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Costimulation and host protection against parasites

Regulating the intestinal mucosal Th1 or Th2 immune response is important for enhancing vaccine delivery and affecting inflammatory bowel disease. B7-1/B7-2 interactions with CD28 provide an important target for regulation of the development of effector Th cells from naive Th cells. We have found that B7-1/B7-2 ligand interactions differentially regulate the Th2 response depending on the particular pathogen. In the immune response to the whipworm, *Trichuris muris*, administration of CTLA4Ig (a B7 ligand antagonist) inhibited the Th2 immune response associated with worm expulsion in BALB/c mice and stimulated an alternative response associated with susceptibility and characterized by decreased IL-4, and increased IFN- γ and IL-13. Similar results were observed using CD28KO or B7-2KO mice. The elevated IL-13 (both gene and protein expression) indicated that this Th2 cytokine was regulated independently from IL-4 and that it could be stimulated during an IFN- γ -dominant response. Further studies showed that the protective response that was inhibited following B7 blockade was restored when IFN- γ was inhibited, using blocking Abs or KO mice. Parasite-induced increases in IL-4 and serum IgE remained low in IFN- γ KO mice following B7 blockade, while IL-13 was elevated. Administration of the IL-13 antagonist (IL-13Ra2Fc) blocked protective immunity. These studies indicate that IL-4-mediated protection is B7-dependent whereas IL-13 expression and host protection is B7-independent during the *T. muris* response.

In marked contrast, our studies of the Th2 immune response to another gastrointestinal nematode parasite, *Nippostrongylus brasiliensis*, have shown that T cell dependent host protection and cytokine production but not humoral immunity is B7-independent. B7-1/B7-2 double knockout (dko) BALB/c mice were inoculated with 500 *Nippostrongylus brasiliensis* (NB) larvae, which trigger a Th2 host protective immune response. Surprisingly, worm expulsion and egg production were comparable at day 8 and day 14 after NB-inoculation of BALB/c B7-1/B7-2 dko and WT mice. ELISPOT and quantitative RT-PCR analyses showed pronounced and comparable increases in IL-4 in mesenteric lymph node (MLN) at day 14 after NB-infection in BALB/c B7-1/B7-2dko and WT controls. Treatment with anti-CD4 mAb blocked host protection and elevations in IL-4 in NB-inoculated BALB/c B7-1/B7-2dko and WT controls. Elevations in total serum IgE and antigen-specific IgG1 were inhibited at day 8 and day 14 after NB infection of BALB/c B7-1/B7-2DKO mice but were pronounced in NB-inoculated WT mice.

Previous findings in other laboratories have suggested that IL-4 signaling may substitute for CD28 signaling during a Th2 response. To address this possibility, we developed the novel CD28/STAT6DKO BALB/c mouse. *N. brasiliensis* inoculation of these mice induced pronounced IL-4 production, demonstrating that Th2 cells can develop in the absence of B7-1/B7-2 ligand interactions and IL-4 signaling. Surprisingly, in unimmunized mice, after 3 months of age, all of the DKO mice, but none of the single KO siblings, spontaneously developed an unexpected phenotype characterized by lymphadenopathy, chronic alopecia, and pruritic skin in the head region and associated infection with the mite, *Demodex*. T cells from draining lymph nodes of these DKO mice cultured with anti-CD3 displayed increased proliferation compared to single KO strains, and DKO lymphocytes produced significant IFN- γ , but did not produce IL-4. Both serum IgG1 and IgG2a were elevated. These findings suggest that the Th2 response develops but is not sustained in the absence of STAT6 and CD28 and that blocking both pathways, but not either alone, results in the development of spontaneous nonatopic chronic dermatitis associated with opportunistic mite infections and a chronic type 1 cytokine response. We speculate that a low-level Th2 response may be required to control *Demodex* in mice and that this may have important implications for control of *Demodex* in humans.