMed: 3494669]
36. Warringa RAJ, Koenderman L, Kok PTM, Kreukniet J, Bruijnzeel PLB. Modulation and induction of eosinophil chemotaxis by granulocyte-macrophage colony-stimulating factor and interleukin-3. Blood 1991;77:2694–2700. [PubMed: 1646045]
37. Kaplan G, Walsh G, Guido LS, et al. Novel responses of human skin to intradermal recombinant granulocyte macrophage-colony-stimulating factor - langerhans cell recruitment, keratinocyte growth, and enhanced wound-healing. J Exp Med 1992;175:1717–1728. [PubMed: 1588289]
38. Dranoff G, Jaffee E, Lazenby A, et al. Vaccination with irradiated tumor-cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting antitumor immunity. Proc Natl Acad Sci USA 1993;90:3539–3543. [PubMed: 8097319]
39. Gangi E, Vasu C, Cheatem D, Prabhakar BS. IL-10-Producing CD4(+)CD25(+) regulatory T cells play a critical role in granulocyte-macrophage colony-stimulating factor-induced suppression of experimental autoimmune thyroiditis. J Immunol 2005;174:7006–7013. [PubMed: 15905543]
40. Kared H, Masson A, Adle-Biassette H, Bach JF, Chatenoud L, Zavala F. Treatment with granulocyte colony-stimulating factor prevents diabetes in NOD mice by recruiting plasmacytoid dendritic cells and functional CD4(+)CD25(+) regulatory T-cells. Diabetes 2005;54:78–84. [PubMed: 15616013]
41. Campbell IK, Bendele A, Smith DA, Hamilton JA. Granulocyte-macrophage colony stimulating factor exacerbates collagen induced arthritis in mice. Ann Rheum Dis 1997;56:364–368. [PubMed: 9227165]
42. Serafini P, Carbley R, Noonan KA, Tan G, Bronte V, Borrello I. High-dose granulocyte-macrophage colony-stimulating factor-producing vaccines impair the immune response through the recruitment of myeloid suppressor cells. Cancer Res 2004;64:6337–6343. [PubMed: 15342423]
43. Glowacki AJ, Yoshizawa S, Jhunjhunwala S, et al. Prevention of inflammation-mediated bone loss in murine and canine periodontal disease via recruitment of regulatory lymphocytes. Proc Natl Acad Sci USA 2013;110:18525–18530. [PubMed: 24167272]
44. Souto GR, Queiroz CM Jr., Costa FO, Mesquita RA. Relationship between chemokines and dendritic cells in human chronic periodontitis. J Periodontol 2014;85:1416–1423. [PubMed: 24605873]
45. Ramaglia L, Di Spigna G, Capece G, Sbordone C, Salzano S, Postiglione L. Differentiation, apoptosis, and GM-CSF receptor expression of human gingival fibroblasts on a titanium surface treated by a dual acid-etched procedure. Clin Oral Investig 2015.
46. Verbeke CS, Gordo S, Schubert DA, et al. Multicomponent injectable hydrogels for antigen-specific tolerogenic immune modulation. Adv Healthc Mater 2017;6.

