PD1 AND LAG3 CONVERGE TO LIMIT POLYFUNCTIONALITY AND SYSTEMIC IMMUNITY

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Background Targeting PD1 with monoclonal antibodies has yielded clinical success across a variety of tumor types, yet overcoming inhibitory receptor (IR)-mediated tolerance is essential to improve immunotherapeutic responses. LAG3 co-expresses with PD1 on CD8+ tumor-infiltrating T cells (TIL), signifying a highly exhausted phenotype, and dual PD1/LAG3 blockade in C57BL/6 mice enhances antitumor immunity. As CD8+ TIL is the dominant LAG3-expressing TIL population, it is hypothesized that PD1 and LAG3 synergizes to limit CD8+ TIL function controlling antitumor immunity.

Methods To understand the cellular and mechanistic basis for PD1/LAG3 synergy, conditional knock-in mice "surgically dissect" *Pdcd1* and/or *Lag3* floxed alleles restricted to CD8+ T cells expressing E8ICre.GFP. These mice were crossed with the *Pmel* transgene to assess PD1 and/or LAG3-sufficient or deficient gp100-specific CD8+ T cell populations. CD8+ *Pmel* cells were isolated and adoptively transferred into C57BL/6 mice harboring a B16-gp100-overexpressing tumor to observe therapeutic benefit, or to assess T cell functionality within the tumor.

Results While little therapeutic benefit was observed with a prophylactic adoptive transfer of wild-type CD8+ *Pmel* cells into mice which then received B16-gp100 tumor, there was reduced tumor growth in mice receiving PD1-deficient CD8+ *Pmel* cells, which was further enhanced in mice receiving PD1/LAG3-deficient CD8+ *Pmel* cells with long-term tumor-free survival. Likewise, a therapeutic administration of PD1/LAG3-deficient CD8+ *Pmel* cells into mice once the tumor has been established showed an initial therapeutic benefit, with enhanced survival, that was not demonstrated with adoptive transfer of PD1 or LAG3-deficient, or wild-type, counterparts. Each PD1 and/or LAG3-sufficient or deficient CD8+ *Pmel* mice were differentially congenically marked to assess each of the four genotypes that can be adoptively transferred into the same host as a "quad transfer" system. Recovery of these populations within the tumor show that the PD1/LAG3-deficient CD8+ *Pmel* cells out-compete PD1 or LAG3-deficient, or wild-type, counterparts due to enhanced proliferation (Ki67/BrdU). Furthermore, PD1/LAG3-deficient CD8+ T cells were more functional with increased IFN γ and GzmB release observed by flow cytometry.

Conclusions Overall PD1 and LAG3 limit anti-tumor immune effects as removal of both IRs on a gp100 antigen-specific CD8+ T cell population results in reduced B16-gp100 tumor growth and enhanced survival in an adoptive transfer model, as a result of enhanced CD8+ TIL functionality and proliferation. These results provide striking evidence that the development of anti-LAG3 agents in the clinic would yield improved responses with anti-PD1.

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