Comment on "Pretreatment Intracerebral and Peripheral Blood Immune Responses in Vietnamese Adults with Tuberculous Meningitis: Diagnostic Value and Relationship to Disease Severity and Outcome"

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Response to Comment on “Cathepsin-L Influences the Expression of Extracellular Matrix in Lymphoid Organs and Plays a Role in the Regulation of Thymic Output and of Peripheral T Cell Number”

In our article published in The Journal of Immunology in June 1, 2005 (1), we reported that lymph nodes from nackt mice (CTSLnkt/CTSLnkt) are hypertrophied, showing a normal absolute number of CD4+ T cells and a marked increase in the number of CD8+ T lymphocytes. Correlatively, extracellular matrix (ECM) components were found to be increased. Contrarily, in nackt thymus, laminin, fibronectin, and collagen I and IV are markedly decreased, with an augmented output of CD8+ cells. We also reported that a mutated form of cathepsin L can be detected in different organs in nackt mice. These results demonstrate that the nacket mutation in the Ctsl gene influences the levels of ECM components in lymphoid organs, the thymic output, and the number of T cells in the periphery, thus broadly affecting the immune system.

In a letter to the editor, Benavides et al. (2) show that it is possible to detect a mutated cathepsin-L (Ctsltm1Cptr) in nacket mice, indeed confirming our results. As stated in our article, it is clear that the nkt mutation produces a mutated Ctsl devoid of its classical proteolytic activity. The 118-bp deletion of nkt mice (2) involves the end of exon 6 and almost all of exon 7, sequences partially coding for the protein H and L chains. As a consequence of this deletion, a stop codon would appear leading to a truncated peptide lacking the last 60 aa, which are included in the active site of this protease (3). However, they claim that the nacket allele clearly behaves as a recessive loss-of-function mutation where heterozygous mice are phenotypically indistinguishable from wild-type mice and homozygous mutants exhibit the same phenotype as Ctsl knockout (KO) mice. Regarding the differences between wild-type and +/nkt mice, Benavides et al. (2) have reported that the skin of +/nkt mice shows no major difference with that of +/+ mice. However, in our hands, significant differences in the level of expression of integrins between +/+ and +/nkt mice can be detected (Fig. 1; I. Piazzon and I. Nepomnaschy, manuscript in preparation). As an example, data on Fig. 1 show that the percentage of lymph node CD4+ cells expressing high levels of $\alpha_6$ integrin chain expression in CD4+ lymph node cells of +/+, +/nkt, and nkt/nkt mice. Lymph node cells from +/+, +/nkt, and nkt/nkt littermates were stained with anti-CD4 and anti-CD49e and analyzed by flow cytometry. Values represent the mean percentages of cells expressing high levels of $\alpha_6$ integrin chain ± SD (n = 6) within CD4+ cells. * Significantly different from +/+ (p < 0.01); from nkt/nkt (p < 0.01).

Figure 1. $\alpha_6$ integrin chain expression in CD4+ lymph node cells of +/+, +/nkt, and nkt/nkt mice. Lymph node cells from +/+, +/nkt, and nkt/nkt littermates were stained with anti-CD4 and anti-CD49e and analyzed by flow cytometry. Values represent the mean percentages of cells expressing high levels of $\alpha_6$ integrin chain ± SD (n = 6) within CD4+ cells. * Significantly different from +/+ (p < 0.01); from nkt/nkt (p < 0.01).

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Comment on “Analysis of the Cellular Mechanism of Antitumor Responses and Autoimmunity in Patients Treated with CTLA-4 Blockade”

In the December 1, 2005, issue of The Journal of Immunology, Maker et al. (1) conclude that the effects of CTLA-4 blockade are due to increased T cell activation rather than inhibition or depletion of T regulatory cells. The authors examined the effects of anti-CTLA-4 administration on T regulatory cells before and at 3 wk after each dose. They saw no reduction in CD4+CD25+ T cells, and to characterize further the T regulatory cell population, they examined Foxp3 gene expression. Increased Foxp3 expression was observed at 3 wk posttreatment compared with pretreatment levels in purified CD4+CD25+ cells. We believe their conclusion that T regulatory cell depletion is not involved in the mechanism of action of anti-CTLA-4 is flawed by a failure to examine its effect at early time points after administration. We postulated that expression of CTLA-4 on T regulatory cells targeted them for Ab-dependent cytotoxicity. PBMCs from patients with advanced malignancy were examined before, within 1–4 days, and 3–4 wk after anti-CTLA-4 administration. At 1–4 days after administration, there was a significant decrease in the number of T regulatory cells as quantitated by expression of CD4, CD25, and CD62L. However, at the time of administration of the next dose, the number of T regulatory cells had increased above baseline, in agreement with the results of Maker et al. These results were confirmed by TaqMan analysis for Foxp3 expression; a decrease at early times was followed by a rebound increase by the next treatment. This depletion followed by increased levels of Foxp3 assayed by TaqMan mirrored the results from flow cytometry. The dynamic changes in T regulatory cells was missed in Maker’s analysis by restricting data collection time points. We agree, but have been unable to demonstrate, that increased T cell activation is responsible for the antitumor effects of anti-CTLA-4 but believe that transient depletion of T regulatory cells permits activation of T cells that recognize self Ags, which in turn produce autoimmunity and tumor regression.

