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Nitric Oxide Production by Dendritic Cells is Associated With Impairment of T Cell Responses

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DENDRITIC cells (DC) are potent antigen presenting cells,[1] with important roles in both immunity and tolerance. Nitric oxide (NO) is an important effector molecule with myriad effects on the immune system. To date there have been no accounts describing NO generation by well-characterized DC. We describe the release of NO by DC propagated from mouse bone marrow in response to GM-CSF and IL-4. IFN-γ and LPS induced DC NO production. This was blocked by the inhibitor of nitric oxide synthase (NOS), N^G-monomethyl-L-arginine (NMMA). In addition, NO was present in the supernatants of primary mixed leukocyte cultures containing relatively high concentrations of DC compared to purified allogeneic T cells. Blockade of NO release by NMMA in these cultures was associated with an increase in T cell proliferation. It was also demonstrated that NO induced apoptosis in DC in a dose-dependent fashion. NMMA inhibited this phenomenon. These observations suggest that NO may be an important modulator of DC-T cell interactions.

MATERIALS AND METHODS

Bone marrow was harvested from B10.BR ($H2^k$, $I\text{-}E^k$) and C57BL/10J (B10; $H2^b$, $I\text{-}A^b$) mice. DC culture was modified after the method described by Inaba et al.[2] DC were propagated in 24 well plates in RPMI-1640 supplemented with 10% v/v fetal calf serum, nonessential amino acids, sodium pyruvate, L-glutamine, penicillin-streptomycin, and 2-ME. 1000 U/mL r mouse GM-CSF, and 1000 U/mL r mouse IL-4 were added at the start of culture. Fresh GM-CSF and IL-4-supplemented media was added to the wells every other day after discarding half of the supernatant. Nonadherent cells released from proliferating cell clusters were harvested after 5 to 7 days of culture.

The DC population was purified by metrizamide column separation as described.[3] Immunophenotypic analysis was performed on a FACScan flow cytometer using the rat MAb NLDC-145, (generously provided by Dr R. M. Steinman, Rockefeller Univ which recognizes a 205-kDa protein DEC-205 expressed on the surface of mouse DC.[4] DC were sorted using a FACStar plus cell sorter to attain a purity >95%.

DC were cultured in the presence of LPS and IFN-γ to induce NO synthesis. NMMA was added to selected cultures (0.5 mM). Sorted DC or fresh splenocytes were irradiated to serve as stimulators in MLR. T cells (2×10^5) purified through nylon wool columns were cultured with varying concentrations of stimulators in complete medium. Selected cultures contained NMMA to block NO production. Cells were labeled with [^{3}H]-thymidine during the final 18 hours of culture, and then harvested to determine the degree of thymidine incorporation. NO production was determined in culture supernatants with a colorimetric assay based on the Griess reaction.[5] Visualization of the inducible NOS protein was performed with a rabbit polyclonal antibody to iNOS. Apoptosis was identified in DC stimulated to produce NO by IFN-γ and LPS in culture using DNA polymerase and biotin-labeled dUTP to identify DNA strand breaks characteristic of apoptosis as described elsewhere.[6] Biotin-dUTP was detected with peroxidase-labeled avidin followed by an enzyme reaction using DAB as substrate.

RESULTS

Purification of DC cultures generated a homogeneous population of >95% purity, free of contaminating macrophages and B cells. Sorted DC were more potent stimulators of naive T cells when compared to presorted cells. When purified DC were cultured for 48 hours in the presence of LPS and IFN-γ, NO release was detected in the supernatant. NO generation induced by these agents was inhibited by addition of the NOS inhibitor NMMA at the start of cultures. The interaction between DC and allogeneic T cells at relatively high stimulator:responder ratios stimulated NO synthesis. The concentration of NO accumulating in the MLR correlated with the number of DC present in the culture. Addition of NMMA at the initiation of culture inhibited NO release. The induction of the iNOS enzyme could be localized to a subpopulation of DC with in situ staining for iNOS. Lymphocyte proliferation in the MLR correlated inversely with the concentration of NO in the supernatant. The presence of NMMA resulted in higher T cell proliferation. No release of NO was detected in the abscence of T cell activation by DC (as in the syngeneic MLR, or when using costimulatory molecule deficient 'immature' DC).

DNA strand breaks were identified by in situ nick translation in DC stimulated to produce NO by IFN-γ and LPS.

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0041-1315/97/$17.00
PII S0041-1345(96)00458-7

Furthermore, addition of NMMA to DC cultures containing these reagents inhibited apoptosis.

DISCUSSION

No study to date has conclusively demonstrated the production of NO by purified DC. We present evidence for the presence of an inducible NOS in DC which can be activated by IFN-γ and LPS. NO synthesis also occurs upon interaction of DC with allogeneic T cells. NO production in this model is inhibited by NMMA. The functional significance of NO production by DC is unclear. Several events which appear to be influenced by NO have been presented here. NO release downregulates T cell proliferation at high DC:T cell ratios in the MLR. It also induces apoptosis in DC, thereby conceivably removing DC from further participation in immune activation. NO may prevent maturation of DC into mature antigen presenting cells, as evidenced by the capacity of alveolar macrophages to inhibit maturation of DC in a NO dependent manner.[7] NO production by DC may have implications for the regulation of immunologic responses.

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