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Comment on "Experimental Arthritis Triggers Periodontal Disease in Mice: Involvement of TNF-α and the Oral Microbiota"

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Letters to the Editor

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Comment on "Innate Immune Collectin Surfactant Protein D Simultaneously Binds Both Neutrophil Extracellular Traps and Carbohydrate Ligands and Promotes Bacterial Trapping"

In a recent study, Douda et al. (1) examined ex vivo–in vivo mouse models to study pulmonary neutrophil extracellular traps (NETs) using LPS-induced lung inflammation and *Pseudomonas* infection and demonstrated that SP-D interacts with bacteria and NETs to augment bacterial trapping. The data from this investigation are interesting, although the roles of these interactions in bacterial killing and clearance were not examined. In another study, SP-D–deficient mice exhibited attenuated bacterial clearance during *Pseudomonas* infection (2). A recent report has shown that NETs are important for *Pseudomonas* killing, ex vivo (3). These findings together suggest that the functions of NETs and the innate immune proteins are more complex in the lungs during bacterial infections than anticipated.

It has been well established that neutrophils are critical contributors to pulmonary host defense, as depletion of neutrophils results in reduction in the clearance of *P. aeruginosa* in the lungs (4). Neutrophils can undergo a necrotic and/or an apoptotic form of cell death. Since 2004, the distinct form of neutrophil death (NETosis) and NET-mediated antimicrobial function have been investigated in detail (5). Although the mechanisms of NETosis are not entirely clear, the roles of PAD4, NADPH oxidase, MAPKs, and IFN-γ are shown to be involved in this process (6). Excessive NETosis may lead to excessive lung damage (7), suggesting that NET formation can be a double-edged sword in infectious settings. Future studies are critical to understand the molecules involved in NETosis to manipulate NETosis for augmenting host defense while attenuating tissue damage via therapies.

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Response to Comment on "Innate Immune Collectin Surfactant Protein D Simultaneously Binds Both Neutrophil Extracellular Traps and Carbohydrate Ligands and Promotes Bacterial Trapping"

Takei et al. (1) of Japan described a novel form of neutrophil death in 1996. This form of neutrophil death was later studied by others and termed as NETosis (death by neutrophil extracellular trap [NET] formation) in 2004 (2–8). Zychlinsky and colleagues (5) conducted many of these studies; they mostly used PMA as an agonist and proved that NADPH oxidases, neutrophil elastases, and myeloperoxidase are important for NETosis. Others showed that peptidyl arginine deiminase PAD4 and citrullination of Histone 3 are also important for NETosis (6). Microbes, microbial components, and other signaling molecules have been considered as biological ligands that could induce NETosis. In vitro studies show that LPS induces NETosis presumably via platelets (7). Our in vivo studies show that LPS and bacteria induce NETosis in the airways (2). Further studies are necessary to identify other NETosis-inducing ligands and NETotic pathways.

Many researchers now accept that a subpopulation of neutrophils undergo NETosis upon encountering specific signals. Physical forces appear to facilitate NETosis. The ability of NETotic neutrophils to kill bacteria under static experimental conditions is similar to that of the live neutrophils, and NET-mediated killing requires physical movement of the cultures (3). These cultures may mimic the situations that neutrophils encounter in circulating blood (7) and breathing lungs (2). Our studies show that soluble fragments of NETs are present in the airways during LPS-induced inflammation and *Pseudomonas aeruginosa* infection of the lungs (2). Whether these NET fragments kill bacteria differently from the elaborate NETs observed during in vitro studies is unknown. As indicated in the comments, it is known that surfactant protein D (SP-D)–defi-

cient mice are defective in clearing *P. aeruginosa* from their lungs. However, the mechanistic details of SP-D–mediated clearance of NET–bacterial complexes are unknown.

Interestingly, Malawista et al. (10) of Yale University conducted a series of studies in the 1980s and showed that enucleated neutrophils "survive" and "phagocytose" microbes. It would be useful to consider these observations in the context of NETosis. Neutrophils have been considered to release soluble proteases, which eventually cause excess tissue inflammation and injury. Recent data show that NETosis also releases "soluble" NET components (2, 4, 5). During NETosis, neutrophil proteases are attached to the chromatin scaffold of the NETs; hence, NETs could be advantageous for the host under physiological conditions. During pathological states, accumulation of excessive NET components including DNA and proteases could be harmful to the tissues [e.g., airways of patients with cystic fibrosis (4)]. Although clearance of NETs has not been systematically studied, DNAse enzyme has been suggested to destroy the NETs (8, 11). Defects in DNAse activity are related to bacterial virulence (8), accumulation of NET components, and autoimmune diseases such as SLE (11, 12). Our studies suggest that SP-D promotes the clearance of NETs by macrophages (9). This could be an alternative way of clearing NETs from the tissues. The field of NETosis is in its infancy and many questions still remain unanswered.

