Could this reflect heterogeneous programs undertaken by V2 thymocytes relative to their rather diverse TCR repertoire[2] or the peripheral polarization of mature γδ T cells in a way similar to that of conventional αβ T cells? If developmental programming accounts for most 'innate-like' functions of γδ T cells, it is likely that a degree of functional plasticity is permitted by the delayed acquisition of some new functional properties, acquisition that is finely tailored by environmental and tissue-derived signals.

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Restraining IL-17: Del-1 deals the blow

Shabaana A Khader

Interleukin-17 (IL-17) induces the recruitment of neutrophils to sites of inflammation. In a model of periodontitis, Del-1 is now described to be an inhibitor of IL-17 expression that suppresses the recruitment of neutrophils and the associated inflammation-mediated pathology.

The recruitment of neutrophils to sites of infection is tightly regulated to mediate host protection and to minimize pathological inflammation. The cytokine interleukin 17 (IL-17) has been well characterized as inducing granulopoietic factors such as granulocyte colony-stimulating factor (G-CSF) and CXC chemokines such as CXCL1, CXCL2 and CXCL5 to mediate the recruitment of neutrophils to sites of infection and inflammation[1]. Furthermore, IL-17-mediated induction of G-CSF also results in the differentiation of $CD34^+$ progenitors into neutrophil progenitors, thereby maintaining the influx of neutrophils into chronic inflammatory sites[1]. Although the factors that mediate and maintain the recruitment of neutrophils during chronic inflammation have been identified, the factors that regulate and restrain the recruitment of neutrophils to limit chronic inflammation and associated disease pathogenesis are not well described. Identifying such factors will allow the design of new therapeutics to decrease chronic inflammation and the resultant disease progression. In this issue of *Nature Immunology*, Chavakis and colleagues identify such a molecule, Del-1 ('developmental endothelial locus 1'), and show that Del-1 inhibits IL-17 expression and suppresses the recruitment of neutrophils and the associated inflammation-mediated pathology in a model of periodontitis[2].

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Periodontitis is a chronic inflammatory condition that affects the tooth-supporting tissues called 'periodontium' and causes tooth loss in about 15% of adults. Although the disease process associated with periodontitis is initiated by bacterial infection of the subgingiva, the resulting inflammatory host immune response, characterized in part by the recruitment of neutrophils, mediates the disease pathology. Thus far, there has been no clear consensus on whether IL-17 has a destructive role or a protective role in periodontitis. For example, a protective role for IL-17 has been proposed in a pathogen model of periodontitis showing that the diminished recruitment of neutrophils in IL-17 receptor–deficient ($Il17ra^{-/-}$) mice is associated with lower host resistance to infection[3]. In contrast, IL-17 can induce several genes encoding molecules involved in osteoclastogenesis and can act in synergy with other proinflammatory cytokines such as tumor-necrosis factor and IL-1β to mediate bone destruction during chronic inflammatory conditions[4]. In addition, IL-17 expression is higher during human periodontitis[4], which suggests a pathological role for IL-17 in periodontitis. The study by Eskan *et al.* sheds new light on this debate by demonstrating that IL-17 production is destructive in periodontitis and highlighting how restraining IL-17 can improve periodontal health[2]. Eskan *et al.*[2] use a mouse model in which old mice develop spontaneous periodontitis[5] characterized by infiltration of neutrophils and the associated jawbone loss, which resembles the natural occurrence of severe periodontitis in humans during old age. With this model, the authors find that the greater influx of neutrophils in the gingiva of old mice is associated with lower expression of Del-1. Del-1 has been shown to negatively regulate neutrophil extravasation by antagonizing β_2 integrin–dependent adhesion of neutrophils onto the vascular endothelium[6]. Accordingly, mice deficient in Del1 ($Edil3^{-/-}$ mice) show more periodontal bone loss associated with more recruitment of neutrophils and a typical 'IL-17 signature' gene profile in the gingiva; that is, induction of IL-17A (IL-17), IL-17F and IL-17C and IL-17-dependent genes encoding molecules such as the CXC chemokines and their receptor CXCR2, as well as G-CSF. In addition, they detect higher expression of the receptor-activator RANKL and more osteoclastic activity in the periodontium of old $Edil3^{-/-}$ mice. Notably, the authors show that lower Del-1 expression and higher IL-17 expression is also associated with diseased gingival tissue, but not healthy gingival tissue, obtained from human patients with periodontitis. These data suggest for the first time that Del-1 can negatively regulate and restrain IL-17, thereby limiting the recruitment of neutrophils and inflammation-mediated bone loss. To definitively test this hypothesis, the authors cross $Il17ra^{-/-}$ mice with $Edil3^{-/-}$ mice and show that the double-deficient $Il17ra^{-/-}Edil3^{-/-}$ progeny have less recruitment of neutrophils and are completely resistant to the spontaneous periodontitis that develops in $Edil3^{-/-}$ mice as they age. The extravasation of neutrophils into inflammatory sites is dependent on interactions between β_2 integrins such as LFA-1 on neutrophils and endothelial receptors such as intercellular adhesion molecules (for example, ICAM-1, the ligand for LFA-1). Consistent with the known role of Del-1 as an antagonist