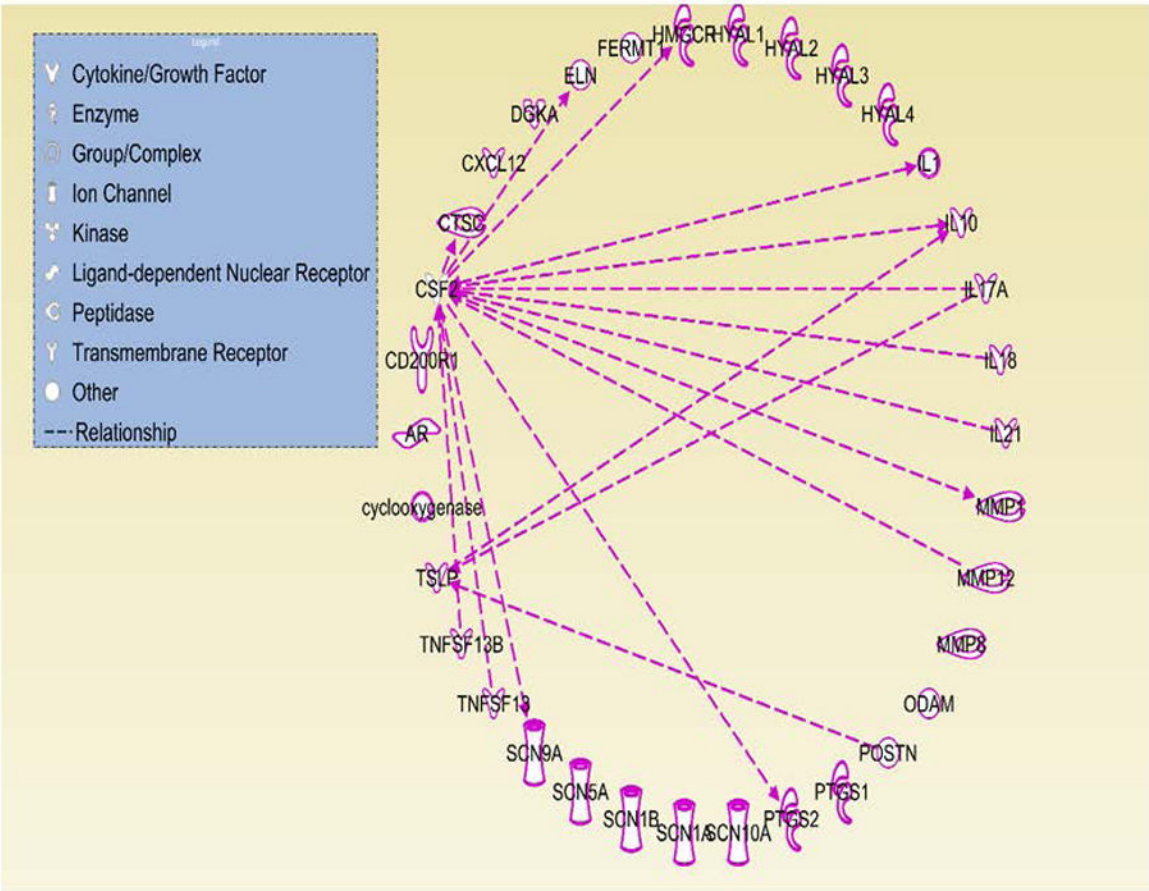

Figure 1. Periodontal disease associated genes and proteins have multiple possible interactions with GM-CSF and TSLP.

Using Ingenuity Pathway Analysis, the genes and proteins associated with periodontal disease were combined with GM-CSF and TSLP nodes. Interacting terms between GM-CSF or TSLP and the periodontal associated protein network were generated and are depicted in the figure. AR, androgen receptor; CD200R1, CD200 receptor 1; CSF2, granulocyte macrophage colony stimulating factor; CTSC, cathepsin C; CXCL12, C-X-C motif chemokine ligand 12; DGKA, diacylglycerol kinase alpha; ELN, elastin; FERMT1, fermitin family member 1; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; HYAL1, hyaluronoglucosaminidase 1;HYAL2, hyaluronoglucosaminidase 2; HYAL3, hyaluronoglucosaminidase 3; HYAL4, hyaluronoglucosaminidase 4; IL1, interleukin 1; IL10, interleukin 10; IL17A, interleukin 17A; IL18, interleukin 18; IL21, interleukin 21; MMP1, matrix metallopeptidase 1; MMP12, matrix metallopeptidase 12; MMP8, matrix metallopeptidase 8; ODAM, odontogenic, ameloblast associated; POSTN, periostin; PTGS1, prostaglandin-endoperoxide synthase 1; PTGS2, prostaglandin-endoperoxide synthase 2; SCN10A, sodium voltage-gated channel alpha subunit 10; SCN1A, sodium voltage-gated channel alpha subunit 1; SCN1B, sodium voltage-gated channel beta subunit 1; SCN5A, sodium voltage-gated channel alpha subunit 5; SCN9A, sodium voltage-gated channel alpha subunit 9; TNFSF13, TNF superfamily member 13; TNFSF13B, TNF superfamily member 13b; TSLP, thymic stromal lymphopoietin.