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Response to Comment on “Analysis of the Cellular Mechanism of Antitumor Responses and Autoimmunity in Patients Treated with CTLA-4 Blockade”

In our article analyzing the cellular mechanisms of antitumor and autoimmune effects of CTLA-4 blockade (1), the cells that were tested were obtained ~3 wk after the last dose of anti-CTLA-4 mAb. All patients had received many doses of anti-CTLA-4 Ab administered every 3 wk, and because the half-life of this Ab is 2–3 wk, it would be expected that any depletion of T regulatory cells would be seen at the 3-wk time point. It is possible, however, as Drs. O’Mahony and Janik point out, that we could have missed a decrease in T regulatory cells at earlier time points. In fact, Drs. O’Mahony and Janik point out that at 1–4 days after administration of anti-CTLA-4 Ab they did see a significant decrease in the number of T regulatory cells.

As a result of their letter, we have performed additional experiments testing the presence of T regulatory cells obtained before and 4 days after administration of anti-CTLA-4 mAb in a patient in our protocol. This patient had received 5 mg/kg anti-CTLA-4 Ab twice and 9 mg/kg twice every 3 wk. Before and 4 days after the fifth dose at 9 mg/kg, blood was obtained and cell analysis was performed simultaneously on these cryopreserved specimens. As in our prior results, there was no evidence of decrease in T regulatory cells. Comparing pre- and post-4-day treatment samples, the CD4+Foxp3+ cells increased from 14.6 to 18.2%, and the percentage of Foxp3+CD25+ cells increased from 23.0 to 34.4%. In addition, we purified CD4+ cells and performed semiquantitative RT-PCR analysis of Foxp3 levels in these cells. Following immunomagnetic bead purification, the CD4 cells isolated by negative selection were 90–94% pure. In samples obtained 4 days after administration of anti-CTLA-4 Ab, the number of Foxp3 mRNA copies per 10^6 β-actin copies increased from 1419 to 1862.

Thus, the results we obtained in this patient studied 4 days after administration of anti-CTLA-4 Ab were similar to those obtained in samples obtained 3 wk following administration of the Ab. Although further studies on samples taken at short times after administration of anti-CTLA-4 Ab are warranted, it seems unlikely to us that the mechanism of action of anti-CTLA-4 Ab administration is due to a decrease of T regulatory cells.

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Comment on “Pretreatment Intracerebral and Peripheral Blood Immune Responses in Vietnamese Adults with Tuberculous Meningitis: Diagnostic Value and Relationship to Disease Severity and Outcome”

Simmons et al. (1) found that in patients with tuberculous meningitis (TBM) CSF concentrations of IL-6 were independently associated with significant neurological deficit (British Medical Research Council grade III). This finding suggested an involvement of IL-6 in the pathogenesis of neurological deficit in TBM. Previous research linked IL-6 to poor outcome in acute ischemic stroke (2), which is a common complication of TBM (3). The authors did not comment on a recent finding in patients with TBM that may explain the increased IL-6 levels in patients with neurological deficit. In a prospective study in children with TBM in Kakinada, India, we found that patients with neurological deficit had significantly higher CSF adenosine deaminase (ADA) levels compared with controls with TBM without this complication (4). ADA is produced by T lymphocytes and monocytes, and it has recently been found that, after binding to the adenosine receptor on dendritic cells, it interacts with CD26 receptors on lymphocytes. This costimulatory signal caused a marked increase in the production of IL-6 by lymphocytes (5). IL-6 and ADA levels correlated significantly in pleural fluid of patients with tuberculous pleuritis (6). Further support for the importance of ADA in IL-6 induction and microvascular compromise comes from trials of ADA inhibition by pentostatin in mice in which ADA inhibition reduced circulating IL-6 levels and parameters of endothelial injury and microvascular dysfunction (7). Future studies need to explore the potential of ADA inhibition with pentostatin, which is approved for use in humans (8), in supportive treatment of TBM.

The author declares that he has no competing interests.

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