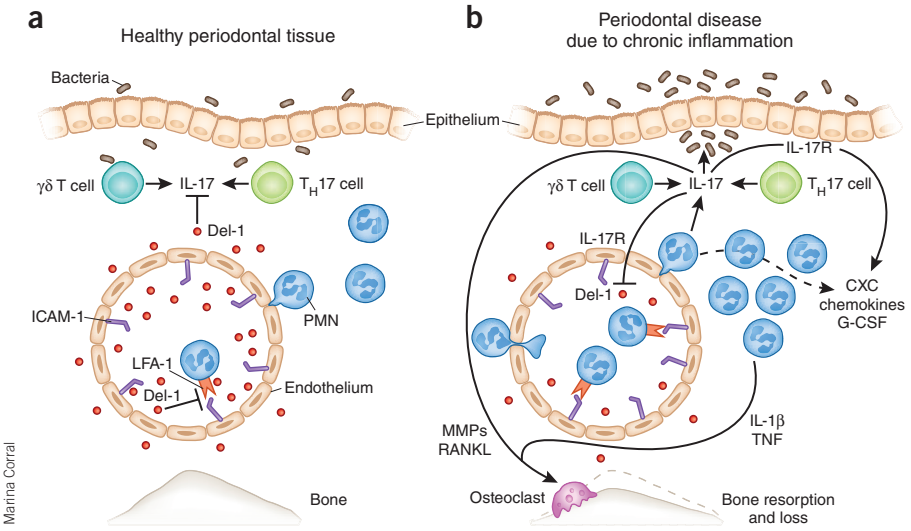

Figure 1 Del-1 restrains IL-17 production, inflammation and bone loss during periodontitis. Higher expression of Del-1 in the periodontal tissue of young mice restrains IL-17 production as well as the trafficking of neutrophils into the tissue (**a**). In old mice or during infection with periodontal pathogens, Del-1 expression is downregulated and IL-17 production by γδ T cells, $CD4^+$ T cells and neutrophils is upregulated (**b**). IL-17 induces G-CSF and CXC chemokines to attract neutrophils into the inflamed tissue. At the same time, IL-17 inhibits Del-1 expression, which promotes the extravasation of neutrophils into the inflamed gingival tissue. IL-17 also acts in synergy with other proinflammatory cytokines to induce matrix metalloproteinases (MMPs) and RANKL; this promotes osteoclatogensis and results in periodontal bone loss. Inflammation-associated bone loss promotes the availability of nutrients and increases bacterial diversity and proliferation, which amplifies the inflammatory cascade. PMN, polymorphonuclear cell; TNF, tumor-necrosis factor.

of LFA-1 (ref. 6), combined deficiency in Del-1 and LFA-1 in old mice results in inhibition of periodontal bone loss and the recruitment of neutrophils into gingiva. Notably, *Il17ra*$^{-/-}$ mice have higher expression of Del-1 mRNA in the gingiva, which suggests that IL-17 also cross-regulates Del-1 expression. Furthermore, the authors generate bone-marrow chimeras and show that absence of the IL-17 receptor on nonhematopoietic cells, probably stromal cells, results in higher Del-1 expression and coincident impairment in the recruitment of neutrophils. These data together clearly show that Del-1 is a newly identified negative regulator that restrains IL-17 production and the recruitment of neutrophils; in return, IL-17 regulates Del-1 expression.

It is thought that chronic inflammation and the associated bone loss during periodontitis perturbs the normal homeostasis of oral microflora by providing additional nutrients and niches for bacterial strains to colonize the periodontium[4]. Consistent with that, young *Edil3*$^{-/-}$ mice have bone heights and number of bacteria in their gingival tissues similar to those of their wild-type littermates but develop a greater bacterial burden than that of the wild-type mice as they acquire the inflammatory phenotype. In addition, the composition of the oral microbiota is qualitatively and quantitatively different in the absence of Del-1 and/or LFA-1 and the receptor IL-17RA, which supports the proposal that host genetics also influence the oral microbiota. Furthermore, the authors confirm that periodontitis is a bacteria-induced disease, as antibiotic treatment inhibits bone loss in old *Edil3*$^{-/-}$mice.