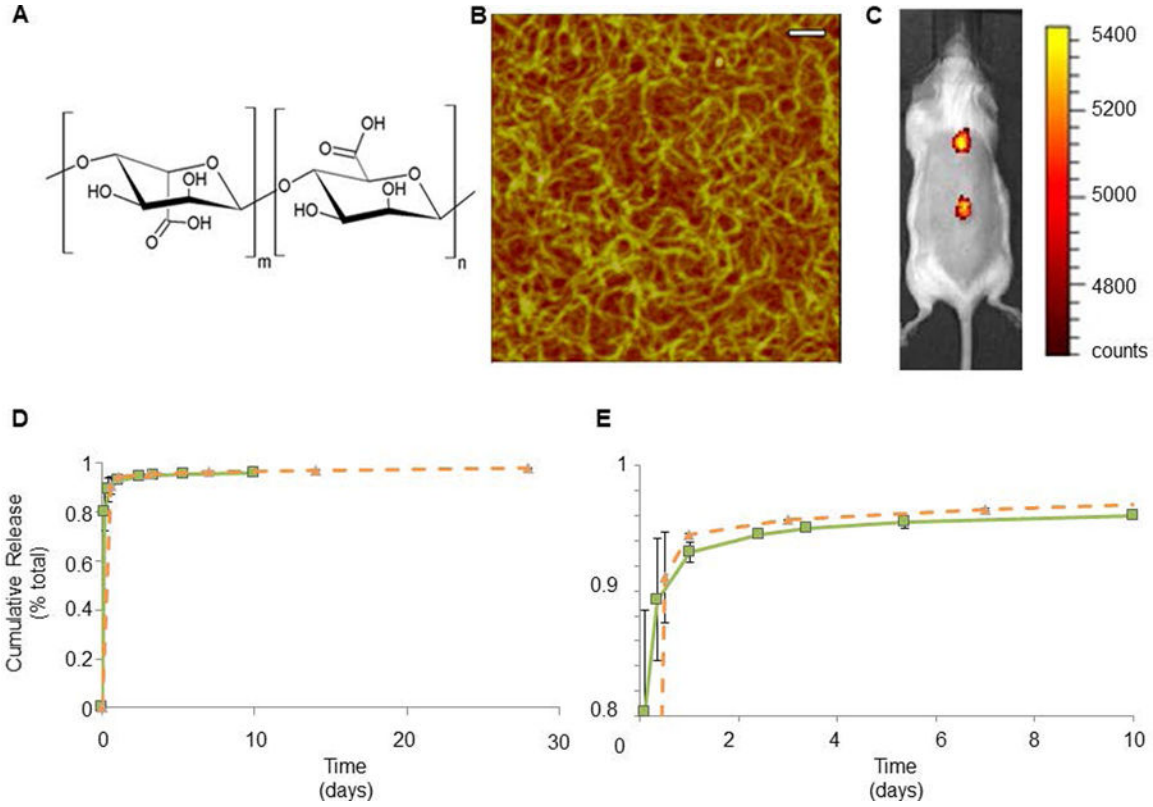

Figure 2. Nanoporous alginate hydrogels rapidly release GM-CSF and TSLP.

(A) Chemical structure of alginate, m and n subscripts represent the guluronic and mannuronic acid monomers, respectively. (B) AFM height image of a peel-transferred alginate hydrogel layer. Scale bar = 100 nm. (C) Fluorescent image of two intradermally injected alginate hydrogels labeled with PE-Cy7 microbeads overlaying a photograph. (D) Release kinetics of GM-CSF (orange triangles with dashed line) and TSLP (green squares with solid line) in PBS at 37°C. (E) Magnified image of (D). Values in (D) and (E) represent mean and SD (n= 4 for GM-CSF or 7 for TSLP).

