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Effects of treatment with azathioprine and cyclosporin A on interferon-γ production by peripheral blood leukocytes of renal allograft recipients

W. Weimar[1], P. Kramer[1], A.M. Dekkers-Bijma[2] and J. Jeekel[1]

[1]*Renal Transplantation Unit, University Hospital Rotterdam-Dijkzigt, and* [2]*Laboratory for Experimental Surgery, Erasmus University Rotterdam, Rotterdam, The Netherlands*

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Summary

We have studied the concanavalin A (ConA)-induced interferon gamma (IFN-γ) production of peripheral blood mononuclear cells (PBMC) of renal allograft recipients. Both under immunosuppressive treatment with azathioprine and with cyclosporin A (CsA) the PBMC of these patients proved deficient for IFN-γ production when compared to those of healthy controls. After conversion from conventional azathioprine to CsA medication the ConA-induced IFN-γ production increased.

interferon-γ; azathioprine; cyclosporin A

Introduction

Viral infections remain a major clinical problem in immunosuppressed patients. Nearly all transplant recipients will sooner or later suffer from infection with one of the members of the Herpetoviridae: herpes hominis type I and II, cytomegalo, varicella zoster and Epstein–Barr (EBV) viruses, or the Papovaviridae: papilloma/polyoma, BK and JC viruses. After primary infection these DNA viruses persist in the body in a latent state, as the result of active immunoregulatory mechanisms. The immunosuppressive regimens used in transplantation medicine disturb these well-balanced host–parasite relationships, allowing the viruses to reactivate and to invade various cells and tissues in their hosts. Primary infections, especially with cytomegalovirus, occur when organs from seropositive donors are transplanted into seronegative acceptors. Although many of the ensuing infections run an asymptomatic course, these viral attacks may result in direct virus disease, in modulation of the immune system and in

oncogenesis. A compromised interferon (IFN) system could be the basis for this virus-related morbidity. Both the IFN-α and the IFN-γ production are deficient in peripheral blood mononuclear cells (PBMC) of renal allograft recipients conventionally treated with azathioprine and steroids [1,2].

Hirsch et al. [3] demonstrated that prophylactic IFN-α administration can prevent viral reactivation syndromes after kidney transplantation, providing further evidence for the prominent role of the IFN system in controlling virus disease.

Cyclosporin A (CsA) is a new potent immunosuppressive agent widely used in transplantation medicine. Recently it has been reported that patients treated with CsA show a significantly lower incidence of cytomegalovirus infection than do those on azathioprine therapy [4]. This could be explained by a relatively less vigorous suppressive effect of CsA on the IFN system. In in-vitro systems CsA has no effect on IFN-α or IFN-β production. However, it can markedly decrease mitogen- or alloantigen-induced IFN-γ production [5,6]. This interference with IFN-γ production could well be of clinical relevance, particularly in view of the increased risk for cancer in immunocompromised patients. Defects of IFN-γ production have been described in patients with EBV-related lymphomas [7]; also this type of malignancy has been reported to occur in transplant recipients treated with CsA [8].

Long-term follow-up studies of large numbers of CsA-treated patients are not yet available. Penn found no statistically significant difference in incidence of lymphomas between patients treated with CsA and conventional therapy, but noticed that these tumors appeared in a remarkably short time (median 6 months) in patients on CsA medication [9]. One could hypothesize that a CsA-induced IFN-γ deficiency is responsible for the occurrence of EBV-related lymphomas and then one would prefer to use the immunosuppressive protocol that least affected IFN-γ production in vivo. Therefore, we compared the IFN-γ production capacity of PBMC of renal allograft recipients treated with the two alternative immunosuppressants: azathioprine and CsA.

Experimental

We measured the IFN-γ production capacity of PBMC of 17 healthy controls and 31 recipients of cadaveric renal allografts with life-sustaining kidney function and treated with azathioprine and steroids. In 16 patients we studied the effect of conversion of the immunosuppressive medication from azathioprine to CsA under the same dosage of steroids. Serum trough levels of CsA were measured by radioimmunoassay and kept between approximately 100 and 200 ng/ml. IFN-γ was induced as described before [2]. In short, 10^6 viable mononuclear cells were cultured for 3 days in the presence of 5, 7.5 and 10 μg/ml concanavalin A (ConA). Antiviral activity of supernatants was measured in a cytopathic effect reduction assay by a dye uptake method [10] using HEp-2 cells (ATCC-CCL23), challenged with vesicular stomatitis virus. Antiviral activity was sensitive to heat and pH 2. Concurrent phytohemagglutinin A (PHA)-induced blastogenesis was measured by [^{3}H]thymidine incorporation. PHA was added in 9 concentrations ranging from 0.075 to 10.0 μg/ml.