One of the best characterized and major populations of cells of the immune response that produce IL-17 are $CD4^+$ T cells of the T_H17 subset of helper T cells[7]. However, a flurry of exciting papers have demonstrated that T_H17 cells are only one of the many sources of IL-17 produced in response to infection and inflammation[8]. In fact, in many disease settings, cells of the innate immune response, such as γδ T cells, lymphoid tissue–inducer cells, natural killer cells and natural killer T cells, are early and potent sources of IL-17 (ref. 8). Consistent with that, Eskan *et al.* find that although both $CD4^+$ T cells and γδ T cells localize together with IL-17 in the gingiva of *Edil3*$^{-/-}$ mice, IL-17$^+$ γδ T cells outnumber IL-17$^+$ $CD4^+$ T cells in the inflamed gingival tissue[2]. Neutrophils are mostly thought of as cells that respond to IL-17, but some reports have proposed that neutrophils can also produce IL-17 (ref. 8). Eskan *et al.* also show that in older *Edil3*$^{-/-}$ mice, when maximal inflammation and the disease phenotype is evident, Ly6G$^+$ neutrophils are one of the predominant cell types that localize together with IL-17 (ref. 2). However, *Il17ra*$^{-/-}$ mice have less recruitment of neutrophils but still have high expression of IL-17 mRNA in the gingiva, which suggests that IL-17 produced by γδ T cells and $CD4^+$ T cells is probably an early source of the IL-17 that recruits neutrophils to the inflamed site. Thus, although both IL-17-producing $CD4^+$ T cells and IL-17-producing γδ T cells depend on IL-23 and use overlapping transcription factors[8], future work should focus on defining the distinct characteristics of IL-17-producing neutrophils in inflammatory conditions.

The search for therapeutic factors that can limit IL-17 production and minimize inflammation will greatly benefit the treatment of several chronic inflammatory diseases, including periodontitis. In a series of convincing experiments, the authors show that microinjection of soluble Del-1 into the gingiva can suppress inflammation not only restraining IL-17 production in old mice but also after infection with the human pathogen *Porphyromonas gingivalis*. Furthermore, through the use of a ligature-induced periodontitis model, the authors demonstrate that systemic or local administration of a fusion protein of Del-1 and the Fc fragment (Del-1–Fc) is much better at diminishing periodontal bone loss than are other inhibitors that interfere with LFA-1–ICAM-1 interactions[2]. These data together indicate a previously unknown role for Del-1 as therapeutic agent in restraining IL-17 production and the recruitment of neutrophils and limiting inflammation.

The new data by Eskan *et al.*[2] indicate that higher expression of Del-1 in the periodontal tissue of young mice restrains IL-17 production as well as the trafficking of neutrophils into the tissue, in part by its antagonistic effects on LFA-1 (**Fig. 1**). In old mice or during infection with periodontal pathogens, Del-1 expression is downregulated and IL-17 production is upregulated (**Fig. 1**). It is possible that γδ T cells are the early innate producers of IL-17, as they can express Toll-like receptors and respond to microbial stimulation[8]. The presentation of antigens derived from oral microbiota also probably results in the accumulation of antigen-specific T_H17 cells that produce IL-17. IL-17 acts on stromal cells to upregulate G-CSF and CXC chemokines to attract neutrophils into the inflamed tissue. At the same time, IL-17 also acts on endothelial cells through its receptor IL-17R to inhibit Del-1 expression, which further promotes the extravasation of neutrophils into the inflamed gingival tissue and IL-17 production by neutrophils. IL-17 and other proinflammatory cytokines together induce matrix metalloproteinases and RANKL to promote osteoclastogenesis, which results in periodontal

bone loss (**Fig. 1**). Inflammation-associated bone loss probably promotes the availability of nutrients and new niches to increase bacterial diversity and proliferation, which further amplifies the inflammatory cascade.

Future studies will no doubt be focused on determining whether the role of Del-1 in restraining IL-17 is specific to the periodontium or whether Del-1 is a universal negative regulator of IL-17 in other tissues, especially those in which chronic inflammation results in bone loss. Furthermore, delineating the specific pathway by which Del-1 restrains IL-17 production will potentially identify additional molecules that can be targeted to restrain IL-17-mediated pathology in inflammatory diseases. In contrast, it is possible that the identification of additional molecules that restrain Del-1 can be targeted to those that increase IL-17-mediated recruitment of neutrophils to mucosal sites to improve host protective immunity to pathogens.