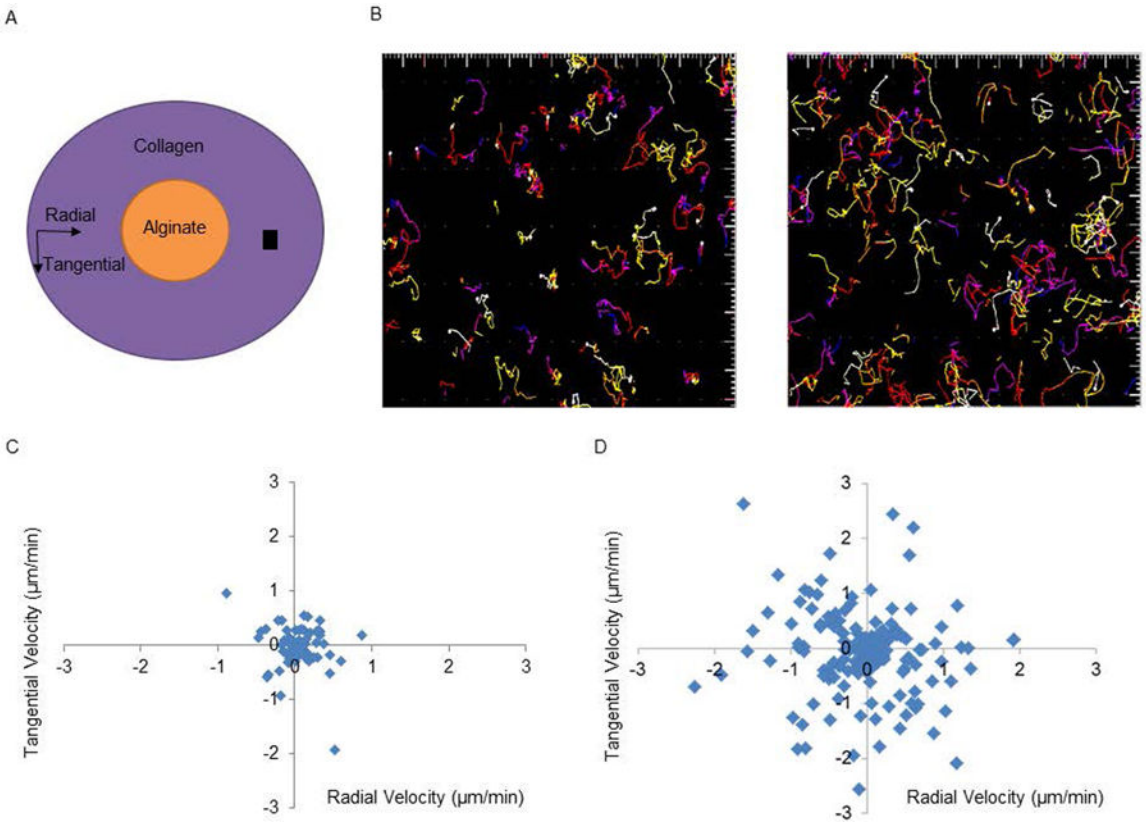

Figure 3. GM-CSF mediated chemokinesis of bone marrow derived dendritic cells *in vitro*.

(A) Alginate gels with or without GM-CSF were placed in a petri dish and surrounded with collagen containing labeled murine bone marrow derived dendritic cells. The cartoon depicts a transverse section of the petri dish with the purple color representing the collagen and DC while the orange color denotes the alginate gel and the black square represents an imaging window as seen in (B). The imaging window was randomly selected. (B) Individual paths of cells in a representative experiment exposed to control (no GM-CSF) or GM-CSF containing alginate hydrogels viewed at 20x. The average velocity of the cells was calculated from initial and final position values and is plotted for control gels (C) and GM-CSF releasing gels (D). Chemotaxis toward the alginate is given as the positive radial coordinate. Each dot reflects the velocity of 1 cell and each plot is representative of three experiments.