PBMC of the 17 healthy controls produced a median of 120 U of IFN-γ per 10^6 cells

(range 40–640) (Fig. 1). This proved significantly different ($P<0.01$; Mann–Whitney–Wilcoxon test) from the values obtained for 31 renal allograft recipients on azathioprine and prednisone medication who produced a median of 15 U of IFN-γ per 10^6 cells (range < 10–160). In 12 of the 31 patients none of the concentrations of ConA was able to induce any measurable IFN-γ. When we leave these 12 non-responders out of the analysis, the yields in the responding PBMC cultures of transplant patients (median 30 U, range 10–160) remained significantly lower than those of normal blood donors. For various reasons 16 patients were switched from azathioprine to CsA medication. This was done in 6 patients with Cushingoid syndromes at their own request and in the expectation that the steroid medication could be discontinued in the rong run, in 6 patients because of low grade humoral rejection and in 4 patients because of liver function disturbances possibly related to azathioprine.

As illustrated in Fig. 2A, one week after this conversion an increase in IFN-γ production was found in 10 of these 16 patients and when the IFN yields were corrected for the absolute number of PBMC per ml of blood, an 8-fold (median 4) increase in production was seen in 13 out of the 16 patients (Fig. 2B). The 3 remaining patients, already totally deficient for IFN-γ under azathioprine therapy, remained non-responsive under CsA. 4 patients, whose PBMC produced no measurable IFN-γ when under azathioprine therapy, became responders after conversion to CsA medication. Although PBMC of 8 patients showed normal IFN-γ synthesis when stimulated with ConA, PBMC of allograft recipients on CsA therapy as a group still produced significantly smaller ($P<0.01$; Mann–Whitney–Wilcoxon test) amounts of IFN-γ per 10^6 cells (median 20 U, range < 10–320) than PBMC of controls (Fig. 1). We found no correlation between maximum ConA-induced IFN-γ production and maximum PHA-induced blastogenesis ($r = -0.01$).

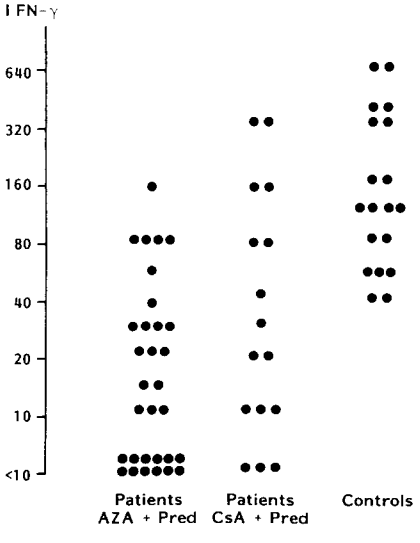

Fig. 1. ConA-induced IFN-γ production by PBMC of renal transplant patients and control blood donors (patient treatments: azathioprine (AZA); prednisone (Pred); cyclosporin A (CsA). Yields expressed as units/10^6 cells.

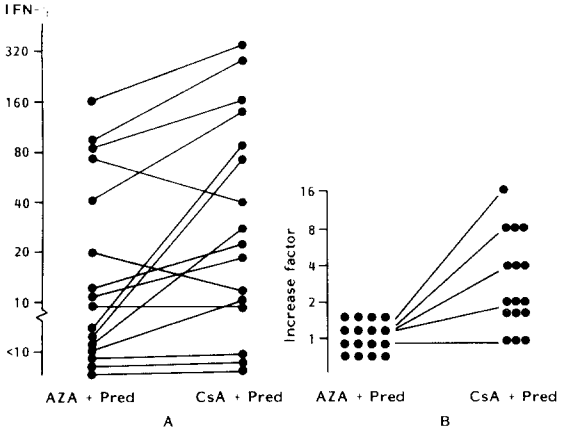

Fig. 2. Effect of converting patients from azathioprine to CsA therapy on IFN-γ production by PBMC. (A) Yields expressed as units/10^6 cells. (B) Yields corrected for number of cells per ml blood and referred to initial value as 1.

Discussion

In this study we found that PBMC of renal transplant recipients under immunosuppression therapy have a low IFN-γ production capacity when stimulated in vitro with ConA. This response to ConA does not seem to be an all-or-none event, as has been described by Vervliet et al. [11] for PBMC of multiple sclerosis patients.