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Comment on "Experimental Arthritis Triggers Periodontal Disease in Mice: Involvement of TNF-α and the Oral Microbiota"

We have read with interest the article by Queiroz-Junior et al. (1) in which TNF was found to mediate periodontal disease during experimental arthritis in mice. The evidence is based on the therapeutic effects of infliximab, a chimeric mAb used in clinic to neutralize human TNF. Although we are not questioning the possible effects of this reagent, we doubt that this could be due to neutralization of murine TNF.

Indeed, initial data on infliximab reported by Centocor (see Refs. 2, 3) indicated that this reagent does not bind and neutralize murine TNF; this is also true for many other neutralizing mAbs against human TNF (A. Kruglov, G. Efimov, and S. Nedospasov, unpublished observations).

In our hands, etanercept, but not infliximab, was able to neutralize murine TNF in the LPS/D-Gal toxicity model in C57BL/6 mice (Fig. 1). However, administration of either infliximab or etanercept was able to rescue transgenic mice expressing human TNF (hTNF/LT Tg^+ mTNF/$LT^{-/-}$) (4) (Fig. 1), showing that infliximab blocks only human TNF in this model. Based on the well-known fact that i.v. injection of

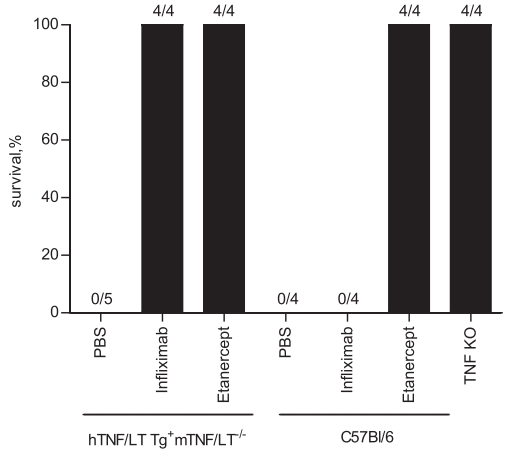

FIGURE 1. Infliximab neutralizes human but not murine TNF in the LPS/D-Gal toxicity model. C57BL/6, hTNF/LT Tg^+ mTNF/$LT^{-/-}$, and TNF KO mice were injected with 10 mcg of LPS and 20 mg of D-galactosamine i.p. Thirty minutes later, infliximab (1 mg/mouse), etanercept (1 mg/mouse), or PBS was administered i.p. Survival of mice was observed during 48 h.

Igs may result in amelioration of inflammation (5) and the fact that Quieroz-Junior et al. used vehicle instead of isotype control Abs for treatment of control group, we suggest that the effects observed in these experiments were not due to TNF neutralization, but perhaps due to the Fc moiety of these chimeric Abs. Other explanations are also possible.

Of note, many experimental studies with arthritis in mice were performed in transgenic model of spontaneous polyarthritis developed by G. Kollias and colleagues (6, 7). In that case, infliximab indeed shows therapeutic effects, but this is due to overexpression of human TNF in this mouse model.

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In our study, an experimental model of Ag-induced arthritis (AIA) in mice was shown to mimic several aspects of the clinical-associated comorbidity periodontal disease (PD), through increased systemic reactivity to auto-Ags in a way dependent on oral microbiota. Moreover, it was shown that TNF-α might play an important role in AIA-induced PD, which was demonstrated by the use of an anti–TNF-α therapy with infliximab (the protocol was based on previous publications listed below).

Infliximab is a chimeric IgG1κ mAb, composed of human and murine regions, raised against TNF-α. Several studies demonstrate that infliximab may neutralize both human and murine TNF-α (1–18). There are many published studies in the literature supporting the effectiveness of using this Ab under different experimental conditions in murine models, including experimental colitis (1, 10), hypothalamic inflammation (2), allergic rhinitis (3), experimental Alzheimer's disease (4), chronic hepatitis C (5), mucopolysaccharidoses (6), aneurysm (7), in vitro conditions by using macrophages (8),murine aortic transplant model (9), HSV-induced inflammation (11), Fas stimulation (12), acute asthma (13), aortic calcification during diabetes (14), and distinct mouse models of joint inflammation (15–18). In several of these studies, the use of isotype controls did not mimic the beneficial effects of infliximab in reducing TNF-α levels and signs of inflammation (4, 7, 9, 15). Under our conditions, although not mentioned in the original study, the use of isotype control Abs in further experiments was not able to prevent signs of AIA and PD. Therefore, we do believe there is enough evidence in the literature to support the use of this tool to investigate the role of TNF-α in mice.

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