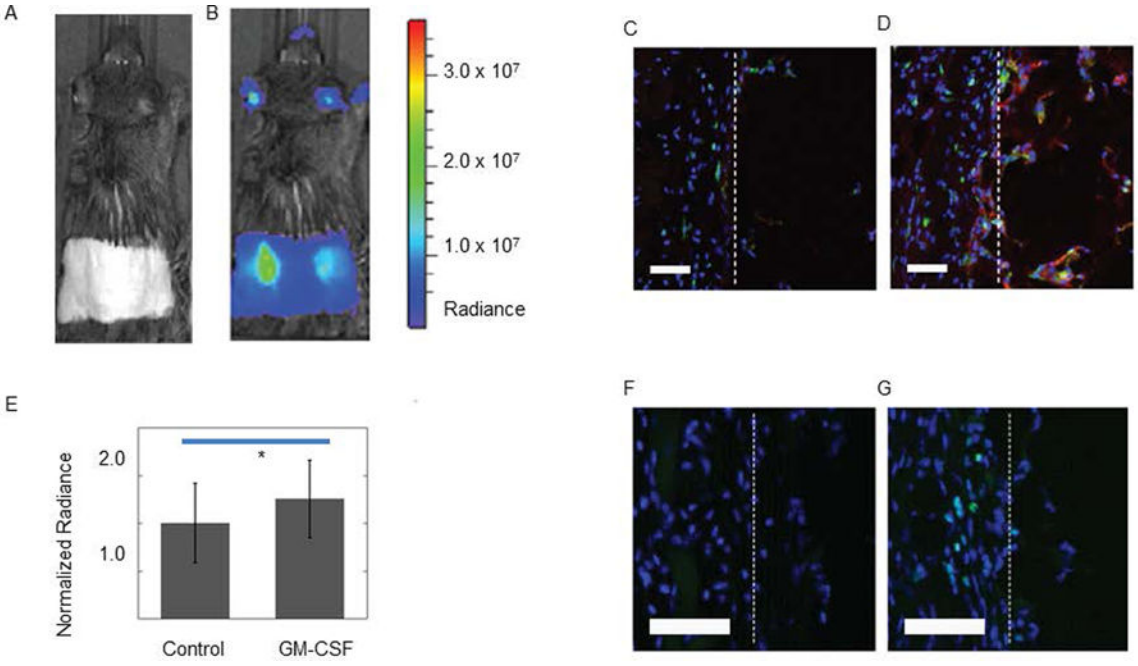

Figure 4. GM-CSF and TSLP delivery enriches DC and Treg cell numbers in the skin.

(A) Photograph and (B) radiant emission in p/s/cm^2/sr of a C57BL/6 *CD11c*-Cre-EGFP x FVB *Stop/loxP* Luc mouse after luciferin administration. Imaging was performed 7 days following injection of alginate gels containing 3 (left) or 0 μg (right) GM-CSF. (C,D) Immunofluorescent stain of sectioned skin 7 days after receiving an injection of blank (C) or GM-CSF containing alginate hydrogels (D), DAPI (blue), MHCII (green), and CD11c (red). White dotted lines indicate the border between the skin (left) and the alginate gels (right). (E) Normalized data comparing radiance surrounding GM-CSF containing hydrogels with blank control hydrogels. (F,G) Immunofluorescent imaging of sectioned skin staining for nuclei (blue) and FOXP3 (green) in animals treated 7 days earlier with alginate hydrogels containing GM-CSF alone (F) or in combination with TSLP (G). White dotted lines indicate the border between the dermal tissue (left) and the alginate gels (right). (immunohistochemistry n=3 animals / condition; live animal imaging n = 5); representative images and animals shown. Scale bars are 50um, *$p < 0.04$ (student's t-test).

