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Blocking of the B7 -CD28 Pathway Increases Apoptosis Induced in Activated T Cells by In Vitro-Generated CD95L (FasL) Positive Dendritic Cells

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Dendritic cells (DC) that express high levels of MHC class II and costimulatory molecules are the most potent antigen-presenting cells and the only population that can activate naive T cells.[1] Evidence has also accumulated, however, that DC can playa role in tolerance induction. Thus, thymic DC induce central tolerance to MHC antigens.2 The ubiquitous presence of donor DC in long-surviving human and animal organ allograft recipients suggests that DC may hold the key to the induction of transplantation tolerance.3›4 We have reported that costimulatory molecule-deficient DC induce alloantigen-specific unresponsiveness in vitro,[5] and prolong allograft survival.[6,7] The mechanisms by which tolerance can be induced by DC, however, remain unclear. A recent report indicates that $CD8^+$ Fas-L^+ ($CD95L^+$) mouse lymphoid tissue DC can induce apoptosis in activated T cells in vitro.[8] The present study was undertaken to evaluate the capacity of in vitro propagated DC to induce allogeneic T-cell apoptosis and to determine the possible regulatory roles of CD95 (Fas/Apo-1) ligand and B7 molecules expressed on DC.

MATERIALS AND METHODS

Mouse bone marrow (BM) cells from B10.BR. B6. and B6-*gld* mice were cultured with GM-CSF+ IL-4 for 5 days as dcscrihed.5 then further purified either on metrizamide columns or by two-color sorting with MAbs directed against DEC 205 and B7-2 (CD86).9 The phenotype of these cultured DC was confirmed by flow cytometry and morphology. Their potent allostimulatory activity was confirmed by MLR. The apoptotic activity of naive and activated B10.BR and B10 T cells was analyzed by DNA fragmentation10 and JAM assay11 respectively, and by in situ nick-end labeling (TUNEL). Fas L mRNA expression by DC was determined by RT-PCR. Fas L protein on DC was detected by flow cytometry and immunocytochemical staining using mouse Fas-Fc fusion protein (Immunex, Seattle, Wash), or polyclonal rabbit anti-Fas L antibody (N-20: Santa Cruz Biotechnology, Santa Cruz, Calif), that recognizes the NH_2 terminus of Fas L. In addition, Fas L was detected by Western blotting using anti-Fas LAb. The fusion protein mCTLA4-Ig (from Dr Peter S. Linsley, Bristol Myers Squibb, Seattle, Wash) was used in blocking experiments to determine the role of B7 molecules in the regulation of DC-induced T cell apoptosis.

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RESULTS AND DISCUSSION

As described previously,[9] the purity of GM-CSF + IL-4 stimulated DC defined by morphology and cell surface immunophenotype was 90% to 95%. These cells were highly efficient inducers of primary allogeneic T cell responses in MLR. Their allostimulatory activity, however, was inhibited in a dose-dependent manner by the addition of mCTLA4-Ig at the start of cultures. The expression of message for Fas L by cultured DC was confirmed by RT-PCR analysis. Levels of Fas L mRNA were similar to those expressed by Con A-activated T cells. Flow cytometric analysis using Fas-Fc showed that the DC were positive for Fas L. Further immunocytochemical analysis of permeabilized cells using anti-Fas L Ab supported the RT-PCR data and gave reactivity: normal spleen cells < Con A blasts = cultured DC. The addition of a competitive peptide, which corresponded to amino acids 2–19 at the NH_2-terminus of Fas L and cross-reacted with mouse Fas L, totally inhibited staining of DC with anti-Fas L antibody, verifying the specificity of the antibody reaction with the cells.[12]

To test the apoptosis-inducing function of the cultured Fas L^+ DC, Fas^+ Jurkat T cells were used as targets in a 18 hour JAM assay. DC propagated from wild-type (B6) mice induced 43.8 ± 1.4% DNA fragmentation at 5:1 ratio (E:T). This was reduced to 27.5 ± 2.7% by adding Fas-Fc fusion protein (10 μg/ml) at the start of the culture. The DC grown from FasL-deficient B6-*gld* mice induced only 1.5 ± 0.5% DNA fragmentation at the same E:T ratio. These results indicated the functional expression of Fas L on DC. B10.BR DC induced comparable low levels of apoptosis both in syngeneic or allogeneic Con A-activated normal T cells (14.4 ± 0.8% DNA fragmentation in 48-hour ConA-BI0 T cells and 12.2 ± 1.7% in ConA-B10.BR T cells, respectively). Naive T cells were resistant to apoptosis induced by DC. Interestingly, CTLA4-Ig (200 ng/mL), which totally inhibited T cell proliferative responses in MLR, enhanced DNA fragmentation of Con A activated target cells by 2- to 4-fold. These data indicated that CD28 costimulation could protect activated T cells from apoptosis and that the apoptotic effect of DC was not MHC restricted.

Compared with DC from wild-type (B6) mice, DC from B6-*gld* mice induced higher allostimulatory responses in MLR, but a lower incidence of apoptosis in activated T cells by TUNEL assay. However, similarly increased levels of apoptosis were induced by B6-DC and *gld*-DC effectors when the CD28/B7 pathway was blocked by CTLA4-Ig. It therefore appears that Fas/Fas L is not the only molecular pathway via which DC can induce apoptosis, (TNF-mediated apoptosis is one potential mechanism13) and that CD28 costimulation may play an important counter-regulating role in T cell survival.

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