Immunosuppressive Agents Enhance the Cytokine-Induced Priming of Inflammatory Cells

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Key Words

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Abstract

The influence of the immunosuppressive substances cyclosporin A (CsA), FK506 and rapamycin on inflammatory mediators from cytokine-primed human leukocytes was studied. The cells were primed with granulocyte/macrophage-colony-stimulating factor, granulocyte-stimulating factor (G-CSF) and interleukin-3 (IL-3), and subsequently stimulated with the chemotactic peptide fMLP. The immunosuppressive agents CsA and FK506 enhanced the release of leukotriene B₄ (LTB₄) from human neutrophil granulocytes dependent on the priming with G-CSF and IL-3. IL-3-primed neutrophils released up to threefold higher amounts of LTB₄ after subsequent stimulation with FK506 and fMLP. In contrast, the immunosuppressant rapamycin inhibited the formation of LTB₄ after subsequent stimulation with fMLP. In addition to the well-documented inhibitory effects on lymphocyte function, our results indicate an enhancing effect of the immunosuppressive agents on the cellular inflammatory response of human peripheral blood neutrophils.

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The molecular mechanisms of immunosuppression induced by cyclosporin A (CsA), FK506 and rapamycin are still not completely elucidated. Their ability to prevent graft rejection obviously resulted from the inhibition of distinct enzymatic phosphorylation/dephosphorylation mechanisms (calcineurin, p70S6-kinase) [1], which regulate cyto-kine gene expression or cytokine-induced cellular responses. These drugs exert their major therapeutic effects by inhibiting T cell activation, cytokine release and cyto-kine receptor expression. However, the relative concentrations of different mediators (including lipid mediators and histamine) determine the induction, support or recovery from acute and chronic inflammation. Therefore, we studied the influence of CsA, FK506 and rapamycin on the generation of arachidonic acid-derived lipid mediators, as well as the release of the neutrophil-activating peptide interleukin (IL)-8 and the release of the preformed inflammatory mediator histamine in a model of cytokine-primed peripheral blood leukocytes (PMNs). The cells were primed with the growth factors granulocyte/macrophage-colony-stimulating factor (GM-CSF), granulocyte-colony-stimulating factor (G-CSF) and IL-3, and subsequently stimulated with fMLP. The immunosuppressive

agents CsA and FK506 enhanced the release of leukotriene B₄ (LTB₄) from human neutrophil granulocytes dependent on the priming with G-CSF and IL-3. IL-3-primed PMNs released up to threefold

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Table 1. Effect of immunosuppressive agents on LTB₄ formation by PMNs

higher amounts of LTB₄ after subsequent stimulation with FK506 and fMLP. In contrast, the immunosuppressant rapamycin inhibited the formation of LTB₄ after priming with G-CSF and IL-3 and a subsequent stimulation with fMLP (table 1). CsA as well as rapamycin enhanced the release of IL-8 from GM-CSF-primed PMNs, spontaneously or after stimulation with fMLP. We observed no effect of FK506 on IL-8 release from cytokine-primed PMNs. Similar effects were obtained after priming the cells of the lymphocyte/ monocyte/basophil (LMB) fraction of peripheral blood. With regard to histamine release from the LMB fraction, enhanced mediator release was observed at lower concentrations of rapamycin (500 pg/ml) and CsA (5 ng/ml); in contrast, there was no significant effect of FK506 (5 ng/ml) on the release of histamine from a cytokine-primed LMB fraction. To elucidate the molecular mechanisms of this enhanced mediator release, we studied the translocation of low-molecular-weight G proteins (p21^G) after stimulation with CsA, FK506 or rapamycin. An increase in translocation of the p21^G protein to the membrane fractions was obtained in stimulated PMNs. The cells from the LMB fraction were about 1,000-fold more sensitive to the immunosuppressive substances than the neutrophils. It was recently demonstrated that CsA exerts anti-inflammatory effects by inhibiting the release of preformed and de-novo-synthesized mediators from human basophils [2, 3]. Dependent on the priming of human neutrophils with a growth factor (IL-3), we detected an enhancing effect of CsA towards a subsequent stimulation with fMLP. Obviously, the observed enhancing as well as suppressive effects of the three immunosuppressive drugs on leukotriene formation from human neutrophil granulocytes are dependent on the pattern of colony-stimulating factors and cytokines in the microenvironment.

PMNs (1 × 10⁶/500 µl) were primed for 20 min with the indicated cytokines. CsA, FK506 or rapamycin (all at 500 ng/ml) were also added. As a subsequent stimulus, fMLP (10 µM) was used for at least 30 min (in the presence of calcium (1 mM) and magnesium (0.5 mM)). LTB₄ was analyzed by reverse-phase HPLC. The means ± SD of at least three independent experiments are shown. * = Significantly different compared to the control without immunosuppressant.

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