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Lipids-Я-Us: peroxisome generation of *i*NKT ligands

Randy R Brutkiewicz & Alexander L Dent

The identification of the natural lipids that select invariant natural killer T cells (*i*NKT cells) *in vivo* has been somewhat elusive and controversial. A new study shows that ether-bonded phospholipids, generated mainly in peroxisomes, are responsible for the development of a major subpopulation of *i*NKT cells.

CD1d molecules present glycolipid and phospholipid molecules to a unique subpopulation of T cells called 'invariant natural killer T cells' (*i*NKT cells). These *i*NKT cells are restricted in terms of which lipids they recognize, and such lipids are considered important for their intrathymic development[1,2]. Facciotti *et al.* report in this issue of *Nature Immunology* that peroxisome-derived lipids are responsible for selecting a large proportion of *i*NKT cells *in vivo*[3]. Which specific lipids are able to select *i*NKT cells *in vivo* has been somewhat controversial. It is clear that the lysosomal glycosphingolipid isoglobotrihexosylceramide (iGb3) is able to stimulate *i*NKT cells in a CD1d-dependent manner[4]. Furthermore, mice deficient in the enzyme β-hexosaminidase b, which is responsible for the synthesis of an iGb3 precursor, lack *i*NKT cells[4]. However, a more specific mutant line of mice that lack iGb3 production is reported to have normal *i*NKT cells[5]. Furthermore, iGb3 has been difficult to detect in normal tissues by some groups[6]. The important question remains of whether there is (are) a natural lipid ligand(s) that can select *i*NKT cells *in vivo*, whose absence would impair *i*NKT cell development.

In the present study, the authors extract lipids from mouse thymocytes, fractionate them by polarity and analyze them by biological activity (activation of *i*NKT cells *in vitro*)[3]. Through the use of mass spectrometry of the active lipid fractions, they find that a class of lipids that contains an alkyl chain with a vinyl ether bond has the *i*NKT cell–stimulating activity. Lipids with this structure constitute a class of lipids found in peroxisomes.

Peroxisomes are important organelles that are responsible for the generation of many ether-bonded lipids[7]. They are also important for the α-oxidation and β-oxidation of fatty acids and glyoxylate detoxification. Ether phospholipids are different from conventional diacyl phospholipids; the sn-1 position of the glycerol backbone contains an ether linkage (rather than an ester linkage). The mass-spectrometry analysis of mouse thymocyte lipids by Facciotti *et al.* suggests the presence of the following two ether-bonded phospholipids: 1-*O*-1′-(Z)-hexadecenyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine and 1-*O*-1′, 9′-(Z,Z)-octadecadienyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine[3]. The synthetic plasmalogen C16-lysophosphatidylethanolamine (pLPE) is able to stimulate *i*NKT cells in terms of upregulation of T cell–activation markers such as CD69 and the secretion of cytokines, which confirms the stimulatory function of the lipid fractions isolated from the thymus. In fact, pLPE-induced stimulation of *i*NKT cells is in the same range as that induced by α-galactosylceramide, the prototypic ligand presented by CD1d to *i*NKT cells.

The absence of glyceronephosphate *O*-acyltransferase (GNPAT), the enzyme that generates such lipids in peroxisomes, causes many specific biological defects, including a severe deficiency in the production of plasmalogens, cataract development, severe hypotonia, and growth and developmental retardation[8]. In humans, GNPAT deficiency is responsible for rhizomelic chondrodysplasia punctata type 2 (ref. 7), a disorder in which patients suffer the development consequences also found in GNPAT-deficient mice. In the Facciotti *et al.* paper, GNPAT-deficient mice have a much lower frequency and absolute number of *i*NKT cells in the thymus, spleen and liver than wild-type mice have[3].

One paradoxical characteristic of *i*NKT cells relates to their ability to recognize and respond to self lipids while properly regulating anti-pathogen immune responses and maintaining self-tolerance[1]. This aspect of *i*NKT cell biology indicates there is a crucial amount of control over the CD1d-mediated presentation of self lipids. However, important questions are how *i*NKT cells are selected in the thymus and, specifically, whether *i*NKT cells are selected by self lipids presented by CD1d. Although these issues have been the subject of intense research, the identification of a specific lipid ligand that promotes the selection of *i*NKT cells in the thymus has been difficult.

Like conventional or 'mainstream' αβ T cells, *i*NKT cells develop and undergo a selection process in the thymus that is instructed by the specificity of the T cell antigen receptor (TCR) on the *i*NKT cell. However, *i*NKT cells use CD1d as their selection ligand rather than the major histocompatibility complex class I or class II molecules used for the selection of mainstream αβ T cells. Published studies have indicated that certain lipids, particularly

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