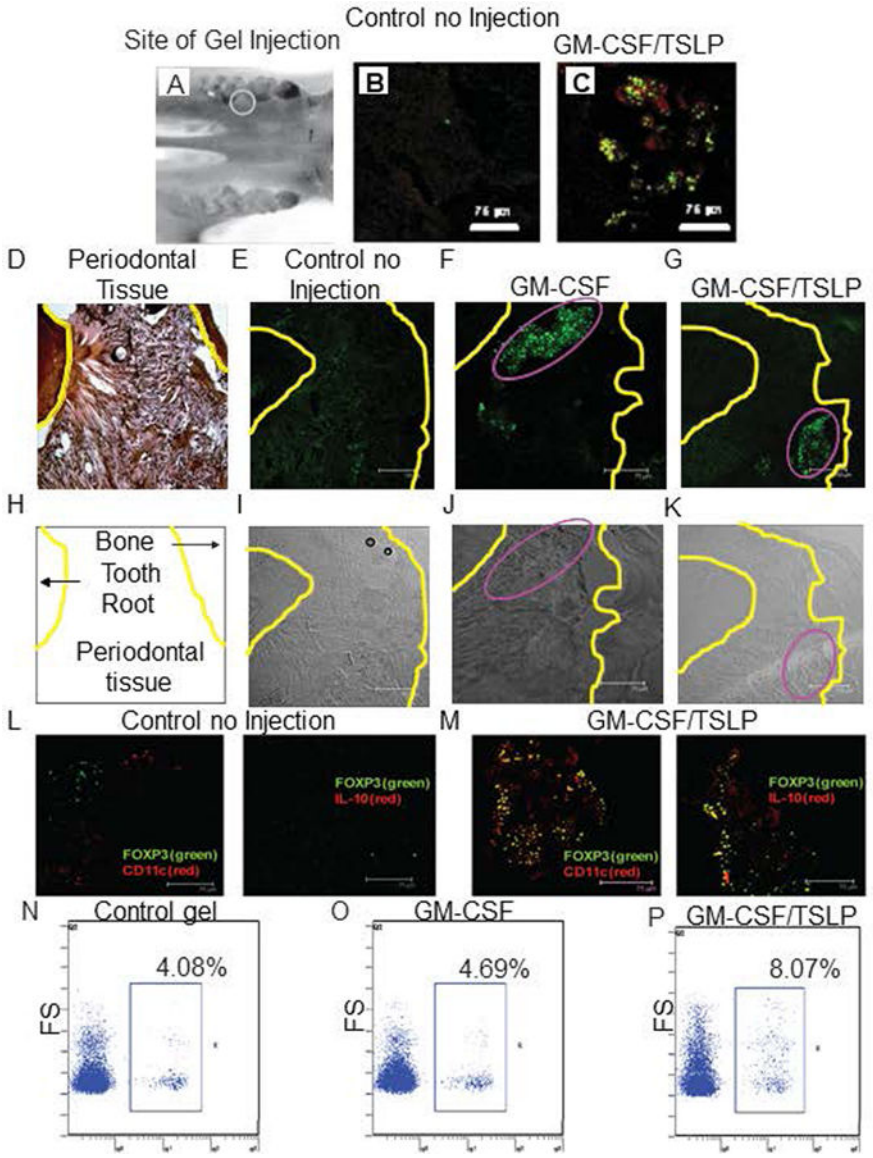

Figure 5. Enhanced recruitment of DC, production of IL-10, and accumulation of FOXP3+ T cells in the periodontal tissue in mice treated with alginate gels containing GM-CSF and TSLP.

(A) Ventral view of the mouse calvarium; circle indicates target location of hydrogel injection. Immunohistochemical staining for IL-10 (red) and CD11c (green) in the periodontal cavity of naïve (B) or GM-CSF and TSLP treated mice (C) 7 days following injection. (D-P) FOXP3-EGFP-KI mice received no injection (E, I, L) or a periodontal injection of a control blank alginate hydrogel (L, N), alginate hydrogel with GM-CSF (F, J, O), or alginate with GM-CSF and TSLP (G, K, M, P). H&E, bright field, or fluorescent images of the maxillary jaws 7 days following injection (D-M). Flow cytometry plot of T cells isolated from the cervical lymph nodes (N-P).