Ciclosporin A Inhibits Mediator Release from Human FcεRI⁺ Cells by Interacting with Cyclophilin

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Ciclosporin A (CsA), a natural cyclic undecapeptide originally used to prevent human organ transplant rejection [1], has more recently proved to be effective in the treatment of such autoimmune diseases as type I diabetes [2], uveoretinitis [3], rheumatoid arthritis [4] and Crohn's disease [5]. The mechanism of action of CsA is largely unknown. Part of the immunosuppressive effect of CsA is generally attributed to the inhibition of lymphokine mRNA transcription [6]; however, CsA appears to have multiple sites of action and could exert its effects by binding to specific cytoplasmic receptor(s). Following the purification by Handschumacher et al. [7] of cyclophilin, a cytoplasmic protein which possesses peptidyl-prolyl-isomerase (PPIase) activity [8, 9], it was suggested that CsA binding to PPIase/cyclophilin is a critical step in the molecular mechanism of action [10]. We examined the effect of CsA and of a series of analogs (CsG, CsC, CsD and CsH), which bind with decreasing affinity to cyclophilin [11], to evaluate the involvement of this protein in the release of chemical mediators from human inflammatory cells possessing high affinity receptors for IgE (FcεRI⁺ cells) (i.e., basophils and mast cells). Pharmacological concentrations (8–250 nM) of CsA concentration-dependently inhibited (5–60%) the release of histamine and the de novo synthesis of peptide leukotriene C₄ (LTC₄) from human basophils (fig. 1) and mast cells isolated from lung parenchyma challenged with anti-IgE. When basophils were activated by the ionophore A23187, which bypasses the channel-operated influx of Ca²⁺ [12], CsA was more potent (93 vs. 60%) and more effective at low concentrations (IC₅₀ = 24 vs. 100 nM). The inhibition of CsA on mediator release was extremely rapid, similar to the rate of inhibition of PPIase activity [8, 9], and was not abolished by washing the cells before anti-IgE or A23187 challenge. The direct activation of different protein kinase C isoforms induced by phorbol myristate (TPA) and bryostatin 1 [13] was unaffected by preincubation with ciclosporins, which suggests that CsA affects biochemical step(s) that are bypassed by direct PKC activation.

The CsA analogs showed a remarkable degree of selectivity in inhibiting the release of histamine from basophils. In particular, CsH has a very low affinity for cyclophilin [11] and did not inhibit the release of histamine and LTC₄ from human FcεRI⁺ cells challenged with anti-IgE or A23187. The rank order of potency CsA > CsG > CsC – CsD > > CsH we observed is consistent with their different affinity for cyclophilin. There was a correlation between the concentrations of CsA isomers that inhibited by 30% histamine release induced by both IgE cross-linking ($r = 0.99$; $p < 0.001$) and Ca²⁺ translocation by A23187 ($r = 0.87$; $p < 0.001$) and their affinity for cyclophilin.

In conclusion, our findings are at variance with the notion that the immunosuppressant effect of CsA necessarily involves the regulation of gene transcription and protein synthesis [6] and suggest that CsA, in addition to its effects on lymphokine gene expression [6], can also interfere with other biochemical events underlying the release of proinflammatory mediators presumably by interacting with PPIase/cyclophilin.

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CONTROL
CsA, 80 μM
CsA, 250 nM
.0025

0 10 20
Elution time (minutes)
20

Fig. 1. Reverse phase HPLC of anti-IgE-stimulated supernatants of purified basophils. Cells were incubated for 5 min with buffer (control) or with CsA (80 and 250 n M) before the addition of anti- $\frac{1}{8}$ E (1 μg/ml). At the end of incubation (45 min) supernatants were extracted and concentrated. Samples were applied to a reverse phase HPLC column and leukotrienes were detected by their UV adsorbance at 280 nm. The migration of synthetic leukotrienes and PGB₂ standards is indicated at their retention times.

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