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The Cystine/Glutamate Antiporter Regulates the Functional Expression of Indoleamine 2,3-Dioxygenase in Human Dendritic Cells

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To the Editor

The cystine/glutamate antiporter controls the biosynthesis of the major cellular antioxidant glutathione (GSH) by transporting cystine, the rate-limiting precursor of GSH synthesis, into the cell in exchange for glutamate [1]. We recently demonstrated that blocking the function of the antiporter depleted GSH and significantly impaired dendritic cell (DC) presentation of exogenous antigen to T cells via MHC class II and the cross-presentation pathway [2]. To explain this defect, here we test the hypothesis that the antiporter regulates DC expression of indoleamine 2,3-dioxygenase (IDO), an immunosuppressive enzyme that has emerged as a key regulator of peripheral immune tolerance. As the suppressive effects of IDO are largely mediated by DCs, a detailed understanding of how IDO is regulated in these cells is critical for the design of targeted strategies to induce robust immune tolerance.

To quantify changes in IDO mRNA transcript levels following antiporter blockade, we used digital mRNA profiling and compared immature DCs, DCs matured by incubation with LPS (mature DCs) and mature DCs cultured in cystine/cysteine-free medium to prevent antiporter uptake of cystine [2]. Consistent with the observation of others, we found that IDO mRNA transcripts were increased in mature DCs relative to immature DCs [3] (Fig. 1A). In line with our hypothesis, IDO mRNA transcripts were significantly increased in mature DCs cultured in cystine/cysteine-free medium when compared to both immature and mature DCs (Fig. 1A).

As IDO is tightly regulated at the translational and post-translational levels, we next quantified kynurenine levels in DC culture supernatants as a measure of IDO enzymatic activity. To do this, we used a well-established colorimetric method [4]. Antiporter uptake of cystine was inhibited by culturing DCs in cystine/cysteine-free medium for 16 and 24 h or by treating DCs with L-homocysteic acid (LHC), a potent competitive inhibitor of the antiporter that does not serve as a substrate for GSH synthesis [5]. While immature DCs did not exhibit IDO enzymatic activity (not shown), LPS induced a modest increase in IDO activity as previously reported [3] (Fig. 1B). In line with our mRNA data, IDO enzymatic activity was significantly increased when antiporter-dependent uptake of cystine was inhibited (Fig. 1B). Both LHC and cystine/cysteine-free medium significantly increased

IDO enzymatic activity at 16 and 24 h relative to non-treated mature DCs. Taken together, these data raise the possibility that the antiporter functions as a regulator of IDO activity in human DCs and thus may directly control the outcome of DC-T cell interactions.

In summary, unraveling the mechanisms by which peripheral tolerance is regulated has direct relevance for the development of innovative clinical approaches to attenuate autoimmunity, allergy and transplant rejection. Here we identify a novel role for the cystine/glutamate antiporter as a regulator of IDO activity in DCs. These data suggest that the functional expression of IDO may be redox regulated and thus extend our understanding of how IDO may be clinically manipulated for therapeutic benefit.

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Figure 1.

Blocking antiporter function increases the abundance of IDO mRNA transcripts and induces IDO enzymatic activity. DCs were treated with or without LPS for 4 h and then incubated for 16 h in complete medium or in cystine/cysteine-free medium (Cys/s-). IDO mRNA was quantified by digital mRNA profiling and normalized to mRNA encoding the housekeeping genes GAPDH and HPRT1 (n=3) (A). Antiporter uptake of cystine was inhibited by treating DCs with L-homocysteic acid (LHC) or by incubating DCs in cystine/cysteine-free medium. Kynurenine was quantified in culture supernatants using Ehrlich's reagent (n=5) (B).