論文の内容の要旨

論文題目：**An Essential Role of DNase II in DNA-sensing by TLR9**

**(TLR9 による DNA 認識における DNase II の役割)**

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Toll-like receptors (TLRs) play critical roles in innate immune defense by recognizing various pathogen-associated molecular patterns (PAMPs). Recent evidence suggests that in addition to detecting PAMPs, they can also cause sterile inflammation by reacting to damage-associated molecular patterns that are generated by cell injury. This is especially important for the TLRs that are designated to detect nucleic acids, which are also present within the host cells. Among these TLRs, TLR9 was initially found to sense bacterial or viral DNA. Upon detection of microbial DNA, TLR9 mounts a robust inflammatory response, including the production of inflammatory cytokines, chemokines and type I interferon (IFN). These cytokines in turn activate lymphocytes and the adaptive immune system important for eradicating the invading pathogen. Similarly, such TLR9-dependent inflammatory response can also be driven by non-microbial stimulant. DNA released from cell injury is able to stimulate TLR9 in the presence of carrier proteins. Chronic inflammation results if

these DNA/protein complexes are not removed. Due to the role of TLR9 in regulating systemic inflammation, it is important to understand the detailed mechanism controlling TLR9 responses. Previous studies have shown that DNase II is a lysosomal DNase important for digesting apoptotic DNA engulfed by phagocytes, such as the macrophages. In the absence of DNase II, the accumulated DNA activates cytosolic DNA sensor, resulting in the production of a huge amount of type I IFN, which is fatal. Unexpectedly, TLR9 does not contribute to the inflammation in the absence of DNase II even though it senses DNA in the endolysosomes. These findings lead to the speculation that TLR9 does not simply respond to any DNA that is accumulated in the lysosome. Instead, DNase II may be needed in DNA sensing by TLR9. In the present study, the role of DNase II in TLR9 responses was investigated. We found that in murine bone marrow-derived conventional dendritic cells (BM-cDCs) and plasmacytoid dendritic cells (BM-pDCs), DNase II was indispensable for TLR9 to mount an inflammatory response towards one of its best-known ligands, CpG-A, but not for its another ligand, the CpG-B. Type I IFN production triggered by CpG-A in BM-pDCs also required DNase II. The ability of DNase II in facilitating TLR9 responses was found to be dependent on its DNase activity. DNase II digested CpG-A (a 20-mer) into shorter fragments of around 11-12 nucleotides in length. CpG-A harbors a central palindrome and a poly-G tail which enable the oligo to form nanoparticles in physiological

conditions. It is this natural conformation of CpG-A that enable a potent induction of IFN. As the central palindrome is nuclease-sensitive, DNase II was likely to cleave the central region of CpG-A to generate the short fragments. Therefore, truncated CpG-A fragments were made in an attempt to mimic the product after cleavage by DNase II. Out of the truncated fragments examined, one of them (named A3'11) was found to stimulate TLR9 even in the absence of DNase II. To examine the localization of DNase II, monoclonal antibodies (mAbs) recognizing the endogenous murine DNase II were established. The literature suggests that DNase II resides in lysosome for DNA digestion, however, using the mAbs, it was found that in addition to the LAMP2-positive lysosome, a more significant amount of DNase II was localized in wheat germ agglutinin (WGA)-positive vesicles, a marker for the *trans*-Golgi. However, upon CpG-A stimulation, DNase II recruitment to the LAMP2-positive lysosomes was dramatically enhanced. This was not observed after CpG-B or the A3'11 oligodeoxynucleotide stimulation. These results affirm the notion that DNase II was required in TLR9-mediated CpG-A responses by generating a short DNA fragment stimulatory to TLR9 in lysosomal compartments. Since CpG-A is also widely used as an adjuvant in anti-tumor or anti-viral vaccines, as well as in treating certain diseases, understanding the role of DNase II in CpG-A responses would contribute to novel vaccine design and treatments in the future.