Farmacologia Cardiovascolare dei Donatori di Ossido d'Azoto (NO): Prospettive Terapeutiche
6° Convegno Monotematico SIF – Milano, 31 Maggio 2002

NITRIC OXIDE-RELEASING NONSTEROIDAL ANTI-INFLAMMATORY DRUGS ABROGATE IMMUNE DYSFUNCTIONS CAUSED BY MYELOID SUPPRESSOR CELLS IN TUMOR-BEARING MICE

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A generalized suppression of CTL anamnestic responses occurs in mice bearing large burden of experimental tumors. Immunosuppression depends upon the accumulation of $CD31^+/CD11b^+/Gr\text{-}1^+$ myeloid suppressor cells (MSC) in secondary lymphoid organs, which in turn is mediated by high systemic concentrations of GM-CSF released by growing tumors[1]. We previously established cloned myeloid suppressor lines as a tool to dissect the inhibitory pathway used to restrain T lymphocyte activation. We found that these lines inhibited mitogen-driven T cell responses by a nitric oxide (NO) dependent mechanism, but we also disclosed a second mechanism employed to block the proliferation of T cells stimulated by allogeneic antigens. By comparing freshly isolated MSC and immortalized cell lines, we showed that enzyme activities of the IL-4-inducible isoform of liver-type arginase (Arg1) and the inducible nitric-oxide synthase (iNOS) were critically required to inhibit alloreactive T lymphocytes generated in mixed leukocyte cultures (MLC). Arg1 over-expression induced in MSC by cytokines released from TH2 lymphocytes augmented the production of super oxide anion via the iNOS reductase domain. Thus, our findings identified a novel category of drugs affecting the L-arginine metabolism of MSC that could be used to subvert the immunosuppressive state in tumor-bearing patients. Unfortunately, several drugs acting upon Arg1 have substantial side effects when administered systemically. We employed simple *in vitro* assays to test rapidly novel molecules interfering with the suppressive activity of MSC. MLC were set up in the absence or in the presence of an amount of γ-irradiated MSC sufficient to suppress completely the proliferation of alloreactive T lymphocytes. In a second assay, γ-irradiated MSC were added to an MLC and the lymphocytes recovered after 5 days of culture were tested for the ability to recognize and kill allogeneic targets. Different nitric oxide-releasing nonsteroidal anti-inflammatory drugs were tested, at various concentrations, to compare their relative efficacy. NCX4060 proved to be the compounds most effective in restoring *in vitro* the proliferative and cytolytic response of lymphocytes stimulated in the presence of different MSC. Moreover, this molecule restored the compromised immune functions in two strains of mice (C57BL/6 and BALB/c) that had been inoculated with tumors, a melanoma and an adenocarcinoma, whose growth induces appearance of MSC and immune unresponsiveness. Finally, when inoculated intraperitoneally in mice bearing an established adenocarcinoma, NCX4060 caused a reduction in the tumor size associated with extensive tumor necrosis, restored the compromised alloreactive response, and allowed the appearance of T lymphocytes recognizing an antigen expressed by the tumor, as revealed by staining with MHC-tetramers. To date, no other single molecule, which can be administered *in vivo*, was found to possess these properties: NCX4060 might thus represent the prototypes of a novel class of immune-modulator drugs to be used alone or in combination with cancer vaccines for the therapy of cancer.

References

1. Bronte, V., P. Serafini, E. Apolloni, and P. Zanovello (2001). Tumor-induced immune dysfunctions caused by myeloid suppressor cells. *J Immunother*. 24, 6:431