

**Regulation of T Cell Homeostasis by LAG-3 (CD223).**

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LAG-3 is a CD4-related, activation-induced cell surface molecule that binds to MHC class II with high affinity. Previous work done by our group suggests that murine LAG-3 negatively regulates the expansion of activated T cells. Here we show that LAG-3 deficient mice (3-6 months of age) have 2-3 times more CD4<sup>+</sup> and CD8<sup>+</sup> T cells than wild type controls, which is consistent with a comparable increase in the number of proliferating, BrdU<sup>+</sup> T cells *in vivo*. Surprisingly, B cell numbers were also increased even though they do not express LAG-3. Similar observations were also made in LAG-3<sup>-/-</sup> and LAG-3<sup>+/+</sup> mice transgenic for the OT-II TCR (ovalbumin 326-339-specific, H-2A<sup>b</sup>-restricted), except that these differences were evident at 5 weeks of age. To evaluate the effect of LAG-3 on homeostatic T cell expansion, MACS purified naïve LAG-3<sup>-/-</sup> and LAG-3<sup>+/+</sup> OT-II<sup>+</sup> T cells were transferred into lymphopenic, RAG-1<sup>-/-</sup> mice. There were ~5 times more LAG-3<sup>-/-</sup> than LAG-3<sup>+/+</sup> OT-II<sup>+</sup> T cells 7 days post transfer. Greater differences were seen with antigen-experienced cells, where there was ~7 times more LAG-3<sup>-/-</sup> OT-II<sup>+</sup> T cells 15 days post transfer than wild type cells. Importantly, this difference was abrogated by ectopic expression of wild type LAG-3 via retroviral transduction but not by a signaling defective mutant. Finally, increased expansion of LAG-3<sup>-/-</sup> OVA<sup>+</sup> T cells (ovalbumin 257-264-specific, H-2K<sup>b</sup>-restricted) compared with wild type controls was also observed 15 days post transfer into RAG-1<sup>-/-</sup> mice. However, this difference was not seen following transfer into MHC class I<sup>-/-</sup> / class II<sup>-/-</sup> mice, demonstrating the importance of LAG-3:MHC class II interaction in mediating this effect. Taken together, these data support a role for LAG-3 in the negative regulation of CD4<sup>+</sup> and CD8<sup>+</sup> T cell homeostasis.

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