In our study the interferon yields in the responding PBMC cultures of patients were significantly lower than those from normal blood donors. The deficiency was apparent in patients under both azathioprine and CsA therapy, but the effect was more pronounced in the azathioprine-treated group. About 40% of the patients in this group showed a total deficiency of IFN-γ production capacity of PBMC, where only 3 of 16 patients showed this defect after conversion from azathioprine to CsA medication.

Our results seem to be in disagreement with most studies on the effect of CsA on IFN-γ synthesis in vitro, where a much more dramatic inhibition of IFN-γ production has been found [5,6,12,13]. This may be explained by the distribution and binding of CsA in blood. At 37°C, 40% of the circulating drug is found in erythrocytes, 5% in the lymphocytes and granulocytes each, and the remaining 50% is distributed in serum [14]. Because of its hydrophobicity, around 98% of CsA in serum is firmly bound to proteins, especially to lipoproteins. Only a small fraction of the total CsA in blood is therefore unbound and bioavailable. However, in in-vitro studies CsA is added into serum-free media or in RPMI supplemented with 5–10% human or fetal calf serum and also often in higher concentrations (500–1000 ng/ml) than aimed at in clinical situations. Obviously, in vitro the amount of free CsA available for its effect on PBMC by far exceeds the amount of unbound CsA in vivo. Therefore, when we compare the results of this study on the IFN-γ production capacity of PBMC from CsA-treated patients we should pay attention to those in-vitro experiments where CsA has been

added in concentrations comparable to the unbound serum levels obtained in patients. Abb et al. [5], Kalman and Klimpel [6] and Reem et al. [15] reported dose–response studies from which can be concluded that CsA only completely inhibits IFN-γ synthesis in unphysiological concentrations. Smaller amounts of CsA only partially affected IFN-γ production, which is in agreement with this study.

The lower incidence of viral infections in transplant recipients treated with CsA as compared to those treated with azathioprine may not have to be explained only on the basis of insensitivity of IFN-α and IFN-β production to CsA, but also by the less profound impairment of IFN-γ production by CsA as compared to azathioprine therapy. The difference in effect between the two drugs could be of a merely quantitative nature. Routinely, because of its nephrotoxicity, CsA is given in a dosage aimed at a 'therapeutic window' of 100–200 ng/ml serum, while azathioprine is administered in a standard dose of 2–3 mg/kg body-weight, titrated according to total peripheral white blood cell count. The latter procedure might lead more easily to over-immunosuppression, which in turn may be reflected in a more profoundly diminished IFN-γ production. However, we found no correlation between IFN-γ production and PHA-induced blastogenesis which is a parameter of immunoreactivity. Reem et al. [12] also reported that mitogen-induced proliferation and IFN-γ synthesis of T-cells are not linked events.

Alternatively, the difference in effect between azathioprine and CsA may have to be explained in terms of specificity of their modes of action. Unlike azathioprine, CsA more selectively inhibits certain immunological reactions, e.g. primary T-cell activation and antigen- of mitogen-triggered release of lymphokines such as interleukin-2 (IL-2) and IFN-γ from activated T-cells. CsA does not interfere with other T-cell effector functions, for example the expression of cytotoxic activity or clonal expansion under the influence of IL-2. It is conceivable that CsA also does not affect IFN-γ synthesis of IL-2-activated T-cells, as has been suggested by Kalman and Klimpel [16], but this has not been confirmed by Reem et al. [15]. Indeed, the dose–response experiments in vitro and the pattern of IFN-γ response frequencies observed in this study make an explanation in terms of specificity unlikely.

Therefore we conclude that the difference between azathioprine and CsA treatment in their effects on IFN-γ production capacity is the result of the more favorable balance of CsA treatment between the doses needed in vivo to inhibit transplant rejection crises and other effects on the immune system including IFN-γ synthesis. In this respect the cell-mediated cytotoxicity to CMV-infected cells is not abrogated by doses of CsA capable of blocking alloreactive reactions in mixed lymphocyte response systems [17]. The antiviral defense against Herpeto- and Papovaviridae is largely dependent on cell-mediated immunity and on the IFN system, and both these immunoreactivities are less affected by CsA than by azathioprine therapy. This may explain the lower incidence of viral infections in transplant recipients when CsA is used for immunosuppression. In view of the bioavailability of CsA in vivo, the results of in-vitro studies on immunosuppressive actions of CsA should be interpreted with care and are not to be directly extrapolated to in-vivo situations.

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