Protective Immunity to Nematode Infection Is Induced by CTLA-4 Blockade

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Materials and Methods

The T cell molecule CTLA-4 is expressed at high levels on the surface 48–72 h after T cell activation (1) and functions as a negative regulator of activation (2–5). The genetic deletion of CTLA-4 in mice results in a profound lymphoproliferative disease with some of the hallmarks of autoimmunity (6, 7). Similarly, blockade of CTLA-4 interaction with its ligands CD80 and CD86 during infection of mice with the nematode, Nippostrongylus brasiliensis, CTLA-4 blockade greatly enhanced and accelerated the T cell immune response to N. brasiliensis, resulting in a profound reduction in adult worm numbers and early termination of parasite egg production. The ability of CTLA-4 blockade to accelerate primary immune responses to a protective level during an acute infection indicates its potential as an immunotherapeutic tool for dealing with infectious agents.
Appropriate dilutions of detecting Ab and then peroxidase-labeled streptavidin were added for 1 h at room temperature. Freshly made 1 mM ABTS (100 μl) in citrate phosphate buffer, pH 9.2, and 0.03% H2O2 was added to each well to develop the reaction. The reaction was stopped by adding 100 μl 2 mM NaN3 and the plates read at 414 nM using an Anthos Hill plate reader. Cytokine production is expressed in Genzyme U/ml; the limit of detection for the IL-4 and IL-5 ELISA was >0.2 U/ml and >70 U/ml, respectively.

Results and Discussion

Anti-CTLA-4 Treatment Enhances IL-4 and IL-5 Production In Vivo. To establish whether T cell effector function is altered by blockade of CTLA-4 signaling, we followed IL-4 and IL-5 production in the draining mediastinal and mesenteric lymph nodes of mice infected with N. brugia and receiving weekly injections of anti-CTLA-4 neutralizing mAb. We found that anti-CTLA-4 mAb treatment induced a profound 20-fold increase in IL-5 production (Fig. 1a) and a massive 40-fold increase in IL-4 production (Fig. 2a) from the draining mediastinal lymph node at day 6 after infection compared to mice given control antibody. Significantly, the peak mediastinal cytokine production of both IL-5 and IL-4 during CTLA-4 blockade occurred earlier.
than that in control mice. Increased cytokine production in response to CTLA-4 blockade was due to increased lymphocyte numbers per mediastinal lymph node as well as increased cytokine production. The mediastinal lymph node obtained from mice given anti–CTLA-4 mAb had nearly fourfold more lymphocytes 6 d after infection than control mice (Fig. 3). Cytokine production in mesenteric lymph nodes was also significantly increased with anti–CTLA-4 mAb treatment (IL-5; Fig. 1 b, IL-4; Fig. 2 b) but it is interesting that in this lymph node peak cytokine production occurred at the same time in control and anti–CTLA-4–treated mice. Increased cytokine production from this lymph node reflected increased IL-4 and IL-5 production only as total mesenteric lymph node cell numbers were not significantly different between groups (data not shown). The observation that increased cellularity and cytokine production only occurred in the lymph nodes draining the site of infection and not the inguinal nodes indicated that the effect of CTLA-4 blockade was antigen driven (IL-5; Fig. 1 c, IL-4; Fig. 2 c).

Blockade of CTLA-4 Signals Decreases Parasite Egg Production. To examine whether the accelerated and increased response during anti–CTLA-4 mAb treatment could enhance immunity to the parasite N. b, we examined fecal egg output throughout a primary infection of N. b (Fig. 4 a). CTLA-4 blockade nearly abolished parasite egg production compared to the tens of thousands of eggs produced per day in control mice. Surprisingly, the numbers of eggs produced during treatment with anti–CTLA-4 mAb more closely resembled the low numbers observed after a secondary infection with N. b (Fig. 4 b). Importantly, blockade of CTLA-4 signaling during a primary N. b infection did not adversely affect development of memory to a subsequent infection with N. b (Fig. 4 b). This observation differs from prediction by others that CTLA-4 signaling is required to facilitate the generation of memory T cells (16). Both groups of mice show strong memory responses against N. b as evidenced by the very low level of fecal egg production.

Anti–CTLA-4 Treatment Decreases the Peak Intestinal Worm Burden. The size and characteristics of the adult worm population found in the small intestine during the peak of infection is often used as an indicator of the efficacy of the protective immune response. We found that the number of adult worms residing in the small intestine at the peak of infection was reduced to a 24% take in mice treated with anti–CTLA-4 mAb compared with a 54% take in control.
mice (Table 1). Microscopic analysis of the worms resident in the small intestine revealed that most of the female worms present in the control mice contained the expected 20–30 eggs, whereas many of the female worms present in the anti–CTLA-4 treated mice contained few or no visible eggs. These data suggest that CTLA-4 blockade enhances the immune response against the migratory larvae resulting in a reduction in the number of healthy adults that reach the small intestine, thus reducing both the worm burden and overall fecundity.

It has been suggested that in vivo blockade of the proposed negative signaling function of CTLA-4 could allow greater expansion of antigen-specific T cells and possibly prolong an immune response. Here we report that treatment of mice with anti–CTLA-4 neutralizing mAb during an acute infection with the extracellular parasite N b allows greater expansion and activation of parasite-specific T cells and this results in greatly enhanced protective immunity similar to that observed during a memory response. In addition to the increase in the magnitude of T cell reactivity, an important aspect of the response induced during CTLA-4 blockade is that the kinetics of the anti-parasite immune response is advanced to the point where it is protective. We postulate that the profoundly enhanced pulmonary response observed with anti–CTLA-4 treatment leads to immune damage of the migrating larvae before they reach the intestine, resulting in a lower worm burden and decreased fecundity. This is in concordance with a previous suggestion that damage to migratory larvae while passing through the lungs may be an important factor in determining the health of the adult worm population (17). The greatly enhanced pulmonary immune response also has the potential to cause increased tissue damage to the lung. However, we did not observe any gross increases in pulmonary damage in mice treated with anti–CTLA-4 mAb. If mice are inoculated with a higher concentration of infective larvae, many of them die at the time that the worms pass through the lungs, presumably due to pulmonary damage. Anti–CTLA-4 mAb treatment did not lead to an increase in the number of deaths observed therefore, if there was any increase in tissue damage it did not equal that obtained by a higher infection dose.

Furthermore, although serum IgE levels in the control infected mice increase more than 100-fold, serum IgE levels in anti–CTLA-4 mAb-treated mice were only very moderately increased (approximately twofold) above that observed in the control animals (data not shown). Blockade of CTLA-4 function may therefore provide an important mechanism for enhancing protective immunity against many infectious agents.

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